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CASWELL FILE

PC 061603

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

006652

APR - 8 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Paraquat Dichloride - Request From Imperial Chemical Industries (ICI) Americas, Inc. that EPA Amend its Previous Conclusion and Not Classify Paraquat as an Oncogen, and Submission of Additional Toxicological Data - EPA Record Nos. 199157, 199158, 199159, and 199160 - EPA Registration Nos. ~~10182-113~~, 10182-113, ~~10182-115~~, and 10182-115 - EPA MRID Nos. 402024-01 Through 402024-05

TB Project No.: 7-0855
Caswell No.: 634

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THRU: Edwin R. Budd, Section Head
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ERB 3/18/88 4/8/88

and

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In 1986, the Toxicology Branch Peer Review Committee classified paraquat as a Category C oncogen. This decision was based on an increased incidence of squamous cell carcinoma, an uncommon tumor, in the head region of the high-dose male Fischer 344 rats, when the treated animals were compared with the concurrent or the historical control male rats (combined toxicity/carcinogenicity study No. 82/ILY 217/328; October 24, 1983). ICI disagreed with this

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decision, maintained that paraquat was not oncogenic and submitted recently the following additional data for consideration by Toxicology Branch (TB) and the Peer Review Committee:

1. Combined chronic toxicity/oncogenicity study (2 years) - rat; no study number; March 10, 1982. EPA MRID No. 402024-03.

2. Combined chronic toxicity/oncogenicity study (2 years) - mouse; no study number; March 10, 1982. EPA MRID No. 402024-03.

3. Mutagenic study: Unscheduled DNA synthesis in rat hepatocytes; No. CTL/P/1550; March 31, 1987. EPA MRID No. 402024-04.

4. Mutagenic study: Chromosomal aberrations in rat bone marrow; No. CTL/P/1560; March 26, 1987. EPA MRID No. 402024-05.

5. Report on the histological review of slides from the head region, for the combined toxicity/oncogenicity rat study No. 82/ILY 217/328, October 24, 1983; No. CTL/P/1894; May 7, 1987. EPA MRID No. 402024-02.

6. Monograph entitled "Oncogenic Potential of Paraquat - Toxicology Data Summary and Overview"; No ID Number; May 1987. EPA MRID No. 402024-01.

The combined chronic toxicity/oncogenicity studies (above reference Nos. 1 and 2) were conducted in Japan under Japanese sponsorship. Paraquat was negative for oncogenicity in both studies. Core Classification: Minimum for the rat study and Supplementary for the mouse study.

The mutagenic studies (above reference Nos. 3 and 4) were conducted by ICI in England. Paraquat was not mutagenic in both studies. Classification for each study: Acceptable.

Slides from the head region (above reference No. 5) were originally examined by a pathologist or pathologists from Life Science Research, the testing laboratory. Although squamous cell carcinomas occurred in different sites of the head region (dorsal cranium, ear, turbinate, snout and hard

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palate), they were morphologically identical and were, therefore, combined by TB for the assessment of oncogenic potential of paraquat as follows:

Paraquat ion (ppm)	0	25	75	150	0	25	75	150
Group	1+2 M	3M	4M	5M	1+2 F	3F	4F	5F
Tumor Type	No. of Tissues with Squamous Cell Carcinoma/ No. Examined							
Squamous carcinoma	3/140	2/70	0/70	6/70	0/138	0/70	3/70	2/70
	Percent of Tissues with Squamous Cell Carcinoma							
Squamous carcinoma	2.14	2.86	0	8.57	0	0	4.28	2.86

According to ICI, there was no justification for combining tumors occurring in separate sites. ICI maintains the following:

1. The skin and oral and nasal cavities have different morphology and physiology and separate biological functions and cannot be considered as a single organ in terms of assessment of carcinogenic effects.

2. When each of these sites was considered independently, there was no statistically significant difference in the incidence of squamous cell carcinomas between the treated and control rats.

In 1987, slides for the head region of male rats were reexamined by ICI (Dr. Ishmael, Head of Pathology) and the incidence of squamous cell carcinomas was reported as follows:

Paraquat Cation (ppm)	0	0	25	75	150
	Incidence of Squamous Cell Carcinoma in Male Rats				
External ear		1/68 = 1.47%			1/69 = 1.45%
Nasal cavity					2/70 = 2.86%
Oral cavity					1/70 = 1.43%

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Paraquat Cation (ppm)	0	0	25	75	150
	Incidence of Squamous Cell Carcinoma in Male Rats				
Skin and Subcutis - head	1/70 = 1/43%	1/68 = 1.47%	2/69 = 2.90%	0	2/70 = 2.86%
Total Number	3		2	0	6
Percent	2.17		2.90	0	8.57

Since Dr. Louis Kasza, Pathologist, TB (now retired), maintained that the above squamous cell carcinomas should be combined and Dr. Ishmael insists that they should not be combined, an independent opinion was sought from Experimental Pathology Laboratories, Inc., Herndon, VA. Upon receipt of their report, the oncogenic potential of paraquat will be reassessed by the TB Peer Review Committee and probably also by the EPA Science Advisory Panel. The additional data, currently submitted by ICI (above reference Nos. 1 through 5) will be considered in these evaluations.

The currently submitted additional data are summarized by ICI in reference No. 6 (above). Included in that monograph are also general remarks about the mutagenicity and "corrosivity" of paraquat. TB acknowledges the receipt of this document.

Attachments

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Reviewed By: Krystyna K. Locke, Toxicologist RRL 3/9/88
Section II, Toxicology Branch (TS-769C) 006652
Secondary Reviewer: Edwin R. Budd, Section Head
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Combined Chronic Toxicity/Oncogenicity (Mouse)

TOX Chem No.: 634
Project No.: 7-0855

Accession No.: 402024-03

Test Material: Paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride)

Synonyms: AT-5, Parakote, Paracote

Study Number(s): None

Sponsor: Asahi Chemical Industry Company, Ltd.
Japan

Testing Facility: Nippon Experimental Medical Research Institute
Japan

Title of Report: AT-5: Chronic Toxicity Study Result - 104-Week Dosing Study in Mouse

Author(s): S. Toyoshima, R. Sato, M. Kashima, and M. Motoyama

Report Issued: March 10, 1982

Conclusions:

Systemic NOEL = 30 ppm (3.92 mg/kg/day*, males)
Systemic NOEL = 30 ppm (3.82 mg/kg/day*, females)
Systemic LEL = 100 ppm (13.09 mg/kg/day*, males and females; HDT); (increased mortality rate in females; decreased total protein, RBC, hemoglobin, hematocrit, and leukocytes in males and females; decreased polymorphonucleocytes in males and GPT and alkaline phosphatase activities in females; increased blood glucose in males and females; decreased absolute and/or relative weights of adrenals, thyroid, liver, and urinary bladder in males; decreased absolute weight of brain in females; and increased absolute and/or relative weights of kidneys, lungs, and heart in males).

Oncogenic NOEL = > 100 ppm (males and females; HDT)

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*Values reported by the testing laboratory.

Gross, non-neoplastic, and neoplastic lesions were observed in various organs of males and females, but did not appear to be treatment related. The most frequent lesions were observed in the lungs (hepatoid changes, congestion, nodes, pneumonia, thickening of alveolar walls, and adenocarcinoma, all in both sexes); liver (turbidity in both sexes, dilatation in females and tumors in males); kidneys (discoloration and coarse surface in both sexes; renal pelvis dilatation and cell infiltration in males; and nephropathy in females); spleen (swelling in both sexes and turbidity and dilatation mostly in females); thymus (atrophy in both sexes and hypertrophy in females); mesenteric lymph node (swelling and cell infiltration in both sexes); eyes (corneal cell proliferation in both sexes and corneal calcification in females); testes (calcification of seminiferous tubules); seminal vesicles (hypertrophy); mammary gland (atrophy and cysts); ovaries (hematoma, cysts, edema, atrophy, and hypoplasia of corpora lutea and follicles); and uterus (edema, atrophy, cysts, and polyps). Leukemia, amyloid degeneration, and leukemia cell infiltration were also observed frequently in males and females.

Classification: Chronic feeding study: Core-Supplementary*
Oncogenic Study: Core-Supplementary*

*Because of omissions and ambiguities, detailed in the review.

A. Materials:

1. Test compound - Technical-grade paraquat dichloride supplied by the sponsor; purity: at least 98%; colorless crystalline powder, readily soluble in water; lot no. 540108.

2. Test animals - Three-week-old JCL:ICR mice, purchased from Japan Clea Laboratories Company, Ltd., Tokyo. Acclimation period: 1 week. Body weight at the initiation of study: 25 to 29 g (males) and 23 to 26 g (females).

B. Study Design:

1. Animal assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 104 Weeks		Interim Sacrifice 26 Weeks		52 Weeks	
		Males	Females	Males	Females	Males	Females
I	0	60	60	10	10	10	10
II	2	60	60	10	10	10	10
III	10	60	60	10	10	10	10
IV	30	60	60	10	10	10	10
V	100	60	60	10	10	10	10

Dose levels used, expressed as paraquat dichloride, were based on the results of preliminary studies (not submitted). Animals were housed 5/sex/cage at 22 °C and relative humidity of 55 percent. The shelves equipped with 30 cages each were rotated to the left of the animal room once every 2 months. Each cage was likewise moved one level down and the lowest level was transferred to the highest level to maintain uniform experimental conditions.

2. Diet preparation - Diet was prepared by Japan Clea Laboratories once every 5 months and pelleted. For every 100 kg of food, 100 g was sent to the sponsor for analysis of paraquat content. At the testing facility, diets were stored at 5 °C and were tested for paraquat stability.

Results - The analytical concentrations of paraquat dichloride in diets ranged from 102 to 106.5 percent of the theoretical (nominal) concentrations.

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Paraquat was stable in diets after storage for 5 months at 5 °C. The concentrations of paraquat dichloride in diets ranged from 95 to 103 percent of the initial concentrations.

The stability of paraquat in diets at room temperature was not tested.

3. Animals received food (Solid Food CE-2 manufactured by Japan Clea Laboratories Company, Ltd.) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

Student's t-test: Body weight, hematology, serum biochemistry, and organ weights. Chi-square test: Mortality and incidence of tumors.

The following levels of significance were used: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

5. Quality assurance statement was not submitted. This study was originally reported in Japanese. ICI Americas, Inc., who submitted the English translation as well as the original report, also included the following Good Laboratory Practice statement:

> The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. After test week 26, each animal was palpated weekly for masses.

Results - Lowered spontaneous mobility, loss of coat luster, and piloerection were observed in moribund animals.

