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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 239-2460. Evaluation of the Following Study: Paraquat: Combined Toxicity and Carcinogenicity Study in Rats. Report No. 82/ILY217/328; Life Science Research, Stock, England; October 27, 1983.

Tox Chem. No. 634
Project No. 73

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and

Theodore Farber, Chief
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Toxicology Branch/HED completed an evaluation of the above study in which Fischer 344 rats, 70 males and 70 females per dose level, were fed unrestricted diets containing technical grade paraquat for 113 to 117 weeks (males) and 122 to 124 weeks (females). The levels of paraquat cation fed were 0 (Group 1), 0 (Group 2), 25 (Group 3), 75 (Group 4) and 150 (Group 5) parts per million. Assuming that, for an

* "Approximate Relation of Parts Per Million in Diet to mg/kg/day." APPRAISAL OF THE SAFETY OF CHEMICALS IN FOODS, DRUGS AND COSMETICS. Published by the Division of Pharmacology, Food and Drug Administration (1959).

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older rat, 1 ppm = 0.05 mg/kg/day*, these values correspond to 0, 0, 1.25, 3.75, and 7.5 mg of paraquat cation per kilogram of body weight, per day, respectively. The incidence of all lesions (except ocular lesions) was reported initially without stating the numbers of organs (or tissues) examined at each dose level. This information was submitted later in a document entitled "Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985. The following findings should be noted:

1. Toxicology Branch/HED regards 25 ppm of paraquat cation (lowest level fed) as an approximate systemic NOEL.* Although some effects (mostly lenticular changes) were observed at that level in the male and female rats, they were either minimal or occurred mostly after 104 weeks of treatment and considered, therefore, to be only an acceleration of the normal aging process (and not a qualitatively different effect). Until that time, or through most of the life span of the animals, a NOEL was in fact 25 parts per million.

   Other effects observed at the 25 ppm level were either minimal (subpleural foci or areas at the termination of the study) or were ambiguous (slight hydrocephalus in the females and spinal cord cysts/cystic spaces in the males, both in rats dying during the study).

   * Considering the above findings, a true systemic NOEL was, therefore, probably very slightly below 25 parts per million. (For the purpose of calculating an ADI, an increased safety factor, higher than the usual 100, may be used in order to compensate for this slight uncertainty in the NOEL).

2. Paraquat was oncogenic in the lungs of rats. There was a dose-related increase in the incidence of pulmonary neoplasms (adenomas and carcinomas), but especially adenomas, in the male and female rats. The incidence of pulmonary neoplasms (number of lungs with neoplasms/number of lungs examined during the course of the study) and the percentage of lungs with neoplasms (shown in parentheses) were 4/139 (2.9), 4/70 (5.7), 6/70 (8.6) and 7/69 (10.1) in the control, low-dose, mid-dose and high-dose male groups, respectively. The corresponding values for the female groups were 0/139 (0), 2/70 (2.9), 3/70 (4.3) and 10/70 (14.3), respectively. A statistically significant ($P < 0.0001$) increase was observed only in pulmonary adenomas and only in the high-dose female rats. Most of the neoplasms were observed at the termination of the study. However, according to Life Science Research (England; the testing laboratory), there were difficulties in characterizing pulmonary lesions as non-neoplastic or

neoplastic, or as adenomas or carcinomas and lung tissue was, therefore, examined microscopically by four independent pathologists.

3. There was a higher incidence of squamous cell carcinomas in the head region (middle ear, hard palate, head tissue and skin), an uncommon tumor, in the paraquat-treated male and female rats, but the incidence was generally low and dose-unrelated. The incidence of these carcinomas (number of rats with carcinomas/number of rats examined during the course of the study) and the percentage of rats with carcinomas (shown in parentheses) were 3/140 (2.1), 3/70 (4.3), 0/70 (0) and 6/70 (8.6) in the control, low-dose, mid-dose and high-dose male groups, respectively. The corresponding values for the female groups were 0/140 (0), 0/70 (0), 3/70 (4.3) and 2/70 (2.9), respectively. All of these carcinomas but one (in a high-dose male rat) occurred between week 53 and termination of the study. (Single tumor was observed at the terminal sacrifice).

4. The incidence (number of organs or tissues with neoplasms/number of organs or tissues examined x 100) of benign pheochromocytomas in adrenal medulla, thyroid parafollicular adenomas and skin tumors of epithelial origin (papilloma and/or squamous cell carcinomas) was increased in the high-dose male rats. There was also an increased incidence of lipomas (subcutaneous fat cell tumor) in the paraquat-treated male rats.

The relationship of these neoplasms and those listed above (3.) to treatment with paraquat is presently unclear. Additional data were therefore requested in order to evaluate adequately the oncogenic potential of paraquat in organs or tissues other than lungs.

5. The predominant ocular lesions detected ophthalmoscopically in the control and the paraquat-treated male and female rats were lenticular opacities and cataracts. These lesions were either not observed or were observed infrequently before the treatment week 103.

Ophthalmoscopical examinations showed that paraquat enhanced the development of ocular lesions in all of the treated groups. At test week 103, dose-related statistically significant ($P < 0.001$) increases in the incidence of ocular lesions were observed only in the mid-dose and high-dose male and female groups. In the case of the mid-dose groups, there were about as many opacities as there were cataracts. However, only one opacity and all cataracts were observed in each of the high-dose groups.

After test week 103, increases in the incidence of ocular lesions (mostly opacities) occurred also in the control and the low-dose groups.

Opthalmoscopic findings were confirmed by histopathological examination.

6. There were increases in the incidence of non-neoplastic lesions in the lungs of the mid-dose and/or high-dose male and female rats when these rats were compared with the controls. These lesions included (in the order of predominance) increased alveolar macrophages, slight peribronchiolar lymphoid hyperplasia and alveolar epithelialization (males only). Increases in the incidence of these lesions were observed in rats which died during the course of the study (unscheduled deaths) and in those sacrificed at the termination of the study. An increase in the incidence of alveolar macrophages was also noted in the high-dose females which were sacrificed after 52 weeks of treatment (scheduled interim sacrifice).

7. The chronic feeding study was classified as Core-Guideline.

8. Because of missing data (additional data were requested; see point 4 above), the oncogenic study was classified as Core-Supplementary. This classification will be updated upon receipt and evaluation of the requested data.

9. The most important findings are summarized on pages 66 to 75 of the review (attached).

Study Type: Chronic feeding/Oncogenic

Study Title: Paraquat: Combined Toxicity and Carcinogenicity Study in Rats. Report No. 82/ILY217/328

Accession Numbers: 252372-252384 (Volumes I, IIA, IIB, III, IVA, IVB, VA, VB, VI, VIIA, VIIB, VIIIA and VIIIB).

Record Number: 122123

Sponsor: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England; for Chevron Chemical Company, Richmond, California.

Testing Lab: Life Science Research, Stock, England

Date of Final Report: October 27, 1983

Test Material:

Paraquat (1,1'-dimethyl-4,4'-bipyridylium) dichloride, Batch No. S 358; technical grade - a brown, aqueous solution containing 32.69% w/w of paraquat cation, pH 3.0, specific gravity 1.138, molecular ratio of the dichloride salt/cation: 1.382. A detailed analysis of the test material was submitted. The test material was stored in brown glass bottles at room temperature and was checked for chemical stability (content of paraquat ion) before the study was started, during the study (after test weeks 8, 40, 50, 84 and 104) and after the study was terminated.

PROTOCOL

I. Experimental Design

Rats used in this study were obtained in two batches, Batch 2 arriving one week after Batch 1. Treatment of male and female rats with paraquat was started on 4/6/78 (Batch 1) and 4/13/78 (Batch 2). The interim sacrifice of the rats from Batch 1 (5 males and 5 females/dose level) and Batch 2 (5 males and 5 females/dose level) occurred on 4/10/79 and 4/18/79, respectively. The terminal sacrifice of the male rats took place during 6/9-7/24/80 and of the female rats during 8/12-8/21/80.

Fischer 344 strain of rats, 70 males and 70 females/dose level, were fed diets containing 0 (Group 1), 0 (Group 2), 25 (Group 3), 75 (Group 4) and 150 (Group 5) ppm of paraquat cation (nominal concentration) for 113-117 weeks (males) and 122-124 weeks (females). Each test group included 35 males and 35 females from Batch 1, and 35 males and 35 females from Batch 2. Additional rats, 5 males and 5 females/dose level, were included in Groups 1, 3, 4 and 5 in order to determine paraquat concentration in tissues after one year of exposure. (These rats were obtained from Batch 1.) The rats were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts, U.S.A. and weighed 60-70 g on arrival to the testing laboratory. The acclimation period was 7 days. The animals were housed in groups of 5/sex/cage and were numbered by ear notching. The temperature of the housing area was 21° ± 2° C and the relative humidity 55% ± 15%.

The rats were fed unrestricted amounts of Spratt's Laboratory Diet 2 (composition was not reported), Spratt's Patent Ltd., Barking, Essex, England. The diet was obtained as a powder and was fed in this form with or without added paraquat. The basic diet was monitored for chlorinated hydrocarbons, polychlorinated biphenyls, organophosphorus and aflatoxin by the suppliers. The experimental diets were analyzed by the testing laboratory as follows: 1) for homogeneity with respect to paraquat cation, using diets prepared for the 1st and 44th treatment weeks; 2) for paraquat stability over 14 days, using diets prepared for the first week of treatment; and 3) for paraquat concentration, every two weeks. During the test weeks 27, 53, 79, 107 and 124, the sponsor analyzed diets for the concentration of paraquat. Water was checked for contamination with chlorinated hydrocarbons, polychlorinated biphenyls and organophosphorus on 7, 6 and 5 occasions, respectively, during the treatment period. These analyses were performed by the testing laboratory.

According to the initial protocol, this study was to be terminated after 104 weeks. However, because of the low number of deaths during that period, the study was extended until at least 113 weeks for the male rats and 122 weeks for the female rats, that is, until survival was reduced to 50% in any one of Groups 1-4. (Group 5, the high-dose group, was excluded from consideration).

According to the testing laboratory, the selection of dose levels was based on the results of a preliminary study referenced as LSR Report Nos. 78/ILY144/111 and 78/ILY144A/388. However, these reports were not submitted.

II. Parameters Examined

1. Observation for toxic signs and mortality

Rats were observed twice daily through test week 118 and once daily thereafter. All rats were also palpated weekly to detect superficial tumors.

2. Food and water consumption

Food consumption was determined weekly for each cage of rats and water intake was observed daily. Quantitative water intake was determined over 3-day periods during weeks 1-4, 13, 26, 41, 52, 65, 78, 92 and 101. Two cages of rats/dose level/sex were used for these determinations.

3. Body weight

Individual body weights were obtained on day 1 of treatment, weekly for the first 12 test weeks, biweekly through week 68 and then weekly until the termination of the study.

4. Food utilization

Food utilization was evaluated from the ratio of food consumed to body weight gained. Weekly calculations were performed through test week 12 and overall calculations for test weeks 1-12, 13-26, 27-40 and 41-52.

5. Achieved dosage

Paraquat intake in terms of mg of paraquat cation/kg body weight/day was calculated for the same intervals as those used for recording individual body weights.

6. Ophthalmoscopy

Both eyes of all rats were examined with a Fison's binocular indirect ophthalmoscope before initiation of treatment and at test weeks 103, 110, 112 or 113 (males) and 118 or 119 (females). Twenty male and 20 female rats were also examined in a similar manner at test weeks 4, 14, 26, 52 and 79. The rats selected were "the last surviving 20 males and 20 females from each group (ten of each sex from each batch of animals)."

7. Hematology

Hematology was performed for 10 male and 10 female rats (5 of each sex from each batch) before initiation of treatment and for 10 males and 10 females/dose level (5 of each sex from each batch) after test weeks 14, 26, 40, 53, 66, 79, 92, 102, 111 or 112 (males) and 118 or 119 (females). The following determinations were performed: hemoglobin and mean cell hemoglobin concentrations; RBC, platelet, reticulocyte, leucocyte and differential leucocyte counts; packed and mean cell volumes; and prothrombin and partial thromboplastin times.

The following additional determinations were also performed:

Determination	Test week	Rats used	Test group
Platelet count and prothrombin and partial thrombo-plastin times	17	5 males	2 and 4
Reticulocyte count	29	5 males & 5 females	1 and 5
		5 females	4
Prothrombin and partial thrombo-plastin times	29	5 females	1 and 4
	54	5 males	1 and 3
		5 females	1, 2, 3, 4 & 5
	96	5 females	2 and 3

Hematology was also performed for rats which were sacrificed in extremis. All procedures were either described or referenced.

8. Clinical chemistry

Urea and glucose concentrations, and alanine aminotransferase and aspartate aminotransferase activities were determined for 10 male and 10 female rats (5 of each sex from each batch) before initiation of treatment and for 10 males and 10 females/dose level (5 of each sex from each batch) after test weeks 14, 27, 40, 53, 66, 79, 92, 102, 111 or 112 (males) and 118 or 119 (females). These determinations were performed on blood serum before the initiation of treatment and on blood plasma after the treatment with paraquat was started. All procedures were referenced.

Clinical chemistry tests were also performed for all rats which were sacrificed in extremis. Additional tests (alkaline phosphatase activity and total and direct bilirubin concentrations) were conducted for some of these rats* because of clinical signs during the closing stages of the study. (*2, 1, 2, 2 and 1 rat from Groups 1M, 2M, 3M, 4M and 5M, respectively and single rats from Groups 1F, 3F and 5F.)

9. Urinalysis

Urine was obtained from 10 male and 10 female rats (5 of each sex from each batch) before initiation of treatment and from 10 males and 10 females/group after test weeks 13, 15, 26, 39, 52, 65, 77, 78, 92, 101, 112-113 (males) and 119 (females). During treatment, rats from Batches 1 or 2, or 1 and 2 were tested at each time interval. During urine collection, rats were placed in the individual metabolic cages and had no food or water.

Samples of urine were centrifuged and the supernatant was examined for volume, pH, specific gravity, total reducing substances, glucose, protein, ketones, bile pigments, urobilin and blood pigments. In all instances, reagents prepared by the Ames Company, Slough, England, were used. The sediment was examined microscopically for epithelial cells, polymorphonuclear and mononuclear leucocytes, RBC, casts, crystals and other abnormalities.

10. Gross necropsy

Gross necropsy was performed on all rats, that is, those sacrificed after 52 weeks of treatment (10 males and 10 females/group); those sacrificed in extremis or found dead; and those sacrificed at the termination of the study. It was reported in a great detail how necropsy was performed. Rats were killed by carbon dioxide asphyxiation.

11. Organ weights

The following organs were weighed at the terminal and interim sacrifices: adrenal, pituitary and thyroid glands, brain, heart, kidneys, liver, lungs, ovaries, testes, spleen and thymus. The ratio of organ weight to body weight was also calculated for each rat at these sacrifices.

12. Histopathology

The following tissues were examined histopathologically: adrenal, Harderian, mammary, pituitary, salivary, thyroid, thymus and prostate glands; epididymides, ovaries, testes, seminal vesicles, urinary bladder, uterus and uterine cervix; cecum colon, duodenum, ileum, jejunum and stomach; aortic arch, heart, sciatic and optic nerves, eyes, kidneys, liver, pancreas, skeletal muscle, skin, bone, spleen, lymph nodes (cervical and mesenteric) and middle ear; brain and spinal cord (cervical, thoracic and lumbar); tongue, esophagus, trachea, lungs and turbinal epithelium; tissue masses, suspected tumors and regional lymph nodes; and blood and bone marrow smears. These tissues were obtained from all animals used in this study, with the exception of the animals which were used for the determination of paraquat concentration in tissues. Tissues found abnormal during gross necropsy were also examined.

After removal, eyes were placed in Davidson's fluid and other tissues in buffered 4% formaldehyde saline. Tissues were subsequently dehydrated, embedded in paraffin, sectioned 5 μ thick and stained with hematoxylin and eosin.

Blood and bone marrow smears were prepared from all rats other than those found dead in their cages. Both smears were air-dried, fixed in absolute methanol and then stained by a May-Grunwald-Giemsa procedure. Blood was obtained from the tail vain and marrow from the femoral bone.

Tumors were classified by the criteria of the International Agency for Research on Cancer (Lyon), under the auspices of W.H.O.

13. Paraquat concentration in tissues, plasma and urine

Paraquat concentrations in tissues and plasma were determined after 52 weeks of treatment, using 5 male and 5 female rats from each test group. In each group, rats with the highest ID numbers were used.

