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

004572

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 239-2186. - Evaluation of a Study Entitled: Paraquat: 1-Year Feeding Study in Dogs. Report No. CTL/P/734; Imperial Chemical Industries (ICI), England; April 20, 1983.

Accession Numbers: 251668 and 251669
Record Number: 110528
Tox. Chem. Number: 634

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and

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Toxicology Branch/HED completed an evaluation of the above study in which Alderley Park beagle dogs, 6 males and 6 females per dose level, were fed diets containing technical grade paraquat for 52 weeks. The cation content of technical paraquat was 32.3% w/w. The amount of food offered daily each dog was 400 g. The levels of paraquat cation fed were 0, 15, 30 and 50 ppm. Based on actual group mean body weights and food consumption, these values corresponded to 0, 0.45, 0.93 and 1.51 mg* of paraquat cation per kilogram of body weight, per day, respectively, in the case of male dogs. For female dogs, these values corresponded to 0, 0.48, 1.00 and 1.58 mg* of paraquat cation per kilogram of body weight, per day, respectively. The following findings were most important:

1. The major effect of paraquat cation was a dose-related increase in the severity and extent of chronic pneumonitis in the mid-dose and high-dose male and female dogs. This effect was noted also in the low-dose male group, but was minimal when compared with the male controls. Chronic pneumonitis was less severe in the low-dose female dogs than in the female controls.

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Because 44 dogs (out of 48 studied), including 6 male and 5 female controls, had some degree of chronic pneumonitis, paraquat had no effect on the incidence of this lesion.

2. There were statistically significant ($p \leq 0.01$) increases in the group mean lung weights (absolute and adjusted for body weight) and statistically insignificant increases in the group mean spleen weights in the high-dose male and female dogs, when the paraquat-treated animals were compared with the controls.

3. There was a dose-related increase in the concentrations of paraquat in urine and lung tissues of the male and female dogs, but no paraquat was detected in the liver. Paraquat was also detected in the kidneys of the mid-dose and high-dose dogs, but the concentrations were not dose-related.

4. Only 1 lens tubular cataract (in a mid-dose female) and 2 benign neoplasms were observed in this study, as follows:

   - o Parafollicular adenoma of the thyroid gland, in a mid-dose female.
   - o Squamous papilloma inside the pinna of the right ear, in a mid-dose male. This growth was detected during week 19 and was removed during week 20 because pus discharging from the mass drained into the ear and caused inflammation.

5. Systemic NOEL was 15 ppm, expressed as paraquat cation. Systemic LEL was 30 ppm. Moderately increased severity and extent of chronic pneumonitis in both sexes, but especially in the male dogs, was observed at this level.

6. Core Classification of this study: Minimum

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* Averages calculated by the reviewer from the data reported in TABLE 2 of the submission.

OPP:HED:TOX:K.LOCKE:sb 6/19/85 X77351 4M-4

TOX. CHEM. NO. 634

Study Type: Chronic Feeding

Study Title: Paraquat: 1-Year Feeding Study in Dogs. Report No.CTL/P/734

Accession Numbers: 251668 and 251669

Record Number: 110528

Sponsor: Chevron Chemical Company, Richmond, California

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

Date of Final Report: April 20, 1983

Test Material: Paraquat (1,1'-dimethyl-4,4'-bipyridylium) dichloride; technical grade - a brown, aqueous solution containing 32.3% w/w of paraquat cation.

PROTOCOL

I. Experimental Design

Treatment of male and female dogs with paraquat was started during 1/6 - 1/15/81 and was terminated during 1/5 - 1/21/82.

Alderley Park beagle-dogs, 6/dose level/sex, were fed diets containing 0, 15, 30 and 50 ppm of paraquat cation (nominal concentration) for 52 weeks. The Alderley Park dogs were used because background information was available for this strain of dogs. The dose levels were selected on the basis of a 90-day oral feeding study with dogs, in which the NOEL was 20 ppm and in which pulmonary lesions were observed at the 60 ppm level of paraquat cation. (No. CTL/C/1027; EPA Accession No. 244873).

The dogs were 20 to 24 weeks old when they were obtained from Imperial Chemical Industries (ICI) breeding colony and were used in the study after the acclimation period of 5 to 6 weeks. The dogs were randomly assigned to the test groups, were identified by tatooed ear numbers and were housed in the indoor pens with the floor area of 345 x 115 cm. Each pen had a heated sleeping area and a separate exercise area. The temperature of the housing quarters was 20 °C.

The basic diet fed was a Laboratory Diet A (composition attached). Appropriate volumes of technical paraquat were added to this diet and 400 grams of the mixtures (apparently not pelletized) were fed daily to each dog (water was unrestricted). The method and dates of diet preparation, and the periods during which these diets were fed, were reported. All diets were fed within 2 weeks of preparation.

Diets were analyzed monthly during the course of the study for the concentration of paraquat cation. The homogeneity and chemical stability of paraquat in the diets, using the standard mixing procedure and dose levels of 15, 30, or 50 ppm, was determined before the initiation of the study.

II. Parameters Examined

1. Observation for abnormalities

The dogs were observed daily for clinical and behavioral abnormalities. They were also examined by a veterinarian before the initiation of the study, after test weeks 13, 26, and 39, and between weeks 48 to 51. This detailed physical examination included ophthalmoscopy.

2. Food consumption

Food consumption was recorded daily, starting 7 days before initiation of the treatment.

