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Technical Information

Summary of Toxicity Studies on Paraquat

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DESCRIPTION OF THE TEST CHEMICAL

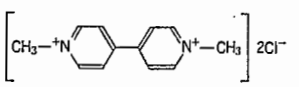
Paraquat, a non-selective contact herbicide, is the methyl diquaternary salt of 4,4′-bipyridyl which kills all green parts of plants rapidly in the presence of oxygen in the atmosphere and light. Its herbicidal properties were first reported in 1958 by Brian *et al.* and it was introduced worldwide in the early 1960s. Since then, the herbicide has been used extensively in about 130 countries in the world including U.K., U.S.A. and Canada in a wide variety of agricultural crops. This article reviews various toxicological studies of paraquat dichloride conducted in Japan.

The chemical structure and physicochemical properties of the herbicide are given below:

Common name: Paraquat

Chemical name: 1,1′-dimethyl-4,4′-bipyridinium dichloride

Structural formula:



Molecular formula: $C_{12}H_{14}Cl_2N_2$

Molecular weight: Cation (186), dichloride salt (257)

Appearance: White crystals, hygroscopic

Melting point: Decomposes at 345°C

Solubility (g/*l*): Water 561, acetone 0.2, methanol 144, ethanol 1.7, insoluble in non-polar organic solvents

Stability: Stable on exposure to hot acids, unstable in alkalis (pH 10 or higher)

ACUTE TOXICITY STUDIES

1. *Technical Material*

Species	Sex	Oral	LD_{50}[a] (mg/kg body weight) Subcutaneous	Intraperitoneal	Dermal
Rat	M	223	27	25	115
	F	258	32	27	79
Mouse	M	360	41	41	—
	F	290	37	39	—

[a] As paraquat dichloride—to convert to paraquat ion divide by 1.38 (Institute of Environmental Toxicology, Tokyo).

Other acute data were published by FAO/WHO on the rat (oral LD_{50} ♂100, ♀110 mg ion/kg, dermal LD_{50} ♂80, ♀90 mg ion/kg), guinea pig (oral LD_{50} ♂30 mg ion/kg), cat (oral LD_{50} ♀35 mg ion/kg) and dog (oral LD_{50} 25–50 mg ion/kg).

2. *Formulated Product*

2.1 *Gramoxone (contains 24% w/w paraquat dichloride)*

Species	Route of administration		LD_{50} (mg/kg)[a]
Rat	Oral	M	585
		F	495
	Dermal	M	2750
		F	3520

[a] As product (Tokyo Dental College).

2.2 *Gramoxone 100 (contains 24% w/w paraquat dichloride)*

Species	Route of administration		LD_{50} (mg/kg)[a]
Rat	Oral	M	910
		F	565
	Dermal	M	>4000
		F	>4000

[a] As product (Medical Scientific Research Laboratory).

SUB-ACUTE TOXICITY STUDIES

1. 13 Week Sub-Acute Toxicity Study in the Rat

Groups of 20 male and 20 female Fischer 344 rats were fed diets containing paraquat dichloride at 0, 10, 30, 100 and 300 ppm for 13 weeks. During the study there were no deaths. There were no clinical signs attributable to the administration of paraquat dichloride.

Body weight gain was reduced markedly in both sexes at the 300 ppm dietary level, at which food consumption, efficiency of food utilisation and water consumption were also slightly reduced. There were also minimal reductions in food and water intake at 100 ppm. There were reduced weights of the brain, pituitary, thyroid, heart, thymus, liver, kidneys, spleen and muscles in the male rats of the 300 ppm group and reduced weights of the heart and liver of the females of the same group, yet no abnormalities associated with administration of paraquat were seen in any of the data obtained in the haematology, blood biochemistry, urinalysis and gross necropsy.

In the histopathological examination, swelling of the alveolar epithelium cells in male rats of the 300 ppm group and increased deposit of brown pigment in the spleen of female rats of the same group were observed. The maximum toxicological no-effect level in this study was 100 ppm in the diet (6.55 mg/kg/day for male rats and 7.10 mg/kg/day for female rats).