Paraquat concentration in urine was determined after 15, 27, 41, 52, 65, 79 and 102 weeks of treatment, using pooled samples obtained from 5 rats/group/batch at each time interval. Samples were collected from 9 AM to 5 PM under conditions of food and water deprivation, and were stored at -4°C until assayed.

Paraquat cation was determined by a radioimmuno assay in plasma, urine, liver, lungs, kidneys and skin.

14. Statistical analysis

Statistical evaluations were performed as follows:

"Inter-group variations in mortality and incidence of palpable masses were assessed by 2 X 2_2 contingency tests (used as two-tailed tests), deriving a Chi^2 value. This test was supplemented, for analysis of mortality in relation to evaluation of tumor distribution, by Cox's test, incorporating Tarone's test for linear trend. The analysis of incidences of pathological change was performed using 2 X 2 contingency tables deriving a value for Chi^2 or by computing the exact probability of the distribution (Fischer's exact test-2 tailed).

Each treated group was compared with the combined incidence in the control groups.

The analysis of incidences of pulmonary tumors was performed by trend test and Fischer's exact test (using the National Cancer Institute Statistics Computer programme).

Statistical evaluation, by Student's t-test using a pooled within-group error variance, was performed on the following:

Overall food consumption
Body weight (at weeks 26, 52, 78, 104, 113 or 117 and 122)
Hematology
Blood chemistry
Absolute organ weights
Body weight-relative organ weights

Some organ weight variations were also assessed by analysis of co-variance with body weight.

Unless stated otherwise, group meant values were not significantly different from controls ($P > 0.05$).

Armitage's test was used to evaluate trends in the normoblasts recorded during the examination of blood smears taken after 113 or 122 weeks of treatment in males and females respectively."

References:

Armitage, P. Biometrics 11, 375-386 (1955).
Cox, D.R. J. Roy. Stat. Soc. Ser. B. 34, 187-220 (1972).
Tarone, R.E. Biometrika 62, 679-682 (1975).

RESULTS

I. Clinical signs

Clinical signs were reported in a tabular form for each test group and also individually for each animal involved in this study. The following signs were observed most frequently in the male and female rats which were found dead or which had to be killed during the study, and in those which were sacrificed at the termination of the study:

Genitourinary staining	Ocular discharge
Anal staining/diarrhea	Necrotic/ulcerated masses
Nasal staining	Palpable masses
Pallor	Eye opacity
Lethargy	Abdominal masses/distension
	Vaginal discharge

Other toxic signs (hypothermia, yellow color of skin, lack of muscle tone and respiratory distress) were observed most frequently in the male and female rats which died or had to be sacrificed during the study. Ptosis and/or swollen eyelids were observed mostly in the female rats at the terminal sacrifice. The following signs were observed predominantly in the male and female rats which were sacrificed after 52 weeks of testing: anal and nasal staining, and ocular discharge. With the exception of eye opacity, respiratory distress and possibly ptosis/swollen eyelids and palpable masses, the observed clinical signs did not appear to be treatment-related. These signs occurred with either the same frequency at all dose levels or with lesser frequency in the paraquat-treated than in the control groups. The incidence of eye opacity, respiratory distress and ptosis is summarized in Table I.

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Table I. Incidence of eye opacity, respiratory distress and ptosis/swollen eyelids in male (M) and female (F) rats[a].

Test Groups[b]	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
	Rats dying during the study[c]									
Number of rats examined	31	29	37	35	27	32	30	31	31	31
Number of rats with eye opacity[d]	1	1	2	1	7	1	1	1	4	8
Percent of rats with eye opacity	3.2	3.4	5.4	2.9	25.9	3.1	3.3	3.2	12.9	25.8
Number of rats with resp. distress	2	2	7	5	7	8	8	8	6	7
Percent of rats with resp. distress	6.4	6.9	18.9	14.3	25.9	25.0	26.7	25.8	19.3	22.6
	Rats sacrificed at termination of the study									
Number of rats examined	29	31	23	25	33	28	30	29	29	29
Number of rats with eye opacity[d]	3	0	1	4	15	2	4	5	10	27
Percent of rats with eye opactiy	10.3	0	4.3	16.0	45.5	7.1	13.3	17.2	34.5	93.1
Number of rats with ptosis/swollen eyelids	0	1	0	0	0	2	2	1	3	4
Percent of rats with ptosis/swollen eyelids	0	3.2	0	0	0	7.1	6.7	3.4	10.3	13.8

a. This table is based on TABLES 3A and 3B and APPENDIXES 2A, B, C, D, E and F of the submission. The values in the table are the numbers of rats showing a given clinical sign at any time during the treatment period.
b. Groups 1, 2, 3, 4 and 5 were fed 0, 0, 25, 75 and 150 ppm of paraquat ion, respectively.
c. Rats which had to be sacrificed or were found dead in their cages.
d. These were cage-side observations, made without use of an ophthalmoscope.

These data show that male and female rats receiving 150 ppm of paraquat and female rats receiving 75 ppm of paraquat had higher incidence of eye opacity than did the controls during the study and at the termination of the study. A slightly higher incidence of eye opacity was also observed in the low-dose females and the mid-dose males when these groups were compared with the controls at the termination of the study.

Paraquat-treated male rats which were found dead or had to be sacrificed during the study had a higher incidence of respiratory distress than did the controls, but the incidence was not dose-related. A slightly higher incidence of ptosis and/or swollen eyelids was observed only in the mid-dose and high-dose female rats at the terminal sacrifice, when these rats were compared with the controls.

Eye opacities were first noted most frequently during the last 26-34 weeks of treatment (test weeks 91-124). In the case of the high-dose females which were found dead or had to be killed during the study and the mid-dose and high-dose females which were sacrificed at the termination of the study, both eyes were generally opaque. Similar findings were reported for the high-dose males at the terminal sacrifice. In the case of the remaining animals with eye opacities, only one eye was generally opaque. These data are summarized in Tables II and III.

Table II. Onset of opacities in single eyes[a]

Test weeks	0-52	53-70	71-80	81-90	91-100	101-110	111-120	121-124
Groups	Rats dying during the study[b]							
1M	1							
2M	1							
3M	1				2			
4M	1							
5M		3	1		1	1	1	
1F		1					1	
2F							1	
3F	1							
4F	1				1	1	1	
5F		3				1	9	
	Rats sacrificed at termination of the study[c]							
1M	1	1				1		
2M								
3M	1							
4M	1			2			1	
5M					2	11	6	
1F	1						1	1
2F			1				3	1
3F	1					2	2	
4F					1	1	8	6
5F		1		1	1	3	39	9

a. This table is based on APPENDIXES 2A, B, E and F of the submission. According to APPENDIXES C and D, only two rats had opaque eyes at the interim sacrifice (after 52 weeks). One rat with an opaque eye was in Group 4M and another in Group 5M. Numbers in this table represent single eyes.

b. Rats which were found dead or had to be sacrificed.

c. Male rats were sacrificed after 113-117 weeks of treatment with paraquat. Female rats were sacrificed after 122-124 weeks of treatment with paraquat.

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Table III. Numbers of male (M) and female (F) rats with one or both opaque eyes[a]

Paraquat ion (ppm)	0	0	25	75	150	0	0	25	75	150
Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Opaque eyes	Rats dying during the study[b]									
Right only	1				6		1			2
Left only		1	1	1	1			1	2	1
Both			1			1			2	5
	Rats killed after 52 weeks (interim sacrifice)									
Right only					1					
Left only				1						
	Rats killed at termination of the study									
Right only	2			1	6			1	1	
Left only	1		1	3	5	1	3	4	3	
Both					4	1	1		6	27

a. This table is based on APPENDIXES 2A, B, C, D, E and F of the submission.
b. Rats which were found dead or had to be sacrificed.

Data reported for the rats with palpable masses included animal's number, location of each mass, week of appearance and disappearance (when a mass could no longer be detected), size at appearance and size at necropsy. These data are summarized in Tables IV, V and VI.

Table IV. Incidence of palpable masses in male rats[a]

Paraquat ion (ppm)	0	0	25	75	150
Group	1M	2M	3M	4M	5M
	Rats dying during study[b]				
° Total number of rats which died[b]	31	29	37	35	27
° Number of rats with masses observed at anytime before death	23	21	28	29	21
° Percent of rats with masses	74	72	76	86	79
° Number of rats with masses observed at necropsy	15	16	20	21	14
° Percent of control[c]	-	-	129	135	90
° Percent of rats with masses	48	55	54	60	52
° Numbers of masses observed in rats at anytime before death	43	40	46	62	46
° Number of masses/rat[d]	1.9	1.9	1.6	2.1	2.2
° Numbers of masses observed at necropsy	21	19	29	32	23
° Percent of control[c]	-	-	145	160	115
° Number of masses/rat[e]	1.4	1.2	1.4	1.5	1.6
	Rats killed at the termination of the study				
° Total number of rats killed	29	31	23	25	33
° Number of rats with masses observed at anytime before sacrifice	22	24	17	24	26
° Percent of rats with masses	76	77	74	96	79
° Number of rats with masses observed at necropsy	16	15	16	17	16
° Percent of control[c]	-	-	103	110	103
° Percent of rats with masses	55	48	70	68	48
° Numbers of masses observed in rats at anytime before sacrifice	50	50	35	60	60
° Number of masses/rat[d]	2.3	2.1	2.0	2.5	2.3
° Number of masses observed at necropsy	26	22	25	32	27
° Percent of control[c]	-	-	104	133	112
° Number of masses/rat[e]	1.6	1.5	1.6	1.9	1.7

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Table IV. (Cont'd) Incidence of palpable masses in male rats[a]

Paraquat ion (ppm)	0	0	25	75	150
Group	1M	2M	3M	4M	5M
	Rats killed after 52 weeks				
° Total number of rats killed	10	10	10	10	10
° Number of rats with masses observed at anytime before sacrifice	1	1	1	1	0
° Number of rats with masses observed at necropsy	1	1	1	1	0
° Numbers of masses observed in rats at anytime before sacrifice	1	1	1	1	0
° Numbers of masses observed at necropsy	1	1	1	1	0
Summary of data					
° Total number of rats examined grossly	70	70	70	70	70
° Number of rats with masses observed at anytime before sacrifice	46	46	46	54	47
° Number of rats with masses observed at necropsy	32	32	37	39	30
° Percent of control[c]	-	-	116	122	94
° Numbers of masses observed in rats at anytime before death	94	91	82	123	106
° Number of masses/rat[d]	2.0	2.0	1.8	2.3	2.3
° Number of masses observed at necropsy	48	42	55	65	50
° Percent of control[c]	-	-	122	144	111
° Percent of all masses	51	46	67	53	47
° Number of masses/rat[e]	1.5	1.3	1.5	1.7	1.7

a. This table is based on APPENDIXES 3A, B, C, D, E and F of the submission.
b. These rats were either found dead or had to be killed (were moribund) during the course of the study. All of these rats were necropsied.
c. An average of values reported for Groups 1M and 2M was used as a control value.
d. Ratio of Numbers of masses observed in rats at anytime before death/Numbers of rats with masses observed at anytime before death.
e. Ratio of Numbers of masses observed at necropsy/Numbers of rats with masses observed at necropsy.

Data summarized in Table IV show the following:

1. When all rats with palpable masses and all palpable masses are considered, there were about 20% more rats* and about 100% more masses** in the controls and the paraquat-treated groups during the course of the study than at the time of death and necropsy. In many instances, masses observed during one week were not observed during the following week, or several weeks later, or at necropsy. In other instances, necropsy revealed that the observed masses were actually fatty tissues and/or prominent mammary tissues.
(* 13% and ** 49% in Group 3M).

2. Among the male rats dying during the study (unscheduled deaths), the low-dose and mid-dose groups had 29 and 35%, respectively, more rats with palpable masses than did the controls. These rats had also 45% (low-dose group) and 60% (mid-dose group) more masses at the time of their death than did the controls. However, the high-dose male group had 10% fewer rat with palpable masses and only 15% more palpable masses than did the controls.

3. Among the male rats sacrificed at the termination of the study, only the mid-dose group had slightly more (10%) rats with palpable masses, observed at necropsy, than did the controls. However, the same group had also 33% more masses when related to the controls.

4. Paraquat caused small, dose-related increases in the numbers of palpable masses per rat, in the male rats dying during the study. There were 1.3, 1.4, 1.5 and 1.6 palpable masses per rat in the control, low-dose, mid-dose and high-dose groups, respectively, when the rats were necropsied. These values represent increases of 7.6, 15.4 and 23.1%, respectively, when related to the control value (1.3 masses/rat).

   Male rats in the control, low-dose, mid-dose and high-dose groups, sacrificed at the termination of the study, had 1.55, 1.60, 1.90 and 1.70 palpable masses per rat, respectively, when they were necropsied. These values represent increases of 3.2, 22.6, and 9.7%, respectively, when related to the control value (1.55 masses/rat).

5. Paraquat had no effect on the incidence of palpable masses in the male rats which were sacrificed after 52 weeks of testing (scheduled sacrifice, 10/group).

During their lives, male rats with palpable masses in the control and the paraquat-treated groups had generally 1-3 masses each, but a few rats (1-2/group) had 4, 5, 6 or 8 masses each. At the time of death, most rats had 1-2 masses each.

Table V. Incidence of palpable masses in female rats[a]

Paraquat ion (ppm)	0	0	25	75	150
Group	1F	2F	3F	4F	5F
	Rats dying during study[b]				
° Total number of rats which died[b]	32	30	31	31	31
° Number of rats with masses observed at anytime before death	22	23	23	22	17
° Percent of rats with masses	69	77	74	71	55
° Number of rats with masses observed at necropsy	15	14	15	15	15
° Percent of control[c]	-	-	103	103	103
° Percent of rats with masses	47	47	48	48	48
° Numbers of masses observed in rats at anytime before death	43	61	42	44	32
° Number of masses/rat[d]	2.0	2.7	1.8	2.0	1.9
° Numbers of masses observed at necropsy	26	25	19	22	22
° Percent of control[c]	-	-	75	86	86
° Number of masses/rat[e]	1.7	1.8	1.3	1.5	1.5
	Rats killed at the termination of the study				
° Total number of rats killed	28	30	29	29	29
° Number of rats observed at any time before sacrifice	22	24	25	27	24
° Percent of rats with masses	79	80	86	93	83
° Number of rats observed at necropsy	21	16	21	20	20
° Percent of control[c]	-	-	114	108	108
° Percent of rats with masses	75	53	72	69	69
° Numbers of masses observed in rats at anytime before sacrifice	53	56	67	67	42
° Number of masses/rat[d]	2.4	2.3	2.7	2.5	1.8
° Number of masses observed at necropsy	32	31	37	35	29
° Percent of control[c]	-	-	117	111	92
° Number of masses/rat[e]	1.5	1.9	1.8	1.8	1.5

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Table V. (Cont'd) Incidence of palpable masses in female rats[a]

Paraquat ion (ppm)	0	0	25	75	150
Group	1F	2F	3F	4F	5F
	Rats killed after 52 weeks				
° Total number of rats killed	10	10	10	10	10
° Number of rats with masses observed at anytime before sacrifice	1	0	0	0	1
° Number of rats with masses observed at necropsy	0	0	0	0	0
° Numbers of masses observed in rats at anytime before sacrifice	1	0	0	0	1
° Numbers of masses observed at necropsy	0	0	0	0	0
Summary of data					
° Total number of rats examined grossly	70	70	70	70	70
° Number of rats with masses observed at anytime before death	45	47	48	49	42
° Number of rats with masses observed at necropsy	36	30	36	35	35
° Percent of control[c]	-	-	109	106	106
° Numbers of masses observed in rats at anytime before death	97	117	109	111	75
° Number of masses/rat[d]	2.2	2.5	2.3.	2.3	1.8
° Numbers of masses observed at necropsy	59	56	56	57	52
° Percent of control[c]	-	-	97	99	90
° Percent of all masses	61	48	51	51	69
° Number of masses/rat[e]	1.6	1.9	1.6	1.6	1.5

a. This table is based on APPENDIXES 3A, B, C, D, E and F of the submission.
b. These rats were either found dead or had to be killed (were moribund) during the course of the study. All of these rats were necropsied.
c. An average of values reported for Groups 1F and 2F was used as a control value.
d. Ratio of Numbers of masses observed in rats at anytime before death/Numbers of rats with masses observed at anytime before death.
e. Ratio of Numbers of masses observed at necropsy/Numbers of rats with masses observed at necropsy.