3. Body weight

Body weights of all dogs were recorded on the first day of dosing and weekly thereafter.

4. Hematology

These determinations (hemoglobin, hematocrit and red blood cell counts; mean cell volume and hemoglobin concentration; total and differential white blood cell counts; platelet count; and kaolin-cephalin and prothrombin times) were performed once before the study was started and then during the treatment weeks 4, 8, 12, 16, 20, 26, 39, and 52. Smears of bone marrow were examined, after staining with a Romanowsky stain, during the test weeks 26 and 52.

5. Clinical chemistry

The following determinations were performed on blood plasma: alanine transaminase, aspartate transaminase, creatine kinase and alkaline phosphatase activities; urea, glucose, albumin, total protein, triglycerides and cholesterol concentrations; and calcium and potassium levels. These determinations were performed once before the study was started and then during the test weeks 4, 8, 12, 16, 20, 26, 39, and 52. Jugular vein blood was used and all dogs were tested.

6. Urinalysis

Urine of all dogs was analyzed for glucose, ketones, urobilinogen, pH, specific gravity and protein, before the study was started and then during the test weeks 8, 16, 24, 39, and 50. Water, but not food, was available during each collection period lasting for about 18 hours.

Urine sediments, obtained by centrifugation, were examined for the presence of crystals, sperm, erythrocytes, leukocytes, squamous epithelial cells, small epithelial cells and casts. These examinations were performed on all dogs before the study was started and then during the test weeks 8, 16, 24, 39, and 50.

7. Organ weights

The following organs were weighed: adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus and thyroids (with parathyroids). The left and right components of paired organs were weighed separately.

8. Pathology

Gross necropsy and histopathology were performed on all dogs. The following tissues were examined histopathologically: adrenals, aorta, bone marrow (rib and costochondral junction), brain, cecum, cervix, colon, duodenum, epididymides, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (bronchial, mesenteric, prescapular), mammary gland, esophagus, ovaries, pancreas, pituitary, prostate, salivary gland (submandibular), sciatic nerves, skin (left flank), spinal cord, spleen, stomach, testes, thymus, thyroids/parathyroids, trachea, urinary bladder, uterus, voluntary muscle (biceps femoris) and all abnormal tissues. After removal and weighing, the lungs were inflated with 10 percent buffered formol saline; the eyes were fixed in Davidson's fixative and the skin in Bouin's fixative; and the remaining tissues were fixed in 10 percent buffered formol saline. For microscopic examination, all tissues were embedded in paraffin and 5 µm sections were stained with hematoxylin and eosin. Sections of kidneys were also stained with Oil Red O in order to demonstrate the presence (or absence) of fat. Selected sections of lung tissue were treated with Perls' stain for the demonstration of hemosiderin.

9. Concentration of paraquat in urine, kidneys, liver and lungs

Samples of urine and tissues, obtained from all dogs, were analyzed for paraquat cation. Urine was obtained by catheterization of the bladder during the test week 29, whereas samples of kidney, liver, and lung tissues were obtained at necropsy.

10. Statistical analysis

Body weight gains (from the start of the study) and organ weights were evaluated by analysis of variance, separately for males and females. Hematology and clinical chemistry data were examined by analysis of covariance at each sampling time, but data from male and female dogs were analyzed together. Organ weights were also examined by analysis of covariance. Each paraquat dose group mean was compared with the control group mean using Student's t-test (two-sided). Group means were adjusted for any missing values.

RESULTS

I. Mortality and Clinical Observations

There were no mortalities. Clinical observations included hyperpnea (increased respiratory rate or depth), increased vesicular sound and reddening of the tongue, especially in the high-dose male and female dogs. These data are summarized in Table I.

Table I. Clinical Observations in Male and Female Dogs[a]

Paraquat cation (ppm)	0		15		30		50	
Observation	Number of dogs affected[b]							
	M	F	M	F	M	F	M	F
Hyperpnea	1	1	1	1	0	2	4	4
Increased vesicular sound	0	0	0	0	1	1	3	4
Reddening of tongue[c]	4	4	2	2	5	3	6	6

a. This table is based on TABLES 3, 4, and 5 of the submission.
b. Out of 6/dose level/sex examined.
M = males; F = females.
c. Tongues were examined once a week.

In the case of the control, low-dose and mid-dosa male and female groups, hyperpnea was observed only once or twice in each affected dog during the treatment weeks 13 to 48. In the case of the high-dose males, hyperpnea was observed 1, 4, 6, or 15 times in the affected dogs during the treatment weeks 13 to 49. Similar results were reported for the female high-dose dogs.

Increased vesicular sound did not occur in the controls and the low-dose male and female groups. In the case of the mid-dose male and female dogs and the high-dose male dogs, each affected dog exhibited this abnormality only once or twice during the treatment weeks 13 to 51. In the case of the high-dose female dogs, two animals exhibited increased vesicular sound once (during weeks 39 and 51), one animal four times (during weeks 13 to 48) and another animal six times (during weeks 26 to 51).

Reddening of the dorsal surface of the tongue was observed in the control and the paraquat-treated male and female groups, but there was a dose-related increase in the incidence and the frequency of observation in the mid-dose and high-dose groups. During the treatment week 9 and termination of the study, 22, 13, 45, and 82 observations of tongue reddening were reported for the control, low-dose, mid-dose and high-dose male dogs, respectively. The corresponding numbers for the female dogs were 16, 10, 38, and 81, respectively.