There were no deaths during the first year of study. At the termination of the study, the mortality rate in the 100 ppm female group was 13 percent higher than that in

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the control group. The mortality rates in other treated groups were similar to those in the control groups. These data are summarized below.

Incidence of Mortality

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Weeks	Number of Animals									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	0	0	0	0	0	0	0	0
53 - 60	0	1	0	1	1	0	1	1	1	0
61 - 70	4	8	8	5	5	2	4	2	5	4
71 - 80	11	16	20	15	17	13	10	10	13	13
81 - 90	26	28	34	27	28	23	23	17	27	30
91 - 100	42	35	40	39	37	29	33	28	37	38
101 - 104	43	37	42	43	42	34	38	32	39	42
Survivors	17	23	18	17	18	26	22	28	21	18
Weeks	Percent Incidence*									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	0	0	0	0	0	0	0	0
53 - 60	0	1.7	0	1.7	1.7	0	1.7	1.7	1.7	0
61 - 70	6.8	13.3	13.3	8.3	8.3	3.3	6.7	3.3	8.3	6.7
71 - 80	18.3	26.7	33.3	25	28.3	21.7	16.7	16.7	27.7	21.7
81 - 90	43.3	46.7	56.7	45	46.7	38.3	38.3	28.3	45	50
91 - 100	70	58.3	66.7	65	61.7	48.3	55	46.7	61.7	63.3
101 - 104	71.7	61.7	70	71.7	70	56.7	63.3	53.3	65	70
Survivors	28.3	38.3	30	28.3	30	43.3	36.7	46.7	35	30

*The interim sacrifices were excluded from calculating incidence.

Amyloid degeneration in various organs, pneumonia, pulmonary adenocarcinoma, leukemia, and leukemia cell infiltration into various organs were reported as major causes of deaths in both sexes and at all dose levels, including controls. In the 2 and 10 ppm female groups, nephropathy was also reported as a major cause of death.

2. Body weight - Animals were weighed weekly from the initiation of study until week 26 and every 2 weeks thereafter.

Results - Body weight gains were not inhibited in any group when the treated animals were compared with the controls. Slight but statistically significant ($p < 0.01$, 0.01, or 0.001) increases in mean body weights in the 30 ppm females (2 to 7% over those of controls) did not appear to be treatment-related. These weight

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increases occurred only during the first year (weeks 3 to 4, 7 to 15, 17, 23 to 25, 30, and 42 to 52) and were not observed in the 30 ppm males or 100 ppm males and females.

3. Food consumption and compound intake - Consumption was determined weekly from the initiation of study until week 26 and every 2 weeks thereafter, and mean daily consumption per animal was calculated. Food efficiency (ratio of mean body weight gain to mean food consumption) for each sex was calculated at weeks 26, 52, and 104. Compound intake was calculated weekly from the food consumption and body weight data until week 26 and every 2 weeks thereafter.

   Results - Paraquat had no effect on food consumption and food efficiency when the treated animals were compared with the controls. The compound intake, calculated as paraquat dichloride, was as follows:

Concentration in Diet (ppm)	Mean Intake (mg/kg of Body Weight/Day)	
	Males	Females
2	0.26	0.26
10	1.31	1.32
30	3.92	3.82
100	13.09	13.03

   According to these data, there was no difference between males and females in the ingestion of the test material at each dose level.

4. Ophthalmological examinations were not performed.

5. Blood was collected from 10 males and 10 females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

   a. Hematology

X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

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Results - Statistically significant ($p < 0.05$) decreases in various parameters were observed only in the 100 ppm male and female groups as follows:

Males

- Erythrocytes (6.1 to 7.7%) and hematocrit (4.1 to 7.4%) at all time intervals tested.
- Leukocytes at weeks 26 and 52 (20.5 and 31.4%, respectively).
- Hemoglobin (4.3%) and lymphocytes (9.2%) at week 26.
- Polymorphonucleocytes (13.5%), at week 104.

Females

- Hemoglobin (6.8 to 10.0%) at all time intervals tested.
- Leukocytes (17.8%) at week 26.
- Erythrocytes (7.8%) at week 52.
- Hematocrit (8.7%) at week 104.

b. Clinical Chemistry

X	Electrolytes:
	Calcium*
X	Chloride*
	Magnesium*
	Phosphorous*
X	Potassium*
X	Sodium*
	Enzyme Activities
X	Alkaline phosphatase
X	Cholinesterase[a]
	Creatinine phosphokinase*
	Lactic dehydrogenase
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*

X	Other:
	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
	Globulins
X	Glucose*
	Total Bilirubin*
X	Total Protein*
	Triglycerides
X	Albumin/globulin ratio

[a]Brain, serum, and corpuscular cholinesterase activities were determined.

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

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Results - Statistically significant ($p < 0.05$, 0.01, or 0.001) changes in various parameters were observed only in the 100 ppm male and female groups as follows:

Males

- Decreases in total protein (7.9 to 11.8%) at all time intervals tested.
- Increase in glucose (47.4%) at week 104.

Females

- Decreases in total protein (7.4 and 9.9%) at weeks 26 and 104, respectively.
- Decreases in GPT (16.7%) and alkaline phosphatase (16.8%) activities at week 52.
- Increase in glucose (17.8 and 29.2%) at weeks 52 and 104, respectively.

6. Urinalysis - Urine was collected from 10 males and 10 females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

	Appearance*	X	Glucose*
	Volume*	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Results - Paraquat had no effect on any of these parameters examined when the treated animals were compared with the controls.

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7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X			
	Digestive System		Cardiovasc./Hemat.		Neurologic		
	Tongue		Aorta*	XX	Brain*		
X	Salivary glands*	XX	Heart*	X	Periph. nerve*		
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)		
X	Stomach*	X	Lymph nodes*	XX	Pituitary*		
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*		
X	Jejunum*	XX	Thymus*		Glandular		
X	Ileum*		Urogenital	XX	Adrenals*		
X	Cecum*	XX	Kidneys*		Lacrimal gland		
X	Colon*	XX	Urinary bladder*		Mammary gland*		
X	Rectum*	XX	Testes*	X	Parathyroids*		
XX	Liver*		Epididymides	XX	Thyroids*		
	Gallbladder*	XX	Prostate		Other		
XX	Pancreas*	XX	Seminal vesicle		Bone*		
	Respiratory	XX	Ovaries	X	Skeletal muscle		
X	Trachea*	XX	Uterus*	X	Skin		
XX	Lung*			X	All gross lesions and masses		

Microscopic examination was done on specimens stained with hematoxylin-eosin. The lung was additionally examined for connective tissue after staining with van Gieson's stain. Eyes were examined after treatment with Bouin fixative. Organs were not weighed for animals that died during the study.

Results

a. Organ weight - Statistically significant ($p < 0.05$ or 0.01) changes in absolute and relative** organ weights were observed in the 30 and 100 ppm male groups and in the 100 ppm female group as follows:

Adrenals - Decreases in absolute (25%) and relative (24%) weights of left adrenal in 30 ppm males at week 26.

Decreases in absolute (37%) and relative (37%) weights of left adrenal in 100 ppm males at week 26.

**Relative weight = organ weight/body weight ratio.

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Thyroid - Decreases in absolute (22%) and relative (23%) weights in 100 ppm males at week 26 and in absolute weight (19%) at week 104.

Liver - Decrease in absolute weight (17%) in 100 ppm males at week 104.

Urinary bladder - Decrease in absolute weight (33%) in 100 ppm males at week 104.

Brain - Decrease in absolute weight (4%) in 100 ppm females at week 104.

Kidneys - Increased relative weight (10%) of left kidney in 100 ppm males at week 104.

Lungs - Increases in absolute (11%) and relative (9%) weights in 100 ppm males at week 26.

Heart - Increase in absolute weight (14%) in 100 ppm males at week 52.

b. Gross pathology - Very few changes were observed at the 26-week interim sacrifice, but the incidence was increased at the 52-week scheduled sacrifice.

At week 26, necropsy revealed changes in the lung, thyroid, and lymph node. These changes consisted of nodules of the lung (one male each in the 30 and 100 ppm group), nodules of the parathyroid (one male in the 10 ppm group) and swelling of the lymph nodes (one female each in the control and 2 ppm groups).

At week 52, gross changes were observed in the lung, kidney, liver, large intestine, and uterus. These changes included lung nodules, liver-like (hepatoid) changes and focal bleeding of the lung, hepatic and kidney discoloration, edema and surface coarseness of kidneys, swelling of the lymph nodes of large intestine, and uterine edema. The total numbers of males and (females) affected in the 0, 2, 10, 30, and 100 ppm groups were 2(4), 3(3), 2(4), 4(3), and 5(3), respectively.

Ten animals/sex/dose level were examined at each interim sacrifice.

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The predominant gross pathological changes in animals sacrificed at the termination of the study (week 104) and in those which died or had to be sacrificed moribund during the course of the study were as follows:

Percent Incidence* of Predominant Gross Changes Observed at the Termination of the Study (Week 104)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	17	23	18	17	18	26	22	28	21	18
Lungs										
Nodes	24	17	39	24	50	23	23	14	38	22
Congestion	12	17	5	29	22	8	0	4	10	6
Hepatoid changes	6	0	0	6	11	12	0	11	10	17
Tumors	0	0	0	0	0	0	0	18	5	0
Liver										
Hypertrophy	6	4	0	6	0	4	9	0	14	6
Filled with bile	0	4	0	0	0	8	9	4	10	11
Spleen										
Hypertrophy	6	0	6	12	6	19	18	7	10	6
Turbidity	0	4	0	6	0	19	14	11	14	11
Dilatation	0	0	0	18	0	19	14	11	10	6
Kidneys										
Pale appearance	29	26	5	18	5	19	18	4	0	22
Thymus										
Hypertrophy	0	0	0	0	0	27	14	11	19	6
Testis										
Soft	29	22	5	18	33	--	--	--	--	--
Seminal vesicle										
Hypertrophy	12	13	22	18	11	--	--	--	--	--

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Percent Incidence* of Predominant Gross Changes Observed at the Termination of the Study (Week 104) (cont'd)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	17	23	18	17	18	26	22	28	21	18
Ovary										
Cyst	--	--	--	--	--	15	5	25	10	11
Hematoma	--	--	--	--	--	4	9	7	0	17
Uterus										
Edema	--	--	--	--	--	35	27	25	24	28

*Percent incidence = Number of organs observed with changes x 100/number of organs examined.

According to the above data, gross changes were observed in various organs, but did not appear to be treatment-related. The percent incidence of these changes was either similar in the control and paraquat-treated groups or a dose relationship was lacking.