Data summarized in Table V show the following:

1. When all rats with palpable masses and all palpable masses are considered, there were about 20% more rats* and about 100% more masses** in the controls and the paraquat-treated groups during the course of the study than at the time of death and necropsy. In many instances, masses observed during one week were not observed during the following week or several weeks later. In other instances, necropsy revealed that the observed masses were actually prominent mammary tissues.
(*10% and **44% in Group 5F).

2. Paraquat had no effect on the incidence of palpable masses in the female rats which either died during the study (unscheduled deaths) or were sacrificed at the termination of the study. In the case of the rats dying during the study, there were as many rats with masses in each of the paraquat-treated groups as in the control groups. However, fewer masses per group and per rat were observed at necropsy in the paraquat-treated groups than in the control groups.

   In the case of the rats sacrificed at the termination of the study, only rats in the low-dose and mid-dose groups had more masses at necropsy than did the controls. However, these increases (17 and 11% in the low-dose and mid-dose groups, respectively) were dose-unrelated. The numbers of masses per rat or 1.7, 1.8, 1.8 and 1.5 masses per rat in the control, low-dose, mid-dose and high-dose groups, respectively, were also dose-unrelated.

During their lives, female rats with palpable masses in the control and the paraquat-treated groups had generally 1-3 masses each. Only 5 rats (one in Groups 1F, 2F, and 3F, and two in Group 4F) had 5-7 masses each. At the time of death, most rats had 1-2 masses each.

Table VI. Onset of palpable masses observed at necropsy[a]

Test Weeks		1-52	53-70	71-80	81-90	91-100	101-110	111-120[b]	121-124[b]
Paraquat ion (ppm)	Group	In male rats dying during the study[c]							
0	1M	1	4	3	4	4	4	1	
0	2M	3	2	4		2	7	1	
25	3M	2	7	3	3	7	7		
75	4M	7	6	6	4	7	2		
150	5M	1	2	6	4	6	3	1	
		In male rats killed at the termination of the study							
0	1M		1	2	3	8	7	5	
0	2M		1	2	3	7	4	5	
25	3M		1	3	2	5	3	11	
75	4M	1	2	6	2	9	7	5	
150	5M	1	1	3	2	9	6	5	
		In female rats dying during the study[c]							
0	1F			3	6	3	8	6	
0	2F		2	5	4	6	3	5	
25	3F	1	2	3	6	5	1	1	
75	4F	1			6	4	5	6	
150	5F			2	6	1	10	3	
		In female rats killed at the termination of the study							
0	1F			1	2	7	3	13	6
0	2F					4	8	12	7
25	3F				2	3	9	18	5
75	4F		1	3	4	8	2	17	
150	5F					4	11	11	3

a. This table is based on APPENDIXES 3A, B, C, D, E and F of the submission. Numbers in the table represent palpable masses.
b. The study was terminated after 113-117 weeks for the males and after 122-124 weeks for the females.
c. These rats were either found dead or had to be killed (were moribund) during the course of the study.

Data Summarized in Table VI show the following:

1. There was no difference in the onset of palpable masses between the control and the paraquat-treated rats, both males and females, which either died during the study or were sacrificed at the termination of the study.

2. Palpable masses appeared earlier in the male rats dying during the study, both the controls and paraquat-treated, than in the remaining male and female rats. For example, within the first 70 weeks of treatment, male rats dying during the study and those sacrificed at the termination of the study had 3-13 and 1-3 masses/group, respectively. The corresponding values for the female rats were 0-3 and 0-1.

3. Palpable masses appeared earlier in the male and female rats dying during the study than in those sacrificed at the termination of the study. In the case of the male rats dying during the study, most masses appeared during the last 40 weeks of treatment (test weeks 71 through 110). However, in the male rats sacrificed at the termination of the study, most masses appeared during the last 27 weeks of treatment (test weeks 91 through 117).

   In the case of the female rats dying during the study, most palpable masses occurred during the last 50 weeks of treatment (test weeks 71 through 120). However, in the female rats sacrificed at the termination of the study, most masses occurred during the last 33 weeks of treatment (test weeks 91 through 123), and especially during the test weeks 111-120.

   Four palpable masses, observed in 4 male rats (Groups 1M, 2M, 3M and 4M) at the interim sacrifice, appeared during the test weeks 23-53*. Rats in the remaining test groups did not have palpable masses at the interim sacrifice.

(*There is some ambiguity here. According to APPENDIX 3C (Vol. IIA, p. 414 of the submission), a palpable mass in rat #13, Group 1M, appeared during the test week 53. However, this rat was sacrificed after 52 test weeks.)

II. Mortality

Paraquat had no effect on the mortality of male and female rats. In the case of the female rats, 41% of both the controls and the paraquat-treated rats survived until the terminal sacrifice. The survival rates for the male rats were 43*, 33, 36 and 47% in the controls, low-dose, mid-dose and high-dose groups, respectively. The slightly decreased survival rates in the low-dose and mid-dose groups, when compared with the controls, were dose-unrelated and did not, therefore, appear to be due to paraquat. The mortality data are summarized in Table VII.
(*Average of values obtained for the two control groups, 1M and 2M).

Table VII. Incidence of mortality among the male and female rats[a]

Paraquat cation (ppm)	Group	MALES (M)				FEMALES (F)			
		Killed	Found dead	Total dead	Percent[b]	Killed	Found dead	Total dead	Percent[b]
		Rats dying during the study							
0	1	23	8	31	44.3	25	7	32	45.7
0	2	24[c]	5	29	41.4	24	6	30	42.9
25	3	27[d]	10	37	52.9	28	3	31	44.3
75	4	28	7	35	50.0	22[c]	9	31	44.3
150	5	23[c]	4	27	38.6	27	4	31	44.3
		Rats sacrificed at the termination of the study							
0	1	29	-	29	41.4	28	-	28	40.0
0	2	31	-	31	44.3	30	-	30	42.3
25	3	23	-	23	32.9	29	-	29	41.4
75	4	25	-	25	35.7	29	-	29	41.4
150	5	33	-	33	47.1	29	-	29	41.4
		Rats sacrificed after 52 test weeks							
0	1	10	-	10	14.3	10	-	10	14.3
0	2	10	-	10	14.3	10	-	10	14.3
25	3	10	-	10	14.3	10	-	10	14.3
75	4	10	-	10	14.3	10	-	10	14.3
150	5	10	-	10	14.3	10	-	10	14.3

a. This table is based on APPENDIXES 2A, B, C, D, E and F of the submission.
b. Based on 70 rats per group.
c. Two rats died at blood sampling.
d. One rat died at blood sampling.

Most of the unscheduled deaths occurred during the last 26 weeks (males) and 30 weeks (females) of the study. The most frequent reasons for sacrificing the rats, both the controls and the paraquat-treated, were loss of body weight, hypothermia, lethargy, poor general condition, shallow or labored respiration, ulcerated and/or necrotic masses (chiefly in the males) and masses affecting locomotion (chiefly in the females). Relevant data are summarized in Tables VIII and IX.

Table VIII. Distribution of unscheduled deaths during the study[a]

Weeks of death		1-52	53-70	71-80	81-90	91-100	101-110	111-120[b]	121-124[b]
Paraquat[e]	Group	MALES							
0	1M		3	2		9	13	4	
0	2M	5[c]	1	2		4	10	7	
25	3M	1	3	1	1	10	10[d]	11	
75	4M		4	2	3	6	11	9	
150	5M	1[d]	2[d]	1	2	7	8	6	
		FEMALES							
0	1F	1		3	2	5	10	8	3
0	2F		1	2	1	1	10	12	3
25	3F	1	5		2	8	5	9	1
75	4F	4[c]	1		3	6	5	12	
150	5F	1	1	1	3	4	11	7	3

a. This table is based on APPENDIXES 2A and 2B of the submission. Numbers in the table represent rats which were either found dead or had to be sacrificed (were moribund).
b. The study was terminated after 113-117 weeks for the males and 122-124 weeks for the females.
c. Two rats died at blood sampling.
d. One rat died at blood sampling.
e. Expressed as ppm of paraquat cation.

Table IX. Most frequent reasons for sacrificing rats during the study (unscheduled deaths)[a]

Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
Group	1	2	3	4	5	1	2	3	4	5
Reason	MALES					FEMALES				
Loss of body weight	12	10	17	12	8	16	13	10	12	11
Hypothermia	6	7	13	7	4	10	8	10	4	7
Lethargy	9	5	10	11	5	8	9	13	8	10
Poor general condition	2	2	3	5	2	4	2	3	2	3
Shallow respiration	1		2			3	3	4	1	1
Labored respiration	1	1	2	2	4	2	2	3	2	2
Ulcerated/necrotic mass	5	4	4	6	6	2	3	1	1	
Mass affecting locomotion		2		2		1	2	2	3	2

a. This table is based on APPENDIXES 2A and 2B of the submission.

III. Food and water consumption

Food consumption was reported for all rats as group mean values (g/rat/week) and as individual values (g/rat/week) for 123 weeks (females; Batches 1 and 2), 113 weeks (males; Batch 1) and 116 weeks (males; Batch 2). Some male rats lived through 117 weeks and their food intake (g/rat/week) was reported as follows: during the test week 114, for 2-3 cages of rats/dose level (Batch 1); during the test weeks 115 and 116, for one cage of rats/dose level (Batch I); and during the test week 117, for one cage of rats/dose level (Batch 2).

Paraquat had no effect on the food intake of the low-dose and mid-dose male and female rats when the treated animals were compared with the controls. In the case of the high-dose males, slight decreases in the food intake occurred during the following test periods: 1-52 weeks (4%); 79-104 weeks (3%) and 105-113 weeks (7%). In the case of the high-dose females, slight decreases in the food intake occurred only during the following test periods: 1-6 weeks (3%); 105-122 weeks (5%); and week 123 (8%). The food consumption of the controls and the high-dose rats is summarized in Table X.

Table X. Food consumption of the controls and the high-dose rats[a]

Test weeks	MALES			FEMALES		
	Controls[b]	High-dose[b]	Percent[c]	Controls[b]	High-dose[b]	Percent[c]
1-26	114	109[d]	96	76	75	99
27-52	117	112[d]	96	77	77	100
53-78	117	116	99	86	85	99
79-104	115	112	97	97	96	99
105-113	111	103	93	-	-	-
105-122	-	-	-	99	94	95
123	-	-	-	87	80	92

a. This table is based on the following data in the submission: TABLE 6B (values for test weeks 1-104); TABLE 6A (values for test weeks 105-122); and APPENDIXES 4A and 4B (values for test week 123).
b. Food consumption is expressed as gram of food ingested/rat/week.
c. Percent of control values.
d. Significantly different from combined controls, $p<0.001$.

Water consumption was reported as ml/rat/day and as ml/kg of body weight/day for the test weeks 1-101, for the male and female rats. With the exception of the test week 101, the controls and the paraquat-treated rats consumed about the same volumes of water/kg of body weight (TABLE 10 of the submission). During the test week 101, the low-dose, mid-dose and high-dose male rats consumed 34, 29 and 32%, respectively, less water than did the controls. The corresponding decreases for the female rats were 36, 21 and 34%, respectively. However, it is unlikely that these dose-unrelated decreases in the water intake so late in the study were caused by paraquat.

IV. Body weight

These data were reported in the following way: 1) as individual body weights through the test weeks 115-117 (males) and 122-123 (females); 2) as group mean body weights, with standard deviations, for 113 weeks (males) and 122 weeks (females); and 3) as group mean body weight gains during the test weeks 1-26, 27-52, 53-78, 79-104 and 105-113 (males only).

Both the controls and the paraquat-treated male rats were gaining weight consistently during the test weeks 1-78 and then were losing weight through the remaining weeks of the study. Whereas the low-dose and mid-dose groups gained as much or more weight during the test weeks 1-78, when compared with the controls, the high-dose rats gained less

weight than did the controls, especially during the test weeks 11-68. During that period, the decreases in the weight gain for the high-dose male rats ranged from 10% to 24%. However, during the treatment weeks 79-113, when rats in all five groups were losing weight, the high-dose male rats generally lost less weight than did the controls, the low-dose and the mid-dose male rats.

Both the controls and the paraquat-treated female rats were gaining weight consistently during the test weeks 1-90 and then were losing weight during the remaining weeks of the study. During the test weeks 1-26, there was no difference in the weight gain between the controls and the paraquat-treated female groups. However, during the test weeks 27-78, while the low-dose group continued to gain as much (or more) weight as did the controls, the mid-dose and the high-dose groups gained 3-9% and 11-34%, respectively, less weight than did the controls. Only during the treatment weeks 41-52 the mid-dose females gained 11% more weight when compared with the controls. During the test weeks 105-122, when both the treated and untreated female rats began to lose weight, the paraquat-treated rats lost more weight, in a dose-unrelated manner, than did the controls. Data showing the weight gain of rats are summarized in Table XI.

Table XI. Group mean body weight gains and losses of male and female rats[a]

Paraquat[b] (ppm)	0			25		75		100	
Group	1		2	3		4		5	
Test	Gain	Average	Gain	Gain	%[c]	Gain	%[c]	Gain	%[c]
Weeks	MALES								
1-10	222	221	220	218	99	219	99	208	94
11-26	78	77.5	77	77	99	73	94	63	81
27-40	51	52	53	51	98	71	136	44	85
41-52	25	24.5	24	25	102	25	102	22	90
53-68	15	17	19	20	118	17	100	13	76
69-78	-2	0.5	3	2		3		4	
79-90	-14	-12	-10	-20	167	-4	33	-6	50
91-104	-31	-36.5	-42	-32	88	-50	137	-33	90
105-113	-20	-23	-26	-33	143	-31	135	-18	78

Table XI. (Cont'd) Group mean body weight gains and losses of male and female rats[a]

	FEMALES								
1-10	87	86	85	88	102	86	100	86	100
11-26	25	26	27	26	100	27	104	26	100
27-40	20	19.5	19	22	113	18	92	17	87
41-52	14	13.5	13	17	126	15	111	12	89
53-68	36	35	34	35	100	32	91	23	66
69-78	22	19.5	17	21	108	19	97	15	77
79-90	22	23	24	17	74	18	78	16	70
91-104	-2	-1.5	-1	2		3		2	
105-122	-3	-5.5	-8	-9	164	-13	236	-10	182

a. This table is based on TABLE 7 of the submission. Weight gains and losses are expressed in grams/rat.
b. Expressed as paraquat cation.
c. Calculated as percent of combined controls.

Group mean body weight values (not gains or losses) were evaluated statistically. In the case of the high-dose male rats, small decreases (6-8%) in group mean body weights, when compared with the control values, were statistically significant only after the test weeks 26, 52, 78 and 104 ($P<0.001$ in each instance), and after the test week 113 ($P<0.05$).

In the case of the high-dose female rats, small decreases (2-10%) in group mean body weights, when compared with the control values, were statistically significant only after the test week 52 ($P<0.05$) and the test weeks 78, 104, 117 and 122 ($P<0.001$ in each instance).

VI. Food utilization

These data were reported as food conversion ratios (group mean values), that is, as ratios of the quantity of food consumed to the body weight gained during the first 52 test weeks. Starting with the test week 13, the high-dose male and female rats utilized food slightly less efficiently than did the controls. During the test weeks 13-26 and 27-52, the food conversion ratios for the male rats were, respectively, 21 and 12% higher than the ratios calculated for the male controls. The corresponding values for the female rats were 11 and 13%, respectively.

VII. Achieved dosages

The actual ingestion of paraquat cation by the male and female rats was reported weekly (group mean values) for the test weeks 1-12 and 69-113 (122), and biweekly for the test weeks 13-68. These data are summarized in Table XII.

Table XII. Actual ingestion of paraquat cation (mg/kg of body weight/day)[a]

Group[b]	3	4	5	3	4	5
Test weeks	Average values			Ranges		
	MALE RATS					
1-5	2.3	6.8	13.2	1.8-2.9	5.4-8.5	10.6-16.8
6-12	1.3	3.9	7.9	1.1-1.5	3.4-4.7	6.9-9.4
13-52	1.0	3.0	6.1	0.9-1.2	2.7-3.7	5.6-7.3
53-113	0.9	2.7	5.4	0.8-0.9	2.4-2.9	4.8-5.9
	FEMALE RATS					
1-5	2.3	6.9	13.7	1.9-2.8	5.9-8.4	11.9-16.2
6-12	1.5	4.6	9.4	1.4-1.8	4.1-5.5	8.4-10.8
13-52	1.3	3.9	7.8	1.2-1.4	3.7-4.3	7.3-8.6
53-113	1.1	3.6	7.2	1.0-1.3	3.2-4.0	6.3-7.9
114-122	1.0	3.3	6.3	0.9-1.1	3.0-3.4	5.6-7.1

a. This table is based on TABLE 9 of the submission.
b. Groups 3, 4 and 5 were fed diets containing 25, 75 and 150 ppm of paraquat cation, respectively.