II. Food consumption

These data were reported only for dogs which did not eat all of their food and included animal's number, weeks of study when food consumption was reduced and amounts of food left uneaten.

Decreased food consumption was observed in 3 control, 3 low-dose, 4 mid-dose and 6 high-dose females, and in 2 high-dose males. In most dogs, including the three female controls, decreases in food intake ranged from small* to moderate**, occurred at various time intervals for a total of 1, 2, 4, 6, 8, 10, 14, 17, 19, or 20 weeks and were not attributed to paraquat. Only in the case of 2 high-dose dogs (male #37 and female #46), the reduced food consumption was regarded as treatment-related. Starting with the test week 15, when hyperpnea was first observed, the male dog #37 left small quantities of food uneaten for a total of 27 weeks. Starting with the test week 1, the female dog #46 left moderate to large*** quantities of food uneaten for a total of 47 weeks.

III. Body weight

These data were reported as group mean body weight gain/week/sex, including approximate 95 percent confidence limits. Group mean initial and final body weights were also reported. Means were based on 6 observations/group.

Paraquat, at the levels tested (15, 30 and 50 ppm), had no effect on the group mean body weight gain of the male and female dogs. After 52 weeks of treatment, the control, low-dose, middose and high-dose male dogs gained 1.22, 0.97, 1.28, and 1.12 kg of weight, respectively. During the same time, the control, lowdose, mid-dose and high-dose females gained 1.80, 2.17, 1.99, and 1.82 kg of weight, respectively. The differences in weight gain between the controls and the paraquat-treated groups were statistically insignificant.

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* Small = up to 15% of food given per week.

** Moderate = 16 to 30% of food given per week.

*** Large = 31 to 60% of food given per week. (Each dog received 400 g of food/day).

However, according to the submission, "several dogs in all groups, including controls, had fluctuating body weights over the course of the study and some showed either little or no overall weight change, and three (male #16, 15 ppm paraquat; males #28 and 30, 30 ppm paraquat; and female #46, 50 ppm paraquat) showed an overall weight loss." Individual body weights or body weight changes were not reported.

IV. Hematology

These data were reported as group mean values for the treatment weeks 0, 4, 8, 12, 16, 20, 26, 39, and 52. Approximate 95 percent confidence limits and statistically significant differences from the control group means were also reported. All means were based on 6 observations per group. Individual data were not reported.

Paraquat, at the dietary concentrations fed, had no effect on any of the hematological parameters examined. Although statistically significant differences from the control means occured in several parameters, they were either small, isolated or dose-unrelated and did not appear to have biological significance. Hermatological parameters that were statistically significantly different in experimental animals versus controls are summarized in Table II.

Examination of bone marrow samples did not reveal any differences between the controls and the paraquat-treated dogs.

V. Clinical chemistry

These data were reported as group mean values for the treatment weeks 0, 4, 8, 12, 16, 20, 26, 39, and 52. Approximate 95 percent confidence limits and statistically significant differences from the control group means were also reported. All means were based on 6 observations per group. Individual data were not reported.

With the exception of plasma glucose levels, which were the same in the control and the paraquat-treated dogs, statistically significant differences from the control means were observed for all of the parameters examined. However, most of these differences were either small, isolated or dose-unrelated and did not appear to result from the ingestion of paraquat.

For example:

- Plasma potassium levels were increased slightly (6 to 11%) throughout the study in the male dogs, mostly in the mid-dose group.

Table II. Statistically Different Hematological Parameters[a]

Parameter	Sex of dogs	Test Week[b]	Percent change[c] Increase	Percent change[c] Decrease	Level of significance (%)[d]
	Low-dose group (15 ppm)[f]				
White blood cell count	M	12	16.0		5
Neutrophil count	M	12	35.8		5
Mean cell hemoglobin con.[e]	M	26	1.4		5
Mean cell volume	M	26		2.0	5
Platelet count	M	39		14.3	5
Platelet count	F	16		11.3	5
Red blood cell count	F	20	5.4		5
Monocyte count	F	26		42.3	5
Kaolin-cephalin time	F	39	8.3		5
	Mid-dose group (30 ppm)[f]				
Mean cell volume	M	4	1.7		5
Eosinophil count	M	4	148.6		5
Eosinophil count	M	8	154.3		1
Monocyte count	M	4	53.4		5
Platelet count	M	12	14.3		5
White blood cell count	M	12	20.9		5
Mean cell hemoglobin con.[e]	M	12		1.5	1
Mean cell volume	M	20		1.9	5
Neutrophil count	M	52	32.2		
Kaolin-cephalin time	F	8		6.9	5
Kaolin-cephalin time	F	39	8.3		5
Monocyte count	F	39		34.4	5
	High-dose group[f] (50 ppm)				
Hematocrit	F	20	6.5		5
Red blood cell count	F	20	7.2		5
Lymphocyte count	F	26		30.8	5
Prothrombin time	F	26		5.9	5

a. This table is based on TABLES 9-36 of the submission.
b. Treatment week during which determinations were made.
c. In relation to controls.
d. Statistically significantly different from the control group mean (t-test; two-sided).
e. Mean cell hemoglobin concentration.
f. Doses are expressed in terms of paraquat cation.

- Plasma urea levels were decreased moderately (20 to 27%) throughout the study in the female dogs, but mostly in the low-dose group.