Percent Incidence* of Predominant Gross Changes Observed in Rats that Died During the Course of the Study (Unscheduled Deaths)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	43	37	42	43	42	34	38	32	39	42
Lungs										
Hepatoid changes	9	8	5	0	0	3	3	13	18	5
Nodes	21	14	5	12	0	0	5	13	8	5
Congestion	14	27	31	21	31	41	39	31	13	38
Tumor	7	16	14	19	17	6	11	6	3	10

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Percent Incidence* of Predominant Gross Changes Observed in Rats that Died During the Course of the Study (Unscheduled Deaths) (cont'd)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	43	37	42	43	42	34	38	32	39	42
Liver										
Turbidity	16	19	12	0	5	6	13	3	5	2
Dilatation	0	0	0	0	0	6	13	3	5	2
Tumor	16	19	12	2	5	0	0	0	0	2
Spleen										
Hypertrophy	16	24	12	14	10	15	16	16	15	14
Kidneys										
Pale appearance	28	22	21	28	26	21	24	0	10	10
Rough surface	7	11	7	9	2	15	11	6	5	7
Edema	2	0	5	5	5	3	0	0	0	0
Divided surface	0	0	0	0	0	3	5	6	5	0
Skin										
Tumor	0	5	2	5	2	6	3	0	3	5
Lymph nodes										
Swelling	12	24	12	19	2	9	13	16	13	10
Uterus										
Atrophy	--	--	--	--	--	18	11	3	13	5
Edema	--	--	--	--	--	12	13	25	13	12
Ovary										
Edema	--	--	--	--	--	26	13	16	5	2

*Percent incidence = Number of organs observed with changes x 100/number of organs examined.

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According to the above data, gross changes were observed in various organs, but did not appear to be treatment-related. The percent incidence of these changes was either similar in the control and paraquat-treated groups or was increased in the treated groups, but a dose relationship was lacking.

Similar pulmonary changes were observed in animals sacrificed at the termination of the study and in those that died or were sacrificed moribund during the course of the study. However, the incidence of pulmonary and skin tumors and swelling in lymph nodes (all dose-unrelated) was higher in animals that did not survive the study.

c. Microscopic pathology

1) Non-neoplastic - The predominant non-neoplastic lesions, observed at the 26-week interim sacrifice were slight to moderate thickening of alveolar walls and bronchodilatation in males and females, swelling of thymus in females, and calculi in urinary bladder in males. The incidence of these lesions ranged from 10 to 40 percent per dose level and was dose-unrelated. Thickening of alveolar walls and swelling of the thymus occurred in every group, including the controls. Bronchodilatation was observed in two males from the 10 ppm group and three males from the 100 ppm group, and in one, one, and two females from the control, 2 and 10 ppm groups, respectively. The incidence of calculi was higher in the control group than in the 30 and 100 ppm groups, where they only occurred.

The predominant non-neoplastic lesions observed in males and females at the 52-week interim sacrifice, were slight to moderate thickening of alveolar walls, cell infiltration (kidneys and salivary gland), renal pelvis dilatation, swelling and atrophy of thymus, swelling of mesenteric lymph node, calculi in urinary bladder, and uterine cysts. The incidence of these lesions ranged from 10 to 40 percent per group, but was dose-unrelated.

The incidence of predominant non-neoplastic lesions observed at the termination of the study (week 104) and in animals that died or were sacrificed moribund during the course of the study, was as follows:

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104**									
	17	23	18	17	18	26	22	28	21	18
Lungs										
Thickening of alveolar walls	12	30	28	47	39	31	55	61	43	28
Kidneys										
Cell infiltration	12	24	17	6	6	0	5	4	0	6
Thymus										
Atrophy	6	30	17	47	44	19	27	11	10	0
Mesenteric lymph node										
Cell infiltration	0	0	0	6	6	8	5	7	5	11
Eyes										
Corneal cell proliferation	6	24	17	6	0	4	14	4	0	6
Corneal calcification	0	0	0	0	0	4	0	7	0	6
Testes										
Calcification of seminiferous tubule	18	30	22	35	33	--	--	--	--	--
Mammary gland										
Atrophy	--	--	--	--	--	42	27	54	38	44
Cysts	--	--	--	--	--	0	14	7	5	11

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104**									
	17	23	18	17	18	26	22	28	21	18
Ovary										
Follicle formation insufficiency	--	--	--	--	--	4	9	11	10	11
Luteinization insufficiency	--	--	--	--	--	4	14	11	14	6
Follicle yellow body formation insufficiency	--	--	--	--	--	23	32	25	29	61
Cysts	--	--	--	--	--	31	18	18	14	11
Uterus										
Atrophy	--	--	--	--	--	0	9	11	19	6
Cysts	--	--	--	--	--	58	59	50	4	78
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Lungs										
Thickening of alveolar walls	35	14	12	12	19	18	24	3	10	10
Pneumonia	28	24	31	23	31	47	37	41	33	48
Spleen										
Swelling	5	3	7	7	2	3	5	0	3	0
Kidneys										
Renal pelvis dilatation	5	3	5	5	7	3	0	0	0	0
Nephropathy	0	0	0	2	0	9	18	16	8	0
Urinary bladder										
Calculi	0	0	2	5	12	0	0	0	0	0
Testes										
Calcification of seminiferous tubule	14	14	14	19	17	--	--	--	--	--

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	6	10	30	100	0	6	10	30	100
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Mammary gland										
Cysts	--	--	--	--	--	3	8	3	3	2
Ovary										
Luteinization insufficiency	--	--	--	--	--	15	5	13	13	10
Atrophy	--	--	--	--	--	3	24	34	28	24
Cysts	--	--	--	--	--	38	32	28	10	17
Uterus										
Atrophy	--	--	--	--	--	38	53	16	31	12
Cysts	--	--	--	--	--	12	21	34	18	26

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined. All animals sacrificed at week 104 and those that died during the study (unscheduled deaths) were examined.
**Percent incidence = number of tissues with lesions x 100/number of tissues examined.

According to the above data, thickening of alveolar walls, calcification of seminiferous tubules, cysts in mammary gland, ovarian cysts, and uterine atrophy and cysts were observed in the animals sacrificed at the termination of the study and in the nonsurvivors. The nonsurvivors also had pneumonia and swollen spleen (males and females), renal pelvis dilatation (mostly males), nephropathy (mostly females), and calculi in urinary bladder. The following lesions were either infrequent or absent in the nonsurvivors, but were observed in both sexes at the termination of the study: atrophy of the thymus, corneal cell proliferation, corneal calcification (females only), and atrophy of mammary gland (females only).

The above lesions did not appear to be treatment-related. In most instances, there were great variations in the incidence of lesions from one group to another and a dose-relationship was lacking.

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Compared with the controls, follicle yellow body formation insufficiency (ovarian lesion) and uterine cysts were increased in the 100 ppm females at the terminal sacrifice, whereas calculi in urinary bladder were increased with dose in the nonsurviving males. However, the incidence of the ovarian lesion did not increase with dose in the 0, 2, 10, and 30 ppm groups, and was not observed in the nonsurvivors. The incidence of uterine cysts (very common lesions) was high in the low-dose groups (2 and 10 ppm), very low in the 30 ppm group, and high again in the 100 ppm group, and did not appear to be treatment-related. Dose-unrelated calculi in urinary bladder were also observed in males at the 26-week interim sacrifice and in males and females at the 52-week interim sacrifice. The dose-related incidence of urinary bladder calculi in the nonsurviving males cannot therefore be unequivocally attributed to treatment.

Another non-neoplastic lesion observed frequently in males and females was amyloid degeneration. This lesion was first noted in the small intestine of two females (one in each 2 and 30 ppm groups) at the 26-week sacrifice. At the 52-week sacrifice, amyloid degeneration occurred mostly in the liver, spleen, kidneys, small intestine, and pancreas of the untreated males. At the terminal sacrifice and in the nonsurvivors, dose-unrelated amyloid degeneration was observed in virtually every organ or tissue examined. At the terminal sacrifice the highest incidence of amyloid degeneration occurred in the small intestine of males (33 to 67%) and females (32 to 64%). In the nonsurvivors (untreated and treated males and females) the highest incidence of amyloid degeneration occurred in the following tissues: liver (9 to 37%), spleen (12 to 33%), kidneys (16 to 48%), small intestine (21 to 56%), thyroid (8 to 40%), and adrenals (12 to 42%).

In summary, for reasons discussed above, the non-neoplastic lesions observed in this study did not appear to be treatment-related. Also, most of these lesions are common in mice, especially after 6 or 12 months of life[1].

Connective tissue, after staining lungs with van Gieson's stain, was observed only in the following animals: 1 female out of 10 examined in the 100 ppm group at the 52-week interim sacrifice; 1 male out of 23 examined (incidence = 4%) in the 2 ppm group at the terminal sacrifice; and in a few males and females that died or were sacrificed moribund during the course of the study (nonsurvivors). The percent incidence of positive findings, very slightly positive (±) or slightly positive (+), in male nonsurvivors was 2, 8, 5, 7, and 2 in the 0, 2, 10, 30, and 100 ppm groups, respectively. The corresponding values for the female groups were 0, 0, 3, 3, and 10, respectively.

Based on the above data, the incidence of this lesion (pulmonary fibrosis) was low in this study and did not appear to be treatment-related, at least in the males. Since there were no other treatment-related non-neoplastic pulmonary lesions in the 100 ppm female nonsurvivors, it could not be concluded with certainty that a slight increase in the incidence of pulmonary fibrosis was treatment-related.

2) Neoplastic - The incidence of neoplastic lesions in this study was as follows:

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 26 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lungs										
Adenoma				10						
Adenocarcinoma					10					

[1] Selected Nonneoplastic Diseases. J.D. Burek, J.A. Molello, and S.D. Warner. In The Mouse in Biomedical Research, Volume II; H.L. Foster, J.D. Small, and J.G. Fox, Editors; Academic Press (1982); pages 425-438.

