AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

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REVIEW OF THE MAMMALIAN TOXICOLOGY

AND

METABOLISM/TOXICOKINETICS

OF

PARAQUAT

Supplement I: TOXICOLOGY

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PREFACE

This report was undertaken by the Office of Chemical Safety (OCS) at the request of the Australian Pesticides and Veterinary Medicines Authority (APVMA), under the Chemical Review Program. Paraquat is under review because of concerns over the potential risks to human health and the environment.

A draft toxicology review was submitted to APVMA in 2003 and was subsequently revised and updated in 2009, to address industry responses and to include additional published data. A report focussing on neurotoxicity was requested of the OCS and submitted to the APVMA in 2010 with additional neurotoxicity studies assessed and incorporated in 2014.

In 2015, the complete review of the toxicology of paraquat was finalised and the report structured into three parts:

- Summary Report
  - *Comprises an overview of all relevant data available on paraquat relating to human health.*

- Supplement I: Toxicology
  - *Comprises a detailed technical report on paraquat toxicology (excluding neurotoxicity) [this report]*.

- Supplement II: Neurotoxicity
  - *Comprises a detailed technical report on paraquat neurotoxicity.*

These three reports should be considered together. This report 'Supplement I: Toxicology', is a complete evaluation of the data provided to the OCS, together with new information available in the public domain since the commencement of the review, with the exception of neurotoxicity.

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1 ABBREVIATIONS

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight

bw	Body weight
g	Gram
kg	Kilogram
mg	Milligram
µg	Microgram
ng	Nanogram
wt	Weight

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
Nm	Nanometre

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg body weight/day

Volume

L	Litre
mL	Millilitre
µL	Microlitre

Concentration

M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry

A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
CPK	Creatine phosphatase (phosphokinase)
ESR	Erythrocyte sedimentation rate
GGT	Gamma-glutamyl transferase

Haematology

APTT	Activated partial thromboplastin time
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
PCV	Packed cell volume
PT	Prothrombin time
RBC	Red blood cell/erythrocyte

WBC	White blood cell/leucocyte
WBC-DC	White blood cells – Differential count

Chemistry

ACTH	Adrenocorticotrophic hormone
DMSO	Dimethyl sulfoxide
DQ	Diquat
eCG	Equine chorionic gonadotrophin
EDTA	Ethylenediaminetetraacetic acid
GC	Gas chromatography
GLC	Gas liquid chromatography
hCG	Human chorionic gonadotrophin
HPLC	High performance liquid chromatography
LSC	Liquid scintillation counting
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine
MS	Mass spectrometry
NAD(PH)	Nicotinamide adenine dinucleotide (phosphate)
PQ	Paraquat
TGAC	Technical grade active constituent
TLC	Thin layer chromatography

Terminology

AUC	Area under the curve
CI	Confidence Interval
F	Female
FEV	Forced expiratory volume
FVC	Forced Vital Capacity
GLP	Good Laboratory Practice
K_m	Michaelis constant (substrate concentration at 1/2 V_{max})
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LOEL	Lowest Observed Effect Level
M	Male
MLD	Maximum lethal dose
MTD	Maximum tolerated dose
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
QA	Quality Assurance
SCE	Sister chromatid exchange
SD	Sprague-Dawley or Standard Deviation
SEM	Standard error of the mean
SPF	Specific pathogen free
UDS	Unscheduled DNA synthesis
V_d	Volume of distribution
V_{max}	Maximum velocity

Organisations

APVMA	Australian Pesticides and Veterinary Medicines Authority
EC	European Commission

FAO	Food and Agriculture Organization (United Nations)
IARC	International Agency for Research on Cancer
MAFF	Ministry of Agriculture, Forestry and Fisheries (Japan)
OCS	Office of Chemical Safety
OECD	Organization for Economic Co-operation and Development
PIC	Poison Information Centre
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

2 EXECUTIVE SUMMARY

Paraquat is a non-selective contact herbicide belonging to the bipyridinium class of compounds which also includes the herbicide diquat. Both compounds share a similar phytotoxic mode of action which involves the inhibition of photosynthesis (specifically photosystem I) thereby generating superoxide free radicals, leading to lipid peroxidation and membrane damage. Plants die rapidly after treatment and subsequent exposure to light.

The main findings of the toxicology assessment are that the mechanism of mammalian toxicity of paraquat, like its mode of action in plants, is *via* the generation of highly reactive free radicals and consequent peroxidation of membrane lipids, sulfhydryl groups, proteins, and DNA, leading to membrane damage and cell death.

The review confirmed the high acute toxicity of paraquat. The acute toxicity profile demonstrates that in laboratory animals, paraquat is of moderate to high acute oral toxicity and high acute inhalational toxicity, but low dermal toxicity. Paraquat is a severe eye irritant, moderate skin irritant, but not a skin sensitiser. Paraquat is moderately to highly acutely toxic to humans by the oral route. The review also confirmed that the repeat-dose toxicity of paraquat, when administered by the oral or inhalational routes, is predominantly characterised by pulmonary lesions due to the preferential uptake of paraquat by the lungs, with other effects seen in kidneys and liver also noted following acute exposures.

From repeat dose studies there was no evidence to indicate that paraquat is carcinogenic in chronic animal studies. The weight-of-evidence indicates that paraquat is non-mutagenic and therefore is not considered to pose a genotoxic hazard to humans. Standard studies showed no reproductive or developmental toxicity in experimental animals. The neurotoxicity potential of paraquat is considered in detail in a separate report – Paraquat Toxicology Review Supplement II – Neurotoxicity. The weight of evidence is that paraquat is not neurotoxic by the oral route of exposure.

2.1 Toxicology Hazard Profile

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of absorption	Poor; in the rat, approximately 10-18% of an orally administered dose is absorbed.
Dermal absorption	Low; 0.3% in humans
Distribution	Distributes to most organs of the body. The highest initial concentrations were found in the kidney and the lungs.
Potential for accumulation	No evidence of accumulation, other than potential accumulation in the lungs
Rate and extent of excretion	Rapid; in the rat 60-90 % in faeces with 10-20% in urine in 72 h,
Metabolism	Not metabolised by rats. Generation of free radicals due to redox-cycling, primarily in the lung, causing oxidative tissue damage.
Toxicologically significant compounds (animals, plants and environment)	Paraquat and 2,2':6',2"-terpyridine (an impurity)

Acute toxicity (Manufacturing concentrate; approx 34% w/w paraquat cation)	
Note: Unless otherwise indicated, units are expressed as paraquat cation	
Rat oral LD_{50} (mg/kg bw)	100-249
Worst oral LD_{50} in other species (mg/kg bw)	22 (guinea pigs)
Lethal human dose (mg/kg bw)†	50 -80
Rat dermal LD_{50} (mg/kg bw)	>1448 (no deaths)
Worst dermal LD_{50} in other species (mg/kg bw)	No data
Rat inhalation LC_{50} (mg/m^3)	0.5
Worst inhalation LC_{50} in other species	No data
Skin irritation	Moderate irritant (rabbit)
Eye irritation	Severe irritant (rabbit)
Skin sensitisation (No evidence of sensitisation in humans)	Non-sensitiser (Buehler method)

Acute toxicity of 2, 2':6', 2"-terpyridine (impurity)	
Rat oral LD_{50} (mg/kg bw)	2.17 - 2.61
Rat dermal LD_{50} (mg/kg bw)	4.31 - 5.04

Short-term toxicity	
Target/critical effect	Lungs/oxidative tissue damage
Lowest relevant oral NOEL (mg/kg bw/d)	0.5 (13-week dietary study in dogs)
Lowest relevant dermal NOEL (mg/kg bw/d)	Not established – no reliable studies
Lowest relevant inhalation NOEC (mg/m^3)	0.01 (21-day rat whole body exposure)

Genotoxicity	Clastogenic effects at cytotoxic concentrations due to free radical damage. Negative *in vivo*. Some *in vitro* equivocal results.
LOEL (mg/kg bw, single dose applied dermally)	6 (induction of micronuclei at this dose and higher in rat bone marrow micronucleus assay)

Long-term toxicity and carcinogenicity	
Target/critical effect	Lungs/oxidative tissue damage
Lowest relevant NOEL (mg/kg bw/d)	0.45 (pulmonary lesions in male dogs, 1-year study)
Carcinogenicity	No evidence of carcinogenicity

Reproductive toxicity	
Reproduction target/critical effect	No effect on reproductive performance. Toxic effects on offspring only at maternotoxic doses (perivascular inflammatory cell infiltration in the lungs of pups).
Lowest relevant reproductive NOEL (mg/kg bw/d, 3-Generation dietary study in rats)	3.75 in pups (perivascular inflammatory cell infiltration in the lungs) 1.25 for parental animals (focal alveolar histiocytosis)

Developmental toxicity	
Developmental target/critical effect	Minor effects (delayed skeletal development at maternotoxic doses)
Lowest NOEL (mg/kg bw/d, oral gavage in rats)	1 - maternotoxicity (increased mortality, clinical signs and reduced body weight gain) 1 – foetotoxicity (reduced mean foetal and litter weights).
Lowest NOEL (mg/kg bw/d, oral gavage in rabbits)	<1 – maternotoxicity (effects on food consumption and body weight gain at all doses tested) 1 - foetal toxicity (increased incidence of post implantation losses and skeletal variations)

Neurotoxicity	Not neurotoxic by the oral route
Delayed neurotoxicity	No evidence of delayed neurotoxicity
Immunotoxicity	No data available

† Dose level at which there are reports of human deaths (Pond, 1990)

Note: Unless otherwise indicated 'paraquat' refers specifically to the paraquat cation

3 SUMMARY TOXICOLOGY REPORT

3.1 Introduction

This toxicological summary covers those studies considered in this review, with the exception of neurotoxicity studies which are included in Supplement II.

3.2 Metabolism and Toxicokinetics

The absorption, distribution, metabolism and elimination of paraquat have been studied in a range of laboratory animal species following administration via different routes. A limited number of *in vitro* studies have also investigated the uptake and metabolism of paraquat.

3.2.1 Oral Administration

Female CD1 and AP strain mice given a single oral gavage dose of paraquat at 200 mg/kg bw (equivalent to the LD_{50}), had maximal concentrations in plasma at 15 minutes after dosing, A second peak was seen at 24 and 7 h after dosing in CD1 and AP strains, respectively. The area under the plasma-time concentration curve (AUC) in the CD1 strain was 55.2 µg.h/mL, and for the AP strain it was 32.7 µg.h/mL (Heylings & Farnworth, 1992).

A single dose of ^{14}C-methyl-labelled paraquat was administered orally to rats at 1 or 50 mg/kg bw, or at 1 mg/kg bw after conditioning at 1 mg/kg bw/d for 14 days. The mean total percentage recoveries, including excreta and tissue residues, were approximately 93-95% of the administered dose. Absorption of paraquat from the gastrointestinal tract (GIT) appeared to be limited because a 50-fold increase in dose did not show any corresponding increase in urinary radioactivity excretion. There was no sex difference in paraquat absorption. Rats excreted approximately 10-20% of the administered dose in the urine in 72 h. Unchanged paraquat in the urine accounted for approximately 93-95% of urinary radioactivity. Based on the mean percentage recoveries, the estimated percentage of the dose absorbed was approximately 12-18%, 10-12% and 18% after dosing at 1 and 50 mg/kg bw, and following conditioning at 1 mg/kg bw/d for 14 days, respectively. Three urinary metabolites, accounting for approximately 0.1-0.8% of the administered radioactivity were detected, but the identity of these was not determined. The percentage radioactivity excreted in the faeces during the 72-h period was approximately 49-70% of the administered dose following administration of 1 mg/kg bw, and approximately 60-80% following the 50 mg/kg bw dose. The absorption, distribution and excretion of paraquat were not affected by conditioning the animals at 1 mg/kg bw/d for 14 days. No paraquat metabolites were identified in the faeces (Lythgoe & Howard, 1995 a,b,c).

Three days after treatment, tissue concentrations of paraquat were very low (~0.02% of the administered radioactive dose). Moreover, the concentrations detected in the carcass ranged from approximately 0.5% to 0.8% of the administered dose, and no paraquat was detected in fat, bone, muscle, blood and plasma. The findings suggest that paraquat does not accumulate in rat tissues (brain, liver, gonads, lungs, heart, spleen and kidneys, and representative samples of abdominal fat, bone and muscle), following a single oral dose of 1 or 50 mg/kg bw or following repeat dosing at 1 mg/kg bw/d for 14 days (Lythgoe & Howard, 1995 a,b,c; Macpherson, 1995).

In female rabbits given ^{14}C-paraquat at 2 or 30 mg/kg bw as a single oral dose, maximum plasma levels were recorded approximately 1 h after treatment. Plasma levels then declined rapidly. However, animals which received 30 mg/kg bw, showed a rebound increase after a slow decline, together with signs of impaired renal function at 72 h post treatment. Clinical signs were observed at 30 mg/kg bw, including inappetance, body weight loss and a marked reduction in urinary and faecal output. The lung concentration of paraquat did not increase with time despite the impaired renal function. The animals at 30 mg/kg bw showed the maximum kidney, liver and lung concentrations approximately 1 h after treatment. The kidney concentrations appeared to be higher than that in the plasma at all observation times. Peak kidney and liver concentrations declined by approximately 80% and 42%, respectively, after 4 h. As in plasma, the levels in both of these tissues showed a rebound increase later. Animals dosed at 2 mg/kg bw, excreted approximately 7% and 87% of the administered dose in the urine and faeces, respectively, in 72 h. At 30 mg/kg bw, the amount excreted in the urine and faeces during this period was low, being approximately 7% and 3%, respectively, probably due to reduced urine and faecal output. After 24 h, the tissue levels (lungs, liver and kidney) were relatively higher (approximately 3- to 20-fold) than in the plasma. The findings suggested that paraquat did not accumulate in the lung following treatment with the high-dose, but caused treatment-related functional and morphological changes in the kidney (Farnworth *et al*, 1993a).

Following administration of a single oral dose of ^{14}C-paraquat at 50 mg/kg bw (equivalent to the LD_{50} dose) to cynomolgus monkeys, maximum plasma levels were measured after approximately 1 h. The levels then declined rapidly during the first day after treatment, and remained relatively constant during days 1 through 7, following dosing. Approximately 21% of the administered dose was excreted in the urine and faeces within 24 h. Paraquat was still detected in the urine and faeces 21 days after dosing (approximately 0.5% of the administered dose). These findings suggested that excretion was very slow (Murray & Gibson, 1974). Forced diuresis appeared to have a limited protective effect in monkeys poisoned with a large dose of paraquat (62 mg paraquat ion/kg bw), as considerable renal damage occurs during the first 4 h after dosing (Purser, 1976).

In a study to assess the acute renal toxicity of paraquat (and diquat), male rats received an oral dose equivalent to 127 mg/kg bw paraquat (91 mg/kg bw paraquat cation) or a subcutaneous (sc) injection of 20 mg/kg bw paraquat (14.4 mg/kg bw paraquat cation). Excretion studies were also performed and it was shown that approximately 10% of paraquat was excreted via the kidneys over 24 h following oral dosing while virtually all of the sc dose was excreted via the kidneys (Lock & Ishmael, 1979).

3.2.2 Inhalational Administration

In a study designed to investigate both the short-term inhalational toxicity and the lung concentrations of paraquat, rats were exposed (whole body) to aerosols of the chemical at concentrations of 0.01, 0.1, 0.5 or 1.0 mg/m^3, 6 h/d, 5 d/wk for 3 weeks. Approximately 0-0.2 µg paraquat/g of tissue was detected in the lung at 0.01 mg/m^3/d, after the 5th or 15th exposure. After one day of recovery from the 15-day exposure period, paraquat was detected only in 1/8 rats at this dose level, and was not detected in the lungs of any animal, when tested 2 and 3 days later. However, slightly higher levels were detected at 0.1 mg/m^3/d after the 5th exposure compared to the 15th exposure. These findings showed that the levels detected in the lung were

dose-related, but no accumulation occurred after repeat dosing. There were no apparent sex differences in the concentration of paraquat in the lungs (Laird *et al*, 1979).