- Plasma cholesterol levels were increased slightly during the study in the high-dose male and female dogs (4.2 to 14.8% and 2.5 to 18.3%, respectively). Slight increases in plasma cholesterol (1.2 to 12.4%) were also observed in the low-dose female dogs.

- Plasma alkaline phosphatase activity was increased throughout the study in the female dogs, but mostly in the high-dose group. At the treatment weeks 4, 8, 12, 16, 20, 26, 39, and 52, the increases in phosphatase activity were 4.8, 14.1, 18.2, 21.7, 39.8, 35.4, 30.9 and 49.5 percent, respectively, when the treated dogs were compared with the controls. There was also a moderate (36.3%) increase in alkaline phosphatase activity at week 52 i the mid-dose female dogs. Since plasma alkaline phosphatase activity was elevated mostly in a high-dose group, especially during the second half of the study, this increase might be treatment-related.

- Plasma triglyceride levels were generally increased throughout the study in the high-dose male and female dogs. In the case of the male dogs, the increases ranged from 12.7 percent to 27.8 percent during the treatment weeks 4 to 52, when the treated dogs were compared with the controls. In the case of the female dogs, the increases in plasma triglyceride levels ranged from 8 percent to 19.5 percent during the same treatment time. Only at the treatment weeks 12 and 26, the increases in plasma triglyceride levels, in the high-dose female group, were 46.6 percent and 85.8 percent, respectively. Plasma lipase activity was not determined.

  Since plasma triglyceride level was increased only in the high-dose groups, this increase might be treatment-related.

Clinical chemistry findings that were statistically significantly different in experimental animals versus controls are summarized in Tables III and IV.

Table III. Statistically Different Clinical Chemistry Findings in Blood Plasma of Male Dogs[a]

Parameters affected[e]	Test week[b]	Percent change[c] Increase	Percent change[c] Decrease	Level of significance (%)[d]
	Low-dose group (15 ppm)[f]			
Alanine transaminase	20	30.1		5
Potassium	16	9.0		1
Potassium	26	6.0		5
Calcium	12	2.1		5
Total protein	8	7.2		5
Total protein	12	7.3		1
Albumin	12		11.1	5
	52		11.5	5
	Mid-dose group (30 ppm)[f]			
Alanine transaminase	8	43.8		5
Potassium	8	10.7		1
Potassium	16	10.3		1
Potassium	20	8.8		5
Potassium	26	6.9		5
Potassium	39	6.2		5
Calcium	8	2.2		5
Total protein	12	7.7		1
Albumin	12		11.7	1
Albumin	39		6.5	5
	High-dose group (50 ppm)[f]			
Potassium	16	10.0		5
Calcium	12	2.2		5
Triglycerides	39	27.8		5

a. This table is based on TABLES 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, and 59 of the submission.
b. Treatment week during which determinations were made.
c. In relation to controls.
d. Statistically significantly different from the control group mean (t-test; two-sided).
e. Alanine and aspartate transaminases, alkaline phosphatase and creatine kinase were reported in the submission as activities (mU/ml). Calcium, urea, cholesterol and triglycerides were reported as mg/100 ml of plasma. Albumin and total protein were reported as g/100 ml of plasma. Potassium was reported as mEq/l.
f. Dose levels are expressed in terms of paraquat cation.

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Table IV. Statistically Different Clinical Chemistry Findings in Blood Plasma of Female Dogs[a]

Parameters affected[e]	Test week[b]	Percent change[c] Increase	Percent change[c] Decrease	Level of significance (%)[d]
	Low-dose group (15 ppm)[f]			
Urea	8		27.1	1
Urea	12		25.5	5
Urea	20		20.2	1
Urea	26		27.7	1
Urea	52		24.2	5
Creatine kinase	39		37.5	5
Creatine kinase	52		42.4	5
Triglycerides	52		33.6	5
Total protein	4	12.4	6.7	5
Cholesterol	12			
	Mid-dose group (30 ppm)[f]			
Urea	8		19.7	5
Urea	12		19.6	5
Creatine kinase	52		37.2	5
Alkaline phosphatase	52	36.3		5
Calcium	8	1.9		5
	26	3.2		5
	39	2.7		5
	High-dose group (50 ppm)[f]			
Urea	8		21.5	5
Urea	52		23.3	5
Albumin	12		8.5	5
Alanine transaminase	26		32.2	5
Aspartate transaminase	8	15.3		5
Alkaline phosphatase	20	39.8		5
Alkaline phosphatase	26	35.4		5
Alkaline phospatase	52	49.5		1
Triglycerides	26	85.8		1
Cholesterol	4	10.6		5
Cholesterol	20	18.3		5
Cholesterol	39	15.6		5
Total protein	20	6.0		5
Total protein	26	7.7		1

a. This table is based on TABLES 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, and 60 of the submission.
b. Treatment week during which determinations were made.
c. In relation to controls.
d. Statistically significantly different from the control group mean (t-test; two-sided).
e. See Table III.
f. Doses are expressed in terms of paraquat cation.

VI. Urinalysis

These data were reported as group mean values for the treatment weeks 0, 8, 16, 24, 39, and 50. Approximate 95 percent confidence limits and statistically significant differences from the control group means were also reported. All means were based on 6 observations per group. Individual data were not reported.

Paraquat, at all levels tested, had no effect on any of the parameters tested.