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 26 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lymph node										
Lymphoma							10			
Number of tissues examined *	Percent Incidence at Week 52 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lungs										
Adenoma			10				10			
Adenocarcinoma		10	10		20					
Uterus										
Polyp						10	10		10	
Leukemia	10									
Number of tissues examined *	Percent Incidence at Week 104 (Terminal Sacrifice)**									
	17	23	18	17	18	26	22	28	21	18
Lungs										
Fibroma		4								
Adenocarcinoma	29	30	28	23	50	31	32	43	38	44
Liver										
Angioma									5	
Adenoma		13			11	4				5
Spleen										
Angioma		4								
Fibrosarcoma										5
Testes										
Seminoma	6		11		6	--	--	--	--	--
Mesenteric lymph node										
Lymphoma				6		8				
Lymphosarcoma										5

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104 (Terminal Sacrifice)**									
	17	23	18	17	18	26	22	28	21	18
Adrenals										
Tumor		4								
Bone										
Osteosarcoma										5
Skin										
Soft tumor								4		
Ovary										
Tumor	--	--	--	--	--		6			
Hypernephromoid	--	--	--	--	--			4		
Uterus										
Polyp	--	--	--	--	--	4		4	5	11
Myoma	--	--	--	--	--			7		5
Fibroma	--	--	--	--	--			4	5	
Leukemia	6			6	6	62	68	64	62	56
T-cell leukemia	6					8	5	4		
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Lungs										
Adenocarcinoma	28	30	21	30	17	9	16	22	10	17
Liver										
Angioma										2
Hepatoadenoma	5	5	2							
Spleen										
Angioma							3			

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined *	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Duodenum										
Carcinoma			2							
Small intestine										
Fibroadenoma										2
Muscle										
Fibroma			2							
Sarcoma										2
Bone										
Osteoma								3		2
Skin										
Fibrosarcoma				5	2	3				2
Spindle cell sarcoma		3								
Adenocarcinoma		3								
Small circular cell sarcoma										2
Squamous cell carcinoma	2									
Basal cell carcinoma							3		3	
Testes										
Seminoma	2			7	2	--	--	--	--	--
Mammary gland										
Fibroadenoma	--	--	--	--	--					2
Adenocarcinoma	--	--	--	--	--		5		3	
Ovary										
Tumor								3		
Fibroma	--	--	--	--	--					2
Carcinoma	--	--	--	--	--					2

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Uterus										
Polyp	—	—	—	—	—	3		3		2
Myoma	—	—	—	—	—		3		3	2
Mesenteric lymph node										
Lymphoma								3		
Leukemia	19	30	24	28	14	50	24	44	41	33
T-cell leukemia										2

**Percent incidence = Number of tissues with lesions x 100/number of tissues examined.

Blank space = Absence of lesion.

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined.

Only three neoplastic lesions were observed at the 26-week interim sacrifice: pulmonary adenoma (30 ppm male), pulmonary adenocarcinoma (100 ppm male), and lymphoma (2 ppm female).

At the 52-week interim sacrifice, a total of 10 neoplastic lesions were noted as follows: 2 pulmonary adenomas (2 ppm female and 10 ppm male); 4 pulmonary adenocarcinomas (2 ppm, 10 ppm, and 2 in 100 ppm males); 3 uterine polyps (0 ppm, 2 ppm, and 30 ppm females); and 1 leukemia in the male control group.

At the terminal sacrifice, the most frequently observed neoplastic lesions were leukemia and T-cell leukemia in females, uterine polyps, and pulmonary adenocarcinoma in males and females. The percent incidence of leukemias and pulmonary adenocarcinoma in females was dose-unrelated, but the incidence of uterine polyps was 7 percent higher in the 100 ppm group than in the controls. The percent incidence of pulmonary adenocarcinoma in males was similar in the 0, 2, and 10 ppm groups; 6 percent lower compared with the controls in the 30 ppm group; and 21

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percent higher, compared with the controls, in the 100 ppm group. In the females, leukemia cells were found in virtually every organ or tissue examined, but were most abundant in lungs, liver, spleen, kidneys, urinary bladder, stomach, thymus, bone marrow, mesenteric lymph node, and salivary glands.

The most frequently observed neoplastic lesions in the nonsurvivors (males and females) were leukemia and pulmonary adenocarcinoma, but the percent incidence was dose-unrelated. Leukemia cells were abundant in both sexes, in the same organs as those listed above, and also in brain and eyes. However, leukemia cell infiltration was low in the thymus of females and was not observed in the thymus of males.

Paraquat dichloride did not appear to be oncogenic in this study. The only tumor of concern was an increased incidence of pulmonary adenocarcinoma in the 100 ppm males observed only at the terminal sacrifice. However, if all pulmonary adenocarcinomas observed in each group during the course of the study are related to 80 animals examined histopathologically in each group, the incidence of this lesion is as follows:

Paraquat dichloride (ppm)	Male Mice Incidence		Female Mice Incidence	
	Numerical	Percent	Numerical	Percent
0	17	21.3	11	13.8
2	19	23.8	13	16.3
10	15	18.8	19	23.8
30	17	21.3	12	15.0
100	19	23.8	12	15.0

Based on the total incidence of pulmonary adenocarcinoma, paraquat did not appear to be oncogenic in the lungs of male mice. This finding is also supported by historical control data submitted by the testing laboratory for four 2-year feeding studies (presumably with the same strain of mice). In these studies, conducted during 1976 and 1982, 99, 44, 75, or 70 mice of each sex per study were used and all were examined histologically. The incidence of

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pulmonary adenocarcinoma in the males ranged from 13.3 to 27.1 percent and in the females from 11.4 to 15.2 percent. The incidence of pulmonary adenocarcinoma in the 100 ppm males was, therefore, within that observed in the historical control males. The highest incidence of tumors occurred during weeks 92 to 104.

Uterine polyps are common in mice, especially aged mice, and the slight increase in the 100 ppm groups was probably not treatment-related.

Leukemia is considered "the most common hematopoietic malignancy in the mouse"[2]. In this study, the incidence of leukemia was higher in the controls than in the 100 ppm group or in some of the other treated groups. Yet, leukemia was reported in only one historical control study in the males (6/44 = 13.6%) and in none of the historical control females.

D. Discussion:

Comments:

This study was conducted between February 27, 1979 and February 20, 1981 in Japan and under Japanese sponsorship, had nothing to do with satisfying data requirements for the U.S. EPA, but was recently submitted to EPA by ICI Americas, Inc., Wilmington, DE. The original report, written in Japanese, has also been submitted along with an English translation.

Most of the submission is handwritten and, therefore, generally difficult to read. The report also contains omissions and ambiguities such as:

1. It is reported on page 12 that "there was only one death in the first half of the study (from the start of treatment to week 52)." Yet, tables on pages 33 and 34 show no deaths during that period.

2. It is stated on page 17 that testicular epithelioma was a frequent lesion and the incidence is quoted in the summary data on page 19. Yet, none was reported

[2] Biology and Diseases of Mice. R.O. Jacoby and J.G. Fox. In Laboratory Animal Medicine; J.G. Fox, B.J. Cohen, and F.M. Loew, Editors; Academic Press (1984); page 82.

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in Table 12A (page 65), Table 12C (page 66), Table 12E (page 69), Table 12G (page 74), Table 14 (page 85), and in the individual data on pages 88 through 101.

3. "Food ingested by individual mouse" is reported on pages 326 through 365. Yet, at the bottom of every page, data concerned with food consumption are summarized for rats.

4. Water intake by individual mice is reported on pages 367 through 406. Yet, each page is entitled "Water intake by individual rats" and "Experimental animal: rat."

5. Neoplastic lesions were reported together with non-neoplastic lesions and each lesion was reported only in terms of numerical incidence and not also in terms of percentage incidence.

6. None of the histopathological lesions was analyzed statistically.

7. No reference was made to MTD, whether or not it was reached. (Considering increased mortality and statistically significant changes in hematology, clinical chemistry, and organ weights at the 100 ppm level in both sexes, an MTD was reached.)

A very striking thing about this study is the apparent perfection with which it was performed. Not a single parameter designated for testing ever failed during the entire course of this study. Not a single blood sample was lost, weight determination missed, tissue not examined or autolyzed.

Considering that this study is not a major mouse oncogenic study (another study conducted by ICI has already been evaluated and accepted), the above missing data will not be required.

Systemic NOEL = 30 ppm (males and females)*
Systemic LEL = 100 ppm (males and females; HDT. See page 1 for findings).

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*Although absolute and relative weights of left adrenals were statistically significantly decreased in males from the 30 ppm group at the week 26 scheduled sacrifice, no weight changes were observed in right adrenals or in both adrenals at other scheduled sacrifices. The observed weight changes at week 26 do not, therefore, appear to be treatment-related.

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Oncogenic NOEL = > 100 ppm (males and females).

Classification: Chronic feeding study: Core-Supplementary**

Oncogenic study: Core-Supplementary**

**Because of omissions and ambiguities listed above, a higher core classification cannot be assigned to this study.

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15909:I:Locke:C.Disk:KENCO:2/8/88:DD:VO:LF:DD:VO:CB

Reviewed By: Krystyna K. Locke, Toxicologist KKL 3/9/88
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Combined Chronic Toxicity/Oncogenicity (Rat)

TOX Chem No.: 634
Project No.: 7-0855

MRID No.: 402024-03

Test Material: Paraquat (1,1'-Dimethyl-4,4'-bipyridylium dichloride)

Synonyms: AT-5, Paracote (Trade names by which paraquat is known in Japan)

Study Nos.: None

Sponsor: Asahi Chemical Industries Company, Ltd.
Japan

Testing Facility: Nippon Experimental Medical Research Institute
Japan

Title of Report: AT-5: Chronic Toxicity Study Result - 104-Week Dosing Study in Rat

Author(s): S. Toyoshima, R. Sato, M. Kashima, M. Motoyama, and A. Ishikawa

Report Issued: March 10, 1982

Conclusions:

Systemic NOEL = 100 ppm (4.15 mg/kg/day*, males)
Systemic NOEL = 100 ppm (5.12 mg/kg/day*, females)
Systemic LEL = 300 ppm (12.25 mg/kg/day*, males, HDT)
Systemic LEL = 300 ppm (15.29 mg/kg/day*, females, HDT)

(Increased mortality in males and females; decreased erythrocytes, hemoglobin and serum protein in males and females; decreased hematocrit, glucose and corpuscular cholinesterase activity in males; decreased leukocytes, albumin to globulin ratio and alkaline phosphatase, GOT and GPT activities in

*Values reported by the testing laboratory.

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females; increased polymorphonucleocytes in males; increased potassium and glucose in females; decreased absolute and/or relative weights of heart [males and females] and liver and brain [females]; and increased absolute weights of kidneys [males and females] and ovaries.)

Oncogenic NOEL = > 300 ppm (males and females; HDT).

Gross, non-neoplastic and neoplastic lesions were observed in various organs of males and females, but did not appear to be treatment-related. The most frequent lesions were observed in the lungs (hepatoid changes, congestion, nodes, peribronchiolitis, pneumonia, suppurative pneumonia and thickening of alveolar walls); liver (bile duct proliferation and fibrosis); kidneys (pale appearance, rough surface, nephritis); pituitary (hypertrophy, hematoma and benign tumors); thyroid (benign tumors); pancreas (benign tumors); adrenals (cysts and hemostasis); spleen (swelling); mesenteric lymph node (swelling and inflammation); testes (atrophy); ovaries (atrophy); uterus (cysts and polyps); and mammary glands (cysts, adenomas, fibromas, fibroadenomas, and adenocarcinomas).