In the inhalational study of Hardy *et al*, (1980), no paraquat residues were detected in the kidney of any high-dose rats after subjecting them to the same exposure regime as Laird *et al* (1979). The estimated half-life of paraquat in the lung in this study was approximately 2 days.

3.2.3 Intrabronchial Administration

Administration of various doses of paraquat directly into the bronchus of rat lung resulted in approximately half being lost to the systemic circulation within 1 h. The half-life of paraquat elimination from the lung ranged from 11 to 76 h, depending on the dose. It was speculated that the dose-related reduction in half-life was related to lung damage, since macroscopic lesions were apparent 24 h after dosing. Of the paraquat diffusing from the lung, most was recovered in the urine (Wyatt *et al*, 1980).

3.2.4 Ocular Administration

Farnworth & Jones (1993) examined the disposition of paraquat following ocular administration to female rabbits at approximately 21 mg/animal. No signs of systemic toxicity were observed. Due to leaking of the dose solution after instillation, the proportions of the dose retained by the animals ranged from 41 to 90% of the administered dose. Paraquat levels in the plasma showed initial peaks approximately 15-30 minutes after dosing, suggesting rapid absorption from the eye.

3.2.5 Percutaneous Absorption

3.2.5.1 In vitro Studies

To investigate the *in vitro* absorption of paraquat from 3 different formulations, low strength Gramoxone (12 g/L paraquat) containing 3 g/L diquat, an Indonesian formulation containing paraquat/diquat at 120/30 g/L, or an aqueous solution of paraquat/diquat at 120/30 g/L were applied to whole rabbit skin. After 4 h of exposure, half of the skin samples were rinsed with distilled water to remove any unabsorbed paraquat. Absorption was low for all test formulations reaching a maximum of 2.5% of the applied dose at 10 h regardless of whether the skin was washed. The low strength Gramoxone formulation was absorbed up to 5-fold more rapidly than the other 2 formulations over the 10-54 h period. Although absorption was not completely prevented, absorption rates were reduced by up to 10-fold over the 10-54 h period following washing. The observation that the amount of paraquat absorbed by washed skin was greater than unwashed skin at 4 and 10 h was difficult to interpret due to the lack of reporting detail (Ward & Heylings, 1993a).

A study by Walker *et al* (date unspecified) compared the *in vitro* dermal absorption for whole human skin to rat, hairless rat, nude rat, mouse, hairless mouse, rabbit and guinea pig skin. Although this study lacked methodological and observational detail, it showed that whole human skin was up to 3 orders of magnitude less permeable to ^{14}C-paraquat than skin obtained from a range of laboratory animals. The implication of this finding is that skin from laboratory animals may not be a good model for predicting percutaneous absorption by human skin, a

conclusion which is supported by numerous other studies conducted using non-Australian paraquat products.

In an *in vitro* study by Dugard (1983), there appeared to be only marginal differences in the absorption of ^{14}C-paraquat from 4 paraquat formulations by whole human skin. Absorption was not detected until 24-30 h for three of these formulations, while the 4th formulation (Gramoxone UK) was not detected until 45-54 h. Absorption over 24-48 h following application of a 1 mg/mL aqueous paraquat solution (equivalent to 0.72 mg/mL paraquat cation) to whole human skin or epidermis was very low, with absorption by epidermis double that by whole skin. These studies suggested that absorption of paraquat during operational exposure to paraquat over a comparable time scale would be minimal.

Scott (1990) examined the effect of Porter (a mineral oil) on the absorption of paraquat by whole human skin following the application of a 2 g/L aqueous paraquat cation solution, or a 1:100 aqueous dilution of 'Gramoxone Export' containing 0, 0.1, 0.2 or 0.5% Porter. Absorption was low and reached 2.49% of the applied amount for the aqueous paraquat solution, 2.36% for the 1:100 dilution of 'Gramoxone Export', 4.65% in the presence of 0.1% Porter, 2.41% in the presence of 0.2% Porter and 1.82% in the presence of 0.5% Porter over 55 h. The absorption rate over the first 1-9 h was 1.4-2.9-fold greater than that over the 24-55 h period for all samples. Absorption of paraquat from the aqueous solution and the 1:100 dilution of Gramoxone was similar while there was a slight increase in the rate and amount of absorption in the presence of 0.1% Porter. However, this effect was not seen at higher concentrations of Porter where the rate and amount of absorption was similar to the aqueous paraquat solution and the diluted Gramoxone. This study revealed that paraquat was poorly absorbed by human skin and Porter did not effect this absorption suggesting that absorption during operational exposure to formulations containing Porter would also be low.

3.2.5.2 In vivo Study

In a human volunteer study, Wester *et al* (1984) applied [^{14}C-methyl]-paraquat to the back of the legs and hands, and ventral forearms of 6 human volunteers in a cross-over design. The proportion of the total dose absorbed over 120 h was 0.29 $\pm$ 0.2%, 0.23 $\pm$ 0.1% and 0.29 $\pm$ 0.1% from the leg, hand and forearm, respectively. The study authors calculated an absorption rate of 0.03 $\mu g/cm^2$ over 24 h. These results indicated that paraquat is poorly absorbed by intact human skin.

3.2.6 Human studies

Beebeejuan *et al* (1971) described the urinary excretion pattern of paraquat in a female patient, who had deliberately ingested approximately 15 g of a commercial preparation containing 5% paraquat dichloride. Clinical observations were consistent with transient renal failure following ingestion. Although paraquat was not detected in plasma 24 h after ingestion, it was detected in the urine until sampling discontinued on the 27th day after ingestion.

Proudfoot *et al* (1979) investigated the time course of plasma concentrations in a study involving 79 human subjects, who had deliberately ingested liquid or granular formulations containing paraquat. It was claimed that measurement of plasma paraquat concentrations was useful in determining the prognosis. Plasma paraquat levels that did not exceed 2.0, 0.6, 0.3,

0.16 and 0.1 mg/L at 4, 6, 10, 16 and 24 h after ingestion, respectively, generally led to a favourable clinical outcome.

Mean plasma concentrations of paraquat measured during the course of 18 acute human poisoning incidents indicated that the elimination half-life was 84 h. The volume of distribution (V_d) was calculated to be approximately 1.2-1.6 L/kg. In a 14-month old boy, who had ingested an unknown quantity of paraquat, the peak plasma level was seen after 4 h. In this patient, the plasma level showed a rapid decline, with 2 rebounds occurring at 76 and 106 days after ingestion. Corresponding rebounds were also noted in the urine and cerebrospinal fluid. Based on these observations, it was suggested that paraquat could be recovered from biological fluids up to 3 months following ingestion. The concentration in the lungs, liver, heart and kidneys was higher relative to a range of other tissues. Generally, the concentration in the kidney was higher than that in the lung 2-8 days after ingestion (Houze *et al,* 1990).

A 15-year-old girl deliberately ingested approximately 50 mL of a commercial paraquat formulation (20% w/v of paraquat dichloride salt), corresponding to nearly 10 g of paraquat ingested (Dinis-Oliveira *et al* 2006). Twenty minutes after ingestion the adolescent vomited. She was taken to hospital about 2 hours 30 minutes after ingestion and immediately treated with a gastric lavage and mineral absorbent. An aggressive therapeutic protocol was instituted. The patient was submitted to haemoperfusion during 4 days, in 7 sessions of 3 hours each. The first session was initiated 4 hours after ingestion. Serum and urinary levels of paraquat were undetectable 20 and 72 hours after ingestion, respectively. A computerized axial tomography (CAT) scan at day 7 revealed some lung damage. The patient was discharged after 22 days of hospitalisation and found to have normal parameters at a 6 month follow up.

3.2.7 *In vitro* Metabolism

The uptake of paraquat by rat lung tissue slices was linear over a 2 hour period and was inhibited by potassium cyanide plus iodoacetate or rotenone suggesting that lung uptake is an energy-dependent process (Rose 1974b). In the study of DeGray *et al* (1991), paraquat (1-5 mM) was rapidly reduced to its radical cation by rat hepatocytes. The study authors concluded that bipyridylium cations generated *in vitro* were largely formed inside the cell, but escaped through the cell membrane.

3.3 Acute Studies

The acute oral toxicity of paraquat is moderate in mice and rats. The oral LD_{50} in mice ranged from 101 to 203 mg/kg bw (Fletcher, 1967; Heylings & Farnworth, 1992), and from 100 to 249 mg/kg bw in male rats (Kimbrough & Gaines, 1970; Duerden, 1994b). The acute oral toxicity in guinea pigs, rabbits and cynomolgus monkeys was high, with LD_{50} values of 22, 40-50 and 50 mg/kg bw, respectively (Murray & Gibson, 1972; Farnworth *et al,* 1993a; Murray & Gibson, 1972). Although difficult to determine with accuracy, the acute oral toxicity of paraquat has been shown to be high in humans, with a lethal dose in the range of 50-80 mg/kg bw reported by Pond (1990).

The lowest dermal LD_{50} value of paraquat in rats was 80 mg/kg bw. This value was determined in an early dermal toxicity study (Kimbrough & Gaines, 1970), in which the purity and experimental conditions were unspecified. A more recent GLP study determined the LD_{50} as > 1448 mg/kg bw in rats (Duerden, 1994c). This value is in good accordance with the finding of

low percutaneous paraquat absorption in studies conducted under both *in vivo* and *in vitro* conditions (see Section 3.2.6). Therefore, the dermal toxicity of paraquat is considered to be low. The lower dermal LD_{50} determined in the study of Kimbrough & Gaines (1970) may have been attributable to an oral component to the toxicity as rats were not prevented from accessing the test site. In addition there may have been the presence of the highly toxic paraquat byproduct, 2,2':6',2-terpyridine in the test material (see Section 4.3.1) used.

Paraquat is highly toxic to rats by the inhalation route (LC_{50} = 0.5 mg/$m^{3,}$, whole body exposure, 4-h) (Hathaway, 1966).

In acute toxicity studies, clinical signs such as decreased activity, dehydration, hypothermia, irregular breathing, reduced faecal output, piloerection, staining around the mouth and upward curvature of the spine have been observed in laboratory animals. Observations from these studies and human poisoning cases with paraquat show that the lungs, liver and kidneys are the main target organs, with the lungs being the most sensitive organ. In an acute oral toxicity study in rats, there were no clear clinical signs of neurotoxicity or of neuropathological changes to a wide range of nervous tissues (Brammer, 2006).

An aqueous solution containing 33% (w/w) paraquat was a severe eye irritant in rabbits (Bugg & Duerden, 1994). It produced slight skin irritation in the same species, when applied for 4 h to intact skin under occluded conditions (Duerden, 1994a). A 28.6% (w/w) paraquat solution was a moderate to severe skin irritant in rabbits when tested undiluted or up to 1:25 (v/v) dilution, but a slight irritant from dilutions of 1:50 (Bullock, 1983). Paraquat was not a skin sensitiser in guinea pigs under the conditions of the maximisation test (Thompson *et al*, 1985).

3.4 Short-term Studies

3.4.1 Oral administration

In a study by Sotheran *et al* (1979b), paraquat (100% purity) was admixed in the diet and fed to mice at 0, 12.5, 25 or 50 ppm (equivalent to 0, 1.88, 3.75 and 7.50 mg/kg bw/d paraquat cation, respectively) for 28 days. The NOEL was 50 ppm (equivalent to 7.50 mg/kg bw/d paraquat cation), the highest dose tested, based on the absence of any treatment-related effects at this dose including deaths, abnormal behavioural or clinical signs, and macroscopic abnormalities. Significant variations in total food consumption, food utilisation and relative lung weights were not considered to be treatment-related as these results were only weakly significant and did not follow a dose-response effect. The experimental design and level of reporting were commensurate with a dose range-finding study.

In another study by Sotheran *et al* (1979c), paraquat (100% purity) or technical paraquat (32.7% paraquat cation) was admixed in the diet and fed to 4-5 week old mice at 0, 100, 125 or 150 ppm (equivalent to 0, 15, 18 and 22.5 mg/kg bw/d paraquat cation, respectively) for 28 days. Seventeen paraquat-related deaths occurred, the majority (4 males and 7 females) observed in the 150 ppm technical paraquat group. Of these, 12 were sacrificed due to clinical signs (weight loss, hunched and subdued appearance, piloerection, laboured or rapid breathing, or suspected cyanosis). Gross (dark red or congested appearance) and histopathological (alveolar wall thickening, congestion and oedema) lung abnormalities were also observed in 15/17 of these deceased mice. In contrast, there was a relatively low incidence of gross lung abnormalities in mice that were sacrificed at the end of the study, however histopathological

lung abnormalities were detected across all treatment groups. The body weight gain of paraquat-treated mice tended to be lower than the controls although a statistically significant effect occurred only in 5-week old females treated with 150 ppm pure paraquat, and 100 and 150 ppm technical paraquat. In the absence of a dose-response relationship these findings were not considered to be treatment-related. The LOEL was 100 ppm (equivalent to 15 mg/kg bw/d paraquat cation), the lowest dose tested, based on the occurrence of histopathological lung abnormalities such as alveolar wall thickening, congestion and oedema. Besides mortality rates which were higher in mice treated with technical paraquat compared to mice treated with pure paraquat, there was no evidence to suggest a difference in effect between pure and technical paraquat, or between mice of 4- or 5-week starting age.

In a study by Hodge *et al* (1980), technical grade paraquat liquor (32.7% paraquat cation) was admixed in the diet and fed to rats at 0, 150, 175 or 200 ppm (equivalent to 0, 15, 17.5 and 20 mg/kg bw/d paraquat cation, respectively) for 28 days. The LOEL was 150 ppm (equivalent to 15 mg/kg bw/d paraquat cation), based on decreased body weight gain (males), decreased food consumption, macroscopic (red or white spots/patches and congestion) and histopathological lung abnormalities (alveolar wall thickening, oedema and congestion) at and above this dose. There were no deaths among control animals while a dose-related increase in mortality was evident in paraquat-treated rats. This study was confounded by the concurrent presence of a respiratory tract infection in rats across all groups, however the results are consistent with other studies and are considered of regulatory value.

In a study by Guttman *et al* (1981), technical grade paraquat liquor (32.7% paraquat cation) was admixed in the diet and fed to female rats at 0 or 150 ppm (equivalent to 15 mg/kg bw/d paraquat cation) for 21 days. No evidence of toxicity was reported. The study authors concluded that a dietary level of 150 ppm paraquat cation (equivalent to 15 mg/kg bw/d) could be used as a maximum tolerated dose (MTD) in a future multi-generation study. This study was suitable only for dose-range finding purposes.

In a study by Farnworth *et al* (1993b), paraquat dichloride (33.0% paraquat cation) was administered orally to female rabbits at 0 or 6.5 mg/kg bw/d paraquat cation for 3 days. The LOEL was 6.5 mg/kg bw/d, based on the occurrence of clinical signs (4/8 animals were slightly subdued and an unspecified number were constipated), inappetance from 48 h, a 5.19 $\pm$ 0.98% loss of body weight over 72 h, significant elevations in plasma urea and creatinine, macroscopic stomach abnormalities (fluid engorgement, reddening and ulceration), mucosal erosion in the glandular area in the majority of rabbits, macroscopic liver (pale, accentuated lobular patterns) and kidney abnormalities (enlarged, pale areas, streaking of the cortex and reddened areas). Gastric lesions and liver abnormalities noted macroscopically were not confirmed histopathologically. There was no treatment-related effect on mortality or organ weights.