These data show that, starting with the test week 6, the female rats at all dose levels ingested more paraquat per kilogram of body weight than did the male rats at the same dose levels. The increases ranged from 15% to 33% and are summarized in Table XIII.

Table XIII. Increased ingestion of paraquat cation by female rats[a]

Group	3	4	5
Test weeks	Percent increase		
6-12	15	18	19
13-52	30	30	28
53-113	22	33	33

a. This table is based on Table XII (above).

VIII. Ophthalmoscopy

These data were reported as a summary of observations and as individual observations. The summary included week of observation, eye structure affected (cornea, iris, lens, conjunctiva, retina and/or anterior chamber), and numbers of animals examined at each time interval and dose level. The individual observations included group number, sex, animal's ID number, eye examined (right, left or both), eye structure affected (as in the summary), week of observation and comments. Using Fischer's Exact Test, Single-Tailed, each paraquat-treated group was compared with the combined control groups. Data concerned with ophthalmoscopy are summarized in Tables XIV, XV and XVI.

Table XIV. Numbers of rats examined ophthalmoscopically[a]

Group	1	2	3	4	5	1	2	3	4	5
Test weeks	MALES					FEMALES				
Pretreatment	75	70	75	75	75	75	70	75	75	75
4, 14, 26, 52 and 79	20	20	20	20	20	20	20	20	20	20
103	44	46	42	43	46	49	51	45	44	47
110	33	37	36	35	30[b]	39	45	37	41	39
112[c] and 113	33	34	33	28	37	-	-	-	-	-
118[c] and 119	-	-	-	-	-	35	36	31	32	33

a. This table is based on TABLE 11 of the submission.
b. According to APPENDIX 7B (pp. 679-681) of the submission, 38 animals were examined in this group. However, data for 8 animals were excluded because of confusion with the identifications of the animals.
c. Rats from Batch 2 were examined at these times. Rats from Batch 1 were examined at the test weeks 113 and 119.

Table XV. Incidence of lenticular opacities and cataracts in male rats[a]

Test week	0[b]	4	14	26	52	79	103	110	112 & 113
	Number of lenticular lesions								
Group 1									
Opacities[c]							1	13	11
Cataracts[d]							4	3	5
Group 2									
Opacities						1	5	6	9
Cataracts						1	1	2	3
Group 3									
Opacities		1	1			1	1	22	22
Cataracts					1	1	4	7	5
Group 4									
Opacities						2	14	22	12
Cataracts							16	16	19
Group 5									
Opacities					2		1		2
Cataracts					1	3	56	40	52
	Number of lenticular lesions/rat[e]								
Group 1							0.11	0.48	0.48
Group 2						0.10	0.13	0.22	0.35
Group 3		0.05	0.05		0.05	0.10	0.12	0.81	0.82
Group 4						0.10	0.70	1.09	1.11
Group 5					0.15	0.15	1.24	1.33	1.46

a. This table is based on TABLE 11 and APPENDIXES 7A, B and C of the submission.
b. Pretreatment period.
c. In each case, the term "opacities" includes the following observations (as they were reported): opacity, posterior capsular opacity, anterior polar opacity, suture line opacity, posterior polar opacity and cloudiness.
d. In each case, the term "cataracts" includes the following observations (as they were reported): posterior polar opacity/cataract, posterior capsular opacity/cataract, posterior capsular cataract, posterior polar cataract, cataract, radial cataract, total cataract and total cataract with resorption.
e. Sum of opacities and cataracts divided by the number of rats examined at each time interval, as listed in Table XIV (above).

Table XVI. Incidence of lenticular opacities and cataracts in female rats[a]

Test week	0[b]	4	14	26	52	79	103	110	118 & 119
	Number of lenticular lesions								
Group 1									
Opacities[c]							1	5	23
Cataracts[d]							3	2	8
Group 2									
Opacities								12	27
Cataracts						1	6	6	9
Group 3									
Opacities								15	24
Cataracts					1	1	5	4	11
Group 4									
Opacities						4	10	24	4
Cataracts							14	21	33
Group 5									
Opacities					1	2	1	1	
Cataracts					1	1	51	47	37
	Number of lenticular lesions/rat[e]								
Group 1							0.08	0.18	0.89
Group 2						0.05	0.12	0.40	1.00
Group 3					0.05	0.05	0.11	0.51	1.13
Group 4						0.20	0.55	1.10	1.16
Group 5					0.10	0.15	1.11	1.23	1.12

a. This table is based on TABLE 11 and APPENDIXES 7A, B and C of the submission.
b. Pretreatment period.
c. and d. The same as in Table XV, except that "cataracts" includes also cloudy radial cataract.
e. Sum of opacities and cataracts divided by the number of rats examined at each time interval, as listed in Table XIV (above).

The predominant ocular lesions in the controls and the paraquat-treated male and female rats were lenticulr opacities and cataracts. These lesions either were not observed or were observed infrequently before the test week 103.

Paraquat enhanced the development of ocular lesions in all of the treated groups. Starting with the test week 110, there was a dose-related increase in the incidence of cataracts in the male and female rats, when the paraquat-treated groups are compared with the controls. However, at test week 103,

dose-related increased incidences of cataracts were observed only in the mid-dose and high-dose groups. Increased incidences of lenticular opacities were inconsistent, dose-unrelated and occurred only in the low-dose and mid-dose groups.

For the purpose of statistical analysis, ocular changes were grouped as follows: a) no abnormality detected and suture lines; b) suture line opacity; c) posterior capsular changes (posterior capsular and polar opacities and cataracts); d) radial cataract; and e) total cataract.

In the case of the male rats, statistically significant increases ($P<0.001$, 0.01 or 0.05) in the incidence of ocular lesions (groups b, c, d and e) were reported for the three paraquat-treated groups during the test weeks 110-112/113. At the test week 103, increases in the incidence of ocular lesions were statistically significant only in the mid-dose and high-dose groups. ($P<0.001$ in most instances).

In the case of the female rats, statistically significant increases ($P<0.001$ in most instances) in ocular lesions (groups e and d) occurred mostly in the mid-dose and high-dose groups during the weeks 103-118/119. Suture line opacity (group b) was increased significantly ($P<0.001$) only in the mid-dose group at the test week 103. Regarding the low-dose female group, there was a statistically significant increase only in the incidence of partial cataracts (group c) and only during the test weeks 110-118/119. However, this increase was regarded as statistically weak ($P<0.05$) and biologically insignificant.

Other ocular changes (lenticular suture line vacuolation, iritis and glaucoma) were observed also during the test weeks 103-112/113 (males) and 103-118/119 (females), and were regarded as secondary changes in eyes with severe cataracts. These changes generally predominated in the high-dose male and female rats and in the case of glaucoma, also in the mid-dose male rats. These data are summarized in Table XVII.

Table XVII. Incidence of suture line vacuolation, iritis and glaucoma in the male and female rats[a]

Group	1	2	3	4	5
Observation	MALES				
Lenticular suture line vacuolation				1	20
Glaucoma			1	5	8
Iritis	1		4	4	11
	FEMALES				
Lenticular suture line vacuolation					8
Glaucoma			1	4	3
Iritis		2	5	4	8

a. This table is based on TABLE 11 of the submission. Numbers in the table represent observations and not rats affected.

In most instances, both eyes were affected with opacities, cataracts and other ocular lesions.

IX. Hematology

These data were reported as individual values for rats dying during the study, and as individual and group mean values (with standard deviations and statistical significance) for the remaining animals. Paraquat had no effect on hematology.

X. Clinical chemistry

These data were reported as individual values for rats dying during the study, and as individual and group mean values (with standard deviations and statistical significance) for the remaining animals. Paraquat had no effect on clinical chemistry.

XI. Urinalysis

These data were reported for individual animals. Paraquat had no effect on urinalysis.

XII. Paraquat concentration in urine

These data were reported as individaul cage values and as group mean values (with standard deviation) for 8 time intervals during the test weeks 15-102. Determinations were performed on pooled urine samples, using one cage of rats (or 5 rats) of the same sex/determination/batch.

Urinary levels of paraquat cation increased with dose, indicating that the ingested paraquat was absorbed by the rats. However, these levels varied widely within each group. Paraquat was not detected in the urine of the control groups. Data concerned with urinary levels of paraquat are summarized in Table XVIII.

Table XVIII. Concentration of paraquat cation in urine of rats[a]

Group	1	2	3	4	5
Level (ppm)[b]	0	0	25	75	150
	Paraquat cation in urine (µg/ml)				
	MALES				
Mean[c]	<0.05	<0.05	1.14	3.74	7.98
SD	-	-	0.64	1.72	4.32
	FEMALES				
Mean[c]	<0.05	<0.05	0.98	3.56	10.17
SD	-	-	0.52	1.31	7.86

a. This table is based on TABLE 14 of the submission.
b. Levels of paraquat cation in diet.
c. Mean of group mean values, obtained for urinary paraquat during the test weeks 15, 27, 41, 52, 65, 79, 92 and 102.
SD Standard deviation.

XIII. Paraquat concentration in tissues and plasma

These data were reported as individual values and are summarized in Table XIX.

Table XIX. Group mean levels of paraquat cation in tissues of rats sacrificed after 52 weeks of treatment[a]

Group and sex	Paraquat in diet (ppm)[b]	Lungs (µg/g)	Liver (µg/g)	Kidneys (µg/g)	Skin (µg/g)	Plasma (µg/ml)
1M	0	ND[c]	ND	ND	ND	ND
3M	25	ND	ND	0.12[e]	ND	0.007[e]
4M	75	0.14[e]	ND	0.28	0.35[d]	0.013
5M	150	0.34	ND	0.71	0.19[e]	0.037
1F	0	ND	ND	ND	ND	ND
3F	25	ND	ND	0.12[e]	ND	0.007[d]
4F	75	0.25	ND	0.36	ND	0.013
5F	150	0.43	0.65	0.12[e]	0 13[e]	0.051

a. This table is based on APPENDIX 22 of the submission. The lower limit of detection was 0.1 µg/g for tissue samples and 0.006 µg/ml for plasma samples. Five rats/dose level/sex were sacrificed.
b. Expressed as cation.
c. None detected (ND).
d. In these groups, paraquat was detected in only 1 or 2 rats.
e. In these groups, paraquat was detected in 3 or 4 rats.

Paraquat was detected in the kidneys and plasma of all three dosage groups and the concentrations were dose-related. Lungs of the mid-dose and high-dose male and female rats also contained paraquat, in a dose-related manner, but none was detected in the low-dose groups. The levels of paraquat in the lungs were lower than those in the kidneys. Livers of male rats and the low-dose and mid-dose female rats did not contain paraquat. In the case of skin samples, only some samples obtained from the high-dose male and female groups and the mid-dose male group contained paraquat.

XIV. Bone marrow and blood smear cytology

Paraquat did not cause abnormalities in bone marrow. However, not all animals were examined at both unscheduled and scheduled sacrifices. These data, reported for individual animals, are summarized in Table XX.

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Table XX. Rats examined for abnormalities in bone marrow[a]

Group	1	2	3	4	5	1	2	3	4	5
	MALES					FEMALES				
	Rats which had to be sacrificed during the study									
Examined	14	11	16	18	16	6	11	7	7	6
Not examined[b]	9	13	10[c]	10	6	19[d]	13	21[c]	13	21
	Rats sacrificed after 52 weeks of treatment									
Examined	5	3	2	2	5	6	5	1	4	4
Not examined[b]	5	7	8	8	5	4[c]	5	9	6	6
	Rats killed at the termination of the study									
Examined	15	30	16	17	13	17	15	15	15	14
Not examined[b]	14[e]	1	7	8	20	11	15	14[c]	14	15

a. This table is based on APPENDIXES 12A, B and C of the submission.
b. The quality of the films obtained for these animals was "too poor for useful examination."
c. Includes 1 animal for whom film was not available for examination.
d. Includes 2 animals for whom films were not available for examination.
e. Includes 3 animals for whom films were not available for examination.

Histological examination of blood smears did not reveal differences between the controls and the paraquat-treated groups. However, there was a reduced incidence of animals with normoblasts in the high-dose male and female groups at the termination of the study. The high-dose male and female groups had, respectively, 5 and 2 rats with one or more normoblasts, whereas the remaining groups, both treated and untreated, had 7-11 normoblasts. The biological significance of this finding is not immediately apparent.

Results of blood smear cytology were reported for individual animals.

XV. Organ weights

Absolute organ weights and ratios of organ weights to body weights were reported for individual animals and as group mean values after 52 weeks of treatment (interim sacrifice), and after 113 weeks (males) and 122 weeks (females) of treatment. Group mean values included standard deviations and P values. Individual, absolute organ weights were also reported for the animals dying during the study. However, because of wide variations in the time of death, these data were not evaluated statistically. The following organs were weighed: brain, heart, lungs, liver, spleen, thymus, kidneys, ovaries (testes), and adrenal, pituitary and thyroid glands.

Small to moderate, but statistically significant, decreases in the absolute and relative weights of livers and testes were observed in the high-dose male rats at the termination of the study (after test week 113). Small decreases in the absolute weights of livers were also noted in the high-dose female rats at the termination of the study (after test week 122), but reflected probably decreases in body weights. However, the relative weights of the lungs of the high-dose male and female rats were greater than those of the controls. Changes in organ weights of the high-dose rats are summarized in Table XXI.

Table XXI. Changes in organ weights of the high-dose rats after 113/122 weeks of treatment[a]

Organs affected	MALES		FEMALES	
	Percent changes in absolute weights			
	Decrease	Increase	Decrease	Increase
Body weight	8[b]		11[d]	
Liver	17[d]		12[d]	
Testes	22[c]		--	
	Percent changes in relative weights[e]			
	Decrease	Increase	Decrease	Increase
Liver	10[c]	-	-	-
Testes	17[b]	-	-	-
Lungs	-	16[b]	-	14[b]

a. This table is based on TABLES 15B and 16B of the submission.
b, c and d. Significantly different from controls; b = $P<0.05$; c = $P<0.01$; and d = $P<0.001$.

e. Relative weight = ratio of organ weight/body weight.

There were either no changes in the absolute and relative weights of the remaining organs or the observed changes were very small and/or statistically insignificant.

XVI. Macroscopic observations

Macroscopic observations were reported for all rats as individual data (APPENDIXES 16A, B, C, D, E and F of the submission), but were not tabulated. (Tabulation was done by the reviewer). The individual tests included ID number of an animal, week of death (for rats which were found dead or had to be sacrificed during the study) and either observations or one of the following notations: "NSL" (No Significant Lesion), "Autolysis" or "Autolysis and cannibalisation". Eyes were not examined macroscopically because there was "adequate ophthalmoscopic and microscopic evidence for the changes observed."

Rats dying or killed in extremis during the study

The predominant observations included emaciation; urogenital and perianal staining; ocular discharge and staining, and nasal staining; pallor of extremities; firm subcutaneous masses; enlarged, hemorrhagic and/or congested pituitaries; prominant and/or congested cervical lymph nodes; lungs with subpleural foci and areas of incomplete collapse; pulmonary congestion or edema; pale and/or swollen livers, and with irregular surfaces; pale, granular and/or cystic kidneys; enlarged and swollen spleens; gastric ulcerations; small or enlarged testes, mostly with subcapsular plaques; small epididymides, seminal vesicles and prostates; and prominent mammary tissues (in males and females). All of these changes occurred with similar frequencies in the controls and the paraquat-treated groups.

Rats sacrificed at termination of the study

The paraquat-treated male and female rats had a higher incidence of pulmonary changes (occasional or multiple, dark or pale subpleural foci/areas) than did the controls, but the incidence was dose-unrelated in the females. The incidence of pulmonary changes (numbers of rats affected/numbers of rats examined) in the controls (combined), low-dose, mid-dose

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and high-dose male groups was 2/60, 4/23, 6/25 and 21/33, respectively. The corresponding values for the female groups were 10/58, 8/29, 7/29 and 22/29, respectively.