Classification: Chronic feeding study: Core-Minimum
Oncogenic study: Core-Minimum

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A. Materials:

1. Test Compound - Technical grade paraquat dichloride, at least 98% pure; colorless crystalline powder; obtained from the sponsor; Lot No. 540108.

2. Test Animals - Three-week-old JCL:Wistar rats, purchased from Japan Clea Laboratories Company, Ltd., Tokyo; Weight at the initiation of study: males, 100 to 130 g and females, 90 to 120 g; acclimation period: 1 week.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 104 Weeks		Interim Sacrifice 26 Weeks		52 Weeks	
		Males	Females	Males	Females	Males	Females
I	0	50	50	6	6	6	6
II	6	50	50	6	6	6	6
III	30	50	50	6	6	6	6
IV	100	50	50	6	6	6	6
V	300	50	50	6	6	6	6

Dose levels used, expressed as paraquat dichloride, were based on the results of preliminary studies (not submitted). Animals were housed 2/sex/cage at 22 °C and relative humidity of 57 percent. The shelves, equipped with 24 cages each, were rotated to the left of the animal room once every 2 months. Each cage was likewise moved one level down and the lowest level was transferred to the highest level to maintain uniform experimental conditions.

2. Diet Preparation - Diet was prepared by Japan Clea Laboratories once every 4 months and pelleted. For every 100 kg of food, 100 g was sent to the sponsor for analysis of paraquat content. At the testing facility, diets were stored at 3 to 5 °C and were tested for paraquat stability.

Results - The analytical concentrations of paraquat dichloride in diets ranged from 99.7 to 103.7 percent of the nominal concentrations.

Paraquat was stable in diets after storage for 4 months at 3 to 5 °C. The concentrations of paraquat dichloride in diets ranged from 94.3 to 106.9 percent of the initial concentrations.

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The stability of paraquat in diets at room temperature was not tested.

3. Animals received food (solid food CE-2 manufactured by Japan Clea Laboratories Company, Ltd.) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

   - Student's t-test: Body weight, hematology, serum biochemistry, organ weights, and urinalysis.

   The following levels of significance were used: *($p < 0.05$), **($p < 0.01$), *** ($p < 0.001$).

5. Quality assurance statement was not submitted.

   This study was originally reported in Japanese. ICI Americas, Inc., who submitted the English translation as well as the original report, included also the following Good Laboratory Practice statement:

   > The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. After test week 26, each animal was palpated weekly for masses.

   Results - Lowered spontaneous motility, loss of coat luster and piloerection were observed in moribund animals.

   At the termination of the study, the mortality rates in the 30 and 300 ppm male groups and in the 300 ppm female group were 16, 26, and 10 percent higher, respectively, than in their control groups. The mortality rates in

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the remaining treated groups were similar to those of the controls. Only one animal died during the first year of the study. These data are summarized below.

Incidence of Mortality

Paraquat Dichloride (ppm)	Males					Females				
	0	6	30	100	300	0	6	30	100	300
Weeks	Number of Animals									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	1	0	0	0	0	0	0	0
53 - 60	0	0	1	0	0	0	0	0	1	1
61 - 70	3	6	12	10	1	1	1	0	4	3
71 - 80	11	8	18	15	17	4	8	4	7	6
81 - 90	15	9	21	19	19	8	14	6	13	11
91 - 100	16	14	22	23	23	15	16	15	17	18
101 - 104	20	22	28	23	33	21	23	19	22	26
Survivors	30	28	22	27	17	29	27	31	28	24
Weeks	Percent Incidence*									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	1.78	0	0	0	0	0	0	0
53 - 60	0	0	2	0	0	0	0	0	2	2
61 - 70	6	12	24	20	2	2	2	0	8	6
71 - 80	22	16	36	30	34	8	16	8	14	12
81 - 90	30	18	42	38	38	16	28	12	26	22
91 - 100	32	28	44	46	46	30	32	30	34	36
101 - 104	40	44	56	46	66	42	46	38	44	52
Survivors	60	56	44	54	34	58	54	62	56	48

*Scheduled deaths were excluded from calculating incidence.

Pneumonia, nephritis, and tumor in pituitary were reported as major causes of deaths in both sexes and at all dose levels, including controls. In the females, various mammary tumors (adenoma, fibroma, fibrosarcoma, fibroadenoma, carcinoma, adenocarcinoma, adenofibroma, and fibrous angioma) were also listed as major causes of deaths. These tumors were also observed at all dose levels, including controls.

2. Body Weight - Animals were weighed weekly from the initiation of study until week 26 and every 2 weeks thereafter.

Results - Females in the 300 ppm group had statistically significant ($p < 0.05$) decreases in body weight gain during weeks 34, 42 to 48, and 54, when compared with the controls. However, these decreases were very small (only 4 to 5 percent).

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No other differences in weight gain were observed when the treated groups were compared with the controls.

3. Food Consumption and Compound Intake - Consumption was determined weekly from the initiation of study until week 26 and every 2 weeks thereafter, and mean daily consumption per animal was calculated. Food efficiency (ratio of mean body weight gain to mean food consumption) for each sex was calculated at weeks 26, 52, and 104. Compound intake was calculated from the food consumption and body weight data, weekly until week 26 and every 2 weeks thereafter.

   Results - Paraquat had no effect on food consumption and food efficiency when the treated animals were compared with the controls. The compound intake, calculated as paraquat dichloride, was as follows:

Concentration in Diet (ppm)	Mean Intake (mg/kg of Body Weight/Day)	
	Males	Females
6	0.25	0.30
30	1.26	1.50
100	4.15	5.12
300	12.25	15.29

   According to these data, females in the 100 and 300 ppm groups ingested about 1 and 3 mg, respectively, more of the test material per kg of body weight, per day, than did the males in the same groups.

4. Ophthalmalogical Examinations - Conjunctiva, cornea, anterior camera oculi, iris, lens, vitreous body, retina, and optic nerve were examined microscopically by means of Hartnach loupe and Neitz right-figure microscope for all animals before treatment and all survivors at weeks 26, 52, and 104.

   Results - Ocular changes were not detected before the initiation of treatment. After the treatment was started, cataracts were observed during each examination in 1 to 4 animals per group, including controls. At test weeks 52

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and 104, corneitis and conjunctivitis were also observed in all groups. The percent incidence* of ocular changes is summarized below.

	Males			Females		
Test Weeks	26	52	104	26	52	104
Control group						
Cataracts	1.6	1.8	6.7	1.6	3.6	13.8
Corneitis	0	3.6	6.7	0	3.6	3.4
Conjunctivitis	0	1.8	6.7	0	1.8	3.4
6 ppm group						
Cataracts	1.6	1.8	7.1	3.2	3.6	14.8
Corneitis	0	1.8	3.8	0	3.6	3.7
Conjunctivitis	0	1.8	0	0	1.8	0
30 ppm Group						
Cataracts	1.6	3.6	13.6	1.6	7.1	6.5
Corneitis	0	3.6	0	0	1.8	3.2
Conjunctivitis	0	5.4	4.5	0	0	6.5
100 ppm Group						
Cataracts	1.6	3.6	3.7	3.2	7.1	14.3
Corneitis	0	1.8	3.7	0	1.8	0
Conjunctivitis	0	1.8	3.7	0	3.6	7.1
300 ppm Group						
Cataracts	4.8	5.4	17.6	3.2	5.3	16.7
Corneitis	0	1.8	5.9	0	3.6	4.2
Conjunctivitis	0	1.8	0	0	0	0

According to these data, the incidence of ocular changes was low, it increased with time, and a dose-relationship was lacking. Ocular changes therefore do not appear to be caused by paraquat.

*Number of animals with changes x 100/number of animals examined.

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5. Blood was collected from six males and six females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)
X	Reticulocyte count	X	Prothrombin time

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

Results - Statistically significant ($p < 0.05$) changes in hematology were observed only in the 300 ppm male and female groups as follows:

- Decreases in erythrocytes (2.9 to 11.4%) and hemoglobin (4.1 to 10.5%) of males and females at all time intervals tested.
- Decreases in hematocrit in males at week 26 (7.6%) and in males and females at week 104 (6.9 and 7.1%, respectively).
- Increases in reticulocytes in males at week 26 (59.8%) and week 104 (43.0%).
- Increase in polymorphonucleocytes in males (50.0%) and decrease in leukocytes in females (12.7%) at week 52.

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b. Clinical Chemistry

X	Electrolytes	X	Other
	Calcium*		Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzyme Activities		Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
X	Cholinesterase[a]		Triglycerides
	Creatinine phosphokinase*	X	Albumin/Globulin ratio
	Lactic dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

[a]Brain, serum, and corpuscular cholinesterase activities were determined.

Results - Statistically significant ($p < 0.05$) changes in clinical chemistry were observed only in the 300 ppm male and female groups as follows:

- Decreases in total protein (5.3 to 8.5%) of males and females at all time intervals tested.
- Decreases in glucose (26.6%) and corpuscular cholinesterase activity (7.9%) at week 52, and decrease in glucose (16.7%) at week 104, all in males.
- Decrease in alkaline phosphatase activity (34.2%) and increase in potassium (13.2%) at week 26; decreases in GOT and GPT activities (17.9 and 23.1%, respectively) and in albumin/globulin ratio (13.0%) at week 52; and decrease in GOT activity (11.0%) and increase in glucose (12.0%) at week 104, all in females.

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6. Urinalysis - Urine was collected from six males and six females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

X		X	
	Appearance*	X	Glucose*
	Volume*	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
		X	Na^+
		X	K^+

Results - Paraquat had no effect on any of these parameters examined when the treated animals were compared with the controls.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive System	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue		Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus*		**Glandular**
X	Ileum*		**Urogenital**	XX	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	XX	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver*		Epididymides	XX	Thyroids*
	Gallbladder*	XX	Prostate		**Other**
XX	Pancreas*	XX	Seminal vesicle		Bone*
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea*	XX	Uterus*	X	Skin
XX	Lung*			X	All gross lesions and masses

Microscopic examination was done on specimens stained with hematoxylin-eosin. The lung was additionally examined for connective tissue after staining with van Gieson's stain. Eyes were examined after treatment with Bouin fixative. Organs were not weighed for animals which died during the study.