VII. Organ weights

These data were reported as group mean weights (absolute and adjusted for body weights) of adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus, and thyroids (with parathyroids). Approximate 95 percent confidence limits and statistically significant differences from the control group means were also reported. All observations were based on 6 animals per group. Individual data were not reported.

With the exception of kidneys, lungs and spleen, there were no statistically significant differences in the weights of organs obtained from the control and the paraquat-treated dogs. The weights of kidneys, lungs and spleen, and the percent differences from control values are summarized in Table V.

According to the above data, there were statistically significant increases in group mean lung weights in the high-dose male and female dogs when the tested animals were compared with the controls. In the case of males, both absolute and adjusted lung weights were increased by 36 percent over the control values. In the case of females, both lung weights were increased by 61 percent.

Group mean spleen weights were increased over the control values in the high-dose male and female dogs. In the case of males, the absolute and adjusted spleen weights were increased by 50 percent and 55 percent, respectively. However, these increases were statistically insignificant and were attributed mainly to one high-dose animal (no. 40) with a high spleen weight. When this animal was excluded from consideration, the absolute and adjusted group mean spleen weights were increased by only 20 percent and 25 percent, respectively, over the control values. These increases, too, were statistically insignificant. The group mean spleen weights were slightly decreased (6 to 12%) in the low-dose and mid-dose male groups.

In the case of high-dose females, the absolute and adjusted group mean spleen weights were increased by 38 percent and 43 percent, respectively, when compared with the control values. However, these weight increases were statistically significant (at the 5% level) only after one mid-dose animal (no. 36) with a high spleen weight was excluded from consideration. The lowest increases in spleen weight (about 18%) occurred in the mid-dose group.

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Table V. Group Mean Weights (g) of Kidneys, Lungs and Spleen, and Differences from Control Values[a]

Paraquat cation (ppm)	0	15	30	50
Male Dogs		**Kidneys**		
Absolute weight	64.2	57.7*	60.8	61.8
percent decrease	-	10.1*	5.3	3.7
Adjusted weight[b]	63.6	57.7**	61.3	61.8
percent decrease	-	9.3**	3.6	2.8
		Lungs		
Male Dogs				
Absolute weight	122	135	135	166**
percent increase	-	10.6	10.7	36.1**
Adjusted weight[b]	122	135	136	166**
percent increase	-	10.7	11.5	36.1**
Female Dogs				
Absolute weight	103	103	109	166**
percent increase	-	3.9	5.8	61.2**
Adjusted weight[b]	103	106	109	166**
percent increase	-	2.9	5.8	61.2**
		Spleen		
Male Dogs				
Absolute weight	46.8	41.1	41.5	70.1
percent change	-	12.2	11.3	49.8
Adjusted weight[b]	45.4	41.1	42.6	70.2
percent change	-	9.5	6.2	54.6
Female Dogs				
Absolute weight	42.4	53.2	49.8	58.5
percent change	-	25.5	17.5	38.0
Adjusted weight[b]	41.1	55.4	48.6	58.8
percent increase	-	34.8	18.2	43.1

a. This table is based on TABLES 71, 73, and 75 of the submission.

b. Group mean weight adjusted for body weight. It was not reported how these adjustments were made.

* Statistically significantly different from the control group mean at the 5% level (t-tested, two-sided).

** Statistically significantly different from the control group mean at the 1% level (t-tested, two-sided).

Decreases in group mean absolute and adjusted kidney weights occurred only in the low-dose male dogs, were small (9 to 10%) and, although statistically significant, did not appear to be biologically significant.

VIII. Gross Necropsy

These data were reported as the following summary:

> "A wide variety of lesions was observed in dogs from all groups including controls. Dogs from treated groups and controls had lesions of the lungs. The most consistently observed lesion was yellow discoloration and consolidation of areas of the lungs. The extent of this lesion varied considerably from lobe to lobe within the same animal, and from animal to animal. Animals from all groups including controls showed this lesion but there was a larger number of animals showing the more severe lesions in the 30 and 50 ppm groups. In addition to the areas of yellow consolidation, a number of dogs showed the presence of other lung lesions. Most of these were small focal lesions of a firm pale nodular nature."

Lesions observed at necropsy were described in detail in the histopathology section of the submission. Lung was the only organ affected by the exposure of dogs to paraquat.

IX. Histopathology

These data were reported as incidences of histopathological findings in various organs, in each test group. The numbers of tissues examined were also reported, but data showing all histopathological findings in each dog were not reported. However, individual data for chronic pneumonitis in the lungs, the primary lesion of concern, were reported for all dogs in the study. These data included numerical grading scores for severity and extent of chronic pneumonitis, and sums of these scores for each dog.

Paraquat caused an increase in the severity of chronic pneumonitis in the mid-dose (30 ppm) and high-dose (50 ppm) male and female dogs. This lesion comprised the following histological changes:

- Peribronchial mononuclear cell infiltration.
- Peribronchiolar fibrosis.
- Interalveolar fibrosis.
- Interalveolar mixed inflammatory cell infiltration generally relatively sparse.
- Hemosiderin-containing macrophages.

- Alveolar cell hyperplasia and hypertrophy epithelialization.
- Limited inflammatory cell infiltration in bronchioles and alveoli.

These changes occurred in close association with each other in the lung tissue and were those diagnosed grossly as "yellow discoloration and consolidation of areas of the lungs." Data concerned with histopathological findings in the lungs and with overall severity of chronic pneumonitis are summarized in Tables VI and VII, respectively.