A number of other short-term repeat-dose oral studies were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4) but not in this summary. These studies are a study in mice by Sotheran *et al* (1979a), a rat study by Farnworth *et al* (1994), rabbit studies by Horner (1992), and two dog studies by Sheppard (1980; 1981a).

3.4.2 Inhalational Administration

In a study by Hardy *et al* (1979), rats were exposed to aerosols of paraquat technical liquor (40% paraquat cation) in distilled water at 0, 0.01, 0.1 and 1.00 mg/m^3 for 6 h/d, on 15 occasions over 3 weeks. The highest dose was reduced to 0.5 mg/m^3 following mortalities after a single exposure (28/36 males and 29/36 females) with signs of rapid respiration and general malaise observed 24-48 h before death. There were no other deaths during the study. The NOEL was 0.01 mg/m^3, based on the occurrence of clinical signs (nasal discharge) and histopathological abnormalities in the larynx (keratinising metaplasia and/or hyperplasia of the epiglottis and arytenoid projections) at 0.10 mg/m^3. These observations also occurred at 0.5 mg/m^3, with the inclusion of ulceration/necrosis in the larynx and histopathological lung abnormalities (macrophages/debris/mucus, alveolar wall thickening and loss of cilia and Clara cells). There was evidence that some of the histopathological anomalies were reversible as rats allowed to recover for 2 weeks showed no signs of lung ulceration/necrosis and a reduction in alveolar wall thickening and the presence of macrophages/debris/mucus.

In a follow-up study by Grimshaw *et al* (1979) that aimed to confirm observations made by Hardy *et al* (1979), rats were exposed to aerosols of paraquat technical liquor (approximately 40% paraquat cation) in distilled water at 0, 0.01 and 0.1 mg/m^3 for 6 h/d, on 15 occasions over 3 weeks. No deaths, clinical signs, treatment-related effects on body weight, food and water consumption or macroscopic abnormalities were observed. The NOEL was 0.01 mg/m^3, based on histopathological evidence of metaplasia and/or hyperplasia, ulceration/necrosis and acute inflammatory cell infiltration at 0.10 mg/m^3 in the larynx of all interim-sacrificed rats.

A number of short-term repeat-dose inhalational studies were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4) but not in this summary. These studies are rat, guinea pig and dog studies by an anonymous author (1965).

3.4.3 Dermal Administration

The short-term repeat-dose dermal studies assessed in this review were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4) but not in this summary. These studies are rat studies by Levin *et al* (1979) and Luty *et al* (1997), and a rabbit study by Cox *et al* (1986).

3.4.4 Subcutaneous Administration

One study by Nagata *et al* (1992) where dogs received subcutaneous injections of paraquat was assessed. This study was considered to have limited regulatory value and in addition, this route is not considered relevant to human exposure.

3.5 Subchronic Studies

In a study by Maita & Saito (1980), mice received paraquat (93.3% purity) for 13 weeks in the diet at 0, 10, 30, 100 or 300 ppm (equivalent to 0, 1.18, 3.65, 11.50 and 35.8 mg/kg bw/d paraquat cation in males and 0, 1.38, 3.91, 13.8 and 41.9 mg/kg bw/d paraquat cation in females, respectively). The NOEL was 100 ppm (equivalent to 11.50 mg/kg bw/d paraquat cation in males and 13.8 mg/kg bw/d paraquat cation in females) based on mortalities, clinical

signs (weight loss, rough hair and emaciation) and macroscopic lung abnormalities (consolidation or dark red areas of the lobes of the lung) in 2/20 females, and decreased body weight gain and histopathological lung abnormalities (eosinophilic swelling of alveolar epitheliocytes of the lung in 17/20 surviving males and 12/18 surviving females) at 300 ppm (equivalent to 35.8 and 41.9 mg/kg bw/d paraquat cation in males and females, respectively). There were no treatment-related effects on haematology, clinical chemistry or urinary parameters.

In a study by Maita *et al* (1980), rats received paraquat (93.3% purity) for 13 weeks in the diet at 0, 10, 30, 100 or 300 ppm (equivalent to 0, 0.678, 1.99, 6.55 and 19.6 mg/kg bw/d paraquat cation, respectively in males and 0, 0.719, 2.11, 7.10 and 21.1 mg/kg bw/d paraquat cation, respectively in females). The NOEL was 100 ppm (equivalent to 6.55 and 7.10 mg/kg bw/d paraquat cation in males and females, respectively) based on a significant decrease in body weight gain and histopathological lung abnormalities (alveolar epithelial hypertrophy) in males and splenic abnormalities (brown pigmentation) in females at 300 ppm (equivalent to 19.6 and 21.1 mg/kg bw/d paraquat cation in males and females, respectively). A slight decrease in food consumption and food conversion efficiency in males, and water consumption in both sexes was also observed at 300 ppm. There were no mortalities, clinical signs or macroscopic abnormalities observed, and no treatment-related effect on any haematology, clinical chemistry or urinary parameter.

In a study by Sheppard (1981b), paraquat technical grade liquor (32.2% purity) was admixed in the diet and fed to Beagle dogs at 0, 7, 20, 60 or 120 ppm paraquat cation (equivalent to 0, 0.175, 0.5, 1.5 and 3 mg/kg bw/d paraquat cation respectively) for 13 weeks. At 120 ppm, 2 males and 2 females were sacrificed in a moribund condition, all exhibiting dyspnoea 0-2 days prior to sacrifice. A significant depression in body weight was seen only in females at 120 ppm. There was no treatment-related effect on food consumption, haematology, clinical chemistry or urinary parameters, ophthalmoscopic or auscultation findings. At 60 ppm, macroscopic lung lesions (described as large, irregular, dark red, depressed areas affecting most lobes) were observed in 1/3 males and 3/3 females. At 120 ppm, all dogs showed gross lung lesions which ranged from slight patchy darkening of all lobes to multi-focal areas of haemorrhage. There was a clear treatment-related effect on the incidence of alveolitis which was detected in all dogs at 120 ppm, and all but one male at 60 ppm. The NOEL was 20 ppm (equivalent to 0.5 mg/kg bw/d paraquat cation) based on macroscopic lung lesions and histopathological signs of alveolitis at and above 60 ppm (equivalent to 1.5 mg/kg bw/d paraquat cation).

3.6 Chronic Studies

Paraquat was admixed in the diet fed to mice at 0, 1.9, 5.6 or 15/18.8 mg/kg bw/d for 97-99 weeks. Mortality was generally elevated in mid-dose males, and mid- and high-dose females, but a dose-related trend was only observed at 99 weeks. Swelling and sores in the genital area, incontinence and alopecia were frequently observed in all groups. The incidence of swelling in the genital area was elevated in the high-dose groups. Incidences of sores in the genital area were elevated in mid- and high-dose males and in high-dose females. A dose-related increase in incontinence was seen in males, with an elevated incidence in treated females. The toxicological significance of this finding, however, was unclear and the number of animals that displayed it did not vary greatly between the control and treated groups. Consistent depressions in food consumption were seen at the high-dose, achieving statistical significance at 36, 52, 56 and 60 weeks in males, and at the majority of observation times in females. Mid-dose animals

showed depressions in food consumption at 40, 56, 60, 72 and 84 weeks with a dose-related trend at 56, 72 and 84 weeks. High-dose groups gained less weight throughout the study compared to controls. At 20, 44, 60 and 80 weeks, the weight gain in high-dose males was depressed by approximately 3-7%. In high-dose females, the depressions were significant at the majority of observation times after week 44. At termination, the body weight gain in the mid-dose group was depressed by approximately 20%. Males at the mid- and high-dose, including those high-dose males that died during the study, showed increased renal tubular degeneration. Mild to marked fatty-type vacuolation of the liver was seen predominantly in mid- and high-dose males that died or were sacrificed during the study. The incidence of pulmonary adenomas was greater in high-dose animals dying during study weeks 79-98 only. However, the overall tumour incidence in treated animals did not show any treatment-related increase. The NOEL was 1.9 mg/kg bw/d based on decreased weight gain and non-neoplastic findings (renal tubular degeneration) at 5.6 mg/kg bw/d (Sotheran *et al*, 1981; Smith, 1986, 1990).

In the study by Toyoshima *et al* (1982a), paraquat dichloride was admixed in the diet and administered to mice at doses of 0, 0.3, 1.5, 4.5 or 15 mg/kg bw/d for 104 weeks. These doses are equivalent to 0, 0.19, 0.95, 2.84 and 9.48 mg of paraquat ion/kg bw per day in males, and 0, 0.19, 0.96, 2.77 and 9.43 mg of paraquat ion/kg bw per day in females. Mortality during the latter part of the study was higher in all groups including controls. The proportions of animals surviving at termination were approximately 28%, 38%, 30%, 28% and 30% for males and 43%, 36%, 47%, 35% and 30% for females in the control, 0.3, 1.5, 4.5 and 15 mg/kg bw/d groups, respectively. Lowered mobility, loss of coat lustre and piloerection were consistently observed in moribund animals. Statistically significant perturbations in haematological parameters were restricted to the high dose (in males, reduced Hct, Hb and RBC, WBC and lymphocyte counts at 26 weeks, and reduced Hct, RBC and WBC counts at 52 and 104 weeks; in females, reduced Hb levels at all 3 observation times and reductions in WBC and RBC counts, and Hct at 26, 52 and 104 weeks). Necropsy revealed approximately a 2-fold increase in nodular abnormalities in the lungs of 15 mg/kg bw/d males at termination.. Statistically significant perturbations in absolute and relative organ weights were noted at 15 mg/kg bw/d at 26, 52 and 104 weeks. The NOEL was 4.5 mg/kg bw/d (2.8 mg/kg bw/d paraquat ion) based on statistically significant decreases in haematological parameters (Hct, Hb, RBC, WBC and lymphocyte counts), perturbations in relative and absolute organ weights (including thyroid, adrenal, lung, heart and liver weights), and reduced body weights at 15 mg/kg bw/d.

In a long-term dietary study, paraquat dichloride was administered to rats at an equivalent dose of 1.25, 3.75 or 7.5 mg of paraquat ion/kg bw per day for 113 (males) and 122 weeks (females). Food consumption and utilisation, and body weight were reduced at the high-dose. The onset and progression of cataracts were accelerated dose-relatedly at the mid- and high-dose, with ocular effects occurring in both sexes at all doses at termination. Relative brain and lung weights in both sexes were significantly increased compared to combined controls. Absolute liver weights in both sexes and relative liver weight in males were significantly depressed compared to combined controls. Absolute heart weights were reduced in both sexes and testes weights in males. These organ weight changes were restricted to the high-dose group, were statistically significant and considered treatment related. The total incidence of chronic pneumonitis was increased in males at the mid- and high-dose. Terminal histopathology showed focal subpleural abnormalities at all dose levels, together with proliferative lesions in alveolar epithelium at the high-dose. Eye lesions (lenticular abnormalities) seen in rats,

regardless of their time of death were attributed to treatment. Although the dose-time relationship of these lesions was not evaluated by the study authors, from the data presented, it was evident that the onset and progression of these lesions occurred after 52 weeks of treatment. There was a dose-related increase in proliferative lesions in the lung alveolar epithelium in mid- and high-dose males. In females, lung lesions were seen only at the high-dose and could be attributed to treatment. Histopathology revealed a progression of paraquat induced proliferative lesions in the alveolar epithelium to the development of adenomas at the mid-dose and above, which then sometimes progressed to pulmonary carcinomas at the high-dose. The data at the mid-dose did not provide unequivocal evidence for such neoplastic transformation. Interpretation of the data was complicated by a supplementary histopathology report, which suggested that adenomatosis was increased at the high-dose, but neoplasia was not increased by treatment. No NOEL was established because of a treatment-related and statistically significant increase in the incidences of both macroscopic and microscopic ocular lesions in both sexes. The LOEL was 2.5 mg/kg bw/d (1.25 mg paraquat ion/kg bw per day), but the study was unable to provide unequivocal evidence of paraquat-induced carcinogenicity (Woolsgrove, 1983; Ashby & Finn, 1983; Ishmael & Godley, 1983; Brown & Whitney, 1984; Woolsgrove & Ashby, 1985; Life Sci Res Inst, 1984; Ishmael, 1987).

In the study of Kalinowski *et al* (1983a, 1983b), paraquat was admixed in the diet and fed to Beagle dogs at 0, 0.45/0.48, 0.9/10 or 1.5/1.6 mg/kg bw/d for one year. Hyperpnoea was observed in some dogs across all groups, however the incidence of this clinical sign and vesicular sounds were elevated only at the high-dose. A dose-related increase in reddening of the dorsal surface of the tongue was seen at the mid- and high-dose. Approximately a 15-30% reduction in food intake in females was seen in all groups, with the effects seen at the high-dose attributed to the test substance. In high-dose females, plasma cholesterol was elevated reaching statistical significance at 4, 20 and 39 weeks. Except on one occasion, plasma triglyceride levels were elevated by approximately 13-28% in males and 8-46% in females at the high-dose, showing statistical significance at 26 weeks. At the high-dose, relative and absolute lung weights were significantly increased by approximately 36% and 61% in males and females, respectively. Relative and absolute liver weights in males were reduced by approximately 6% and 7%, respectively. Absolute spleen weights were higher in both sexes, with increases of approximately 49% and 38% in males and females, respectively. Chronic pneumonitis associated with yellow discolouration and consolidated areas of the lung together with histological effects were consistently observed in all animals, including controls. The prevalence of these lesions, however, was higher in the mid- and high-dose groups. The NOEL was 0.45 mg/kg bw/d, based on the occurrence of pulmonary lesions associated with chronic pneumonitis at and above 0.9 mg/kg bw/d.

A number of chronic repeat-dose dietary studies were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4.5.1) but not in this summary. These studies are a study in mice by Fletcher *et al* (1972) and in rats by Toyoshima *et al* (1982b).

3.7 Reproductive Studies

In the study by Igarashi (1980), paraquat was administered to rats at 0, 2.0, 10 or 20 mg/kg bw/d in the diet for 2 parental generations of animals and their offspring throughout all phases of the study. Food consumption and body weights of the F0 generation were depressed at 20 mg/kg bw/d. Lung lesions characteristic of paraquat toxicity were also seen at this dose level.

Body weights of the F1 generation pups were significantly depressed at 20 mg/kg bw/d. Paraquat had no effects on reproductive performance or the development of the reproductive organs up to 20 mg/kg bw/d. The NOEL in the offspring was 10 mg/kg bw/d (7.2 mg paraquat ion/kg bw/d) based on a significant depression in F0 and F1 pup body weights, and the increased incidence of hydrourethrosis in F2b pups, at 20 mg/kg bw/d. The NOEL for parental animals was also 10 mg/kg bw/d (7.2 mg paraquat ion/kg bw/d)based on significantly reduced body weights in F0 and F1 parents at 20 mg/kg bw/d.

In the study by Lindsay *et al* (1982 a,b), paraquat dichloride was administered to rats at 0, 2.5, 7.5 or 15 mg/kg bw/d in the diet (equivalent to a dose of 0, 1.25, 3.75 or 7.5 mg of paraquat cation/kg/day), for 3 parental generations of animals and their offspring throughout all phases of the study. The majority of treatment-related mortalities in each generation occurred in high-dose females. Paraquat had no effects on reproductive performance or the development of the reproductive organs up to 15 mg/kg bw/d. The NOEL in pups was 7.5 mg/kg bw/d (3.75 mg/kg bw/d paraquat ion) based on perivascular inflammatory cell infiltration in the lungs of F1b pups at 15 mg/kg bw/d (7.5 mg/kg bw/d paraquat ion). The NOEL for parental animals was 2.5 mg/kg bw/d (1.25 mg/kg bw/d paraquat ion), based on the dose-related increase in the incidence and severity of focal alveolar histiocytosis at and above 7.5 mg/kg bw/d (3.75 mg/kg bw/d paraquat ion).