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Results

a. Organ Weight - Statistically significant ($p < 0.05$ or 0.01) changes in absolute and relative* organ weights were observed only in males and females from the 300 ppm group as follows:

Liver - Decreases in absolute weight in females at week 52 (11%) and in absolute (14%) and relative (11%) weights at week 104.

Heart - Decreases in absolute weights in females at week 52 (13%) and week 104 (10%), and in absolute and relative weights in males at week 104 (15 and 14%, respectively).

Kidneys - Increases in absolute weight of right kidney in males (13%) and females (17%) at week 26, and in absolute weight of both kidneys in males at week 52 (10 to 12%).

Ovaries - Increases in absolute weight of both ovaries at week 26 (35 to 39%) and in absolute weight of left ovary at week 52 (32%).

Brain - Decrease in relative weight in females at week 52 (8%).

b. Gross Pathology - Very few pathological changes were observed at interim sacrifices (weeks 26 and 52). At week 26, lung hepatoid changes were noted in two males (one in 30 ppm and one in 100 ppm group) and kidney edema was observed in two males (one in 6 ppm and another in 100 ppm group) and in one control female.

At week 52, kidney edema was observed in one male (300 ppm group); pituitary hemostasis, in two females (one in 30 ppm and one in 100 ppm group); and edematous uterus, in two females (one in control and another in 100 ppm group). Six animals/sex/dose level were examined at each interim sacrifice.

*Relative weight = Organ weight/bwt ratio.

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The predominant gross pathological changes in animals sacrificed at the termination of the study (week 104) and in those that died during the course of the study were as follows:

Percent Incidence* of Predominant Gross Changes Observed at the Termination of the Study (Week 104)

Paraquat Dichloride (ppm)	Males					Females				
	0	6	30	100	300	0	6	30	100	300
Number of rats (organs) examined	30	28	22	27	17	29	27	31	28	24
Lungs										
Hepatoid changes	50	36	23	48	29	28	41	32	21	42
Nodes	7	1	0	0	2	17	19	16	11	4
Congestion	17	36	41	33	24	17	33	48	46	54
Metachromatism	0	0	0	0	0	14	7	6	7	21
Spleen										
Hypertrophy/ swelling	3	7	0	7	6	3	4	0	4	4
Kidneys										
Pale appearance	37	32	45	30	12	0	19	0	11	4
Rough surface	20	18	32	22	12	0	7	0	11	4
Divided surface (3 layers)	23	11	36	15	12	0	7	0	0	4
Pituitary										
Hypertrophy	10	7	5	4	12	3	7	3	14	0
Hematoma	13	21	0	7	12	28	30	35	43	13
Testis										
Soft	10	21	14	19	24	--	--	--	--	--
Atrophy	10	18	14	15	24	--	--	--	--	--
Lymph node										
Swelling	7	4	9	7	6	0	7	3	4	0

*Percent incidence = Number of organs observed with changes/number of organs examined x 100.

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According to the above data, gross changes were observed in various organs, but did not appear to be treatment related. The incidence of these changes was either similar in the control and the paraquat-treated groups or a dose-relationship was lacking.

Percent Incidence* of Predominant Gross Changes Observed in Rats that Died During the Course of the Study (Unscheduled Deaths)

Paraquat Dichloride (ppm)	Males					Females				
	0	6	30	100	300	0	6	30	100	300
Number of rats (organs) examined	20	22	28	23	33	21	23	19	22	26
Lungs										
Hepatoid changes	0	14	7	9	18	0	9	16	14	12
Nodes	45	18	54	39	45	0	9	11	5	4
Congestion	0	27	32	57	15	29	17	32	23	23
Metachromatism	40	18	54	39	52	0	9	16	14	23
Suppuration	0	14	14	0	9	0	9	5	5	4
Liver										
Metachromatism	5	14	18	0	18	19	4	5	14	0
Turbidity	10	9	21	4	18	10	9	5	5	0
Pituitary										
Hypertrophy	15	27	14	22	12	14	17	37	9	15
Hematoma	35	14	11	9	6	48	30	26	45	35
Testis										
Atrophy	5	9	18	26	12	--	--	--	--	--
Seminal vesicle										
Atrophy	0	5	14	13	6	--	--	--	--	--

*Percent incidence = Number of organs observed with changes/number of organs examined x 100.

According to the above data, gross changes observed in the liver, pituitary, and testes, and some pulmonary changes (nodes, metachromatism, and suppuration in males and congestion in females)

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did not appear to be treatment-related because a dose-relationship was lacking. The relationship of other pulmonary changes (hepatoid changes in males and females, congestion in males, and nodes, metachromatism, and suppuration in females) to treatment is harder to interpret because these changes were not observed in the control groups, but occurred in a dose-unrelated manner in the paraquat-treated groups.

Similar pulmonary changes were observed in animals sacrificed at the termination of the study and in those which died during the study (unscheduled deaths). High incidence of pituitary hypertrophy and hepatoma was also observed in both groups, in treated and untreated animals.

c. Microscopic Pathology

1) Non-neoplastic

The predominant non-neoplastic lesions observed at the 26- and 52-week interim sacrifices were slight to moderate thickening of alveolar walls in males and females, bile duct proliferation in females and atrophy of the thymus in males. The incidence of bile duct proliferation in the 0, 6, 30, 100, and 300 ppm groups was 0, 0, 16.7, 16.7, and 33.3 percent, respectively. The incidence of other lesions ranged from 16.7 to 50.0 percent per group, but a dose relationship was lacking.

The percent incidence (number of tissues with lesions x 100/number of tissues examined) of predominant non-neoplastic lesions observed at the termination of the study (week 104) was as follows:

Paraquat Dichloride (ppm)	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined*	30	28	22	27	17	29	27	31	28	24
Organ	Percent Incidence at Week 104**									
Heart Fibrosis	23	18	9	0	6	0	0	0	0	0
Lungs										
Peribronchiolitis	77	57	59	78	82	55	48	71	68	58
Pneumonia	37	50	32	70	41	48	41	42	61	67

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Paraquat Dichloride (ppm)	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined*	30	28	22	27	17	29	27	31	28	24
Organ	Percent Incidence at Week 104**									
Lung (cont'd)										
Suppurative pneumonia	3	14	14	15	18	10	15	10	14	17
Fibrosis	3	0	14	4	12	3	0	3	4	0
Liver										
Bile duct proliferation	37	32	45	33	53	38	22	45	18	33
Kidneys										
Nephritis	67	46	77	41	29	52	37	35	36	33
Thymus										
Atrophy	37	25	54	18	29	55	67	68	61	42
Adrenals										
Cyst formation	0	0	0	0	0	0	4	6	14	4
Hemostasis	3	0	0	0	0	0	0	6	11	0
Testes										
Atrophy of seminiferous tubule	13	21	9	18	23	--	--	--	--	--
Mammary gland										
Cyst formation	--	--	--	--	--	7	4	16	18	0
Ovary										
Atrophy	--	--	--	--	--	7	0	10	43	21
Luteinizing insufficiency	--	--	--	--	--	14	18	6	4	8
Uterus										
Cyst formation	--	--	--	--	--	28	0	10	18	4

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Paraquat Dichloride (ppm)	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined*	30	28	22	27	17	29	27	31	28	24
Organ	Percent Incidence at Week 104									
Mesenteric lymph node										
Catarrhal inflammation	3	21	18	15	0	7	7	13	4	17
Trachea										
Inflammation	0	14	14	7	12	3	0	0	0	0
Eyes										
Atrophy of retina[a]	0	0	0	0	12	3	0	0	0	4
Atrophy of retina[b]	0	0	0	0	0	10	4	0	7	4
Loss of retina[a]	0	0	0	0	12	0	0	0	0	0
Small eyeball[a]	0	0	0	0	6	0	0	0	0	0

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined and numbers of animals sacrificed.

a = both eyes b = one eye

With the exception of lesions in the kidneys, thymus, and testes, lesions in other organs were rated as slight. Lesions in kidneys, thymus and testes were rated as follows: nephritis (kidneys), slight to moderate in females and slight to severe in males; atrophy of thymus, slight in males and moderate in females; and atrophy of seminiferous tubules, slight to severe.

According to the above data, the highest incidence of lesions was observed in lungs (peribronchiolitis and pneumonia), liver, kidneys, and thymus of both sexes, but a dose-relationship was lacking in all instances. In some organs, the incidence of lesions was 1) similar in the treated and untreated groups (lung fibrosis in females); 2) lower in the

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treated than in the control groups (heart fibrosis in males); or 3) observed in only one sex (heart fibrosis and some eye lesions were absent in females, whereas adrenal cysts and hemostasis, and atrophy of retina were absent in males).

Connective tissue, after staining lungs with van Gieson's stain, was not observed at the 26- and 52-week interim sacrifices. At the terminal sacrifice, the combined incidence of positive (+) and marginally positive (±) findings in the 0, 6, 30, 100, and 300 ppm male groups was 10, 3, 18, 18, and 12 percent, respectively. The corresponding values for the female groups were 7, 4, 6, 7, and 4 percent, respectively. This lesion does not appear to be treatment-related.

The percent incidence (number of tissues with lesions x 100/number of tissues examined) of predominant non-neoplastic lesions observed in animals which died during the course of the study (unscheduled deaths) was as follows:

Paraquat Dichloride (ppm)	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined*	20	22	28	23	33	21	23	19	22	26
Organ	Percent Incidence in All Nonsurvivors**									
Lungs										
Peribronchiolitis	20	14	4	9	9	38	26	26	23	31
Pneumonia	60	41	64	74	58	29	30	32	50	35
Suppurative pneumonia	5	18	25	0	27	19	13	26	14	15
Thickening of alveolar walls	5	4	4	13	3	9	17	10	14	23
Liver										
Bile duct proliferation	10	0	11	9	12	0	4	5	0	0
Fibrosis	5	4	7	9	12	15	4	5	0	4
Spleen										
Swelling	5	14	7	4	18	14	26	5	14	8
Kidneys										
Nephritis	40	68	54	52	45	52	35	10	18	15

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Paraquat Dichloride (ppm)	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined*	20	22	28	23	33	21	23	19	22	26
Organ	Percent Incidence in All Nonsurvivors									
Adrenals										
Cyst formation	0	0	4	0	0	33	26	5	0	0
Hemostasis	0	0	4	0	0	5	13	26	23	46
Testes										
Atrophy of seminiferous tubule	5	9	7	13	9	--	--	--	--	--

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined and numbers of animals which died during the study.

With the exception of lesions in liver and adrenals which were rated as slight, other lesions were rated as slight to moderate (pneumonia, swelling of spleen, and atrophy of seminiferous tubules) or slight to severe (nephritis).