These data show that various histological lung lesions were observed in the paraquat-tested and control dogs, but most were treatment-unrelated. However, increases in the incidence of slight focal granuloma (4/6 vs. 2/6) and focal pleural fibrosis (3/6 vs. 2/6) in the high-dose male dogs might be treatment-related.

Regarding chronic pneumonitis, there was no increase in the incidence of this lesion because 44 dogs out of 48 studied had some degree of chronic pneumonitis. This lesion was absent only in one control and two low-dose females, and in one low-dose male. However, the severity of chronic pneumonitis was increased with dose. In the case of the controls and the low-dose male and female dogs, chronic pneumonitis was minimal to slight. In the mid-dose group, three males and five females had also minimal to slight chronic pneumonitis, but this lesion was moderate in the remaining mid-dose dogs. In the high-dose groups, four males and all of the females had marked chronic pneumonitis. A numerical assessment of an overall severity of chronic pneumonitis in individual dogs and test groups is shown in Table VII.

Individual total scores and especially group scores in the above table show that chronic pneumonitis was most severe in the high-dose female dogs and then in the high-dose male dogs, when these scores are compared with those obtained for the controls.

In the case of the mid-dose dogs, chronic pneumonitis was more severe in the males than in the females. The overall severity scores for the males and females were 115 percent and 63 percent higher, respectively, than those obtained for the controls.

Females in the low-dose group did not show any increase in the severity and extent of chronic pneumonitis over the controls, but there was an increase in the low-dose male group. In that group, there were 3 dogs with slight chronic pneumonitis (numerical score 11 to 20), whereas 2 dogs in the control group had this lesion. An overall severity score for the low-dose males was, therefore, 19 percent higher when related to that obtained for the controls.

Table VI. Histopathological Findings in The Lungs of Male and Female Dogs[a]

Paraquat Cation (ppm)	0	15	30	50	0	15	30	50
Number of Tissues Examined	6	6	6	6	6	6	6	6
	MALES				FEMALES			
• Chronic pneumonitis								
Lesions absent		1			1	2		
Minimal	4	2	1		4	4	3	
Slight	2	3	2	1	1		2	
Moderate			3	1			1	
Marked				4				6
• Focal granuloma								
Minimal	2	2	1		3	3	3	1
Slight	2	1	2	4	1	1		1
Moderate		1	3	1	1		2	
• Focal calcification								
Minimal	1	3	2	2		1	2	
Slight	2		1				1	1
• Focal acute pneumonia		1	2		2		2	1
• Focal chronic pneumonia			1					
• Focal pleural fibrosis	2	1	1	3	2			2
• Focal subpleural chronic pneumonitis	1	3	1	2	1	1	1	
• Dilated vessels in pleura containing mononuclear cells					1			
• Focal necrotizing pneumonia						1		
• Moderate emphysema								1

a. This table is based on TABLE 78 of the submission.

Only one lenticular cataract (minimal, in a mid-dose female) and two neoplasms were observed in this study. One mid-dose female had a parafollicular adenoma of the thyroid gland and one mid-dose male had a benign squamous papilloma inside the pinna of the right ear. Lenticular cataract and parafollicular adenoma were detected at the termination of the study. Papillomatous growth was detected during treatment week 19 and was removed surgically during week 20 because pus discharging from the mass drained into the ear and caused inflammation. (This growth was examined histopathologically.) Neither the cataract nor the two neoplasms were attributed to treatment with paraquat.

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Table VII. Assessment of Overall Severity of Chronic Pneumonitis in Individual Dogs and Test Groups[a]

Males				Females			
Animal No.	Severity score[c]	Extent score[c]	Total[b]	Animal No.	Severity score[c]	Extent score[c]	Total[b]
0 ppm							
1	5	5	10	7	2	2	4
2	4	4	8	8	0	0	0
3	8	10	18	9	6	7	13
4	5	6	11	10	5	4	9
5	2	1	3	11	4	5	9
6	1	1	2	12	4	4	8
Total	25	27	52	Total	21	22	43
15 ppm							
13	3	3	6	19	2	2	4
14	6	7	13	20	3	2	5
15	11	9	20	21	0	0	0
16	0	0	0	22	4	4	8
17	3	3	6	23	4	4	8
18	8	9	17	24	0	0	0
Total	31	31	62	Total	13	12	25
30 ppm							
25	12	13	25	31	2	2	4
26	4	5	9	32	5	4	9
27	12	15	27	33	10	12	22
28	8	8	16	34	6	10	16
29	7	5	12	35	5	8	13
30	11	12	23	36	3	3	6
Total	54	58	112	Total	31	39	70
Animal No.	Severity score[c]	Extent score[c]	Total[b]	Animal No.	Severity score[c]	Extent score[c]	Total[b]
50 ppm							
37	16	17	33	43	18	17	35
38	7	10	17	44	29	21	40
39	13	15	28	45	26	20	36
40	16	20	36	46	20	28	48
41	17	15	32	47	19	15	34
42	18	18	36	48	18	16	34
Total	87	95	182	Total	110	117	227

a. This table is essentially TABLE a, APPENDIX 12 of the submission.
b. Overall severity (sum of severity and extent scores).
c. The following scoring system was employed:

No abnormality detected	= numerical score 0
Minimal chronic pneumonitis	= numerical score 1 - 10
Slight chronic pneumonitis	= numerical score 11 - 20
Moderate chronic pneumonitis	= numerical score 21 - 30
Marked chronic penumonitis	= numerical score 31 - above

Each category of chronic pneumonitis was described in detail in the submission.