(Institute of Environmental Toxicology)

2. 13 Week Sub-Acute Toxicity Study in the Mouse

Groups of 20 male and 20 female ICR-CRJ mice were fed diets containing paraquat dichloride at 0, 10, 30, 100 and 300 ppm for a period of 13 weeks. Two female mice of the 300 ppm group died from pulmonary damage. Reduced bodyweight gains and a slight reduction in the food utilisation efficiency were recorded in both sexes of the same group. There was no evidence of effects of treatment on the food and water consumptions. No treatment-related abnormalities were seen in any of the data obtained in the haematology, blood biochemistry and urinalysis. There were a few small changes in the organ weights, the most notable of which were increased lung weights and/or lung to body weight ratios in the 300 ppm group.

In the histopathological examination, eosinophilic swelling of the alveolar epithelium cells was observed in the males and females of the 300 ppm group. The maximum no-effect level of this chemical in the diet was determined as 100 ppm (11.5 mg/kg/day for males and 13.8 mg/kg/day for females) in this study.

(Institute of Environmental Toxicology)

CHRONIC TOXICITY STUDIES

1. Two Year Chronic Toxicity/Carcinogenicity Studies in the Rat

1) Groups of 80 male and 80 female Fischer (F344) rats were fed on diets containing paraquat dichloride at 0, 10, 30, 100 and 300 ppm for a period of 104 weeks. At weeks 26, 52 and 78 of the dosing period, 8 males and 8 females per group were sacrificed and were subjected to detailed examinations, in addition to those surviving to 104 weeks and those dying during the course of the study.

There were no clinical signs associated with administration of paraquat dichloride in any of the treatment groups and there was no significant difference in overall cumulative mortalities between the treatment and control groups. There was a clear reduction in body weight gain of males and a slight reduction in body weight gain of females of the 300 ppm group. Slightly reduced food and water consumptions were recorded in the male and female rats of the 300 ppm group throughout the dosing period.

Reduced white blood cell counts, reduced globulin values and increased A/G ratio was observed in the male rats of the 300 ppm group. Also, towards the end of the study, males and females of the 300 ppm group only showed an increased incidence compared with the control group of cataracts which were morphologically identical to the spontaneous cataracts seen in ageing rats on this study. Measurements of organ weights revealed reduced weights of the brain, heart, liver, kidneys, adrenals, ovaries and skeletal muscles in the male and female rats of the 300 ppm group and increased lung and testis to body weight ratio

in the male rats of the same group at weeks 26 and 52.

Histopathological examination of lung tissue showed a significant increase in the incidence of proliferation of the interalveolar septum and hyperplasia of alveolar epithelium observed in male and female rats of the 300 ppm group and male rats of the 100 ppm group. These pulmonary changes were ascribed to the administration of the chemical.

A higher incidence of a lung lesion classified as pulmonary adenoma was noted in female rats of the 300 ppm group. This observation in conjunction with the non-neoplastic lung lesions observed in this and other dose groups can be regarded as elements of an overall proliferative and hyperplastic lung lesion which is a reparative response to chronic lung damage by paraquat.

However, the authors concluded that the observation of pulmonary adenoma in the 300 ppm females could not be attributable to treatment with paraquat because the incidence was not clearly outside the historical control range for this type of tumour in F344 rats at that laboratory. There was no evidence that any dose level of paraquat induced lung adenocarcinoma. Thus it is concluded that whilst paraquat can induce a chronic proliferative and hyperplastic lung lesion, it was not tumorigenic in the rats used in this study. The maximum no-effect level of paraquat dichloride in the rats of this strain was determined as 30 ppm (1.06 mg/kg/day) for males and 100 ppm (4.32 mg/kg/day) for females in this study.

(Institute of Environmental Toxicology)

2) Groups of 50 male and 50 female JCL: Wistar rats were fed diets containing paraquat dichloride at 0, 6, 30, 100 and 300 ppm for 104 weeks. Further groups of 6 males and 6 females received the same diets for 26 weeks and for 52 weeks.

The distribution of mortality and of clinical abnormalities were unaffected by treatment. There was approximately 50% mortality across the groups at the end of the study.

At 300 ppm paraquat dichloride there was a slight transient inhibition of body weight gain between weeks 34 and 54 of the study. Food consumption, efficiency of food utilisation and water consumption were not affected. No obvious treatment-related effect on the eyes was noted during the study.

Haematological changes which the investigation attributed to the administration of paraquat at 300 ppm included reduced erythrocytes and haemoglobin in both sexes, and reduced haemotocrit and increased reticulocytes in males. Total serum protein levels were reduced in both sexes at the highest dietary inclusion rate. However, in all cases the extent of the changes was of little, if any, toxicological significance. No effect attributable to paraquat was seen in organ weights or during gross and histopathological examinations at autopsy. There was no tumourous finding due to the administration of paraquat.