Hausburg *et al* (2005) selected virgin pubertal female mice of the outbred non-Swiss albino mice [Hsd: NSA™ (CF-1®)] strain for a study of paraquat treatment on reproduction in mice. They were synchronised and superovulated by ip injection of 10IU equine chorionic gonadotrophin (eCG) followed 44-48 hours later, and just prior to placing the females with proven breeder males, by ip injection with 5IU of human chorionic gonadotrophin (hCG). The mice were treated with saline (vehicle) or paraquat (30 mg/kg bw) by ip injection of 100 µL/10g bw on the day of ovulation. When compared to control mice, there were no significant differences in paraquat treated mice for body, liver or uterine weight in dams, or the number of foetuses per dam, the number of resorptions per dam, the total foetal weight per dam, individual foetal weight, or the number of foetal malformations. However, the percent of dams that were pregnant on day 17 was significantly reduced (24%) by paraquat exposure. A decrease in the number of pregnant dams without an increase in foetal resorptions suggested that paraquat exposure adversely affected preimplantation development or very early post-implantation development.

D'Souza *et al* (2006) reported that dermal application of paraquat was cytotoxic to male germ cells in adult rats (SD). Treatment groups received 6, 15 or 30 mg/kg bw/d for 4 h/d for 5 days and were sacrificed 7, 14, 28 and 42 days after the last day of treatment. The study reports that treatment of rats with paraquat caused cytotoxicity in germ cells, mainly in epididymal sperm (day 7) and late spermatids (day 14) and thus decreased the sperm count. The lack of effect on post treatment days 28 and 42 suggests that treatment did not affect spermatocytes and spermatogonia. The cytotoxicity was also recorded as increased sperm mortality. Sperm motility was not significantly affected by paraquat. Paraquat treatment also apparently affected the morphogenesis of rat spermatozoa at all dose levels on all sampling days, indicating that there was interference in the metamorphosis of germ cells into mature sperm. However, this interference was diverse in nature and without either dose-dependence or consistency in findings. Although this study presented weak evidence that paraquat may be cytotoxic to male germ cells in the rat, the OCS considered that this was not of sufficient regulatory value to regard this as evidence for cytotoxicity to germ cells.

3.8 Developmental Studies

Pregnant mice were treated by oral gavage with paraquat technical at 0, 1, 5 or 10 mg/kg bw/d from days 6 through 15 of gestation. There was no evidence of developmental toxicity at levels up to 10 mg/kg bw/d. Maternotoxicity characterised by reduced body weight gain was evident at 5 and 10 mg/kg bw/d, but there was no clear dose-response relationship. The NOEL for maternotoxicity was 1.0 mg/kg bw/d. The NOEL for foetotoxicity was >10 mg/kg bw/d (Hodge *et al*, 1978a).

In a dose range-finding study, mice were given aqueous solutions of paraquat by oral gavage at 0, 10, 20, 30 or 40 mg/kg bw/d, once daily from days 6 to 15 of gestation. Treatment-related maternal mortalities and clinical signs occurred at 30 and 40 mg/kg bw/d. Food consumption and body weight gain were depressed dose-relatedly at 30 and 40 mg/kg bw/d. Necropsy revealed dark red lungs in 1, 2 and 6 dams at 20, 30 and 40 mg/kg bw/d, respectively. Statistically significant increases in absolute and relative lung and trachea weights, and relative kidney weights were seen at 30 and 40 mg/kg bw/d. At 20 mg/kg bw/d, absolute and relative lung and trachea weight were elevated by approximately 20% and 25%, respectively. Foetal body weights of all treatment groups were depressed by approximately 4-20%, achieving statistical significance at 30 mg/kg bw/d for all foetuses and at 10 mg/kg bw/d for male foetuses. However, the data at 10 and 20 mg/kg bw/d were within the historical control ranges. Based on significant depressions in foetal body weights at 30 mg/kg bw/d, the dose levels of 7.5, 15 and 25 mg/kg bw/d were chosen for the main developmental toxicity study (Palmer, 1992a).

In the study of Palmer (1992b), administration of paraquat once daily to timed-mated female mice by oral gavage at 0, 7.5, 15 or 25 mg/kg bw/d from days 6 through 15 post coitum resulted in no malformations in the offspring. Maternotoxicity, characterised by mortality, clinical signs, reduced food consumption, body weight gain, and increased lung and relative kidney weights were observed at the high-dose and therefore the NOEL for maternotoxicity was 15 mg/kg bw/d. The effects on embryonic/foetal development, characterised by reduced foetal weight, increased incidences of retarded ossification of a number of skeletal variants were only apparent at the maternotoxic dose level (25 mg/kg bw/d) and therefore the NOEL for foetotoxicity was 15 mg/kg bw/d.

In the study of Hodge *et al* (1978b), paraquat was administered once daily to mated female rats by oral gavage at 0, 1, 5 or 10 mg/kg bw/d on days 6 through 15 post-coitum. There were 12 premature mortalities, 4 due to inadvertent dosing. Clinical signs such as hypersensitivity, piloerection, weight loss, hunched back appearance and staining around the eyes, nose, head or genital area were seen in the majority of the mid- (16/29) and high-dose (25/30) dams, with respiratory distress seen in some dams at the latter dose. Treatment-related and statistically significant reductions in weight gains were noted at 5 and 10 mg/kg bw/d (approximately 24% and 29%, respectively). A treatment-related reduction (33%) in the number of litters was noted at the high-dose. Foetal weight was depressed by approximately 4% and 6%, while the mean litter weight was reduced by approximately 3.5% and 4.5% at the mid- and high dose, respectively, possibly consequent to the maternotoxicity seen at these 2 dose levels. The NOEL for maternotoxicity was 1 mg/kg bw/d based on increased mortality, clinical signs and reduced body weight gain at 5 and 10 mg/kg bw/d. The NOEL for foetotoxicity was also 1 mg/kg bw/d based on reduced mean foetal and litter weights at 5 and 10 mg/kg bw/d.

Female rats were dosed orally with paraquat at 0, 1, 3 or 8 mg paraquat cation/kg bw/d on days 7 through 16 post-coitum. High-dose dams, which had the highest initial group mean body weight showed a slight weight loss, achieving statistical significance at the majority of observation times. Paraquat showed no developmental toxicity. Statistically significant, isolated minor foetal and skeletal abnormalities were seen, with some falling within the historical control data ranges and/or not showing any association with treatment. The maternotoxicity NOEL was 3 mg/kg bw/d based on significant weight loss in dams at 8 mg/kg bw/d. The NOEL for foetotoxicity was 8 mg/kg bw/d, the highest tested dose (Hodge, 1992b).

In three separate studies, Tinston (1991a,b,c) investigated the developmental toxicity of paraquat in female rabbits following oral dosing at 0, 1.0, 1.5, 2.0, or 2.5 mg/kg bw/d from days 7 through 19 of gestation. Except for minor skeletal variations in foetuses, which occurred at maternotoxic dose levels, no malformations were observed at any dose level. Decreased food consumption and body weight gain were seen at 1.5 mg/kg and above, together with abortion at 2.0 mg/kg bw/d and above. Foetotoxicity characterised by non- or retarded ossification of the skeleton with an increased incidence of developmental variations (extra ribs, microphthalmia) was observed at 1.5 mg/kg bw/d and above. In one study, treatment-related mortalities and clinical signs were seen in dams at all 3 dose levels (1.0, 1.5 and 2.0 mg/kg bw/d), resulting in an inadequate number of litters to provide conclusive evidence on the teratogenic potential of the chemical. Based on the findings of these studies, the maternotoxicity NOEL was <1.0 mg/kg bw/d. The NOEL for foetal toxicity was 1.0 mg/kg bw/d, based on the increased incidence and skeletal variations at and above 1.5 mg/kg bw/d.

Hausburg *et al* (2005) examined the effect of paraquat exposure on the development of preimplantation embryos *in vitro* and *in vivo*. In *in vitro* studies mouse embryos cultured in medium containing paraquat at 0, 8, 40, 200, or 1000 µM for 24 h. After culturing, embryos were evaluated by light microscopy for indication of fertilisation, stage of development, quality of embryos, and abnormal features (Laub *et al* 2000). In the *in vivo* studies embryos were either isolated on day 1 from female mice that were treated ip with saline or paraquat (30 mg/kg) on the day of ovulation (day 0) or embryos were isolated on day 3 from female mice that were treated with saline or paraquat (30 mg/kg) on day 2. In the *in vitro* and *in vivo* studies paraquat significantly decreased the successful development of preimplantation embryos to the 8-cell stage and beyond. The percentages of embryos successfully developing to later stages decreased as the paraquat concentration increased. Both studies support the conclusion that the developmental period from the 4-cell to the 8-cell embryo is the most sensitive window for exposure to paraquat.

A number of developmental studies were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4) but not in this summary. These studies are two published study in mice by Bus *et al* (1975), and Bus & Gibson (1975), a study in mice by Hodge (1992a) providing additional information for the Hodge *et al* (1978) study, and an embryotoxicity study in rabbits (Hodge, 1990).

3.9 Genotoxicity Studies

The majority of *in vitro* gene mutation assays conducted in prokaryotes (Longstaff *et al,* 1976; McGregor, 1977; Shirasu *et al (*date not stated); Critchton *et al,* 1978; Benigni *et al,* 1979; Levin *et al,* 1982) and eukaryotes (Longstaff *et al,* 1976; Clay & Thomas, 1985; Kitahara *et al,* 1996; Speit *et al,* 1998) to assess the mutagenicity of paraquat were negative. A study by Longstaff and Callander (1992) that reported forward mutation in *S. typhimurium* strain TA 92, and studies by Longstaff *et al* (1985b & c) that reported the absence of forward mutations in mouse lymphoma L5178Y cells, were considered to be of limited regulatory value due to the lack of reporting detail. Studies by Benigni *et al* (1979), reported a concentration-related increase in 8-azaguanine mutants of *S. typhimurium* strains TA 1535 and TA 92 following treatment with paraquat. Benigni *et al* (1979) also reported forward mutation in *S. typhimurium* strain G46, but a study by Shirasu *et al* (date unspecified), which used the same strain, was negative. An *in vitro* gene mutation assay using *S. typhimurium* strains TA 98, 100, 1535, 1537 and 1538 (Longstaff 1985) indicated that the study of Benigmi (1979) was invalid due to the presence of a mutagenic antifoaming agent in the incubation media.

A modified liquid incubation assay was employed by Moody and Hassan (1982) to determine the mutagenicity of paraquat in *S. typhimurium* strains TA 98 and TA 100. Paraquat was concluded by the study authors to be 'highly mutagenic' based on a concentration-related increase in the number of revertants/10^8 viable cells. Paraquat was up to 8.6-fold more mutagenic to *S. typhimurium* strain TA 100 than TA 98, with the effect on both strains up to 6-fold greater in the presence of limited histidine than in the total absence of histidine. The latter observation suggested that active growth was important for the expression of the mutagenic effect. In TA 98, 1.0 mM paraquat was approximately 3-fold more mutagenic than the control 30 μM proflavine, but at least 300-fold less mutagenic than mitomycin C. For TA 100, 1.0 mM paraquat was at least 4-fold more mutagenic than 2 μg/mL sodium azide but at least 250-fold less mutagenic than mitomycin C. An approximately 10-fold lower incidence of revertants was observed under anaerobic conditions suggesting that the mutagenicity of paraquat was in part dependent on the presence of oxygen (ie O_2^-).

In vitro differential toxicity assays were conducted using *B. subtilis* strains H17 and M45 at 20-500 μg/disk in the absence of metabolic activation (Shirasu *et al* date not stated), and using *S. typhimurium* strains TA 1538 and TA 1978 at 100 μg/plate in the presence and absence of metabolic activation (Benigni *et al,* 1979). Both studies were negative.

No unscheduled DNA synthesis (UDS) was reported in primary rat hepatocytes (Trueman *et al,* 1985). A study reporting UDS in human epithelial-like cells (Benigni *et al,* 1979) was considered to be equivocal due to the absence of a dose-response effect and lack of statistical significance. Gene conversion was reported at cytotoxic concentrations of Gramoxone (100 g/L ai) in S. *cerevisiae* (Parry, 1973). A more recent study by El-Abidin Salem *et al* (1993) reported reversion, conversion and crossing over in S. *cerevisiae* following treatment with cytotoxic concentrations of Gramoxone (20% paraquat). Parry (1977) reported that paraquat caused a statistically significant increase in the number of S. *crevasse* convertants at and above 0.02 μg/mL relative to the control. This finding was considered to be equivocal due to the absence of any dose-response effect, the absence of a positive control and the fact that the test strain and method were not validated (ie the study was aimed at developing a potential test method for assaying mutagenic compounds).

In vitro cytogenetic tests conducted in the absence of metabolic activation revealed clastogenicity at non-cytotoxic concentrations in Chinese Hamster fibroblasts (CHL) (Sofuni & Ishidate, 1988) and at cytotoxic concentrations in Chinese hamster cells (V79) (Speit *et al,* 1998). However, results of the later study were not confirmed in a comet assay (Speit *et al,* 1998). Speit *et al* (1998) also reported that although paraquat-induced chromosomal aberrations were observed at high concentrations that totally prevented cell survival, paraquat did not cause any significant DNA lesions to induce mutations in V79 cells. In the study by Sofuni & Ishidate (1988), almost all aberrations were of the chromatid type and extensively involved gaps and breaks. In this same study, the number of chromosomal aberrations was increased in the presence of diethyldithiocarbamate (an inhibitor of superoxide dismutase) and diethyl maleate (a glutathione scavenger).

Paraquat was clastogenic at cytotoxic concentrations in human lymphocytes both in the presence and absence of metabolic activation (Sheldon *et al* 1985a) Gramoxone (20% paraquat) was shown to be clastogenic to human lymphocytes in the absence of metabolic activation (El-Abidin Salem *et al,* 1993). A study by Ribas *et al* (1997/98) revealed no clastogenicity in human lymphocytes using the cytogenetic test, while the comet assay showed a positive result, with the effect greater in the absence of metabolic activation. In the same paper, paraquat was negative in a micronucleus test performed on human lymphocytes.

Paraquat was negative in *in vivo* micronucleus tests (Sheldon *et al,* 1985b). A micronucleus test conducted in Swiss mice (Ortiz *et al,* 2000) reported a reproducible time-related increase in the number of micronuclei/polychromatic erythrocytes in both peripheral blood and bone marrow. In this same study, melatonin was shown to significantly decrease the occurrence of micronuclei. D'Souza *et al* (2005) examined the effect on micronuclei of the bone marrow of male Sprague Dawley rats administered dermally at 6, 15 and 30 mg/kg bw in a single application. Paraquat increased the number of micronuclei in a dose-dependent manner. Micronucleated polychromatic erythrocytes were detected at each time point with the maximum number present at 48 h.

Paraquat was reported to have no clastogenic activity in rats (Howard *et al,* 1987). Paraquat was shown to be clastogenic to bone marrow and germ cells in BALB/c mice (Rios *et al,* 1995). The effect on bone marrow cells was only seen in high-dose females given multiple doses, while cytotoxicity (ie decreased mitotic indices) was seen at all doses in females, and at the mid- and high-dose in males. The effect on germ cells did not follow a dose-response pattern although a statistically significant increase in sperm head abnormalities was observed at some concentrations when cells were treated at 3 different stages (spermatozoa, spermatids and spermatogonial cells in preleptone).

Paraquat was negative in two dominant lethal studies performed in mice (Anderson *et al,* 1976; Pasi *et al,* 1974) with the study by Pasi *et al* (1974) reporting reduced gestation rates during the 3rd mating week. The anti-fertility effect of paraquat observed during this study was thus restricted to post-meiotic spermatids. A recessive lethal study performed in *Drosophila melanogaster* (El-Abidin Salam *et al,* 1993) reported a significant increase in sex-linked recessive lethals in the 2nd and 4th broods, with a significant overall difference between the control and treatment group.