According to the above data, the highest incidence of lesions was observed in lungs (peribronchiolitis in females and pneumonia in both sexes) and kidneys (nephritis), but a dose-relationship was lacking. In general, the incidence of lesions observed in various organs fell into one of the following patterns:

- Was similar in the control and paraquat-treated groups;
- Was lower in the paraquat-treated than in the control groups;
- Increased only in one or two treated groups;
- Increased in the treated groups, in a dose-unrelated manner; or
- Increased slightly with dose (liver fibrosis in males).

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Paraquat Dichloride (ppm)	PERCENT INCIDENCE*									
	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined**	Termination of the Study at Week 104 (cont'd)									
	30	28	22	27	17	29	27	31	28	24
Kidneys										
Cell infiltration (leukemia)	0	0	0	3.7	0	0	0	0	0	0
Lipoma	0	3.6	0	0	0	0	0	0	0	0
Myxomatoid lipoma	3.3	0	0	0	0	0	0	0	0	0
Small intestine										
Fibrosarcoma	0	0	0	0	0	3.4	0	0	0	0
Large intestine										
Myoma	0	0	0	0	0	0	0	3.2	0	0
Pancreas										
Tumor in islands of Langerhans (benign)	3.3	0	9.1	7.4	17.6	0	0	0	0	0
Adenoma in islands of Langerhans	0	3.6	0	7.4	5.9	3.4	3.7	0	3.6	0
Adenocarcinoma in islands of Langerhans	0	0	0	0	0	3.4	0	9.7	3.6	0
Adenoma	0	0	4.5	0	0	3.4	0	0	3.6	0
Adenocarcinoma	0	3.6	0	0	0	0	0	0	0	0
Fibroma	6.7	3.6	0	7.4	0	0	14.8	3.2	3.6	4.2
Pituitary										
Tumor (benign)	33.3	39.3	4.5	18.5	35.3	41.4	37.0	41.9	60.7	16.7
Thyroid										
Tumor (benign)	0	0	0	0	11.8	0	0	0	0	0
Adenoma	3.3	7.1	0	0	5.9	3.4	0	0	0	4.2
Parathyroid adenoma	3.3	0	0	0	0	0	0	0	3.6	0
C cell carcinoma	0	3.6	0	3.7	0	3.4	11.1	3.2	0	4.2

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Paraquat Dichloride (ppm)	PERCENT INCIDENCE*									
	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined**	Termination of the Study at Week 104 (cont'd)									
	30	28	22	27	17	29	27	31	28	24
Adrenals										
Tumor (benign)	6.7	3.6	0	3.7	0	3.4	0	0	0	0
Medulla tumor (benign)	0	0	0	0	0	3.4	0	0	0	4.2
Medulla adeno-carcinoma	0	0	0	0	0	0	0	3.2	0	0
Skin										
Fibroma	3.3	0	9.1	7.4	0	0	0	0	0	0
Myosarcoma	0	0	0	3.7	0	0	0	0	0	0
Fibrosarcoma	0	0	4.5	0	0	0	0	0	0	0
Epithelial carcinoma	0	0	0	0	0	0	0	3.2	0	4.2
Testes										
Seminoma	3.3	7.1	0	0	5.9	--	--	--	--	--
Mammary gland										
Lipoma	--	--	--	--	--	0	0	0	3.6	0
Papillary adeno-myoma	--	--	--	--	--	3.4	0	0	0	0
Adenoma	--	--	--	--	--	6.9	22.2	38.7	35.7	12.5
Fibroma	--	--	--	--	--	0	7.4	9.7	10.7	0
Fibroadenoma	--	--	--	--	--	6.9	3.7	3.2	0	8.3
Adenofibroma	--	--	--	--	--	20.7	0	3.2	0	0
Fibrosarcoma	--	--	--	--	--	0	0	0	7.1	0
Adenocarcinoma	--	--	--	--	--	6.9	3.7	9.1	10.7	0
Ovary										
Vesicle tumor-like adenoma	--	--	--	--	--	0	0	0	0	4.2
Granulosa cell tumor	--	--	--	--	--	3.4	0	0	0	0
Uterus										
Polyp	--	--	--	--	--	10.3	14.8	6.5	14.3	20.8
Myosarcoma	--	--	--	--	--	0	0	0	3.6	0

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Paraquat Dichloride (ppm)	PERCENT INCIDENCE*									
	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined**	Termination of the Study at Week 104 (cont'd)									
	30	28	22	27	17	29	27	31	28	24
Mesenteric lymph node										
Cell infiltration (leukemia)	3.3	0	0	3.7	0	0	0	0	0	0
Fibroma	3.3	0	0	0	5.9	0	0	0	0	0
Fibrosarcoma	0	0	4.5	0	5.9	0	0	0	0	0
Leukemia	3.3	0	0	3.7	0	0	0	0	0	0
Number of tissues examined**	Unscheduled Deaths									
	20	22	28	23	33	21	23	19	22	26
Lungs										
Fibrosarcoma	3.3	0	0	0	0	3.4	0	0	0	0
Cell infiltration (leukemia)	0	0	0	0	0	0	0	0	3.6	0
Liver										
Small cell sarcoma	0	0	0	0	0	0	0	3.2	0	0
Kidneys										
Fibrosarcoma	3.3	0	0	0	0	0	0	0	0	0
Cell infiltration (leukemia)	0	0	0	0	0	0	0	0	3.6	0
Duodenum										
Small cell sarcoma	0	0	0	0	0	0	0	3.2	0	0
Pancreas										
Tumor (benign)	0	0	0	0	0	0	7.4	0	0	0
Pituitary										
Tumor (benign)	36.7	32.1	27.3	29.6	41.2	44.8	37.0	35.5	42.9	50.0

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Paraquat Dichloride (ppm)	PERCENT INCIDENCE*									
	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined**	Unscheduled Deaths (cont'd)									
	20	22	28	23	33	21	23	19	22	26
Adrenals										
Tumor (benign)	0	0	9.1	0	0	0	0	0	0	0
Adenoma	0	0	0	0	0	3.4	0	0	0	0
Small cell carcinoma	0	0	0	0	0	0	0	3.2	0	0
Skin										
Tumor (benign)	0	0	0	3.7	0	0	0	0	0	0
Cartilage tumor	0	3.6	0	0	0	0	0	0	0	0
Fibroma	0	3.6	0	0	5.9	0	0	0	0	0
Lipoma	0	0	0	0	5.9	0	0	0	0	0
Cartilage fibrosarcoma	0	0	0	0	5.9	0	0	0	0	0
Fibrosarcoma	0	0	4.5	3.7	0	0	0	0	0	4.2
Mammary gland										
Lipoma						6.9	0	0	0	0
Adenoma						13.8	11.1	3.2	3.6	0
Fibroma						6.9	11.1	3.2	3.6	8.3
Cartilage fibroma						3.4	0	0	0	0
Fibrous angioma						0	3.7	0	0	0
Fibroadenoma						13.8	11.1	3.2	0	12.5
Adenofibroma						6.9	3.7	3.7	14.3	29.2
Fibrosarcoma						3.4	3.7	0	3.6	0
Small cell sarcoma						0	0	3.2	0	0
Adenocarcinoma						3.4	11.1	0	3.6	0
Uterus										
Polyp	--	--	--	--	--	0	0	0	3.6	4.2
Fibrosarcoma	--	--	--	--	--	0	0	0	3.6	0
Small circular cell sarcoma	--	--	--	--	--	0	0	0	3.6	4.2

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Paraquat Dichloride (ppm)	PERCENT INCIDENCE*									
	Male Rats					Female Rats				
	0	6	30	100	300	0	6	30	100	300
Number of tissues examined**	Unscheduled Deaths (cont'd)									
	20	22	28	23	33	21	23	19	22	26
Mesenteric lymph node										
Lymphoma	0	0	0	3.7	0	0	0	0	0	0
Fibroma	3.3	0	0	0	0	0	0	0	0	0
Fibrosarcoma	6.7	3.6	0	0	0	0	0	0	0	0
Small cell sarcoma	0	0	0	0	0	0	0	3.2	0	0
Metastasized pituitary tumor	6.7	0	0	0	0	3.4	0	0	0	0
Leukemia	6.7	0	0	0	0	3.4	0	0	3.6	0

*Number of tissues with lesions x 100/number of tissues examined.

According to the above data, pituitary tumors in males and females, and various mammary gland tumors in females predominated during the study. Compared with the controls, the incidence of pituitary tumors was slightly (about 5%) increased in both sexes in the 300 ppm group, whereas the incidence of mammary gland adenofibroma was increased in the 100 ppm (7.4%) and 300 ppm (22.3%) groups. Skin tumors (single incidences of fibroma, lipoma and/or cartilage tumor) were observed only in the 6, 100, and 300 ppm male groups.

Pancreatic, pituitary, thyroid, skin, testicular, mammary gland, uterine, and mesenteric lymph node tumors were observed most frequently at the terminal sacrifice. Compared with the controls, dose-unrelated increases were observed in the incidence of pancreatic tumor (males), mammary gland adenoma and fibroma, uterine polyps (females), thyroid adenoma (males), skin fibroma (males), mesenteric lymph node fibrosarcoma (males), and thyroid tumors. The incidence of pituitary tumors was increased only in the 6 ppm male group and 100 ppm female group. Skin tumors (single occurrence of myosarcoma or fibrosarcoma) were noted only in the 30 and 300 ppm groups,

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whereas epithelial carcinoma (also single occurrences) was observed in the 30 and 300 ppm female groups. The highest incidence of tumors occurred during weeks 92 to 104.

Based on the above data, paraquat was not oncogenic in this study. Although the incidence of certain neoplasms was occasionally higher in the 300 ppm group than in the control group (benign tumors in the pancreas and thyroid of males; benign tumor in the pituitary of males and females; fibrosarcoma in mesenteric lymph node, skin lipoma, and cartilage fibroma in males; uterine polyps; and mammary gland adenofibroma in females), these tumors are common, especially in aged rats. This is evident from the historical control data submitted by the testing laboratory for three 2-year studies with rats, conducted during 1976 to 1981, and from open literature (The Laboratory Rat, Vol. I, Biology and Diseases; H.J. Baker, J.R. Lindsey, and S.H. Weisbroth, Editors; Academic Press, Inc.; 1979; pages 334 to 371).

D. Discussion:

Comments

This study was conducted during February 27, 1979 and February 20, 1981 in Japan and under Japanese sponsorship, had nothing to do with satisfying data requirements for the U.S. EPA, but was recently submitted to EPA by ICI Americas, Inc., Wilmington, DE. The original report, written in Japanese, has also been submitted with an English translation.