There was a dose-unrelated increased incidence of erythrophagocytosis in bronchial lymph node in the mid-dose and high-dose male and female dogs, and this lesion might be due to treatment. In the control, low-dose, mid-dose, and high-dose male groups, 1, 1, 4, and 4 dogs, respectively, had erythrophagocytosis (out of 6 dogs examined in each group). The numbers of dogs affected in the corresponding female groups were 2, 1, 4, and 4, respectively (also out of 6 dogs examined in each group). Two high-dose females also had hyperplasia of bronchial lymph node, but none was observed in other dogs (males or females).

In a scientifically sound study with animals, the controls should remain healthy during most of their lives in order to permit meaningful assessment of the effects of treatment. In this study, most control dogs were not healthy because all of the control males and five females had chronic pneumonitis, although there were no visible signs of poor health. There is nothing in the submission to indicate what could have caused chronic pneumonitis in such young dogs (about 1 year and 7 months old when the study was terminated).

However, this study does not have to be rejected because the major effect of paraquat ingestion was an increase in the severity and extent of chronic pneumonitis in the mid-dose and high-dose dogs of both sexes. A small increase in the overall severity of chronic pneumonitis occurred also in the low-dose male dogs. Based on these data, it appears that paraquat suppressed, in a dose-related manner, resistance to disease in these dogs.

X. Concentration of Paraquat Cation in Urine, Kidneys, Liver, and Lungs

There was a dose-related increase in the concentration of paraquat in urine and lung tissues of the male and female dogs. Paraquat was also detected in the kidneys of the mid-dose and high-dose dogs, but the concentrations were not dose-related. Paraquat was not detected in the following tissues: kidneys, liver and lungs of all control dogs; kidneys of the low-dose male and female dogs; and livers of all paraquat-treated dogs. Samples of urine from the control dogs also did not contain paraquat. Data showing the actual levels of paraquat in lungs, kidneys, and urine are summarized in Table VIII.

Table VIII. Concentration of Paraquat Cation in Tissues (µg/g) and Urine (µg/ml) of dogs.[a]

Dietary Paraquat Cation (ppm)	0	15	30	50
	MALES			
Lung	ND	0.15 - 0.20	0.36 (0.16)	0.63 (0.32
Kidney	ND	ND	0.17 (0.05)	0.18 (0.06)
Urine	ND	0.67 (0.17)	2.64 (1.67)	3.70 (1.03)
	FEMALES			
Lung	ND	0.13 - 0.16	0.77 (1.10)	1.04 (0.42)
Kidney	ND	ND	0.12 - 0.17	0.19 (0.08)
Urine	ND	0.91 (0.49)	2.23 (0.83)	5.74[b] (1.75)

a. This table is based on TABLES 79 and 80 of the submission. Numbers in the table are means based on 6 observations per group. Numbers in parentheses are standard deviations.
b. Mean based on 5 observations per group.
ND = Not detected. The limits of detection were 0.05 µg paraquat cation/ml of urine and 0.1 µg/g of tissue.

XI. Stability and Homogeneity of Paraquat in Diet

Paraquat was stable in diets over an eight-week period. Longer storage intervals were not studied. However, all diets were fed within two weeks of preparation.

Diets were also homogenous with regard to the concentration of paraquat cation.

XII. Achieved Concentrations of Paraquat in Diets and as mg/kg of Body Weight

Data reported for achieved concentrations of paraquat in diets included batch number, date of diet preparation, date of analyses and results of duplicate determinations. Thirteen batches of the control, low-dose and mid-dose diets and 15 batches of the high-dose diets were analyzed.

Ingestion of paraquat in terms of mg/kg of body weight (group mean values) was reported for each 4-week period of the entire study. These data and achieved concentrations in diets are summarized in Table IX.

Table IX. Achieved Concentrations of Paraquat in Diet and as mg/kg of Body Weight

Nominal concentration of paraquat cation in diet (ppm)	15	30	50
Achieved concentration of paraquat cation in diet (ppm)[a]			
Range	14-16	28-31	48-54
Average	14.9	29.7	50.0
Mg/kg of body weight (ICI)[b]			
Range - males	0.44 - 0.47	0.90 - 0.99	1.46 - 1.60
- females	0.45 - 0.57	0.94 - 1.10	1.48 - 1.79
Average - males	0.45	0.93	1.51
- females	0.48	1.00	1.58
Mg/kg of body weight (EPA)[c]	0.38	0.75	1.25

a. Based on APPENDIX 8 of the submission. Range = results obtained for 13 or 15 batches of diets that were analyzed.

b. Based on TABLE 2 of the submission. Range = calculations performed by ICI for individual 4-week periods. Paraquat was not detected in control diets. Limit of detection was 2.0 ppm.

c. As would be calculated by Toxicology Branch/HED, assuming that 1.0 ppm = 0.025 mg/kg of body weight/day (for dog). (APPRAISAL OF THE SAFETY OF CHEMICALS IN FOODS, DRUGS, AND COSMETICS. Food and Drug Administration; 1959).

According to the above table, the actual (determined) concentrations of paraquat cation in the diets were very similar to nominal concentrations. Also, based on the mg/kg of body weight basis, male and female dogs ingested similar amounts of paraquat cation.