No *in vivo* UDS in rats was reported by Trueman & Barber (1987). A host-mediated assay performed by Shirasu *et al* (date unspecified) in mice was negative despite the observation of a dose-related increase in cytotoxicity.

Two somatic mutation and recombination tests (SMART) performed in two different strains of *Drosophila melanogaste*r reported positive results. In one strain, there was a significant increase in the frequency of small single spots and total spots at 2, 6 and 8 mM, but not at 4 mM (Torres *et al,* 1992). In the other strain, there was a significant difference in the dose-response regression compared to the control, with a concomitant dose-related increase in cytotoxicity (Gaivao *et al,* 1999).

The mutagenicity studies of paraquat generally showed little activity. The occasional finding of clastogenicity is weakened by the generally low regulatory standard of the evaluated studies. Despite the occasional positive finding, the overall weight of evidence is that paraquat is not genotoxic.

3.10 Neurotoxicity Studies

Consideration of the neurotoxicity of paraquat is considered in detail in a separate technical report 'Supplement II: Neurotoxicity'. The overall conclusion of this report was that paraquat does not induce neurotoxicity via the oral, dermal or intranasal routes; routes of relevance to human exposure to this herbicide.

3.11 Human Studies

3.11.1 Occupational Exposure

A study involving plantation workers in Malaysia was conducted to obtain quantitative dermal and respiratory exposure estimates for paraquat during normal working conditions. Spray operators appeared to be the most exposed group, while carriers and tappers were only exposed to low levels. Paraquat residues were detected in the urine of 9/19 spray operators and 1/7 carriers, with levels ranging from 0.05 to 0.76 mg/mL (Chester & Woollen, 1981).

To investigate the effects of regular long term exposure to low concentrations of aqueous paraquat spray solutions, a group of spray operators, who worked in a rubber and palm oil estate in Malaysia, were examined. The respiratory, liver and renal function, and haematology and clinical chemistry data of the study group were compared with those from a group of factory workers and another group of general workers. Skin irritation or rashes on the hands, legs or groin associated with spraying, were commonly seen in spray operators and could generally be attributed to leaky knapsacks. All skin reactions resolved quickly with minimal therapy, usually a steroid cream. There was no evidence of any adverse health effects from regular long-term use of low concentration paraquat spray, despite the lack of adequate personal protective equipment (PPE) (Howard *et al,* 1981).

In a study conducted in Israel, some clinical parameters of a group of 15 agricultural workers who were accidentally exposed to paraquat following skin or eye splashing, were evaluated. Local skin reactions such as contact dermatitis, vesicles and grade II or III burns, and/or eye lesions such as irritation, conjunctivitis and chemosis, were seen in 12 workers. Paraquat was detected in both the plasma (25 to 50 ng/mL) and urine (0.025-0.15 µg/mL) of 3 persons, and

in the urine only in 2 workers (0.05 and 0.07 µg/mL, respectively). From the data it appeared that a single exposure of healthy skin or eyes to low concentration paraquat caused local lesions but did not result in any systemic effects (Hoffer & Taitleman, 1989).

A Sri Lankan study examined the serum and urine of 12 paraquat sprayers. No paraquat was detected in any of the subjects. Urinary volumes and urinary creatinine concentrations were generally consistent during the 12-day sampling period (Woolen, 1989).

An epidemiological study was conducted on a group of male tea plantation workers in Sri Lanka. These workers sprayed paraquat for a minimum of 5 and an average of 12 years, and did not wear any PPE. There were 2 control groups, one a group of non-exposed male factory workers and a group of general workers, who were matched for their age and length of service. Although serum aspartate aminotransferase (AST) levels showed a positive correlation with the duration and extent of paraquat exposure, these were consistently within the normal range. Except for a marginal increase in the incidence of nosebleeds, no significant differences were determined between exposed and non-exposed groups with regard to any of the study parameters. Under the conditions of the study, long term exposure of unprotected workers to low concentrations of aqueous solutions of paraquat did not reveal any adverse health effects (Gurutharan *et al,* 1990).

Dalvie *et al* (1999) calculated that a clinically relevant oxygen desaturation of 5% will result from a lifetime of paraquat exposure.

These following two independent studies (in South Africa and Costa Rica), conducted in different study populations and in different crop sectors, both found similar results and supported the idea that chronic long term paraquat exposure results in sub-clinical respiratory impairment.

Schenker *et al* (2004) investigated whether low level paraquat exposure in workers employed on 338 banana, coffee, or palm oil farms throughout Costa Rica causes restrictive lung function with gas transfer impairment. The findings of small changes in V_E/V_{CO_2} at maximal exercise and change in oxygen desaturation (ΔSp_{O_2}) from rest to peak exercise suggested that subclinical function changes may be present, but long term, low level paraquat exposure in the population studied was not associated with clinically significant interstitial lung disease or impairment of gas exchange. Changes of chronic airflow obstruction with paraquat exposure were not observed, but the study authors indicated that self-reported respiratory symptoms associated with cumulative paraquat exposure (chronic cough, shortness of breath with wheeze) warranted further study.

Dalvie *et al* (2005) supported the observations of Schenker *et al* (2004) that an association between long term paraquat exposure and inefficient ventilation and oxygen desaturation during exercise suggested that paraquat may cause subclinical gas exchange abnormalities. They commented that the relationship between paraquat exposure and exercise induced oxygen desaturation amongst Costa Rican banana, coffee, and palm oil farm workers was consistent with what they found amongst South African deciduous fruit farm workers (Dalvie *et al*, 1999). This was despite the fact that in the Costa Rican study the handlers had a substantially shorter work history (median 8.5 compared with 16 years in the South African study) and also used protective equipment while in the South African study population they generally did not wear PPE.

3.11.2 Poisoning Incidences

A large number of human poisoning incidents have been reported in many parts of the world following deliberate ingestion of commercial preparations. In Australia there is no harmonised systematic method for recording paraquat poisonings; these events are often classified under the category of herbicide or weed killer. Additionally very little information is available on the clinical outcome of patients who made paraquat-related phone inquiries to Poisons Information Centres or who presented at hospital emergency departments. Regardless of these limitations, the available evidence indicates that deaths from paraquat poisoning are rare, and that the majority of hospital admissions or inquiries to Poisons Information Centres relate to occupational accidents involving predominantly young to middle aged males.

3.12 Mechanisitic Studies

Oliveira *et al* (2005) investigated whether paraquat induced lipid peroxidation is accompanied by changes in blood pressure and heart rate in rats. Groups of adult male Wistar rats were studied 2 and 12 h after paraquat (35 mg/kg bw ip) administration. The lipid peroxidation was evaluated by monitoring thiobarbituric acid reactive substances (TBARS) in the kidneys, liver and lungs, and validated by including a group treated with an antioxidant, superoxide dismutase (CuZnSOD 50,000 IU/kg), in the study. The TBARS levels were significantly higher ($p < 0.05$) in the kidneys of the rats studied 2 h after paraquat than in their respective controls. Similarly, systolic and diastolic blood pressure were higher ($p < 0.05$), while heart rate was lower ($p < 0.05$) than basal levels 2 and 12 h after paraquat administration. In contrast, the group treated simultaneously with paraquat and CuZnSOD exhibited lower levels of TBARS ($p < 0.05$) in all studied organs compared to the control group, while the mean arterial pressure and heart rate did not differ from those seen in the control group. It was concluded that acute paraquat poisoning symptoms in rats includes high blood pressure, when administered by the ip route.

Podprasat *et al* (2007) investigated the repeat low dose effect of paraquat on liver function and xenobiotic-metabolizing enzyme activities, and correlated the effects with tissue accumulation. Male Wistar rats recieved daily subcutaneous doses of 0, 4.0, 5.0, and 6.0 mg of paraquat/kg bw/day for seven consecutive days. The body weight and clinical signs of toxicity were observed. Two other groups of ratsreceived 0 or 4.0 mg paraquat/kg bw/d, sc for 3, 7, or 10 days. Piloerection and impairment of respiratory function (sunken thorax) were observed in treated animals at all doses after four injection days. Reduced locomotion and response to the stimuli with slight ataxia was observed after 6 days of treatment and significant weight loss in 30% of treated animals occurred over the 7-day treatment period. Plasma ALT and ALP tended to decrease with an increase in the dose of paraquat and a dose dependent decrease in both albumin and total bilirubin was observed. A statistically significant decrease in BUN and serum creatinine levels, but not the total plasma protein level, was observed at 6.0 mg/kg day only. Low dose paraquat given up to 10 days did not affect BUN, creatinine, total protein, total bilirubin, and plasma albumin. Paraquat was cleared from the plasma within 24 hours following each dose, regardless of frequency of dosing, however levels in the lung and liver were two to three-fold greater following repeat dosing. This study indicated that paraquat may affect liver function when administered daily by sc injection, however this is a route not relevant to human exposure.

Dinis-Oliveira (2008) reviewed the impact of paraquat on the lung. Independent of the route of administration, the lung and the kidney are the organs showing the highest concentrations of paraquat. Paraquat pulmonary concentrations can be 6-10 times higher than those in the plasma and the compound is retained in the lung even when blood levels start to decrease. It has been shown that the lung is able to accumulate paraquat against a concentration gradient and that the mechanism of uptake is an adenosine triphosphate (ATP)-driven process which exhibited saturation kinetics. Paraquat is neither metabolised by the lung nor becomes covalently bound to any degree and it is apparent that its accumulation is mediated through binding to and subsequently translocating into cells by a carrier system. This involves the participation of the polyamine transport system abundantly expressed in the membrane of alveolar cells of type I, II, and Clara cells.The main molecular mechanism of paraquat toxicity is based on redox cycling and intracellular oxidative stress generation.

The acute renal toxicity of paraquat (and diquat) was studied in male rats that received an oral LD_{50} of paraquat at 680 μmol/kg bw (equivalent to 127 mg/kg bw paraquat or 91 mg/kg bw paraquat cation) or a sc LD_{50} of paraquat at 108 μmol/kg bw (equivalent to 20 mg/kg bw paraquat or 14.4 mg/kg bw paraquat cation). Excretion studies using ^{14}C-paraquat (and diquat) were also performed. The study did not report on mortalities or clinical signs. Renal damage occurred following dosing via both routes as shown by the significant increase in urinary glucose, protein and albumin, increased urinary shedding of renal cells, and increased plasma urea. Mild focal hydropic degeneration in proximal tubules was also reported. Oral dosing also resulted in a significant increase in urinary β-D-glucosaminidase activity. Paraquat inhibited the accumulation of N'-methylnicotinamide, and inhibited glucose oxidation and fatty acid synthesis in renal cortical slices, however none of these effects occurred in cortical slices isolated from rats that had been treated *in vivo* with paraquat. As the magnitude of all of these effects was markedly lower than that seen with $HgCl_2$ (positive control), the study authors concluded that paraquat was mildly damaging to the kidneys. Approximately 10% of paraquat was excreted via the kidneys over 24 h following oral dosing while virtually the entire sc dose was excreted (Lock & Ishmael, 1979).

To determine the effect of paraquat (and diquat) on renal function, fasted male rats received an oral LD_{50} of paraquat at 680 μmol/kg bw (equivalent to 127 mg/kg bw paraquat or 91 mg/kg bw paraquat cation) or a sc LD_{50} of paraquat at 108 μmol/kg bw (equivalent to 20 mg/kg bw paraquat or 14.4 mg/kg bw paraquat cation). Renal function was adversely affected as evidenced by the following significant effects: reduced excretion of paraquat, diquat, inulin, urea, p-aminohippurate and N'-methylnicotinamide; decreased urine flow; increased plasma urea; decreased plasma volume; increased Hct. Paraquat dehydrated the blood, liver and lungs when given subcutaneously, while the GIT actually retained water when rats were dosed orally. Renal toxicity was also shown to increase over time and was exacerbated by fasting (Lock, 1979).

A number of additional studies were not considered suitable for regulatory purposes or were of limited regulatory value. These studies are included in the main toxicology report (Section 4) but not in this summary. These studies are an investigation on the effect of paraquat on rat liver mitochondrial function by Gage (1967 & 1968) and a study on the effects on adrenal corticosteroid production/liver glycogen utilisation by Crabtree *et al* (1973); Rose *et al* (1974).

3.12.1 Antidote Studies

Numerous studies have been undertaken in laboratory animals, and in humans who have been admitted to hospital following intentional or accidental poisoning. Collectively these studies have not identified an effective antidote or treatment regimen for paraquat poisoning and therefore the current approach used in the treatment for paraquat poisoning is supportive.

Most compounds that have shown antidotal activity in laboratory animals have been administered prior to, immediately or soon after dosing with paraquat. This is not a realistic scenario for human poisoning cases, where hospital admission and treatment typically occur several hours after exposure. Various sodium sugar sulphates (Tsuchiya *et al*, 1989) and aromatic sulphonates (Farnworth & Heylings, 1994) have been shown to increase survival in paraquat-treated mice, when administered immediately or up to one hour, respectively, after paraquat dosing. The antidotal activity of aromatic sulphonates was confirmed in rats where survival was increased and paraquat levels in the plasma, liver, lung and kidney reduced (Trebilcock & Heylings, 1995). $MgSO_4$ was shown to improve survival in rats and to reduce plasma, lung and kidney paraquat levels, when administered up to 3 hours after poisoning (Heylings & Trebilcock, 1994).

Given that the mechanism of paraquat toxicity involves the generation of free radicals, a variety of antioxidants have been tested for their antidotal potential. Niacin or a combination of niacin and thiamine given to rats 24 hours after paraquat poisoning was shown to increase survival times and reduce weight loss, while not completely preventing death or respiratory signs (Brown *et al,* 1981). Melatonin was found to increase the LD_{50} in rats from 79 to 251 mg/kg bw when given 30 minutes prior and up to 14 hours after paraquat administration (Melchiorri *et al,* 1996). N-acetylcysteine was reported to reduce lung oedema and inflammatory cell infiltration but not increase survival in rats (Wegener *et al,* 1988; Hoffer, 1993). Human poisoning case studies have suggested the potential benefit of early antioxidant therapy using deferoxamine and acetylcysteine (Lheureux *et al,* 1995).

Numerous other classes of compounds have been tested for their antidotal potential, including those which block paraquat uptake by the lungs (eg polyamines, D-propanolol, chlorpromazine), anti-inflammatory agents (eg cytokines, corticosteroids) and collagen inhibitors (eg dehydroproline), but none of these have proven efficacious.

A range of standard treatment procedures have been assessed in laboratory animals and utilised in human poisoning cases. Some treatments involve the removal of unabsorbed paraquat from the gastrointestinal tract following oral dosing, however dermal and inhalational exposures are not amenable to this type of approach. Some treatments involve the removal of paraquat from the plasma using haemodialysis (following renal failure) or haemoperfusion. Administration of Fuller's earth or activated charcoal has been shown to improve survival and reduce plasma, lung and kidney paraquat levels in rats (Trebilcock & Heylings, 1995). A combination of Fuller's earth/$MgSO_4$ and charcoal haemoperfusion improved survival and removed up to 20% of the applied dose in dogs (Widdop *et al,* 1975 & 1977). Single early haemoperfusion was suggested to serve some benefit in a canine model of paraquat poisoning while repeated haemoperfusion was not considered useful (Hampson *et al,* 1990). Overall, the majority of studies in laboratory animals and humans have shown no clear benefit of any of these treatment therapies. Often, the interpretation of the effectiveness of an antidote/treatment in humans has been obscured by the fact that multiple compounds and treatments have been given.