Other prominent observations included emaciation; firm or soft subcutaneous masses; enlarged, hemorrhagic and/or congested pituitaries; enlarged adrenals; prominent and/or congested cervical lymph nodes; pulmonary congestion and petechiae; livers with irregular surfaces; kidneys with pitted, granular surfaces and/or cysts; enlarged and swollen spleens; gastic ulcerations; enlarged testes, mostly with subcapsular plaques; small epididymides; prominent mammary tissues (mostly in the females); and areas of uterine distension. All of these findings occurred with similar frequencies in the control and the paraquat-treated groups.

XVII. Histopathology: non-neoplastic lesions

These data were reported as incidences of lesions (with P values) in each test group and as individual data. The individual data included ID number, week of death (for rats which were found dead or had to be sacrificed during the study) and detailed description of microscopic findings. The summary data (Tables 17A, B, C, and D of the submission) included numbers of rats examined in each group and pathological changes detected in each organ. It was not reported initially how many organs or tissues were examined at each dose level. However, this information was submitted later in a separate document (Chevron Response to EPA Comments On the Rat Chronic/ Oncogenicity Study - Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985).

Assuming that all tissues were examined in each animal, there were no treatment-related changes in rats dying during the first year of study and in those killed after 52 weeks (interim sacrifice).

Many different changes were observed in rats dying between week 53 and termination, but these changes either occurred with similar frequency in the controls and the paraquat-treated groups or prevailed in the control groups. Exceptions were as follows:

1. Dose-unrelated, increased incidence of hydrocephalus in the female groups.

2. Dose-unrelated increased incidence of cysts or cystic spaces in the spinal cord of the male and female rats.

3. Dose-related increase in the incidence of degeneration of sciatic nerve fibers of the male rats.

Observations 1, 2 and 3 were regarded by the testing laboratory as possibly treatment-related.

Wide range of non-neoplastic changes was also reported for rats killed at the termination of the study. However, most of these changes also either occurred with similar frequency in the controls and the paraquat-treated groups or prevailed in the control groups. The only change considered by the testing laboratory to be treatment-related was a higher incidence of hydrocephalus in the high-dose female rats than in the controls. Data concerned with treatment-related or possibly treatment-related non-neoplastic lesions are summarized in Table XXII. Pulmonary and ocular lesions are discussed separately.

Table XXII. Incidence of hydrocephalus, degeneration of sciatic nerve fibers and spinal cord cysts/cystic spaces*

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat ion (ppm)	0	0	25	75	150	0	0	25	75	150
Rats dying between week 53 and termination of study	Number of rats with lesions									
Number of rats examined	30	24	36	35	26	31	30	30	27	30
Hydrocephalus**	4	1	2	6	0	3	2#	8[a]	9[a]	9[a]
Degeneration of sciatic nerve fibers	6	4	8	17[c]	19[c]	15	11	10	11	11
Spinal cord cysts/ cystic spaces	0	0+	6[b]	4[a]	7[a]	5	1	6	3	7
	Percent of rats with lesions									
Hydrocephalus**	13.3	4.2	5.6	17.1	0	9.7	6.9	26.7[a]	33.3[a]	30.0[a]
Degen. of sciatic nerve fibers	20.0	16.7	22.2	48.6[c]	73.1[c]	48.4	36.7	33.3	40.7	36.7
Spinal cord cysts/ cystic spaces	0	0	16.7[b]	11.4[a]	26.9[c]	16.1	3.3	20.0	11.1	23.3

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Table XXII. (Cont'd) Incidence of hydrocephalus, degeneration of sciatic nerve fibers and spinal cord cysts/cystic spaces*

Rats sacrificed at termination of study	Number of rats with lesions									
Number of rats examined	29	31	23	25	33	28	30	29	29	29
Hydrocephalus***	0	1	2	2	2	4	0	1	3	11[b]
Degeneration of sciatic nerve fibers	13	7	11	15	13	5	7	2	6	10
Spinal cord cysts/ cystic spaces	0	0	0	0	1	0	0	0	0	0
	Percent of rats with lesions									
Hydrocephalus***	0	3.2	8.7	8.0	6.1	14.3	0	3.4	10.3	37.9[b]
Degen. of sciatic nerve fibers	44.8	22.6	47.8	60.0	39.4	17.9	23.3	6.9	20.7	34.5
Spinal cord cysts/ cystic spaces	0	0	0	0	3.0	0	0	0	0	0

* This table is based on TABLES 17C and D, and Text Tables 2a, b and c of the submission. Unless indicated otherwise, the numbers of rats examined equal the numbers of tissues or organs examined.

a, b and c. Significantly different from combined controls; $P<0.05$; $P<0.01$ and $P<0.001$, respectively.

Twenty-nine (29) rats were examined for the presence of hydrocephalus in this group.

+ Twenty-three (23) rats were examined for the presence of spinal cord cysts/cystic spaces in this group.

** Includes dilatation of the fourth ventricle.

*** Essentially slight hydrocephalus.

Data summarized in the above table show that male rats were unaffected by hydrocephalus at all levels of paraquat tested. Since the distribution of mortality was unaffected by treatment, the incidence of hydrocephalus was also evaluated by the testing laboratories as a combined (total) incidence for all rats. On this basis, the incidence of hydrocephalus in the female groups 1F, 2F, 3F, 4F and 5F was 7/59, 2/59, 9/59, 12^{a}/56 and 20^{c}/59, respectively, and there was no statistically significant difference between the control and the low-dose groups.

The total incidence of degeneration of the sciatic nerve fibers in the male groups 1M, 2M, 3M, 4M and 5M was 19/59, 11/55, 19/59, 32^{c}/60 and 32^{c}/59, respectively. The corresponding values for the female rats were 20/59, 18/60, 12/59, 17/56 and 21/59, respectively. On this basis, only the mid-dose and high-dose males were significantly affected by degeneration of the sciatic nerve fibers.

With the exception of one occurrence in the high-dose male rats, spinal cord cysts or cystic spaces were not observed at terminal sacrifice.

XVIII. Histopathology: neoplastic lesions

These data were reported as follows: 1) as incidences of neoplastic changes with P values in each test group; 2) as numbers of benign and malignant neoplasms/sex/group; and 3) as histopathological findings for individual rats. The individual data included ID number, week of death (for rats which were found dead or had to be sacrificed during the study) and detailed description of the microscopic findings. The summary data (TABLES 19A, B, C, D and E of the submission) included numbers or rats examined in each group and neoplastic changes detected in each organ. It was not reported initially how many organs or tissues were examined at each dose level. However, this information was submitted later in a separate document (see pg. 38 for reference.) The incidence of all neoplastic lesions, excluding ocular lesions, is summarized in Table XXIII.

Table XXIII. Incidence of neoplastic lesions, excluding ocular lesions, in male and female rats[a]

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
	Rats dying during the first 52 weeks of treatment									
Number of rats examined	1	5	1	0	1	1	0	1	4	1
Number of benign neoplasms	1	0	0	0	0	0	0	1	0	0
Number of malignant neoplasms	0	2	1	0	0	0	0	0	1	0
Total number of neoplasms	1	2	1	0	0	0	0	1	1	0
	Rats dying between week 53 and termination									
Number of rats examined	30	24	36	35	26	31	30	30	27	30
Number of benign neoplasms	41*	47*	67	65*	47*	27	38	41*	38	39
Number of malignant neoplasms	22*	16*	26	20*	18*	21	18	18*	16*	19
Total number of neoplasms	63	63	93	85*	65	58	56	59	54*	58
Number of neoplasms/ rat	2.1	2.6	2.6	2.4	2.5	1.9	1.9	2.0	2.0	1.9
Ratio of benign/ malignant neoplasms	1.9	2.9	2.6	3.2	2.6	1.8	2.1	2.3	2.4	2.1
	Rats killed after 52 weeks of treatment b									
Number of rats examined	10	10	10	10	10	10	10	10	10	10
Number of benign neoplasms	0	1	1	2	0	0	1	1	1	1
Number of malignant neoplasms	0	0	0	0	0	0	0	0	0	0

Table XXIII. (Cont'd) Incidence of neoplastic lesions, excluding ocular lesions, in male and female rats[a]

	Rats killed at termination of study									
Number of rats examined	29	31	23	25	33	28	30	29	29	29
Number of benign neoplasms	65	66	52	71	86	51*	45*	43*	46*	55
Number of malignant neoplasms	8	12	16	13	14	14*	14*	20*	11*	11
Total number of neoplasms	73	78	68	84	100	65	59	63	57	66
Number of neoplasms/rat	2.5	2.5	3.0	3.4	3.0	2.3	2.0	2.2	2.0	2.3
Ratio of benign/malignant neoplasms	8.1	5.5	3.2	5.5	6.1	3.6	3.2	2.1	4.2	5.5

a. This table (compiled by the reviewer) is based on TABLES 19A, B and C of the submission. Values marked with asterisks do not agree with those reported in TABLE 19E of the submission ("Numbers of benign and malignant neoplasms"). However, the differences are small, generally one or two neoplasms in either direction.

b. Interim sacrifice.

These data show that there were only a few neoplasms during the first 52 weeks of treatment and that they were not treatment-related. However, rats which died or were sacrificed during the second year of the study and those sacrificed at the termination of the study had many neoplasms. The incidence of total neoplasms in the male and female rats did not appear to be treatment-related when numbers of neoplasms/rat are considered. Although the paraquat-treated male rats had 20-36% more neoplasms/rat than did the controls at the termination of the study (that is, 3.0-3.4 vs 2.5 neoplasms/rat), the incidence was not dose-related. The mid-dose male and female rats dying during the study (Groups 4M and 4F) and the mid-dose and high-dose female rats sacrificed at the termination of the study (Groups 4F and 5F) had higher ratios of benign/malignant neoplasms than did the controls. In the case of the remaining rats, these ratios were similar for the untreated and the paraquat-treated rats.

The predominant neoplasms in rats dying between week 53 and termination of the study were pituitary adenomas and carcinomas, thyroid parafollicular adenomas, benign pheochromocytomas, pancreatic islet cell adenomas, thyroid follicular adenomas, monocytic leukemia, mammary gland benign fibroepithelial tumors, testicular interstitial cell tumors, skin and subcutis fibromas, lipomas and papillomas, and squamous cell carcinomas in the head region (an uncommon tumor). The incidence of these neoplasms is summarized in Table XXIV.

Table XXIV. Incidence of predominant neoplasms in rats dying between week 53 and termination of study[a]

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
Number of rats which died	30	24	36	35	26	31	30	30	27	30
Neoplasms	Incidences of neoplasms[a]									
	30	24	36	35	25	31	30	30	27	30
Adrenal: benign pheochromocytoma		4	5	3	3	1				
	30	23	36	35	24	31	29	29	27	29
Pancreas: islet cell adenoma	1	1	3	2	1		1			
	27	23	35	34	26	28	27	28	27	28
Pituitary: adenoma	4	7	13	12	4	15	11	16	22[b]	18
carcinoma		1		2		3	2	4	2	4
	29	21	31	31	25	31	30	30	26	28
Thyroid: follicular adenoma	1		1	1	1		1	2		3
parafollicular adenoma	3	2	2	2	2	3	2	5	1	3
	30	24	36	35	26	31	30	30	27	30
Mammary gland: benign fibroepithelial tumor		1	1	1	2	10	13	13	12	9
	29	24	36	35	26	--	--	--	--	--
Testis: interstitial cell tumor	24	21	31	26	21	--	--	--	--	--
	30	24	36	35	26	31	28	30	27	30
Skin and subcutis:										
Fibroma	1	1	4	7[c]	3	1	2	2	1	1
Lipoma				1	4[c]			1		
Papilloma		1		2	3					
Squamous cell carcinomas in head region:										
Hard palate					1					
Middle ear[e]										1
Skin	1	2	1		1					
Head tissue			2		3				3	1
	30	24	36	35	26	31	30	30	27	30
Monocytic leukemia	14	11	12	13	9	5	9	4	3	7

Table XXIV. (Cont'd) Incidence of predominant neoplasms in rats dying between week 53 and termination of study[a]

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
	Percent of tissues with neoplasms									
Adrenal: benign pheochromocytoma		17	14	9	12	3				
Pancreas: islet cell adenoma	3	4	8	6	4		3			
Pituitary: adenoma	15	30	37	35	15	54	41	57	81[b]	64
carcinoma		4		6		11	7	14	7	14
Thyroid: follicular adenoma	3		3	3	4		3	7		10
parafollicular adenoma	10	10	6	6	8	10	7	17	4	10
Mammary gland: benign fibroepithelial tumor		4	3	3	8	32	43	43	44	30
Testis: interstitial cell tumor	83	87	86	74	81	--	--	--	--	--
Skin and subcutis:										
Fibroma	3	4	11	20[c]	11	3	7	7	3	3
Lipoma				3	15[c]			3		
Papilloma		4		6	11					
Squamous cell carcinomas in head region:										
Hard palate					4					
Middle ear[e]										3
Skin	3	8	3		4					
Head tissue			5		11				11	3
Monocytic leukemia	47	46	33	37	35	16	30	13	11	23

a. This table is based on TABLE 19B of the submission and on Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985. Pulmonary and ocular lesions are discussed separately.

b. and c. Significantly different from controls; $P<0.01$ and $P<0.05$, respectively.

d. For each organ or tissue, the top line represents the numbers of tissues examined and the remaining lines the numbers of neoplasms observed.

e. In groups 1M, 2M, 3M, 4M, 2F, and 4F, tissues from 29, 22, 34, 34, 29, and 26 rats, respectively, were examined.

These data show that the controls and the paraquat-treated rats which died during the study had in most instances, about the same incidence of neoplasms. Exceptions were as follows:

1. The mid-dose and high-dose male rats had more skin lipomas and papillomas than did the controls. However, the incidences, although dose-related, were low.

2. The paraquat-treated male rats had more skin fibromas than did the controls, but the incidence was dose-unrelated.

3. There was an increased incidence of pituitary adenomas in the paraquat-treated female rats and the low-dose and mid-dose male rats, when compared with the controls, but the incidence was dose-unrelated.

4. Squamous cell carcinoma in the head region, an uncommon tumor, occurred in 3 control, 3 low-dose and 5 high-dose males, and in 3 mid-dose and 2 high-dose females. Although the incidence (percent of rats with squamous cell carcinoma) was higher in the paraquat-treated rats than in the controls, it was dose-unrelated.

5. The incidence of follicular adenoma in the thyroid was increased with dietary levels of paraquat in the low-dose and high-dose female groups, but no adenomas were observed in the mid-dose female group.

The predominant neoplasms in rats sacrificed at the termination of the study were, in most instances, the same as those occurring in rats which died during the study. Also, in most instances, the controls and the paraquat-treated rats had about the same incidence of neoplasms (Table XXV). Exceptions were as follows:

1. There was an increased incidence (dose-related) of benign pheochromocytomas in the paraquat-treated male rats when compared with an incidence in the controls.

2. The high-dose male group had about twice as many parafollicular adenomas as did the controls. An increased incidence of parafollicular adenomas occurred also in the high-dose female group.

3. Only one squamous cell carcinoma in the head region was observed at terminal sacrifice. This uncommon neoplasm occurred in the high-dose male group.

4. There were single incidences of malignant lymphomas (in the lymphoreticular system) in each of the paraquat-treated male and female groups, but no malignant lymphomas were observed in the male and female controls.