SUMMARY

Alderly Park beagle dogs, 6 males and 6 females/dose level, were were fed diets containing 0, 15, 30, and 50 ppm of paraquat cation for 52 weeks. The amount of food fed daily to each dog was 400 grams. The following parameters were examined: observation for clinical and behavioral abnormalities, food consumption, body weight gain, hematology, histopathology, and concentration of paraquat cation in urine, kidneys, liver and lungs. Body weight gains, organ weights, hematology and clinical chemistry data were evaluated statistically. The following results were obtained:

1. None of the dogs died.

2. Clinical observations included hyperpnea, increased vesicular sound and reddening of tongue, especially in the high-dose male and female dogs.

3. Out of 48 dogs studied, 44 (including 6 male and 5 female controls) had some degree of chronic pneumonitis. This lesion comprised peribronchial and peribronchiolar fibrosis, alveolar epithelialization and mononuclear cell infiltration. These changes occurred in close association with each other in the lung tissue and were diagnosed at necropsy as "yellow discoloration and consolidation of areas of the lungs."

The major effect of paraquat ingestion was a dose-related increase in the severity and extent of chronic pneumonitis in the mid-dose and high-dose male and female dogs. This effect was noted also in the low-dose male group, but was minimal when compared with the controls. Females in the low-dose group did not show any increae in the severity and extent of chronic pneumonitis.

4. Only one lenticular cataract (in a mid-dose female) and two benign neoplasms were observed in this study, as follows:

- Parafollicular adenoma of the thyroid gland, in a mid-dose female.
- Squamous papilloma inside the pinna of the right ear, in a mid-dose male. This growth was detected during week 19 and was removed during week 20 because pus discharging from the mass drained into the ear and caused inflammation.

5. There were statistically significant ($P \leq 0.01$) increases in the group mean lung weights (absolute and adjusted for body weight) and statistically insignificant increases in the group mean spleen weights in the high-dose male and female dogs, when the paraquat-treated animals were compared with the controls.

Decreases in group mean kidney weights occurred only in the low-dose male dogs, were small (9% or 10%) and, although statistically significant ($P \leq 0.05$ or 0.01), did not appear to be biologically significant.

Paraquat had no effect on the weights of liver, adrenals, brain, gonads, heart, pituitary, thymus, and thyroid.

6. There was a dose-related increase in the concentration of paraquat cation in urine and lung tissues of the male and female dogs. Paraquat was also detected in the kidneys of the mid-dose and high-dose dogs, but the concentrations were not dose-related. Paraquat was not detected in the livers of all treated dogs. Tissues other than lung, kidney, and liver were not studied.

7. Treatment-related decreased food consumption was observed only in one male and one female high-dose dog.

8. Paraquat, at all levels tested, had no effect on the group mean body weight gain of the male and female dogs. However, according to the submission, body weights of several dogs fluctutated during the study and one low-dose male, two mid-dose males and one high-dose female "showed an overall weight loss." Individual data were not reported.

9. Paraquat, at all levels tested, has no effect on hematology, clinical chemistry and urinalysis. Although statistically significant ($P \leq 0.05$ or 0.01) differences from the control group means occurred in several hematological and clinical chemistry parameters studied, they were either small, isolated or doseunrelated and did not appear to result from the ingestion of paraquat or to have biological significance.

10. With the exception of severity and extent scores for chronic pneumonitis, individual data were not reported for any of the parameters examined.

11. Paraquat was stable in diets for 8 weeks. Longer storage times were not studied, but all diets were fed within 2 weeks of preparation.

NOEL and CORE Classification

Systemic NOEL = 15 ppm* (expressed as paraquat cation)

Systemic LEL = 30 ppm (moderately increased severity and extent of chronic pneumonitis in both sexes, but especially in the male dogs).

CORE Classification: MINIMUM

Although individual data were not reported for most of the parameters examined, animals with clinical toxic signs, weight loss or histological findings different from controls were always identified by number, sex and test group. A request for individual data is therefore, in the judgment of this reviewer, unnecessary at this time.

* Although the overall severity score for chronic pneumonitis was 10 points higher in the 15 ppm (low-dose) male group than in the control male group (that is, 62 vs. 52, respectively), this increase is regarded by the reviewer as insignificant. Regarding the low-dose female group, the overall severity score for chronic pneumonitis was lower in that group than in the female controls (that is, 25 vs. 43, respectively).

LABORATORY DIET A*

Ingredients (supplied by BP Nutrition (UK) Limited)

Whole wheat
Whole maize
Wheatfeed pellets
Meat and bonemeal
Jet-sploded full fat soya bean meal
Unextracted dried yeast
Spray dried whey powder
Lecithin premix
Lab Diet A Mastermix SQC

Major constituents analysed (by BP Nutrition (UK) Limited)

Moisture
Crude fat
Crude protein
Crude fibre
Ash
Calcium
Phosphorus
Sodium
Chlorine
Potassium
Magnesium
Iron
Copper
Manganese
Zinc
Vitamin A
Vitamin E

Each batch of diet was assayed (by BP Nutrition (UK) Limited) for the following contaminants: arsenic, cadmium, lead, mercury, nitrite, nitrate, selenium, fluorine, total DDT, Dieldrin, Lindane, Heptachlor, Malathion, total PCBs, total aflatoxins, total viable count, Salmonella species, presumptive coliforms, E coli type 1, mesophilic spores and fungal units. Percentage of each ingredient/ constituent in diet was not reported.

* APPENDIX 2 in the submission.