The current therapy for paraquat poisoning involves a combination of gastric lavage (following oral exposure) and/or haemodialysis and/or haemoperfusion. Ventilation with hypoxic breathing mixtures (eg nitric oxide) may be employed in severe cases to reduce lung damage caused by oxygen radicals. However, in severe or late-stage poisoning when breathing becomes difficult, oxygen therapy may actually be necessary.

A reliable indicator of likelihood of survival following poisoning appears to be the exposure dose which can be estimated from paraquat concentrations in the plasma and urine (Proudfoot *et al*, 1979). A study has shown that the initial measurement of routine laboratory parameters of renal and hepatic function, as well as acid-base status, could be used to predict patient outcome following paraquat poisoning (Hong *et al*, 2000). The rate of increase in plasma creatinine over a five hour period has also been used as a biological index to predict patient outcome in paraquat poisoning (Ragoucy-Sengler & Pileire, 1996). The other important determinate of survival is how soon after exposure treatment is initiated (Fletcher & Cavalli, 1976).

4 MAIN TOXICOLOGY REPORT

4.1 Introduction

The toxicological database for paraquat is extensive and consists of unpublished reports generated by industry, in addition to an extensive range of published studies. The database was considered adequate.

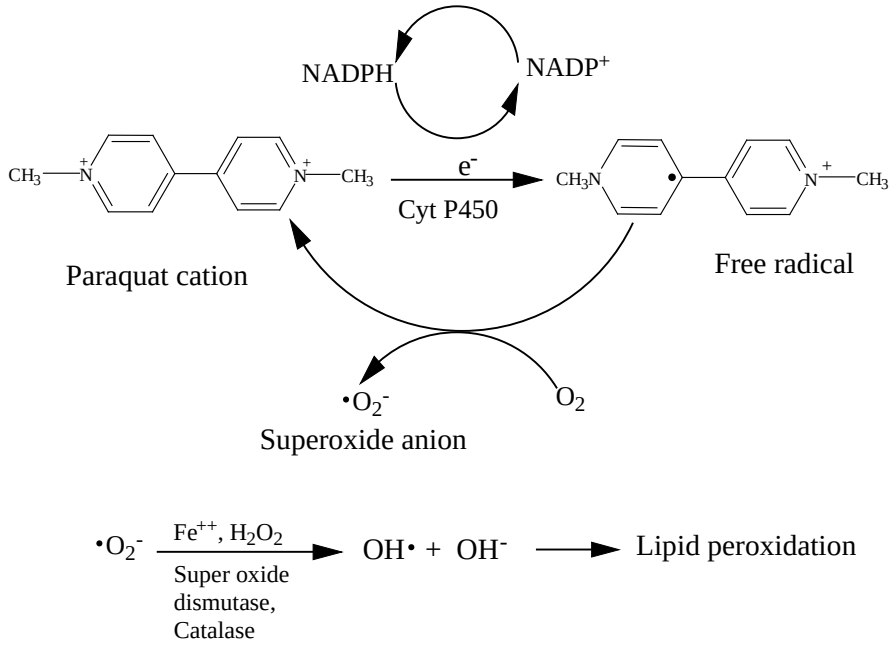
Paraquat is a member of the bipyridinium group of chemicals which also includes the herbicide diquat. There are currently two types of combination products registered in Australia, one containing both paraquat and diquat and the other paraquat and amitrole. Although both paraquat and diquat have similar oral LD_{50} values, paraquat produces lung lesions, while diquat does not. The lungs are the primary target for paraquat toxicity due to the presence of an active uptake mechanism. During its metabolism, paraquat is reduced to form a free radical, which then reacts with molecular oxygen to reform the cation and a superoxide anion. The latter then reacts with cellular H_2O_2 to form two hydroxyl radicals. The intracellular generation of free radicals causes oxidative damage to type I and II pneumocytes, followed by desquamation. This leads to oedema, alveolitis and exudation of granulocytes, all of which usually occurs within a few hours of an acute exposure. A regenerative phase then proceeds approximately 7-14 days after ingestion which is characterised by the proliferation of fibroblasts leading to fibrosis and possibly death. Renal damage is also a feature of paraquat intoxication, while multi-organ failure can also occur at higher doses.

4.2 Toxicokinetics and Metabolism

4.2.1 Metabolic Pathway

Oxidative metabolism of paraquat primarily occurs in the lung, following an energy-dependent uptake into this organ. Selective channels for polyamines mediate lung uptake of paraquat. Oxidation-reduction and auto-oxidation occur inside type I pneumocytes, and are mediated by the cytochrome P450 group of enzymes. During metabolism, paraquat is reduced by nicotinamide adenine dinucleotide phosphate (NADPH) and undergoes a single-electron reduction to form a free radical, which then reacts with molecular oxygen to reform the cation and a superoxide anion. The latter then interacts with cellular H_2O_2 to form two hydroxyl radicals ($OH^{\bullet}$ and OH^{+}) and oxygen, the reaction which is catalysed by the enzymes superoxide dismutase and catalase, in the presence of ferrous (Fe^{++}) ion (Harber-Weiss reaction, Figure 1) (Bismuth *et al*, 1990, Honroé *et al*, 1994).

Figure 1. Oxidation-reduction of paraquat and formation of free radicals [developed from Bus & Gibson (1984); Bismuth *et al* (1990) Honroé *et al* (1994)]



4.2.2 Mice

4.2.2.1 Oral Administration

Heylings JR & Farnworth MJ (1992) Paraquat: acute oral toxicity and absorption in the mouse. CTL Study no: XM2378, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals. Study duration: not stated. Report no: CTL/R/1119, Report date: June 29, 1992.

Non-quality assured study. No GLP statement was provided.

Study & observations: A single dose of paraquat dichloride (38.2% paraquat, w/v, ref no: Y00061/160, ICI Polymers & Chemicals) in water was administered by gavage to groups of female, CD1 and Alderley Park strain (AP) mice (ICI Pharmaceuticals, bw: 18-20 g) at 200 mg paraquat/kg bw. Based on the findings of the acute toxicity study, the dose administered to the animals was equivalent to the LD_{50} of paraquat (phase 1 of the study). The animals were acclimatised to the laboratory conditions for one week. Groups of 5 mice/strain were sacrificed by halothane overdose at 0.25, 0.5, 1, 2, 4, 7, 12 and 24 h post treatment. Blood samples were collected by cardiac puncture for plasma paraquat determination (by RIA), BUN and creatinine. A Student's t-test was used to compare the data between the two strains.

Findings: One CD1 mouse died within 24 h of treatment. There were no mortalities among the AP strain mice during this period. It was reported that the treated animals showed signs of multi-organ failure resulting death within 4 days. (According to the findings of the acute toxicity study, the animals that received paraquat at 150-175 mg/kg bw, showed clinical signs such as piloerection, inappetance and panting). Plasma paraquat levels of the two mouse strains at different sampling times are given in Table 1 below. In both strains, the plasma profile was characterised by rapid absorption, achieving plasma levels of approximately 7 µg/mL, within approximately 15 minutes of dosing. Plasma paraquat profiles in both strains were similar over

the first 4 h after dosing. Time-related, rebound increases in plasma paraquat level were seen in CD1 mice at 24 h (last sampling time, 3.4 µg/mL), and in AP mice at 7 h post dosing (1.72 µg/mL). At 24 h post dosing, CD1 mice had significantly higher plasma paraquat levels compared to that in AP strain of mice ($p \leq 0.05$). The study authors hypothesised that the secondary rise in plasma paraquat levels could be directly due to an alteration in the GIT absorption/renal clearance balance, following paraquat-induced gastro-intestinal stasis. Plasma paraquat area-under-curve (AUC) values for CD1 and AP mice were 55.2 and 32.7 µg.h/mL, respectively. It was reported that the plasma AUC values in excess of 30 µg.h/mL approached the lethal absorbed dose of paraquat.

Table 1: Plasma paraquat levels (µg/mL)* for CD1 and AP mice following a single oral dose

Strain	Sampling time after dosing (h)							
	0.25	**0.5**	**1**	**2**	**4**	**7**	**12**	**24**
CD1	6.81 ± 0.31	5.77 ± 0.32	4.38 ± 0.45	4.16 ± 0.31	2.09 ± 0.42	0.72 ± 0.3	1.75 ± 1.2	3.43 ± 1.46
AP	6.85 ± 0.18	6.03 ± 0.66	4.55 ± 0.36	3.24 ± 0.56	1.61 ± 0.37	1.72 ± 0.41	1.13 ± 0.54	0.28 ± 0.13

*Mean ± SEM for 5 animals.

BUN and creatinine data for the two strains of mouse following dosing are given in the Table 2 below. Two- to 3-fold, parallel increases in BUN was seen in CD1 mice, commencing from 7 h post dosing, whilst about 3-fold increases in this parameter were recorded in the AP strain at 7 and 12 h post treatment, which then declined. Increases in BUN suggests functional damage to the kidneys, which could be attributed to the test substance. It appeared that the CD1 strain was more susceptible to paraquat-induced renal damage than the AP strain. Plasma creatinine levels were generally, unaffected by treatment.

Table 2: BUN and creatinine levels (µg/mL)* in CD1 and AP mice

Strain	Sampling time after dosing (h)							
	0.25	**0.5**	**1**	**2**	**4**	**7**	**12**	**24**
BUN								
CD1	62.3 ± 1.33	75.2 ± 6.3	66.0 ± 2.74	79.2 ± 6.1	68.8 ± 2.08	130.2 ± 17.7	146.0 ± 41.8	193.0 ± 59.8
AP	55.8 ± 4.7	63.8 ± 5.68	71.7 ± 0.85	81.8 ± 4.04	74.8 ± 5.9	168.0 ± 21.0	148.0 ± 39.0	85.2 ± 13.3
Creatinine								
CD1	1.1 ± 0.15	0.6 ± 0.03	0.63 ± 0.02	0.65 ± 0.06	0.82 ± 0.15	0.62 ± 0.04	0.85 ± 0.35	0.9 ± 0.25
AP	1.24 ± 0.12	0.62 ± 0.02	0.6 ± 0.0	0.84 ± 0.19	0.54 ± 0.04	0.75 ± 0.05	0.58 ± 0.05	0.58 ± 0.03

*Mean ± SEM for 5 animals.

Conclusions: Maximum plasma paraquat levels (approximately 7 µg/mL) were seen at 15 minutes after dosing. However, it was unclear whether the maximum plasma level was achieved within 15 minutes following administration of the LD_{50} dose. Time-related, secondary rises in the plasma level of paraquat were seen at 7 and 24 h post dosing in CD1 and AP mice, respectively. The AUC in CD1 strain was 55.5 µg.h/mL, while for the AP strain it was 32.7 µg.h/mL. Two- to 3-fold parallel increases in BUN were seen in CD1 mice, commencing from 7 h post dosing, whilst approximately 3-fold increases were recorded in the AP strain at 7 and 12 h post treatment.

4.2.3 Rats

4.2.3.1 Oral Administration

Lythgoe RE & Howard EF (1995a) Paraquat: Excretion and tissue retention of a single oral dose (1 mg/kg) in the rat. Study no: URO475, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca. Study duration: March-July, 1995. Report no: CTL/P/4683, Report date: September 08, 1995,

Lythgoe RE & Howard EF (1995b) paraquat: Excretion and tissue retention of a single oral dose (50 mg/kg) in the rat. Study no: URO476, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca. Study duration: February-July, 1995. Report no: CTL/P/4684, Report date: September 21, 1995, and

Lythgoe RE & Howard EF (1995c) Paraquat: Excretion and tissue retention of a single oral dose (1 mg/kg) in the rat following repeat dosing. Study no: URO477, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca, Study duration: March-July, 1995. Report no: CTL/P/4685, Report date: September 21, 1995.

Quality assured studies. Conducted in accordance with the US EPA, UK, OECD and EC GLP standards, and Japanese-MAFF regulations.

Study & observations: These three studies monitored urinary and faecal excretion, and tissue residue levels of paraquat following oral (po) administration to fasted rats. A single po dose of ^{14}C-methyl-labelled paraquat dichloride (CTL ref: Y00061/230) in deionised water was administered to SD rats (5/sex, bw: 176-206 g, Biological Services Section, Zeneca Pharmaceuticals) by gavage at 1 or 50 mg/kg bw.

Another group of rats (5/sex) were conditioned with 14 daily oral doses of 1 mg/kg bw unlabelled paraquat prior to dosing with radiolabelled paraquat at 1 mg/kg bw. After dosing, rats were individually housed in metabolism cages under standard laboratory conditions. Urine and faeces were collected at 6 (urine only), 12, 24, 36, 48 and 72 h after dosing. Three days after dosing, rats were sacrificed. In addition to sampling a range of tissues for the tissue retention assay (brain, liver, gonads, lungs, heart, spleen and kidneys, and representative samples of abdominal fat, bone and muscle), a sample of blood was collected and a portion of it was centrifuged to separate plasma. Following oxidation or solubilisation, the radioactivity in the tissue samples was analysed using TLC and LSC. When calculating urinary paraquat levels, results were added to the recovery data obtained in the terminal cage wash.

Findings: During the first 24 h after dosing, urinary radioactivity levels were approximately 9-19% and 10-19% of the administered dose in males and females, respectively (see Table 3 below). The amount of radioactivity excreted in the faeces during the first 24 h was approximately 55-68% of the administered dose. In both sexes, greater than 90% of the administered dose was excreted in the urine and faeces within 72 h.

Table 3: Excretion (percentage of the administered dose) of ^{14}C-paraquat in the urine and faeces*

Time after dosing (h)	Urine			Faeces		
	Dose (mg/kg bw)					
	1	50	1 (after conditioning)	1	50	1 (after conditioning)
Males						
0-12**	16.6	7.5	17.1	23.9 ± 17.3	6.1 ± 6.3	4.2 ± 5.8
12-24	1.2 ± 0.6	1.7 ± 0.2	1.7 ± 0.5	39.1 ± 12.9	48.4 ± 5.5	64.1 ± 4.3
24-36	0.4 ± 0.2	1.1 ± 0.3	0.5 ± 0.2	3.5 ± 3.4	17.2 ± 3.6	1.4 ± 0.7
36-48	0.4 ± 0.5	0.4 ± 0.3	0.4 ± 0.2	3.6 ± 2.3	5.9 ± 3.3	0.7 ± 0.5
48-72	0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.1	2.1 ± 1.1	3.6 ± 1.7	0.6 ± 0.2
0-72	19.1 ± 7.8	10.9 ± 1.1	19.9 ± 5.7	72.4 ± 5.9	81.2 ± 2.6	71.1 ± 6.3
Females						
0-12**	10.0	9.8	17.0	16.6 ± 16.0	6.1 ± 6.3	4.2 ± 5.8
12-24	0.8 ± 0.4	1.7 ± 0.6	1.7 ± 0.5	57.5 ± 14.8	48.4 ± 5.5	64.1 ± 4.3
24-36	0.4 ± 0.1	1.0 ± 0.8	0.5 ± 0.2	3.3 ± 2.1	17.2 ± 3.6	1.4 ± 0.7
36-48	0.3 ± 0.1	0.6 ± 0.5	0.4 ± 0.2	0.8 ± 0.4	5.9 ± 3.3	0.7 ± 0.5
48-72	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	1.6 ± 0.7	3.6 ± 1.7	0.6 ± 0.2
0-72	12.5 ± 1.8	12.5 ± 1.8	19.9 ± 5.7	79.8 ± 4.0	81.2 ± 2.6	71.1 ± 6.3

*****Values were rounded up to the nearest decimal point. The radioactivity found in cage wash for the groups of 1, 50 and 1 (after conditioning) mg/kg bw were 1.4 ± 0.2, 0.4 ± 0.1 and 1.4 ± 0.6% of the administered dose.
** Calculated total radioactivity excreted during the period based on the data provided.