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Table XXV. Incidence of predominant neoplasms in rats sacrificed at termination of study[a]

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
Number of rats sacrificed	29	31	23	25	33	28	30	29	29	29
Neoplasms	Incidence of neoplasms[b]									
Adrenal: benign pheochromocytoma	3	5	4	5	9	1	1		1	1
Pancreas: islet cell adenoma	3	3		4	2	4	2	1	3	1
Pituitary:[c] adenoma	5	6	5	6	7	17	15	15	10	16
carcinoma				1		2	1	2	3	1
Thyroid: follicular adenoma	1	2		1	2	1	2		2	
parafollicular adenoma	5	4	4	4	11	5	4	1	3	7
parafollicular carcinoma		2	3	2			2	3	1	1
Mammary gland: benign fibroepithelial tumor	6	5	3	4	5	17	17	16	17	18
Testis: interstitial cell tumor	29	30	21	25	33	--	--	--	--	--
Preputial gland: adenoma	1	2	1			1			3	1
Skin and subcutis:										
Fibroma	5	4	4	7	4	1	1	2	2	2
Lipoma	1	1	3	2	1		1			
Papilloma	3	1	2	2	4			1		
Basal cell tumor	1	2	1	3	2	1	1	1		1
Fibrosarcoma				1	1			2		
Squamous cell carcinomas in head region: Middle ear[d]					1					
Monocytic leukemia	5	6	8	5	4	4	3	6	3	2
Malignant lymphoma (Lymphoreticular system)			1	1	1			1	1	1

Table XXV. (Cont'd) Incidence of predominant neoplasms in rats sacrificed at termination of study[a]

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
	Percent of tissues with neoplasms									
Adrenal: benign pheochromocytoma	10	16	17	20	27	4	3		3	3
Pancreas: islet cell adenoma	10	10		16	6	14	7	3	10	3
Pituitary:[c] adenoma	17	20	22	27	21	61	52	55	36	55
carcinoma				4		7	3	7	11	3
Thyroid: follicular adenoma	3	6		4	6	4	7		7	
parafollicular adenoma	17	13	17	16	33	18	13	3	10	24
parafollicular carcinoma		6	13	8			7	10	3	3
Mammary gland: benign fibroepithelial tumor	21	16	13	16	15	61	57	55	59	62
Testis: interstitial cell tumor	100	97	91	100	100	--	--	--	--	--
Preputial gland: adenoma	3	6	4			4			10	3
Skin and subcutis:										
Fibroma	17	13	17	28	12	4	3	7	7	7
Lipoma	3	3	13	8	3		3			
Papilloma	10	3	9	8	12			3		
Basal cell tumor	3	6	4	12	6	4	3	3		3
Fibrosarcoma				4	3			7		
Squamous cell carcinomas in head region: Middle ear[d]					3					
Monocytic leukemia	17	19	35	20	12	14	10	21	10	7
Malignant lymphoma (Lymphoreticular system)			4	4	3			3	3	3

a. This table is based on TABLE 19C of the submission and on Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985. Pulmonary and ocular lesions are discussed separately.

b. Unless indicated otherwise, the numbers of rats sacrificed equal the numbers of tissues examined.

c. In groups 2M, 4M, 2F, 3F, and 4F, tissues from 30, 22, 29, 27, and 28 rats, respectively, were examined.

d. In group 3M, tissues from 22 rats were examined.

The incidence of predominant neoplasms in the entire study is summarized in Tables XXVI and XXVII. Based on these data, the following observations can be made:

1. The incidence of benign pheochromocytomas in adrenal medulla was increased in the high-dose male rats.

2. The incidence of thyroid parafollicular adenomas was increased in the high-dose male rats.

3. The incidence of skin tumors of epithelial origin (papilloma and squamous carcinoma) was increased in the high-dose male rats.

4. The incidence of lipoma (subcutaneous fat cell tumor) were increased in the treated male rats.

The potential relationship of the above neoplasms to treatment with paraquat is unclear. Additional data were therefore requested in an effort to clarify this relationship and to evaluate adequately the oncogenic potential of paraquat.

Table XXVI. Incidence of predominant neoplasms in male rats: Summary[a]

Test Group	1M and 2M				3M				4M				5M			
Paraquat cation (ppm)	0				25				75				150			
Neoplasms	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]
	80	60	140		33	37	70		35	35	70		43	26	69	
Adrenal: benign pheochromocytoma	8	4	12	8.6	4	5	9	12.9	5	3	8	11.4	9	3	12	17.4
	80	58	138		33	37	70		35	35	70		43	25	68	
Pancreas: islet cell adenoma	6	2	8	5.8		3	3	4.3	4	2	6	8.6	2	1	3	4.4
	74	56	130		33	35	68		31	34	65		41	27	68	
Pituitary: adenoma	11	11	22	16.9	5	13	18	26.5	6	12	18	27.7	7	4	11	16.2
carcinoma		1	1	0.8					1	2	3	4.6				
TOTAL	11	12	23	17.7	5	13	18	26.5	7	14	21	32.3	7	4	11	16.2
	80	55	135		33	32	65		35	31	66		43	26	69	
Thyroid: parafollicular adenoma	9	5	14	10.0	4	2	6	9.2	4	2	6	9.1	11	2	13	18.8
parafollicular carcinoma	2		2	1.5	3		3	4.6	2		2	3.0				
TOTAL	11	5	16	11.9	7	2	9	13.8	6	2	8	12.1	11	2	13	18.8
follicular adenoma	3	1	4	3.0		1	1	1.5	1	1	2	3.0	2	1	3	4.3
	80	60	140		33	37	70		35	35	70		43	27	70	
Mammary gland: benign fibroepithelial tumor	11	1	12	8.6	3	1	4	5.7	4	1	5	7.1	5	2	7	10.0
	80	59	139		33	37	70		35	35	70		43	27	70	
Testis: interstitial cell tumor	59	45	104	74.8	21	31	52	74.3	25	26	51	72.9	33	21	54	77.1
	80	60	140		33	37	70		35	35	70		43	27	70	
Preputial gland: adenoma	4	4	8	5.7	1	1	2	2.9		2	2	2.9				

Table XXVI. (Cont'd) Incidence of predominant neoplasms in male rats: Summary[a]

Test Group	1M and 2M				3M				4M				5M			
Paraquat cation (ppm)	0				25				75				150			
Neoplasms	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent
	80	60	140		33	37	70		35	35	70		43	27	70	
Skin and subcutis:																
Fibroma, fibrosarcoma and mesenchymal fibrosarcoma	9	4	13	9.3	4	4	8	11.4	8	7	15	21.4	5	4	9	12.
Lipoma	2		2	1.4	3		3	4.3	2	1	3	4.3	1	4*	5	7.
Papilloma and squamous cell carcinoma[c]	4	8	12	8.6	3	4	7	10.0	3	2	5	7.1	6	9	15	21.
Basal cell tumor	3	1	4	2.9	1	2	3	4.3	3	1	4	5.7	2		2	2.
Unidentified carcinoma														1	1	1.
	80	60	140		33	37	70		35	35	70		43	27	70	
Lymphoreticular system																
Malignant lymphoma		1	1	0.7	1	1	2	2.9	1		1	1.4	1		1	1.
Monocytic leukemia	11	25	36	25.7	8	12	20	28.6	5	13	18	25.7	4	9	13	18.

a. This table is based on TABLES 19A, 19B and 19C of the submission and on Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985. For each organ or tissue, the top line represents the numbers of tissues examined and the remaining lines the numbers of neoplasms observed. Pulmonary and ocular lesions were considered separately.

b. Of total tissues examined.

c. Includes squamous cell carcinoma in head region.

S = Scheduled sacrifices (interim and final).

D = Moribund sacrifices and deaths.

* Significantly different from controls (P<0.05).

Table XXVII. Incidence of predominant neoplasms in female rats: Summary[a]

Test Group	1F and 2F				3F				4F				5F			
Paraquat cation (ppm)	0				25				75				150			
Neoplasms	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent
	78	62	140		39	31	70		39	31	70		39	31	70	
Adrenal: benign pheochromocytoma	2	1	3	2.1					1		1	1.4	1		1	1.
	78	61	139		39	30	69		39	31	70		39	30	69	
Pancreas: islet cell adenoma	6	1	7	5.0	1		1	1.4	3		3	4.3	1		1	1.
	74	56	130		36	29	65		37	31	68		39	28	67	
Pituitary: adenoma	33	26	59	45.4	16	17	33	50.8	11	22*	33	48.5	16	18	34	50.
carcinoma	3	5	8	6.1	2	4	6	9.2	3	2	5	7.4	1	4	5	7.
TOTAL	36	31	67	51.5	18	21	39	60.0	14	24	38	55.9	17	22	39	58.
	78	62	140		39	31	70		39	30	69		39	29	68	
Thyroid: parafollicular adenoma	9	5	14	10.0	1	5	6	8.6	3	1	4	5.8	7	3	10	14.
parafollicular carcinoma	2		2	1.4	3		3	4.3	1		1	1.4	1		1	1.
TOTAL	11	5	16	11.4	4	5	9	12.9	4	1	5	7.2	8	3	11	16.
follicular adenoma	3	1	4	2.9		2	2	2.9	2		2	2.9		3	3	4.
	78	62	140		39	31	70		39	31	70		39	31	70	
Mammary gland: benign fibroepithelial tumor	34	23	57	40.7	16	13	29	41.4	17	12	29	41.4	18	9	27	38.

Table XXVII. (Cont'd) Incidence of predominant neoplasms in female rats: Summary[a]

Test Group	1F and 2F				3F				4F				5F			
Paraquat cation (ppm)	0				25				75				150			
Neoplasms	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]	S	D	Total	Percent[b]
	78	60	138		39	31	70		39	31	70		39	31	70	
Skin and subcutis:																
Fibroma and fibrosarcoma	2	4	6	4.3	4	3	7	10.0	2	2	4	5.7	2	2	4	5.
Lipoma	1		1	0.7		1	1	1.4								
Papilloma and squamous cell carcinoma[c]					1		1	1.4		4	4	5.7		2	1	2.
Basal cell tumor	2	1	3	2.2	1		1	1.4		1	1	1.4	1		1	1.
	78	62	140		39	31	70		39	31	70		39	31	70	
Lymphoreticular system																
Malignant lymphoma		3	3	2.1	1	1	2	2.9	1	1	2	2.9	1	1	2	2.
Monocytic leukemia	7	14	21	15.0	6	4	10	14.3	3	3	6	8.6	2	7	9	12.

a. This table is based on TABLES 19A, 19B and 19C of the submission and on Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985. For each organ or tissue, the top line represents the numbers of tissues examined and the remaining lines the numbers of neoplasms observed. Pulmonary and ocular lesions were considered separately.

b. Of total tissues examined.

c. Includes squamous cell carcinoma in head region.

S = Scheduled sacrifices (interim and final).

D = Moribund sacrifices and deaths.

* Significantly different from controls ($P<0.05$).

XIX. Histopathology: non-neoplastic and neoplastic lesions of the lungs

It was difficult to characterize pulmonary lesions as non-neoplastic or neoplastic, or as adenomas or carcinomas. For this reason, lungs showing foci, alveolar epithelialization and/or neoplastic changes during an initial examination were re-examined independently by four pathologists: 1) Dr. J.B. Finn, the Director of Pathology at Life Science Research (testing laboratory); 2) Dr. J. Ishmael, the sponsor's pathologist; 3) Dr. Robert A. Squire, a consultant pathologist from Ruxton, Maryland; and 4) Dr. Donald Dungworth from University of California, School of Veterinary Medicine, Davis, California. The final assessment was prepared by Dr. Finn, taking into account the findings of other pathologists. The reports of the pathologists, showing differences in their respective diagnosis, were included in the submission (TABLE 20 and Appendixes 18, 19 and 21). The incidence of treatment-related or possibly treatment-related pulmonary lesions is summarized in Tables XXVIII and XXIX.

Table XXVIII. Incidence of treatment-related or possibly treatment-related non-neoplastic lesions in the lungs of rats*

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
Rats killed after 52 weeks of treatment	Number of rats with lesions									
Number of rats examined**	10	10	10	10	10	10	10	10	10	10
Alveolar epithelialization	0	1	1	1	1	0	0	1	1	1
Alveolar pigmented macrophages	0	3	1	1	0	2	0	1	1	4
	Percent of rats with lesions									
Alveolar epithelialization	0	10	10	10	10	0	0	10	10	10
Alveolar pigmented macrophages	0	30	10	10	0	20	0	10	10	40
Rats dying between week 53 and termination	Number of rats with lesions									
Number of rats examined**	30	24	36	35	25	31	29	30	27	30
Alveolar epithelialization	2	1	1	4	5	0	4	1	4	2
Accumulations of alveolar macrophages	8	7	8	10	4	3	4	8	6	10[c]
Increased numbers of alveolar macrophages	1	0	1	6[a]	7[b]	2	3	1	3	0

Table XXVIII. (Cont'd.) Incidence of treatment-related or possibly treatment-related non-neoplastic lesions in the lungs of rats*

	Percent of rats with lesions									
Alveolar epithelialization	6.7	4.2	2.8	11.4	20.0	0	13.8	3.3	14.8	6.7
Accumulations of alveolar macrophages	26.7	29.2	22.2	28.6	16.0	9.7	13.8	26.7	22.2	33.3[c]
Increased numbers of alveolar macrophages	3.3	0	2.8	17.1[a]	28.0[b]	6.5	10.3	3.3	11.1	0
Rats killed at termination of study	Number of rats with lesions									
Number of rats examined**	29	31	23	25	33	28	30	29	29	29
Alveolar epithelialization	0	0	2	2	4	3	3	4	3	0
Peribronchiolar lymphoid hyperplasia (slight)	15	17	11	10	26[a]	14	13	6[a]	7	15
Focal accumulation of alveolar macrophages	11	6	8	10	19	6	8	5	8	12
Increased numbers of alveolar macrophages	0	0	1	2	3	1	0	3	0	1
	Percent of rats with lesions									
Alveolar epithelialization	0	0	8.7	8.0	12.1	10.7	10.0	13.8	10.3	0
Peribronchiolar lymphoid hyperplasia (slight)	51.7	54.8	47.8	40.0	78.8[a]	50.0	43.3	20.7[a]	24.1	51.7
Focal accumulation of alveolar macrophages	37.9	19.4	34.8	40.0	57.6	21.4	26.7	17.2	27.6	41.4
Increased numbers of alveolar macrophages	0	0	4.3	8.0	9.1	3.6	0	10.3	0	3.4

* This table is based on TABLES 17 A, B and C of the submission and on Amended Supplement to LSR Report No. 8/ILY217/328; August 23, 1985.

** Number of rats examined equals number of lung tissues examined.

a, b and c. Significantly different from combined controls; $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Treatment-related, non-neoplastic pulmonary changes were not observed in the male and female rats dying during the first year of study and in the male rats killed after 52 weeks of treatment (interim sacrifice). However, there was an increased incidence of alveolar pigmented macrophages in the lungs of the high-dose female rats killed after 52 weeks of treatment.

In the rats dying between weeks 53 and termination, there was a dose-related increased incidence of alveolar epithelialization and alveolar macrophages in the mid-dose and high-dose male groups. However, an increased accumulation of alveolar macrophages was observed only in the high-dose females.

At the termination of the study, an increased incidence of pulmonary changes was noted mostly in the high-dose male rats.

Pulmonary neoplastic lesions

Adenomas and carcinomas (predominant lesions) were observed in the rats dying during the second year of the study and in those killed at the termination of the study. In both instances, there were more adenomas than carcinomas. There were no pulmonary adenomas or carcinomas in rats dying during the first 52 weeks of treatment, but 2 adenomas were noted at the interim sacrifice. The incidence of adenomas and carcinomas is summarized in Table XXIX.

Table XXIX. Incidence of pulmonary adenomas and carcinomas*

Test Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Paraquat cation (ppm)	0	0	25	75	150	0	0	25	75	150
Rats killed after 52 weeks of treatment	Number of rats with neoplasms									
Number of rats examined**	10	10	10	10	10	10	10	10	10	10
Adenoma	0	0	0	1	0	0	0	0	0	1
Rats dying between week 53 and termination										
Number of rats examined**	30	24	36	35	25	31	29	30	27	30
Adenoma	1	1	2	0	1	0	0	0	0	2
Carcinoma	1	0	0	1	1	0	0	0	0	1
Rats killed at termination of study										
Number of rats examined**	29	31	23	25	33	28	30	29	29	29
Adenoma	0	1	1	4	3	0	0	1	2	5
Carcinoma	0	0	1	0	2	0	0	1	1	1
Number of rats examined during study #	70	69	70	70	69	70	69	70	70	70
Adenoma	1	2	3	5	4	0	0	1	2	8[c]
Carcinoma***	1	0	1	1	3	0	0	1	1	2
Total neoplasms	2	2	4	6	7	0	0	2	3	10[c]
Percent of rats with neoplasms	2.9	2.9	5.7	8.6	10.1	0	0	2.9	4.3	14.3[c]

* This table is based on TABLES 19A, B and C and Text Table 4 of the submission, and on Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985.

** Number of rats examined equals number of lung tissues examined.

*** This term denotes the following carcinomas: 1) bronchioloalveolar carcinoma (single rats in Groups 3M, 3F, 4F and 5M, & 2 rats in Group 5F); 2) squamous cell carcinoma (single rats in Groups 4M & 5M); and 3) both bronchioloalveolar and squamous cell carcinomas in same mass (single rats in Groups 1M and 5M).

\# Including rats dying during the first 52 weeks of treatment. The numbers of rats (and lung tissues) examined during the first year of treatment were 1, 4, 1, 0, and 1 in Groups 1M, 2M, 3M, 4M, and 5M, respectively. The numbers of rats (and lung tissues) examined in Groups 1F, 2F, 3F, 4F, and 5F were 1, 0, 1, 4, and 1, respectively.