On the basis of this study the maximum no-effect level was 100 ppm paraquat dichloride (4.15 mg/kg/day for male rats and 5.12 mg/kg/day for female rats).

(Nippon Experimental Medical Research Institute)

2. Two Year Chronic Toxicity/Carcinogenicity Study in the Mouse

Groups of 60 male and 60 female JCL: ICR mice were fed diets containing paraquat dichloride at 0, 2, 10, 30 and 100 ppm for 104 weeks. Further groups of 10 males and 10 females received the same diet and were sacrificed at 26 and 52 weeks. The distribution of mortality and abnormalities in general condition were unaffected by treatment. There was approximately 60–70% mortality across the groups at the end of the study.

Apart from a transient effect on body weight gain at weeks 34–54, there was no abnormality in reduced body weight gains or clinical signs associated with administration of paraquat in any of the treatment groups, and there was no significant difference in the food consumption, food utilisation efficiency, water consumption and mortalities between the treatment and control groups.

In the animals of the 100 ppm group, there were reduced total serum protein and increased glucose in both sexes, and reduced red blood cell counts and haematocrit values in the male mice of the group, reduced haematocrit value and haemoglobin volume and lower levels of GOT and alkalinephosphatase activity in fe-

male mice. However in all cases the extent of the findings was of little, if any, toxicological significance. Measurements of organ weights revealed minor effects due to paraquat; reduced pituitary, liver and urinary bladder weights in the male mice of the 100 ppm group and reduced brain weight in the female mice of the same group.

There was no change associated with administration of paraquat in the gross necropsy investigations and histopathological examinations. There was no tumourous finding due to administration of paraquat.

The maximum no-effect level of paraquat in the mice of this strain was determined as 30 ppm (3.92 mg/kg/day for males and 3.82 mg/kg/day for females) in this study.

(Nippon Experimental Medical Research Institute)

REPRODUCTION STUDY IN THE RAT

Groups of up to 30 male and 30 female Wistar strain rats were fed diets containing paraquat dichloride at 0, 20, 100 and 200 ppm over 2 generations and effects of this chemical on the reproductive performance were investigated. Teratological examination of foetuses from the F_{1c} and F_{2b} generations was performed.

There were deaths from respiratory disturbance among dams of the 200 ppm groups in both F_0 and F_1 generations during the lactation period or immediately after weaning. These dead animals had increased lung weight and were found, as a result of gross necropsy investigations and histopathological examination, to have developed pulmonary fibrosis. Yet there was no such finding in examinations of F_2 females and males of each generation. Lung weights were increased in surviving F_0 females in the 200 ppm group. Two F_1 dams in the 200 ppm group died due to parturition difficulties but this was not considered to be related to treatment.

There was reduced body weight gain throughout most of the dosing period in the male and female rats of the 200 ppm group of all generations. Also transient reductions in body weight gain occurred in F_0 and F_1 male rats in the 100 ppm group. Food and water consumptions in treated groups were not significantly different from control values.

There was no evidence that paraquat dosing had any effects on the gonadal function of both male and female parent animals, or pregnancy and lactation of the dams, and there was no effect of administration of paraquat on the numbers of corpora lutea, implantations, live foetuses, mortalities of embryos, sex ratio, weights of live foetuses at birth, external and internal abnormalities or skeletal abnormalities in the foetuses. Reduction in body weight gain of F_{1a} and F_{2a} pups up to 21 days and some evidence of foetotoxicity were seen in the 200 ppm group.

Thus, although there was some evidence of maternal and foetal toxicity at 200 ppm, it was concluded that paraquat had no effect on the reproductive performance or development and differentiation of successive generations of the rats at this dose level.

(Imamichi Institute for Animal Reproduction)

TERATOGENICITY STUDY IN THE RAT

Groups of 23 pregnant Wistar strain rats were dosed with paraquat dichloride orally by gavage at the rates of 0, 1.5, 4.5 and 13.5 mg/kg/day from day 7 to day 17 of the gestation period. Observation of the general conditions of all rats were made daily and measurements of body weights and food consumption were conducted weekly. At day 21 of the gestation period foetuses were taken by cesarean section and were examined for foetal toxicity and teratogenicity. Various other maternal parameters were also measured at this time.