Most of the submission is handwritten and, therefore, generally difficult to read. The report also contains omissions such as: 1) neoplastic lesions were not separated from non-neoplastic lesions and all were reported only in terms of numerical incidence and not also in terms of percentage incidence; 2) none of the histopathological lesions were analyzed statistically; and 3) no reference was made to maximum tolerated dose (MTD), whether or not it was reached. (Considering increased mortality and statistically significant changes in hematology, clinical chemistry, and organ weights at the 300 ppm level in both sexes, an MTD was reached.)

A very striking thing about this study is an apparent perfection with which it was performed. Not a single parameter designated for testing ever failed during the entire course of this study. Not a single blood sample was lost, weight missed, tissue not examined or autolyzed!

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Considering that this study is not a major combined chronic toxicity/oncogenicity rat study (a more recent and fully acceptable study is available) and that it was conducted some 7 years ago, the above missing data will not be required, and the study is being accepted as follows:

Chronic feeding study: Core-Minimum
Oncogenic study: Core-Minimum

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15908:I:Locke:C.Disk:KENCO:1/28/88:DD:VO:EK:DD

Reviewed By: Krystyna K. Locke, Toxicologist KKL 3/9/88 KD 8/4/88 652
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head
Section II, Toxicology Branch (TS-769C) Budd 3/25/88

Attachments

DATA EVALUATION REPORT

Study Type: Mutagenic (In vivo bone marrow chromosomal aberration study in rats)

TOX Chem No.: 634
Project No.: 7-0855

MRID No.: 402024-05

Test Material: Paraquat Dichloride (Technical); Lot No. 460; Purity: 33.07% (w/w) ion

Study No(s).: CTL/P/1560

Sponsor: Imperial Chemical Industries (ICI) PLC
Plant Protection Division
Fernhurst, England

Testing Facility: ICI Central Toxicology Laboratory
Alderley Park, England

Title of Report: Paraquat Dichloride (Technical): An Acute Cytogenetic Study in the Rat

Author(s): C.A. Howard, C.R. Richardson, I. Pate, T. Sheldon, J. Wildgoose, S. Beck, and P.B. Banham

Report Issued: March 26, 1987

Conclusions:

Paraquat dichloride (technical) did not induce an increased level of chromosomal aberrations in the rat bone marrow test system. Single doses up to 150 mg paraquat ion/kg, which approximated a maximum tolerated dose (MTD), did not produce a significant, reproducible, increased aberration frequency in either males or females. Positive results were obtained with cyclophosphamide, a well-referenced clastogen.

Classification of Study: Acceptable

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Experimental Procedures:

Alderley Park Wistar-derived (Alpk:AP) rats received, by gavage, single doses of paraquat dichloride equivalent to 0, 15, 75, or 150 mg of paraquat ion/kg body weight. Cyclophosphamide, 30 mg/kg body weight (single dose by gavage) was used as a positive control. The animals were sacrificed for bone marrow sampling as follows:

Group	1		2		3		4		5	
Dose*	0		15 P		75 P		150 P **		30 C	
Hours After Treatment	Number of Rats Sacrificed									
	M	F	M	F	M	F	M	F	M	F
12	12	12	-	-	-	-	8	8	-	-
24	12	12	8	8	8	8	8	8	12	12
48	12	12	-	-	-	-	8	8	-	-

*Mg/kg of body weight; P = paraquat ion; C = cyclophosphamide.
**Maximum tolerated dose (MTD), established in a preliminary study. In that study, rats of the same strain (5 males and 5 females/group) received by gavage single doses of technical paraquat dichloride, equivalent to 0, 100, 125, 150, or 175 mg of paraquat ion/kg body weight, and were observed for toxic signs and mortality for 5 to 6 days.

Paraquat dichloride was dissolved for dosing in deionized water and cyclophosphamide in physiological saline. In all instances, 1 mL of dosing solution/100 g of body weight was used. Paraquat solutions were analyzed for paraquat ion concentration prior to dosing.

The rats were:

1. Obtained from the breeding colony maintained by the testing laboratory;

2. Six to 8 weeks old and weighed 195 to 258 g when supplied;

3. Acclimated for 4 days;

4. Assigned randomly to groups and location on the rack;

5. Housed 4/cage/sex at 20 ± 2 °C and relative humidity of 54 to 57 percent; and

6. Fed pelleted Porton Combined Diet (PCD) and water ad libitum, before and after treatment. (Composition of diet and analysis for nutrients and contaminants, as well as water analysis, were submitted.)

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Two hours before sacrifice, each animal received an intraperitoneal injection of colchicine (2 to 3 mg/kg body weight) to arrest dividing cells in c-metaphase. Bone marrow was obtained from both femurs and processed as described in Attachment I. In most instances, 50 cells from each animal were examined for chromosomal damage. Data were analyzed statistically as detailed in Attachment II.

Results:

The mean concentrations of paraquat ion in the dosing solutions were within 2 percent of target levels.

At the 12- and 48-hour sampling times, there were no statistically significant increases in the frequencies of chromosomal aberrations in the 150 mg/kg male and female groups when compared with the negative control groups. Animals from other test groups, including positive control, were not tested at these time intervals.

At the 24-hour sampling time, small but statistically significant ($p < 0.05$ or 0.01) increases in the frequencies of chromosomal aberrations were observed in the 75 mg/kg male and female groups and in the 150 mg/kg female group when compared with the negative control groups. The proportion of abnormalities, including and excluding gaps, and the proportion of animals with each individual abnormality was significantly increased ($p < 0.01$) in the positive control group for both sexes, indicating that the system was sensitive in identifying a clastogenic agent.

At the 24-hour sampling time, there was a statistically significant ($p < 0.05$ or 0.01) decrease in mitotic index in the positive control group. Decreases in mitotic indices were also observed in male and female rats treated with 150 mg/kg of paraquat, at both the 24- and 48-hour sampling times, although these were not statistically significant compared with the negative control groups. A reduction in mitosis indicated that paraquat had reached bone marrow, the target organ. (For detailed results on mitotic indices and chromosomal aberrations, see Attachment III.)

It was concluded that technical paraquat dichloride was nonclastogenic in the rat bone marrow system. Small statistically significant but dose-unrelated increases in the frequencies of chromosomal aberrations, observed in both sexes at the 75 mg/kg dose level and in females at the 150 mg/kg dose level, in samples taken 24 hours after dosing, were attributed to the abnormally low aberration frequencies observed in the negative control groups. It was also stated, but data not submitted, that all values obtained for chromosomal aberrations in the paraquat-treated groups fell

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within historical control frequencies.* Statistically significant increases in chromosomal aberrations were not observed in other paraquat-treated groups or at the 12- and 48-hour sampling times.

In summary, Toxicology Branch accepts the conclusion of the testing laboratory that technical paraquat dichloride was not mutagenic in this study.

Classification of Study: Acceptable

Quality Assurance Statement, dated February 25, 1987, was submitted.

Attachments

*Richardson, C.R.; Howard, C.A.; Wildgoose, J; Sheldon, T. (1985) Cytogenetic observations on > 30,000 cells from both positive and negative control animals in the in vivo rat bone marrow system. Poster presented at 4th International Conference on Environmental Mutagens, Stockholm, June 24-28.

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Reviewed By: Krystyna K. Locke, Toxicologist KKL 3/9/88
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head ERB 3/9/88
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Mutagenic (Unscheduled DNA Synthesis) - Rat

TOX Chem No.: 634
Project No.: 7-0855

MRID No.: 402024-04

Test Material: Paraquat dichloride (Technical); Lot No. 460;
Purity: 33.07% (w/w) ion

Study No(s).: CTL/P/1550

Sponsor: Imperial Chemical Industries (ICI) PLC
Plant Protection Division
Fernhurst, England

Testing Facility: ICI Central Toxicology Laboratory
Alderley Park, England

Title of Report: Paraquat Dichloride (Technical): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo

Author(s): R.W. Trueman and G. Barber

Report Issued: March 31, 1987

Conclusions:

Technical paraquat dichloride at dose levels of 45, 75, or 120 mg/kg of body weight did not induce unscheduled DNA synthesis in rat hepatocytes exposed in vivo. Unscheduled DNA synthesis was induced under the same conditions by 6-dimethylaminophenylazobenzthiazole (6BT), the positive control. The selection of dose levels was governed by the need to evaluate paraquat at adequate concentration and that these levels should not induce toxicity in the hepatocytes. Although hepatocytes from animals in the 120 mg/kg group showed marked signs of toxicity, as evidenced by rounded shape and deeply stained nuclei, sufficient cells of normal morphology were available to be examined for unscheduled DNA synthesis.

Classification of Study: Acceptable

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Experimental Procedures:

This study was conducted according to the procedure of Mirsalis et al. (1,2), modified by Ashby et al. (3,4) and Lefevre et al. (5).

Male Alderley Park (Alpk:AP, specific pathogen-free) albino rats, two to three per group per experiment, received by gavage single doses of paraquat dichloride (0, 45, 75, or 120 mg/kg of body weight) and were sacrificed at 4 or 12 hours after dosing. 6BT (40 mg/kg of body weight) was used as a positive control because it consistently gave positive results in this assay system. Two separate experiments were performed for each time point. For dosing, paraquat was dissolved in distilled water and 6BT in corn oil, and all solutions were administered in volumes of 10 mL/kg of body weight. Negative control animals were dosed with distilled water. The hepatocytes were isolated and processed as summarized in Attachment I.

The rats were:

1. Obtained from the breeding colony maintained by the testing laboratory;

2. Assigned randomly to groups and location on the rack;

3. Housed 4 to 6/cage before treatment and singly thereafter, at 20 to 23 °C and relative humidity of about 50 percent; and

4. Fed pelleted Porton Combined Diet and water ad libitum, before and after treatment. (Composition of diet and analysis for nutrients and contaminants were submitted.)

The rats weighed between 242 and 440 g at the beginning of treatments.

Results:

No increase in unscheduled DNA synthesis was observed at any dose level of technical paraquat dichloride at either time point when compared with concurrent negative controls. At the 4-hour time interval, the mean percentages of cells in repair in the 0, 45, 75, and 120 mg/kg paraquat-treated groups were 1.0, 1.0, 1.6, and 0.8, respectively. The corresponding percentages for the 12-hour time interval were 3.5, 1.8, 1.0, and 4.0, respectively. In the 6BT (positive control) group, the mean percentages of cells in repair at the 4- and 12-hour time intervals were 50.5 and 94.0, respectively. (For detailed results, see Attachment II.)

Classification of Study: Acceptable

Quality Assurance Statement, dated March 30, 1987, was submitted.

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