[c] Significantly different from combined controls ($P<0.001$).

The summary portion of Table XXIX (excluding "Percent of rats with neoplasms") is identical with Text Table 4 ("Animals bearing primary pulmonary neoplasms in the lungs") of the submission. Using the NCI computer program, Life Science Research (the testing laboratory) performed statistical analyses on these data. The analyses "consisted of a trend test (an exact generalization of Fisher's Exact Test) and a series of pairwise comparisons of treated groups against pooled controls using Fisher's Exact Test. Males and females were considered separately." The results of these analyses and their interpretation by the testing laboratory appear in Table XXX.

Table XXX. Results of statistical analysis (p values) and their interpretation*

	Males				Females			
	Trend test	Pooled Control G3	G4	G5	Trend test	Pooled Control G3	G4	G5
Adenoma	0.10	0.32	0.08	0.17	<0.0001	0.33	0.11	0.00012
Carcinoma	0.054	0.054	0.57	0.11	0.056	0.33	0.33	0.11

In summary:

"i) In females there was a significant trend ($P < 0.0001$) between increasing dosage of paraquat and the incidence of pulmonary adenoma.

ii) The pairwise comparisons demonstrated in females a highly significant ($P = 0.00012$) incidence of adenoma at a dosage of 150 ppm.

iii) The comparisons for the low and intermediate dosage groups for either sex did not reach significance at the $P < 0.05$ level.

iv) There was no treatment-related incidence of carcinoma."(1)

* This is essentially Text Table 6 (page 43) of the submission.

G3, G4 and G5: rats fed 25, 75 and 150 ppm of paraquat cation, respectively.

(1) Paraquat: Combined Toxicity and Carcinogenicity Study in Rats. No. 82/ILY 217/328; October 27, 1983; Volume I, page 43.

XX. Histopathology of the eyes

These data were reported as incidences (with P values) of pathological changes in the eyes of rats and as individual observations on rat eyes. The summary data (TABLES 18A, B and C of the submission) included numbers of eyes examined and pathological changes observed in each group of rats as follows: 1) rats dying during the treatment period; 2) rats sacrificed after 52 weeks of treatment (interim sacrifice); and 3) rats killed at the termination of the study. The individual data (Appendixes 17A, B, C, D, E and F of the submission) included ID numbers of rats and microscopic pathology (separately for each eye) as follows: 1) for rats killed after 52 weeks of treatment and at the termination of the study; and 2) for rats dying during the study (treatment weeks 1-52, 53-104, 105-116 and 117-termination). It was also indicated in the individual data for which rats histopathological examination was not performed and why. Data concerned with predominant ocular changes are summarized in Tables XXXI through XXXIV.

Table XXXI. Incidence of predominant ocular changes at termination of study*

Test Group	1+2M	3M	4M	5M	1+2F	3F	4F	5F
Paraquat cation (ppm)	0	25	75	150	0	25	75	150
Number of rats examined	60	23	25	33	58	29	29	29
Number of eyes examined	120	44	50	66	114	56	56	58
Number of eyes not examined**	0	2	0	0	2	2	2	0
Ocular change	Percent of eyes with change***							
° Peripheral Morgagnian corpuscles****	62.5	77.3	90.0	98.5	77.2	89.3	94.6	98.3
° Peripheral lenticular degeneration****	2.5	22.7	84.0	65.2	26.3	55.4	46.4	56.9
° Mid-zonal lenticular degeneration****	5.0	13.6	70.0	109.1	28.1	51.8	119.6	108.6
° Pear-shaped posterior peripheral lenticular change	3.3	9.1	70.0	69.7	27.2	33.9	58.9	58.6

Table XXXI. (Cont'd) Incidence of predominant ocular changes at termination of study*

Test Group	1+2M	3M	4M	5M	1+2F	3F	4F	5F
Paraquat cation (ppm)	0	25	75	150	0	25	75	150
Number of rats examined	60	23	25	33	58	29	29	29
Number of eyes examined	120	44	50	66	114	56	56	58
Number of eyes not examined**	0	2	0	0	2	2	2	0
Ocular change	Percent of eyes with change***							
° Central lenticular degeneration	0	0	0	0	0	0	0	5.2
° Lens capsule fibrosis	0.8	2.3	4.0	15.2	0	1.8	1.8	1.7
° Lens capsule rupture	0	0	0	18.2	0	0	0	6.9
° Peripheral retinal degeneration: of outer nuclear layer#	6.7	4.4	22.0	19.7	7.0	7.1	1.8	10.3
° Displacement of retina by bony metaplasia#	2.5	2.2	2.0	6.1	0.9	1.8	1.8	3.4
° Synechia (posterior)#	0.8	2.2	2.0	12.1	0.9	0	0	5.2
° Iritis (slight)#	0	6.7	4.0	6.1	0.9	0	0	8.6
° Proteinaceous aqueous humor#	0	2.2	6.0	18.2	0.9	0	0	10.3
° Vitreous cellularity#	0	2.2	2.0	12.1	0	0	0	3.4

* This table is based on TABLE 18A and APPENDIX 17A of the submission.

** Due to missing eyes, absent or damaged lenses, and/or poor or damaged tissue sections.

*** Calculated as follows: number of eyes with change x 100/number of eyes examined in each group.

**** Includes slight, moderate and marked changes. In the case of mid-zonal lenticular degeneration, "heart-shaped" changes are also included. Where percent of eyes with change exceeds 100, this means that different degrees of pathological changes (for example, slight and moderate) were observed in the same eye, in some eyes, in that group.

In the case of Group 3M, 45 eyes were examined to obtain these findings. However, 44 eyes were available for the examination of lenticular tissues.

These data show that there was an increased incidence of lenticular changes (not always dose-related) in the paraquat-treated male and female rats when rats were compared with the controls. When slight, moderate and marked changes are considered together, there was a dose-related increase in the incidence of peripheral Morgagnian corpuscles, mid-zonal lenticular degeneration (males only) and lens capsule fibrosis (males only). Other lenticular changes, which occurred with greater frequency in the paraquat-treated groups than in the controls, were either dose-unrelated or were observed only in the high-dose groups. Moderate to marked changes generally prevailed in the mid-dose and high-dose groups, whereas slight to moderate changes prevailed in the low-dose group. In the control groups, slight changes were most frequent. In most instances, the differences in the incidence of lenticular lesions between the controls and the paraquat-treated mid-dose and high-dose groups were statistically significant at the $P<0.001$ level. The incidences of lenticular changes in the low-dose group and their statistical significance is summarized in Table XXXII.

Table XXXII. Incidence of lenticular changes (significantly different from controls) in the low-dose (25 ppm) groups of rats killed at the termination of study*

Lenticular change	Sex	Percent of eyes with lenticular change**			P value
		Control group	Low-dose group	Percent increase	
° Peripheral Morgagnian corpuscles:					
moderate	Males	22.5	40.9	18.4	<0.05
marked	Females	7.9	23.2	15.3	<0.01
° Peripheral lenticular degeneration:					
slight	Males	2.5	18.2	15.7	<0.01
moderate	Females	4.4	23.2	18.8	<0.01
° Mid-zonal lenticular degeneration:					
moderate	Males	0	6.8	6.8	<0.05
moderate	Females	7.9	23.2	15.3	<0.05

* This table is based on TABLE 18A of the submission.

** Based on the following numbers of eyes examined: 120 (Groups 1M and 2M; combined male controls); 44 (Group 3M; low-dose males); 114 (Groups 1F and 2F; combined female controls); and 56 (Group 3F; low-dose females).

Other ocular (non-lenticular) changes in which an effect of paraquat was implicated, observed at the termination of study, were either dose-unrelated or occurred mostly in the high-dose groups (see last 5 entries in Table XXXI). According to the testing laboratory, "it is likely that these changes were secondary to the development of advanced cataract, and therefore they do not represent a primary effect of treatment with paraquat." Dr. Louis Kasza, Pathologist (Hazard Evaluation Division/ Toxicology Branch) agrees with this comment. According to Dr. Kasza, when an eye is inactive (as is an eye with cataract), various degenerative changes can occur.

Based on TABLE 18C (summary table) of the submission, ocular changes observed in rats dying during the study were similar in type and incidence to those observed at the termination of the study. Changes that were attributed to paraquat were confined to the lens and were dose-related in both males and females. In most instances, moderate to marked changes predominated in the mid-dose and high-dose groups, whereas slight to moderate changes predominated in the low-dose and the control groups. In most instances, the differences in the incidence of lenticular lesions between the controls and the high-dose groups were statistically significant at the $P<0.001$ level. In the case of the mid-dose groups, these differences were significant mostly at the $P<0.01$ or $P<0.05$ levels. The non-lenticular changes were either dose-unrelated or occurred mostly in the high-dose groups.

Data concerned with ocular changes in rats dying during the study are summarized in Tables XXXIII and XXXIV. In order to demonstrate that ocular changes were most abundant after the treatment week 104, the incidence of ocular changes was summarized separately for the treatment weeks 1-104 and 105-termination.

Table XXXIII. Incidence of predominant ocular changes in rats dying during the treatment period*

Test Weeks	1. - 104				105 - Termination				1 - 104				105 - Termination			
Paraquat cation (ppm)	0	25	75	150	0	25	75	150	0	25	75	150	0	25	75	150
Test Group	1 and 2M	3M	4M	5M	1 and 2M	3M	4M	5M	1 and 2F	3F	4F	5F	1 and 2F	3F	4F	5F
Number or rats examined	36	20	18	16	24	17	17	11	23	17	16	16	39	14	15	15
Number of eyes examined	63	38	34	27	36	29	30	22	40	28	23	26	72	28	28	30
Number of eyes not examined**	9	2	2	5	12	5	4	0	6	6	9	6	6	0	2	0
Ocular Change	Number of eyes with ocular change															
Peripheral Morgagnian corpuscles****	20	21	10	16	19	24	25	20	10	6	8	21	57	28	26	30
Peripheral lenticular degeneration****	3	8	6	9+	19	17	13	5	13	8	12	6	45	11	11	6
Mid-zonal lenticular degeneration****	0	0	0	6	1	3	5+	16#	1	0	0	4	8	7	15	27
Pear-shaped posterior peripheral lenticular change	0	2	2	10	2	5	14	17	0	0	0	16	11	13	15	24
Peripheral lenticular degeneration: loss of outer nuclear layer	0	0	0	0	6	6	3	5	1	3	4	4	24	14	11	16
Synechia: posterior	1	1	3	1	1	3	1	2	4	0	2	1	4	2	6	1
Synechia: anterior	12	7	3	7	13	6	11	8	7	5	4	2	12	4	9	12
Proteinaceous aqueous humor	0	0	3	2	3	3°	4°°	4	0	0	0	0	6	0	6	2
Vitreous: protein in vitreous	0	2	0	2	2	2	0	5	0	0	0	0	1	1	2	2
Ocular Change	Percent of eyes with ocular change***															
Peripheral Morgangnian corpuscles****	31.7	55.3	29.4	59.3	52.8	82.8	83.3	90.9	25.0	21.4	34.8	80.8	79.2	100	92.9	100
Peripheral lenticular degeneration****	4.8	21.1	17.6	33.3	52.8	58.6	43.3	22.7	32.5	28.6	52.2	23.1	62.5	39.3	39.3	20.0
Mid-zonal lenticular degeneration****	0	0	0	22.2	2.8	10.3	16.7+	72.7#	2.5	0	0	15.4	11.1	25.0	53.6	90.0

Table XXXIII. (Cont'd) Incidence of predominant ocular changes in rats dying during the treatment period*

Test Weeks	1 - 104				105 - Termination				1 - 104				105 - Termination			
Paraquat cation (ppm)	0	25	75	150	0	25	75	150	0	25	75	150	0	25	75	150
Test Group	1 and 2M	3M	4M	5M	1 and 2M	3M	4M	5M	1 and 2F	3F	4F	5F	1 and 2F	3F	4F	5F
Number or rats examined	36	20	18	16	24	17	17	11	23	17	16	16	39	14	15	15
Number of eyes examined	63	38	34	27	36	29	30	22	40	28	23	26	72	28	28	30
Number of eyes not examined**	9	2	2	5	12	5	4	0	6	6	9	6	6	0	2	0
Pear-shaped posterior peripheral lenticular change	0	5.3	5.9	37.0	5.6	17.2	46.7	77.3	0	0	0	61.5	15.3	46.4	53.6	80.0
Peripheral lenticular degeneration: loss of outer nuclear layer	0	0	0	0	16.7	20.7	10.0	22.7	2.5	10.7	17.4	15.4	33.3	50.0	39.3	53.3
Synechia: posterior	1.6	2.6	8.8	3.7	2.8	10.3	3.3	9.1	10.0	0	8.7	3.8	5.6	7.1	21.4	3.3
Synechia: anterior	19.0	18.4	8.8	25.9	36.1	20.7	36.7	36.4	17.5	17.9	17.4	7.7	16.7	14.3	32.1	40.0
Proteinaceous aqueous humor	0	0	8.8	7.4	8.3	11.1°	14.3°°	18.2	0	0	0	0	8.3	0	21.4	6.7
Vitreous: protein in vitrous	0	5.3	0	7.4	5.6	6.9	0	22.7	0	0	0	0	1.4	3.6	7.1	6.7

* This table is based on APPENDIXES 17C, D, E and F of the submission. "Rats dying during the treatment period" means those rats which were found dead in their cages and those which had to be killed because they were moribund.

** Due to missing eyes, absent or damaged lenses, poor or damaged tissue sections, or insufficient tissue for useful study.

*** Based on numbers of eyes examined in each group. In the case of mid-zonal lenticular degeneration, "heart-shaped" changes are also included.

**** Includes slight, moderate and marked changes.

° 27 eyes were examined in this case.

°° 28 eyes were examined.

\+ One "heart-shaped" change is included.

\# Six "heart-shaped" changes are included.

Table XXXIV. Incidence of lenticular changes (significantly different from controls) in the low-dose (25 ppm) groups of rats dying during the treatment period*

Lenticular change	Sex	Percent of eyes with lenticular change**			P valu
		Control Group	Low-dose Group	Percent Increase	
° Peripheral Morgagnian corpuscles: marked	Males	7.1	22.4	15.3	<0.05
° "Pear-shaped" posterior peripheral lenticular change	Males	2.0	10.4	8.4	<0.05
	Females	9.8	23.2	13.4	<0.05

* This table is based on TABLE 18C of the submission.

** Based on the following numbers of eyes examined: 99 (Groups 1M and 2M; combined male controls); 67 (Group 3M; low-dose males); 112 (Groups 1F and 2F; combined female controls); and 56 (Group 3F; low-dose females).

No treatment-related ocular changes were observed in rats sacrificed after test week 52 (interim sacrifice).

SUMMARY

Fischer 344 strain of rats, 70 males and 70 females per dose level and weighing initially 60-70g, were fed diets containing 0 (Group 1), 0 (Group 2), 25 (Group 3), 75 (Group 4) and 150 (Group 5) ppm of paraquat cation for 113-117 weeks (males) and 122-124 weeks (females). Additional rats, 5 males and 5 females per dose level, were included in Groups 1, 3, 4 and 5 in order to determine paraquat concentration in tissues after one year of exposure. The animals were housed in groups of 5/sex/cage and were numbered by ear notching. The temperature of the housing area was 21 ± 2°C and the relative humidity 55 ± 15%. The test material was a technical grade paraquat (1,1'-dimethyl-4,4'-bipyridylium) dichloride containing 32.69% w/w of paraquat cation, which was stable in diets and during storage at room temperature. The diet fed in unrestricted amounts was Spratt's Laboratory Diet 2 (composition not reported). The diet was obtained as a powder and was fed in this form without or with added paraquat.

According to the initial protocol, this study was to be terminated after 104 weeks. However, because of low number of deaths during that period, the study was extended until at least 113 weeks for the male rats and 122 weeks for the female rats, that is, until survival was reduced to 50% in any one of Groups 1-4. (Group 5 was excluded from consideration). The following parameters were examined:

- Toxic signs and mortality
- Food and water consumption
- Body weight
- Food utilization
- Ophthalmoscopy
- Achieved dosage in terms of mg/kg of body weight
- Hematology
- Clinical chemistry
- Urinalysis
- Gross necropsy
- Organ weights
- Histopathology
- Paraquat concentration in tissues, plasma and urine

Most of the data were analyzed statistically.