The dams of the 13.5 mg/kg group showed reduced movement for about 30 minutes immediately after dosing, and two animals died. These two animals showed reduced body weight and increased respiration frequency prior to death, and severe congestion was observed in the lungs in gross necropsy investigations. In addition, mean body weight gains and food consumptions were reduced for survivors in this group compared with control values. At 4.5 mg/kg/day one rat died and body weight gain was slightly reduced during the dosing period. No unusual effect was seen at 1.5 mg/kg/day.

There was a slight increase in pre-implantation loss in all groups which was considered unrelated to treatment.

There was no abnormality of pregnancy or effects on foetal survival morphology, sex ratio and body weights associated with administration of paraquat at doses up to, and including 13.5 mg/kg/day.

Based on the results, it was concluded that paraquat had neither foetal toxicity nor teratogenicity in the test rats even at the dose rate of 13.5 mg/kg/day, even though maternal toxicity was induced at this dose level.

(Imamichi Institute for Animal Reproduction)

MULTIGENERATION REPRODUCTION/ TERATOGENICITY STUDY IN THE RAT

Groups of 30 male and 30 female CRJ: CD (SD) strain rats were fed diets containing paraquat dichloride at 0, 100, 200 and 400 ppm during 3 generations and effects of this chemical on the reproductive performance of parent animals and foetal toxicity or teratogenicity of this chemical were investigated.

Reproduction: In the 200 ppm group, F_0 male parent animals showed slightly reduced food consumption from the commencement of dosing period till week 4–5, although there was no significant difference in other data between the treatment and control groups. In the 400 ppm group, increased mortalities, respiratory disturbance, reduced body weight gains, slightly reduced food and water consumptions and hyperplasia in alveolar walls and some lung fibrosis were seen in the parent animals of most if not all generations. There were reduced survival rates at day 21 and weight loss at day 0 of lactation and retarded aperture of the vagina in some or most offspring generations. The maximum reproductive toxicity no-effect level of this chemical in the rats of this strain was determined as 200 ppm (16.6 mg/kg/day for males and 18.1 mg/kg/day for females) in this study.

Teratogenicity: There were skeletal anomalies in higher incidences than control in groups receiving paraquat at 100 ppm or over among F_{1b} animals, although the difference did not attain statistical significance. There was no such change among the F_{2b} pups. In the 400 ppm group, there was retarded ossification among F_{1b} and F_{2b} pups and reduced weight gain among F_{2b} male pups indicative of foetotoxicity. Yet, there was no evidence of effects of dosing of paraquat in any other data. It was concluded that paraquat had no teratogenic activity on the rats of this strain even at 400 ppm, the highest dose rate adopted in this study.

(Bozo Research Centre)

MUTAGENICITY STUDIES

1) Rec-assay: The rec-assay, using the recombination-wild (H-17) and DNA deficient (M45) strains of *Bacillus subtilis* was carried out to survey the DNA-damaging capabilities of paraquat at concentrations 20–500 μg/disk. The result showed that paraquat induced similar length of inhibition zones in both of these strains, which were also similar to the inhibition zones caused by the negative control. Based on the result, it was judged that paraquat had no DNA-damaging capability.

2) Reverse mutation test: The reverse mutation tests, with and without S-9 Mix using histidine auxotrophic *Salmonella typhimurium* five strains and one strain of *Escherichia coli* requiring tryptophan, were conducted in accordance with the technique of Ames *et al.* to investigate the mutagenic potential of paraquat. The concentrations of paraquat adopted in these tests were 0.5–500 μg/plate. The result showed that paraquat induced no increase in the numbers of revertant colonies in any strains even at the highest dose rate with or without a metabolic activation system. Based on the result, it was judged that paraquat had no reverse mutation inducing capability.

3) Host-mediated assay: Groups of ICR male mice (6 mice/group) were administered paraquat by gavage in two equal doses over a 24 hr period at rates of 5 and 20 mg/kg. Immediately after the second administration, one strain of histidine auxotrophic *S. typhimurium* (G-46) was inoculated intraperitoneally to investigate mutagenic potential of the test chemical. The result showed that there was no significant increase in the mutation frequency in the paraquat treated groups. Based on the result, it was judged that paraquat had no

reverse mutation inducing capability.

(The Institute of Environmental Toxicology)

SUMMARY

A wide variety of toxicological studies on paraquat dichloride have been conducted in Japan to assess its safety. The results of these studies support the view that this herbicide will be safe if used following the recommended use instructions.

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