Based on the percentage mean recovery data, the estimated percentage of the dose absorbed was approximately 12-18%, 10-12% and 18% after dosing at 1 and 50 mg/kg bw, and following conditioning at 1 mg/kg bw/d for 14 days, respectively. Up to approximately 0.02% of the administered radioactivity was detected in various tissues that were analysed. Three days after dosing, no radioactivity was detected in fat, bone, muscle, blood and plasma of either sex. The level of radioactivity detected in the carcass was approximately 0.5-0.8% of the administered dose.

Conclusions: Absorption, distribution and excretion of paraquat was unaffected by conditioning rats daily for 14 days at 1 mg/kg bw/d. Absorption of paraquat appeared to be limited since a 50-fold increase in dose showed no corresponding increase in urinary excretion. There was no sex difference in paraquat absorption. No evidence of tissue accumulation was observed.

Macpherson D (1995) Paraquat: Biotransformation in the rat. Study no: UR0510, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca. Study duration: September-October, 1995. Report no: CTL/P/4806, Report date: December 15, 1995.

Quality assured study. Conducted in compliance with the US EPA, UK, OECD and EC GLP standards, and Japanese-MAFF regulations.

Study & observations: This study investigated the metabolic fate of orally administered paraquat in rats by monitoring the excretion of the parent compound and its metabolites in the urine and faeces. Rats (strain, age, bw and source unspecified) were administered with ^{14}C-methyl-labelled paraquat dichloride (CTL Ref no: Y00061/230) in deionised water, either as a single oral dose at 1 or 50 mg/kg bw (1/sex).

Another group of rats (2/sex) was conditioned with 14 daily doses of 1 mg/kg bw unlabelled paraquat prior to dosing with radiolabelled paraquat at 1 mg/kg bw. It was stated that for each of the dosing regimes, weighed sub-samples of urine were taken and combined to obtain separate representative pooled samples for each sex. This procedure was also adopted for freeze-dried faecal samples. According to the study authors, there were a total of 12 sets of pooled samples. The sample collection period was 0-72 h. Samples from each pool were used in the analysis by LSC. Paraquat and its metabolites in faecal samples were extracted by a sequential procedure, which involved methanol, then 1M HCl in methanol, followed by 1M aqueous HCl extraction. Urine samples were concentrated by freeze-drying. TLC and HPLC were used to identify and quantify the test substance in the samples. Metabolites in HPLC fractionated samples were detected and quantified by LSC. For faecal extracts, the data were corrected for extraction efficiency.

Findings: Approximately 10-19% of the administered dose was excreted in the urine following the single or repeat dosing. Unchanged paraquat accounted for 93-95% of urinary radioactivity (approximately 12-15% of the administered dose). Metabolite I, accounted for 1.4% of the urinary radioactivity and 0.2% of the administered dose. This was detected only in a male rat that received a single dose of paraquat at 1 mg/kg bw. Metabolite II was detected in all rats, irrespective of the dose and accounted for approximately 3-6% of the urinary radioactivity and 0.4-0.8% of the administered paraquat dose, and appeared to be the major urinary metabolite. Although metabolite III was not detected in any animal after a single dose of 1 mg/kg bw, it was detected after repeat dosing at the same dosage, and also after dosing at 50 mg/kg bw. It accounted for approximately 0.1-0.2% of the administered paraquat dose and approximately 1-2% of the urinary radioactivity.

After 72 h, the percentage extraction of radioactivity in the faeces ranged from approximately 63-71% (approximately 70-71% and 63-65% in males and females, respectively) of the administered dose following both single and repeat administration of paraquat at 1 mg/kg bw. At 50 mg/kg bw, approximately 92% and 80% of the administered radioactivity was recovered in the faeces in males and females, respectively. Percent radioactivity excreted in the faeces was approximately 49-53% of the administered dose following administration of 1 mg/kg bw dose, and approximately 72% and 60% of the administered dose, in males and females following the 50 mg/kg bw dose. The faecal metabolites were not identified.

Conclusions: The findings of this study were in agreement with those of Lythgoe & Howard (1995a,b,c). Three urinary metabolites of paraquat were detected and quantified. These metabolites accounted for approximately 0.1-0.8% of the administered dose.

4.2.3.2 Inhalational Administration

Laird WJD, Moore DJ & Woolen BH (1979) Paraquat concentrations in rat lungs following exposure to paraquat aerosols. Study no: ICI 254/7949, Lab: ICI Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Ltd. Study duration: not specified, Report no: CTL/P/460, Report date: August 17, 1979.

Quality assured pre-GLP study.

Study & observations: In a 3-week whole-body exposure, inhalational study, SD rats (168 males and 169 females, Charles River UK Ltd, bw: 64-98 and 72-96 g for males and females,

respectively, 26-32 days old) were exposed to paraquat (batch: Y0061/009/002, paraquat content: 40% w/v) aerosols at concentrations of 0 (control), 0.01, 0.1, 0.5 or 1.0 mg paraquat /m^3, 6 h/d, 5 days/week for 3 weeks (a total of 15 exposures). The average aerodynamic diameter of particles was approximately 2 µm. Full details of the main inhalation study (Hardy *et al*, 1979, Report no: ICI 254/7949) are presented under short-term repeat-dose studies of this report. Given that after one 6-h exposure session a large proportion of the animals at 1.0 mg/m^3/d died, further treatment at this dose level was discontinued. Instead, an additional group of 32 rats/sex was exposed to 0.5 mg paraquat/m^3. The representative animals used were taken from those groups of rats participating in the above main inhalation study. Four rats/sex at 0.01 and 0.1 mg paraquat/m^3/d were sacrificed by an ip injection of sodium pentabarbitone immediately after the 5th and 15th exposures, and also at intervals of 1, 2, and 3 days after the 15th exposure. The chest was opened and the trachea plus the heart and lungs were collected for paraquat analysis using an RIA.

Findings: Mean concentrations of paraquat in lung samples of the animals at the 2 dose groups are given in Table 4 below. No paraquat was detected in 4 rats at 0.01 mg/m^3/d when they were tested immediately after the 5th (2 males) or 15th (2 females) exposure. Whereas in the remaining animals the levels detected were between 0.11-0.22 µg/g of lung tissue. After one day of recovery from 15 days of exposure, paraquat was detected only in the lung of 1/8 rats, and was not detected in the lungs of any animal at this dose level after 2 and 3 days of recovery. At 0.1 mg/m^3/d, slightly higher levels were detected after the 5th exposure compared to the 15th exposure (mean values were 1.85 vs 1.73 and 2.33 vs 1.60 µg/g of lung tissue for males and females, respectively). Overall, the results indicate that the levels detected in the lung were dose-related, and paraquat did not accumulate in the lung when rats were repeatedly exposed at 0.01 or 0.1 mg/m^3/d for 3 weeks. During the recovery period, lung paraquat concentrations in rats at 0.1 mg/m^3/d decreased by approximately 20% in the first 24 h; >50% within 48 h; and by more than 75% within 72 h, thus showing rapid elimination of paraquat from the lung. There were no apparent sex differences in lung concentrations following repeat exposure to paraquat aerosols.

Table 4: Paraquat concentrations in the lung

Target dose (mg/m^3/d)	Number of exposures		Day after the 15th exposure		
	5	15	1	2	3
Males					
0.01	0.13 ± 0.02	0.08 ± 0.13*	0.03 ± 0.10*	ND	ND
0.1	1.85 ± 0.47	1.73 ± 0.46	1.35 ± 0.24	0.63 ± 0.11	0.38 ± 0.17
Females					
0.01	0.09 ± 0.12*	0.10 ± 0.13*	0.01 ± 0.10	ND	ND
0.1	2.33 ± 0.36	1.60 ± 0.27	1.33 ± 0.30	0.68 ± 0.07	0.32 ± 0.06

ND = not detected, *Data represent the extreme limits of the mean, and were calculated assuming that for the samples where paraquat was not detected, the concentration was either 0 (lower limit) or 0.1 µg/g of tissue (upper limit).

Conclusions: Either no or very low levels (up to approximately 0.22 µg/g of tissue) of paraquat were detected in the lungs of animals that were dosed at 0.01 mg/m^3/d (whole body exposure). Lung concentrations increased at higher dose levels. There was no evidence of tissue accumulation following repeat exposure. Further, there was no sex difference in the concentration of paraquat in the lungs.

Hardy CJ, Clark GC, Woolen BH & Laird W (1980) Assessment of accumulation of paraquat in the lungs. Study no: unspecified, Lab: Department of Inhalation Toxicology, Huntingdon Research Centre, Huntingdon, Cambridgeshire & ICI Ltd, Central Toxicology Laboratory. Sponsor: ICI Ltd. Study duration: unspecified. Report no: CTL/C/965. Report date: August 22, 1980.

Quality assured GLP study.

Study & observations: This study was performed to determine whether paraquat attains a steady state, identify any sex differences in lung deposition and estimate the elimination half-life in the lung. SD rats were exposed to aerosols of paraquat (batch: 0061/009/002, paraquat content: approximately 40%, w/v) in distilled water at 0 (control), 0.01, 0.1 or 0.5 mg paraquat/m^3, 6 h a day, 5 days a week for 3 weeks. There were 10 male rats in the control group, whilst the treatment groups had 85 males and 10 females/group (bw: 114-146 and 116-136 g, for males and females, respectively, Charles River UK Ltd).

Rats were observed twice daily for clinical signs. For low- and mid-dose groups, 5 males/group were sacrificed following 1, 2, 3, 4, 5, 10 and 15 exposures. Five females per group were sacrificed after the 5th and 15th exposures. For the high-dose group, the number of rats sacrificed after the 10th and 15th exposure was reduced due to mortalities. After the first exposure, 5 males/group were sacrificed at 7 h, and 1, 2, 3 and 6 days post exposure to investigate the half-life of the test substance following a single dose. At the end of the 15th exposure, 5 male rats/group were sacrificed at similar sampling intervals to examine the half-life of paraquat after repeat exposure situation. The number of high-dose rats sacrificed was again reduced due to mortalities in that group. Control rats were sacrificed on day 1 of the study, and the lungs and kidneys removed for analysis. The data from all rats, except for those that died, were used to assess paraquat tissue accumulation and its half-life using an RIA. Data were analysed using a Student's t-test and an ANOVA.

Findings: There were 14 deaths in the high-dose group. One female and 12 males died during the 2-day period between the 6th and 7th exposures, and 1 male died 2 days after the 15th exposure. During the 2-day period between the 6th and 7th exposures, piloerection, tachypnoea and brown nasal staining were noticed in the majority of rats at the high-dose. No clinical abnormalities were noticed in animals in the other dose groups. The mean percentage of particles of less than approximately 0.5 µm in aerodynamic diameter were 87%, 65% and 39% for low, intermediate and high dose atmospheres, respectively. All particles in each atmosphere were considered to be <0.7 µm in aerodynamic diameter, and therefore, considered to be respirable. Mean chamber aerosol concentrations for the mid- and high dose groups were within 10% of the target levels, whereas the mean concentration for the low-dose was approximately 40% higher than the nominal value. It was stated that no paraquat was detected in the kidney of high-dose rats at any of the sampling times, suggesting that lung paraquat was not due to the uptake of paraquat from the plasma following GIT absorption. At the low-dose, paraquat levels found in the lung were near the limit of detection (ie 0.1 µg/g). The amount of paraquat in the lungs and the half-life data for the mid- and high-dose groups are given in Table 5 below. The study authors stated that at the high-dose, lung paraquat levels were maximal after 4 exposures, and was 5 µg/g of tissue. These levels decreased within a few days to approximately 2 µg/g of tissue after 10 exposures, remaining at that level until completion of the study. An increase in lung weight was seen between 5th and 15th exposures. However, no supporting data pertaining to any of these findings were provided.

Table 5: Concentration and elimination half-life of paraquat in the lung

Dose group	Initial mean lung concentration (µg/g)	Estimated half-life (days)	Approximate 95% confidence interval
0.1 mg/m^3/d 15 exposures	1.42	1.98	1.23-5.08
0.5 mg/m^3/d 1 exposure	1.51	1.65	1.32-2.21
0.5 mg/m^3/d 15 exposures	2.43	1.89	1.35-3.17

Conclusions: The estimated lung half-life of paraquat was approximately 2 days. Initial lung paraquat concentration was related to the dose administered. Paraquat was not detected in the kidneys of any of high-dose rats, at any of the sampling times. There was no evidence of tissue accumulation following repeat exposure to paraquat at levels up to 0.5 mg/m^3/d for 15 days.

4.2.3.3 Intrabronchial Administration

Wyatt I, Doss AW, Zavala DC & Smith LL (1980) Intrabronchial instillation of paraquat in rats: Lung morphology and retention study. Study no: HR0046, Lab: Central Toxicology laboratory, ICI Ltd, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI, Study duration: not stated. Report no: CTL/R/563, Report date: September 24, 1980.

Pre GLP, non-quality assured study.

Study & observations: This report provided data for 3 studies conducted in male Wistar rats (bw: 180-200 g, age: unspecified). In the first lung distribution and retention study, ^{3}H-paraquat (as paraquat dichloride) in saline was instilled directly into the left bronchus of rats (3 rats/dose/time point) at 0.0001, 0.01, 0.1, 1 or 10 µg/animal. The dose volume was 0.1 mL. The instillation procedure involved surgical deposition of paraquat in the bronchus of the anaesthetised animal, through an incision (between 2 cartilage segments) of the exposed trachea. After dosing, the incisions were sutured, the animals were allowed to recover, and given food and water. A further 1, 3, 6, 24, 48 or 72 h after dosing, 3 rats/dose were sacrificed by halothane overdose. Controls were treated identically but were instilled with 0.1 mL of saline. These animals were sacrificed 1 h after instillation. Following sacrifice, the abdomen was opened and the hepatic artery was severed. The heart and lungs of each animal were removed, and the lobes of the lungs were divided into the following 3 groups: left lobe, posterior lobe and the remaining 3 lobes. After weighing and examination for any macroscopic lesions, the radioactivity of these lung lobes was determined by LSC. The elimination half-life of paraquat in the lung was determined by the least square method using the log concentrations of paraquat at 6, 24, 48 and 72 h after dosing.

In the second experiment, a 10 ng quantity of ^{3}H-paraquat was instilled into the lungs of rats. The number of animals used and the method of dosing were unspecified. Rats were sacrificed as previously described at 15 and 60 minutes after dosing, and the amount of radioactivity in blood (obtained by cardiac puncture), urine, kidneys, trachea and the lung determined.

The third study involved the gross and histopathological examination of the lung tissue following instillation of paraquat at 0.0001, 0.01, 0.1, 1.0 or 10 µg/animal in 0.1 mL of saline into the lungs of rats (6 rats/dose). Controls consisted of 6 unoperated and 12 sham operated rats. One half of the rats in each group were sacrificed at 2 days after dosing, whilst the remainder were sacrificed 12 days later. The heart and lungs were removed and the latter was inspected for any macroscopic abnormalities, and then processed for histological examination.

The data were presented as group means ± SEM, and analysed for statistical significance by covariance analysis or a Student's t-test.

Findings:

Macroscopic abnormalities and wet weight changes: Instillation of 10 µg of paraquat into the left bronchus resulted in macroscopic lesions in the left lung 24 h after dosing, with 50% of the lung affected. By 72 h, the left lung lobes of all treated rats appeared 'plum coloured' and were of 'jelly-like consistency', showing increasing tissue damage with time. The study authors claimed that the lung damage was less severe in rats that received the 1.0 µg dose. In these animals, the lung lesions were described as patchy, and their intensity did not increase with time. Following instillation of 100 ng of paraquat, abnormally enlarged lungs were noted in 2 rats at 48 and 72 h post dosing, respectively. Given that all lung lobes were affected, the study authors did not attribute these changes to the test substance, and were not considered as typical paraquat lung lesions. The study authors suggested that those lesions may have occurred due to Sendi virus infection, which was identified a few weeks later in the colony of rats in the performing laboratory. Further, it was stated that an additional study using the same dose did not provide any evidence of an increase in lung weight.