The following findings were most important:

1. With the exception of eye opacity, respiratory distress (males) and possibly ptosis/swollen eyelids (females) and palpable masses, the most frequently observed clinical signs (lethargy, pallor, hypothermia, lack of muscle tone, ocular discharge, skin discoloration, abdominal masses/distension, necrotic/ulcerated masses, and nasal, genitourinary and anal staining) did not appear to be treatment-related.

2. Male and female rats receiving 150 ppm of paraquat and female rats receiving 75 ppm of paraquat had higher incidence of eye opacity than did the controls during the study and at the termination of the study. A slightly higher incidence of eye opacity was also observed in the low-dose females and the mid-dose males when these animals were compared with the controls at the termination of the study. Eye opacities occurred most frequently during the treatment weeks 91-124 and both eyes were generally opaque in the mid-dose and high-dose groups. (These were cage-side observations, made without an ophthalmoscope).

3. The predominant ocular lesions, detected ophthalmoscopically, in the controls and the paraquat-treated male and female rats were lenticular opacities and cataracts. These lesions were either not observed or were observed infrequently before the treatment week 103.

   Paraquat enhanced the development of the ocular lesions in all of the treated groups. Starting with the treatment week 110, there was a dose-related increase in the incidence of cataracts in the male and female rats, when the paraquat-treated groups were compared with the controls. There was also a dose-related increase in the numbers of male and female rats with lenticular lesions (cataracts and opacities), when the controls were compared with the treated animals. However, at test week 103, dose-related increased incidences of cataracts were observed only in the mid-dose and high-dose groups. Increased incidences of lenticular opacities were inconsistent, dose-unrelated and occurred only in the low-dose and mid-dose groups.

   In the case of the male rats, increases in the incidence of ocular lesions (opacities and cataracts) in the paraquat-treated groups were statistically significant ($P<0.001$, 0.01 or 0.05) at the test weeks 110 and 112/113. At the treatment week 103, increases in the incidence of ocular lesions were statistically significant ($P<0.001$ in most instances) only in the mid-dose and high-dose groups. In the case of female rats, statistically significant ($P<0.001$ in most instances) increases in cataracts occurred mostly in the mid-dose and high-dose groups during the treatment weeks 103-118/119. Ophthalmoscopic findings were confirmed by histopathological examination.

4. Among the male rats dying during the study, the low-dose and mid-dose groups had proportionally more rats with palpable masses and more palpable masses than did the control groups. However, the high-dose male group had fewer rats with palpable masses and only slightly more palpable masses than did the controls.

Among the male rats sacrificed at the termination of the study, only the mid-dose group had more palpable masses than did the controls.

Paraquat had no effect on the incidence of palpable masses in the female rats which either died during the study or were sacrificed at the termination of the study.

There was no difference in the onset of palpable masses between the control and the paraquat-treated rats, both males and females, which either died during the study or were sacrificed at the termination of the study.

5. There was a higher incidence of respiratory distress in the paraquat-treated male rats dying during the study than in the controls, but the incidence was dose-unrelated.

6. Paraquat had no effect on mortality, hematology, clinical chemistry and urinalysis.

7. Food intake of the low-dose and mid-dose male and female rats was unaffected by paraquat. In the case of the high-dose male rats, slight decreases (3-7%) in the food intake occurred during the treatment weeks 1-52 and 79-113. In the case of the high-dose female rats, slight decreases (3-8%) in the food intake occurred only during the treatment weeks 105-123. Water consumption was unaffected by paraquat.

8. Starting with the test week 13, the high-dose male and female rats utilized food slightly less efficiently than did the controls. (Food utilization was reported for the first 52 test weeks).

9. Paraquat had no effect on group mean body weights of the low-dose and mid-dose male and female rats. In the case of the high-dose male rats, small decreases (6-8%) in group mean body weights, when compared with the control values, were statistically significant only after the treatment weeks 26, 52, 78 and 104 ($P<0.001$ in each instance), and after the treatment week 113 ($P<0.05$). In the case of the high-dose female rats, small decreases (2-10%) in group mean body weights, when compared with the control values, were statistically significant only after the treatment week 52 ($P<0.05$) and the treatment weeks 78, 104, 117 and 122 ($P<0.001$ in each instance).

10. Lungs, liver, kidneys, skin, plasma and urine were examined for the concentration of paraquat cation. Dose-related concentrations of paraquat were detected in the following tissues: kidneys and plasma (all treated groups); lungs (male and female mid-dose and high-dose groups); liver (only in some rats of the high-dose female group); and skin (only

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in some rats of the mid-dose male group and the high-dose male and female groups). Urinary levels of paraquat cation increased with dose, indicating that some of the ingested paraquat was absorbed by the rats. (Paraquat is poorly absorbed from the gut).

11. Bone marrow and blood smear cytology did not reveal differences between the controls and the paraquat-treated groups. However, due to technical difficulties, bone marrow cytology was not performed for all animals.

12. The following organs were weighed at the interim and terminal sacrifices: adrenal, pituitary and thyroid glands, brain, heart, kidneys, liver, lungs, ovaries, testes, spleen and thymus. Small to moderate, but statistically significant, changes in the weights of liver, kidneys and testes were observed only in the high-dose groups at the termination of the study. In the males, there were group mean decreases in the absolute and relative weights of liver and testes ($P<0.001$, 0.01 or 0.05) and an increase in the relative weight of the lungs ($P<0.05$). In the females, there was a group mean decrease in the absolute weight of liver ($P<0.001$) and an increase in the relative weight of the lungs ($P<0.05$). Organ weights were also reported for rats dying during the study, but, because of wide variations in the time of death, these data were not evaluated statistically.

13. Macroscopic changes occurring in rats which died or were killed in extremis during the study included emaciation; urogenital and perianal staining; ocular discharge and staining, and nasal staining; pallor of extremities; firm subcutaneous masses; enlarged, hemorrhagic and/or congested pituitaries; prominent and/or congested cervical lymph nodes; lungs with subpleural foci and areas of incomplete collapse; pulmonary congestion or edema; pale and/or swollen livers, and with irregular surfaces; pale, granular and/or cystic kidneys; enlarged and swollen spleens; gastric ulcerations; small or enlarged testes, mostly with subcapsular plaques; small epididymides, seminal vesicles and prostates; and prominent mammary tissues (in males and females). All of these changes occurred with similar frequencies in the controls and the paraquat-treated groups.

    Similar changes and occurring with similar frequencies in the controls and the treated groups were also observed in rats sacrificed at the termination of the study. However, the paraquat-treated male and female rats had a higher incidence of pulmonary changes (occasional or multiple, dark or pale subpleural foci/areas) than did the controls, but the incidence was dose-unrelated in the females.

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14. The incidence of non-neoplastic and neoplastic lesions was reported initially without stating how many organs or tissues were examined at each dose level. It was only reported how many rats were examined (those dying during the study and those killed at scheduled times). However, this information was submitted later in a document entitled Amended Supplement to LSR Report No. 82/ILY217/328; August 23, 1985.

Wide range of non-neoplastic changes were observed in rats dying during the study and in those sacrificed at the termination of the study. However, most of these changes either occurred with similar frequency in the controls and the paraquat-treated groups or prevailed in the control groups. The following statistically significant non-neoplastic changes, observed in rats dying between week 53 and termination of study, were regarded as possibly treatment-related:

- Dose-unrelated increased incidence of hydrocephalus in the female rats ($P<0.05$).
- Dose-unrelated increased incidence of cysts or cystic spaces in the spinal cord of the male and female rats ($P<0.001$, 0.01 or 0.05).
- Dose-related increased incidence of degeneration of sciatic nerve fibers in the male rats ($P<0.001$).

The only statistically significant non-neoplastic change, regarded as treatment-related in rats killed at the termination of the study, was a higher incidence of hydrocephalus in the high-dose female rats than in the controls ($P<0.01$).

15. Only a few neoplastic lesions were observed during the first 52 weeks of treatment and they were not treatment-related. However, rats dying between week 53 and termination and those killed at termination of the study had a wide range of neoplasms (detailed in Tables XXIV and XXV of this review). In most instances, the controls and the paraquat-treated rats had about the same incidence of neoplasms. Exceptions were as follows:

In rats dying between week 53 and termination

- Skin lipoma - dose-related increased incidence in mid-dose and high-dose male rats.
- Skin papilloma - dose-related increased incidence in mid-dose and high-dose male rats.

- Squamous cell carcinoma in the head region (hard palate, middle ear, skin or head tissue), an uncommon tumor - occurred in 3/54 control males (5.6%), 3/36 low-dose males (8.3%), 5/26 high-dose males (19.2%), 3/27 mid-dose females (11.1%) and 2/30 high-dose females (6.7%).

In rats sacrificed at the termination of the study

- Benign pheochromocytoma (tumor of adrenal medulla) - dose-related increased incidence in male rats.
- Parafollicular adenoma (thyroid tumor) - increased incidence in high-dose male and female groups.
- Squamous cell carcinoma in the head region (middle ear), an uncommon tumor - one carcinoma was observed in the high-dose male group.

When the incidence of neoplastic lesions, other than lung lesions, is considered in the entire study and in all animals (Tables XXVI and XXVII of this review), the following observations can be made:

- The incidence of benign pheochromocytomas in adrenal medulla was increased in the high-dose male rats.
- The incidence of thyroid parafollicular adenomas was increased in the high-dose male rats.
- The incidence of skin tumors of epithelial origin (papilloma and squamous cell carcinoma) was increased in the high-dose male rats.
- The incidence of lipoma (subcutaneous fat cell tumor) was increased in the treated male rats.

The potential relationship of the above neoplasms to treatment is unclear. Additional data were therefore requested in an effort to clarify this relationship and to evaluate adequately the oncogenic potential of paraquat.

16. It was difficult to characterize pulmonary lesions as non-neoplastic or neoplastic, or as adenomas or carcinomas. For this reason, lungs showing foci, alveolar epithelialization and/or neoplastic changes during an initial examination were reexamined by four pathologists, of which two were independent consultants. The final assessment was prepared by Director of Pathology at Life Science Research (testing laboratory), taking into account the findings of other pathologists. The

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following findings were regarded as treatment-related or possibly treatment-related:

- Alveolar pigmented macrophages (non-neoplastic change) - increased incidence in the lungs of high-dose female rats, killed after 52 weeks of treatment (interim sacrifice).
- Alveolar epithelialization and alveolar macrophages (non-neoplastic change) - dose-related increased incidence in the mid-dose and high-dose male rats dying between week 53 and termination of study.
- Above and other non-neoplastic pulmonary changes (summarized in Table XXVIII of this review) - increased incidence was noted mostly in the high-dose male rats, killed at the termination of study.

17. The predominant pulmonary neoplastic lesions were adenomas and carcinomas, and were observed in rats dying during the second year of the study and in those killed at the termination of the study. However, 2 pulmonary adenomas were noted at the interim sacrifice.

The incidence of total neoplasms (adenomas and carcinomas) in the control, low-dose, mid-dose and high-dose male groups was 4/139 (2.9%), 4/70 (5.7%), 6/70 (8.6%) and 7/69 (10.1%), respectively. The corresponding numbers for carcinomas were 1, 1, 1, and 3, respectively.

The incidence of total neoplasms in the control, low-dose, mid-dose and high-dose female groups was 0/139 (0%), 2/70 (2.9%), 3/70 (4.3%) and 10/70 (14.3%), respectively. The corresponding numbers for carcinomas were 0, 1, 1, and 2, respectively.

Life Science Research analyzed the incidences of adenomas and carcinomas by various statistical tests and the following results were obtained (Table XXX) in this review):

- In females, there was a significant trend ($P<0.0001$) between increasing dosage of paraquat and the incidence of pulmonary adenoma.
- The incidence of adenoma in the high-dose female rats was highly significant ($P=0.00012$).
- There was no treatment-related incidence of carcinoma.
- Based on these data, Toxicology Branch/HED concluded that paraquat appeared to be oncogenic in the lungs of rats.

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18. During the treatment weeks 6-113, the female rats at all dose levels ingested more paraquat (15-33%) per kilogram of body weight than did the male rats at the same dose levels.

19. The lowest level of paraquat tested (25 ppm, expressed as cation) was considered "to lie close to the no-effect-level for lenticular change." The following differences were observed between the low-dose male or female groups and their controls:

    - Increased incidence of lenticular changes (opacities and cataracts) at treatment weeks 110 and 112/113 (males) and 118/119 (females). For example, at week 110, the ratios of numbers of lesions observed/numbers of rats examined were 0.34 and 0.81 for the control and low-dose male groups, respectively. The corresponding values for the female rats were 0.30 and 0.51, respectively (Tables XV and XVI in this review). Since these lesions are associated with an aging process and were present in the untreated rats, paraquat then accelerated the aging process. (These lenticular changes were detected ophthalmoscopically).

    - Increased incidence of lenticular changes (detected microscopically) in the male and female rats dying during the study or killed at the termination of the study (Tables XXXII and XXXIV in this review). These included (significantly different from the controls) slight or moderate peripheral lenticular degeneration, marked or moderate peripheral Morgagnian corpuscles, marked mid-zonal lenticular degeneration and "pear-shaped" changes. The percentage of eyes with changes (based on the numbers of eyes examined) in the control and the low-dose male groups ranged from 0 to 22.5 and from 6.8 to 40.9, respectively. This means that, for the low-dose male rats, increases in the incidence of lenticular changes ranged from 6.8% to 18.4% over the control values.

      The percentage of eyes with changes in the control and the low-dose female groups were 4.4-9.8 and 23.2 (in all instances), respectively. This means that, for the low-dose female rats, increases in the incidence of lenticular changes ranged from 13.4% to 18.8% over the control values.

      (There were no statistically significant increases in the peripheral lenticular degeneratione in the low-dose male and female rats which died during the study).

- Increased incidence of macroscopic pulmonary changes (occasional or multiple, dark or pale subpleural foci or areas) in the male and female rats sacrificed at the termination of the study (APPENDIXES 16E and F of the submission). The incidence of pulmonary changes (number of rats affected/number of rats examined) in the control and the low-dose male groups was 2/60 (3.3%; combined controls) and 4/23 (17.4%), respectively. The corresponding values for the female groups were 10/58 (17.2%) and 8/29 (27.6%), respectively.

- Increased incidence of slight hydrocephalus in the female rats dying between week 53 and termination of study (Table XXII in this review). The incidence of hydrocephalus (number of rats with lesion/number of rats examined) in the control and the low-dose groups was 5/60 (8.3%; combined controls) and 8/30 (26.7%), respectively. However, similar increases were observed in the mid-dose and high-dose female groups or 9/27 (33.3%) and 9/30 (30.0%), respectively.

- Increased incidence of spinal cord cysts/cystic spaces in the male rats dying between week 53 and termination of study (Table XXII in this review). The incidence of these lesions (number of rats with lesions/number of rats examined) in the control and the low-dose groups was 0/53 (combined controls) and 6/36 (16.7%), respectively. However, a smaller increase was observed in the mid-dose male group (4/35 or 11.4%).

NOELs and CORE Classification of Study

Toxicology Branch/Hazard Evaluation Division has concluded that, based on evidence in this study, paraquat is oncogenic in the lungs of rats. There is also some concern that paraquat may possibly be oncogenic in other tissues. Additional data were therefore requested from the registrant in order to evaluate adequately the oncogenic potential of paraquat.

Approximate Systemic NOEL: 25 ppm*

* Although some effects (mostly lenticular changes) were observed ophthalmoscopically and microscopically at that level in the male and female rats, they were either minimal or occurred mostly after 104 weeks of treatment and appeared, therefore, to be only an acceleration of the normal aging process (and not a qualitatively different effect). Until that time, or through most of the life span of the animals, a NOEL was in fact 25 ppm.

Other effects observed at the 25 ppm level (lowest fed) in the male and female rats were either minimal (subpleural foci or

areas at the termination of the study) or were ambiguous (slight hydrocephalus in the females and spinal cord cysts/cystic spaces in the males, both in rats dying during the study). For example, the incidence of spinal cord cysts/cystic spaces in the low-dose group was higher than that reported in the mid-dose group, and the incidence of hydrocephalus was similar in all of the paraquat-treated groups.

Considering the above findings, a true systemic NOEL was, therefore, probably very slightly below 25 ppm. (For the purpose of calculating an ADI, a safety factor higher than the usual 100 may be used in order to compensate for this slight uncertainty in the NOEL).

Core Classification of study - Guideline as a chronic feeding study and Supplementary as an oncogenic study. This classification for the oncogenic study will be updated upon receipt and evaluation of the requested data.