Relative wet left lung weights following treatment are presented in Table 6 below. Time-related and statistically significant increases in the left lung weight were seen in the 10 µg dose group commencing from 24 h post dosing. At the 1.0 µg dose level, a similar and consistent effect was noted at 48 and 72 h after dosing, whereas in rats receiving the 100 ng (0.1 µg) dose, this was evident only at 72 h post treatment. No significant lung weight changes were seen in the other dose groups.

Table 6: Relative left lung weights at various times after treatment with paraquat[a]

Time of sacrifice after dosing (h)	Dose				
	10 µg	**1.0 µg**	**100 ng**	**10 ng**	**100 pg**
0 (controls)	352 (6)	356 (6)	400 (5)	389 (120)	385 (6)
1	383 (3)	413 (3)	348 (3)	379 (6)	351 (3)
3	347 (3)	411 (3)	304 (2)	344 (6)	376 (3)
6	383 (3)	421* (3)	347 (3)	360 (6)	378 (3)
24	491**(3)	411 (3)	361 (3)	361 (6)	345 (3)
48	593** (3)	499** (3)	477 (3)	376 (3)	373 (3)
72	674** (3)	529** (3)	498* (3)	378 (2)	394 (3)

[a]Values in parentheses represent the number of animals tested. The mean values adjusted for body weight.
*Significantly different from the controls ($p \leq 0.05$); **Significantly different from the controls ($p \leq 0.001$)

Distribution and retention: The distribution of ^{3}H-paraquat in different lobes of the left lung, expressed as the percentage of the instilled dose is given in Table 7 below. At all dose levels, approximately 80% of the administered dose was found in the left lobe 1 h after dosing, whilst the remainder was somewhat evenly distributed between the posterior and the remaining lobes.

Table 7: Distribution of ^{3}H-paraquat in different lung lobes

Dose	Lung lobes		
	Left	Posterior	Rest
10 µg	81.1 ± 4.7	10.0 ± 2.5	8.9 ± 2.4
1.0 µg	89.8 ± 2.4	4.6 ± 1.1	5.6 ± 1.4
100 ng	71.7 ± 6.1	16.9 ± 3.9	11.4 ± 2.9
10 ng	75.5 ± 4.0	14.7 ± 2.6	9.9 ± 1.8
100 pg	82.7 ± 3.5	10.5 ± 3.4	6.5 ± 1.4

The study authors stated that, based on individual animal data in some rats, quantities as small as 40% of the administered dose was found in the left lobe. For this reason, the retention of paraquat in the whole lung with time was determined (see Table 8 below). For all dose levels, approximately 50% of the administered radioactivity was present in the lung 1 h after dosing. Based on these data, at least 2 phases of clearance from the lung were identified; a rapid initial phase and a much slower phase between 6 and 72 h post treatment, which obeyed first order elimination kinetics.

Table 8: Paraquat concentrations in the lung (% of initial dose)

Dose	Time after instillation (h)					
	1	3	6	24	48	72
10 µg	47.9 ± 6.6	49.6 ± 7.1	49.9 ± 7.1	18.1± 2.5	5.4 ± 2.2	0.7 ± 0.1
1.0 µg	59.0 ± 2.1	50.0 ± 5.7	42.0 ± 7.8	37.0 ± 2.8	21.9 ± 5.9	10.0 ± 4.7
100 ng	63.1 ± 1.3	59.7 ± 4.8	38.0 ± 4.8	35.2 ± 5.2	20.4 ± 11.6	18.8 ± 3.1
10 ng	56.4 ± 6.2	58.6 ± 5.0	48.9 ± 4.9	38.4 ± 2.9	30.7 ± 4.5	21.2 ± 1.1
100 pg	52.2 ± 1.9	36.2 ± 7.2	40.3 ± 3.3	35.6 ± 5.8	29.3 ± 0.8	22.0 ± 3.2

An increase in elimination half-life was seen in the slower phase (6 – 72 h) with decreasing amounts of paraquat instilled in the lung. At 10, 1.0 µg, 100, 10 ng, and 100 pg levels, paraquat elimination half-lives were 11, 28, 53, 58 and 76 h, respectively. Shorter half-lives noted at higher paraquat concentrations may have been due to the loss of radioactivity as a result of oedema formation and tissue damage during the absorption and initial elimination phases.

Distribution in other tissues: The distribution of ^{3}H-paraquat in different tissues and body fluids is given in Table 9 below. Approximately 90% of the administered dose was found in different tissues examined 15 minutes after dosing, with approximately 50% of that amount being found in the lung (the amount of paraquat present in the plasma was corrected to the amount found in the total body water). Although the heart of each treated animal was sampled, no radioactivity data for this organ were provided.

Table 9: Distribution of a 10 ng dose of paraquat in different tissues and body fluids*

Time after dosing (min)	(ng paraquat)						
	Plasma (per mL)	Total body H_2O	Left lung	Trachea[a]	Kidney[b]	Urine[c]	% Recovery
15 (6)	0.025 ± 0.005	3.46 ± 0.68	4.9 ± 0.3	0.12 ± 0.04	0.15 ± 0.04	0.17 ± 0.05	88.9 ± 6.3
60 (5)	0.009 ± 0.001	1.25 ± 0.13	5.2 ± 0.45	0.15 ± 0.02	0.05 ± 0.01	0.82 ± 0.23	74.8 ± 2.5

*Mean ± SEM. Values in parentheses represent the number of animals tested; [a]The part lying between the point of insertion of the cannula and the lung lobes; [b]Amount present in both left and right kidneys; [c]Amount for the total urinary output for the duration.

Two days after dosing, histopathology revealed perivascular oedema and polymorphonuclear cell infiltration in the lungs of 2/3 rats which received the 10 µg paraquat dose. At this observation time, one rat in this dose group showed alveolar wall thickening, collapse of the alveoli, marked congestion and perivascular oedema. At 1 µg, perivascular polymorphonuclear cell infiltration and focal alveolar wall thickening were observed in some lung sections at 2 days post treatment, with no histological evidence of paraquat toxicity in rats in the other dose groups. It was stated that the lung lesions seen in these animals were consistent with those seen after acute paraquat toxicity.

Conclusions: Time and dose-related lung lesions (gross and histological) were noted at 1 and 10 µg dose levels. Approximately 80% of the administered paraquat dose was found in the left lung one hour after dosing, whilst the remainder was evenly distributed between the posterior and remaining lung lobes. The concentration in the left lung was independent of the dose administered. Elimination from the lung involved two phases, namely, initial rapid phase followed by a slow secondary phase. The half-life of the latter was inversely proportional to the dose instilled.

4.2.4 Rabbits

4.2.4.1 Oral Administration

Farnworth M, Foster J & Lock E (1993a) The toxicity of paraquat to rabbits following oral administration. Study No's: XB 2434, 2567, 2607 and 2610, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor Zeneca Agrochemicals, Study duration: not specified, Report no: CTL/R/1164, Report date: September 20, 1993.

No GLP or QA statements were provided.

Study & observations: A single dose of ^{14}C-paraquat dichloride (batch: YF6219, paraquat ion content, w/w: 33%, ICI) in deionised water was orally administered to 5 groups of female NZW rabbits (4-5/group, bw: approximately 4 kg, Alderley Park) at 30 mg/kg bw. Another group of 4 rabbits were treated at 2 mg/kg bw similarly, as there were moderate signs of toxicity after 72 h. Four rabbits treated similarly with water served as controls. The high-dose animals were sacrificed by an iv over dose of Euthatal at 1, 4, 24, 48 or 72 h post dosing, whilst those at the low dose were sacrificed 144 h after dosing. Rabbits were examined for clinical signs at least 4 times/d. Food consumption was monitored daily. Blood samples were collected prior to dosing and at 1, 2, 4, 7, 12, 24, 48, 72, 96, 120 and 144 h post treatment. Urine and faeces were collected at 3 and 7 (urine only) and 12, 24, 48, 72, 96, 120 and 144 h after dosing. At necropsy, tissues were examined for macroscopic lesions and the lungs, liver and kidneys were removed, weighed and sampled for radioactivity determination and histopathology. Plasma paraquat levels were determined by RIA, whilst tissue levels were measured by LSC. Plasma urea and creatinine levels were also determined.

Findings: At 2 mg/kg bw, no treatment-related clinical signs were observed. The maximum plasma paraquat level was recorded one hour after dosing, and was below the limit of detection after 7 h. The AUC was 3.76 ± 0.59 µg.h/mL. Over 72 h, 7% and 87% of the administered dose was excreted in the urine and faeces, respectively (estimated from graphs). Approximately 6% of the administered dose was excreted in the urine during the first 24 h. The mean paraquat

concentrations in the liver, lung and kidney were 0.029, 0.076 and 0.023 µg/g of wet tissue, respectively. Histopathology did not reveal any treatment-related effects.

At 30 mg/kg bw, clinical signs such as inappetance, decreased food or water consumption and loss of body weight were observed. It was stated that this dose of paraquat reduced the urinary output by approximately 50%, together with a marked reduction in faecal output. However, no supporting numeric data were provided for any of these parameters. There was a progressive increase in both plasma urea and creatinine levels commencing at approximately 24 h post dosing, and therefore the study was terminated at 72 h. Plasma paraquat levels peaked at 1 h after dosing, declined rapidly during the next 5 h, and then more gradually decreased up to 48 h. The plasma paraquat level again increased at 72 h post dosing, probably as a result of reduced renal clearance. The AUC was 29.7 ± 3.4 µg.h/mL. As a result of reduced renal function and faecal output, a small proportion of the administered dose was excreted by the treated animals. Approximately 7% and 3% of the administered dose was eliminated in the urine and faeces, respectively, within 72 h of dosing. Approximately, 4% of the administered dose was excreted in the urine in the first 24 h. The level of paraquat in plasma, lung, liver and kidney at different sampling times is given in Table 10 below. Although there was a rebound effect in plasma paraquat level at 72 h post treatment, the data showed that the concentration of paraquat in the lung did not increase with time, despite the treated animals having impaired renal function. Tissue paraquat levels appeared relatively higher compared to that in the plasma from 24 h post-treatment onwards.

Table 10: Tissue and plasma paraquat levels (µg/g of wet tissue or mL) at different sampling times

Tissue	Time after dosing at 30 mg/kg bw*				
	1 h	4 h	24 h	48 h	72 h
Plasma	5.39 ± 0.22	2.01± 0.23	0.35 ± 0.13	0.11 ± 0.08	0.48 ± 0.14
Kidney	14.7 ± 4.8	3.03 ± 0.51	1.23 ± 0.14	2.48 ± 0.51	2.67 ± 0.5
Liver	3.76 ± 1.10	2.16 ± 0.21	1.48 ± 0.10	1.75 ± 0.15	1.94 ± 0.24
Lung	1.85 ± 0.91	1.48 ± 0.69	1.23 ± 0.16	1.12 ± 0.11	1.00 ± 0.21

*Values represent mean ± SEM for at least 4 rabbits/time point.

Histopathological examination of one rabbit showed submucosal oedema of the stomach wall and squamous metaplasia of the mucosa at 72 h post treatment. Signs of hydropic changes in the S2 segment of the proximal renal tubule was seen in 3/5 rabbits at 24 h post treatment. In 2/4 rabbits, these renal lesions had progressed to multifocal, regional renal necrosis, with the presence of tubular dilatation and luminal casts at 72 h.

Conclusions: The maximum plasma paraquat level was recorded 1 h after dosing. At 30 mg/kg bw, a rebound increase in plasma paraquat was seen at 72 h following a slow decline, together with impaired renal function. Paraquat did not appear to accumulate in the lung of high-dose animals, but there were treatment-related functional and morphological changes in the kidney.

4.2.4.2 Ocular Administration

Farnworth MJ & Jones BK (1993) Paraquat: Distribution in the rabbit following eye instillation. Study no: FB4743, Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor Zeneca. Study duration: February-March, 1993. Report no: CTL/P/4007, Report date: June 02, 1993.

Quality assured GLP study.

Study: A single nominal dose of 25 mg of ^{14}C-paraquat (Zeneca Chemicals & Polymers) in deionised water was instilled into one eye of each of 3 female NZW rabbits (Conventional Animal Breeding Unit, Alderley Park, age & bw: unspecified). Two solutions were prepared for dosing, each comprising a mixture of unlabelled (batch: YF6219, 33% paraquat, w/w, Zeneca Chemical & Polymers) and labelled paraquat, formulated in the dose vehicle. Dose preparation 1 was comprised of an 82.7% (v/v) solution of unlabelled paraquat diluted in deionised water (final pH of 4.22), into which ^{14}C-paraquat was added. Dose solution 2 was prepared similarly, but was adjusted to pH 7.08 with 1 M sodium hydroxide solution. The dosage volume was 0.1 mL (20 µCi/animal). Dose solution 1 was instilled into the left eye of each of 2 rabbits (the second rabbit received a local anaesthetic in both eyes prior to treatment, volume unspecified), whereas the third rabbit received the dose solution 2 similarly. Excess paraquat that leaked from the eye following application was quantified. Rabbits were individually housed in metabolism cages under standard conditions. The animals were sacrificed at 48 h post treatment by an intravenous overdose of Euthatal and necropsied.

Observations: The eyes of each rabbit were examined pre-experimentally and then for irritation responses (graded as slight, moderate or extreme) at 1, 24 and 48 h post treatment. Signs of toxicity were observed during the first hour after dosing and then at least 4 times/d. Food consumption was monitored daily for the duration of the study. Blood samples were collected before treatment, and then at 15 and 30 minutes, and 1, 2, 4, 7, 12, 24 and 48 h post treatment. Urine and faeces were collected at 3, 7 (urine only), 12, 24 and 48 h after dosing. After removal of animals, each metabolism cage was washed with 500 mL of water, and its radioactivity was determined. At necropsy, approximately 1.5 g of each of the liver, the lungs and the left kidney were removed for radioactivity determination. The remaining parts of the above tissues were processed for histopathology. Plasma paraquat concentration was determined by a RIA, whilst urea and creatinine levels were assayed using a KONE specific analyser. Tissue, urinary and faecal radioactivity were determined by LSC following digestion or oxidation.

Findings: The dose instilled into each eye was determined by subtracting the amount of radioactivity recovered in gauze pads from the nominal dose. The proportions of the administered dose retained by 3 rabbits were 90.44% (rabbit 1), 40.97% (rabbit 2) and 58.14% (rabbit 3), respectively (equivalent to 20.8, 9.4 and 12.2 mg, respectively).

The two animals that did not receive the local anaesthetic in their eyes showed a moderate pain response. Conjunctival redness, chemosis and a mucoid discharge were seen in all rabbits, which progressively increased with time. Fluorescein did not highlight any eye lesions. There were no signs of systemic paraquat toxicity. It was stated that the animals appeared to be healthy, but all showed reduced food consumption and weight loss during the study (no supporting data were provided). The study authors attributed this finding to frequent blood sampling, which led to a loss of appetite and body weight loss.

Plasma creatinine and urea levels fluctuated over time, but were considered to be within normal ranges by the study authors. The plasma paraquat concentration versus time data showed 2 sharp initial peaks approximately 15-30 minutes after dosing in rabbits 1 and 3 (the two non-anaesthetised rabbits), with two subsequent broader peaks occurring at approximately 1-2 h post treatment (plasma levels being 2.8 and 1.2 µg/mL for rabbits 1 and 3, respectively). The two initial peaks were considered to be due to absorption of the test substance through the eye and the surrounding tissue, while the subsequent peaks were attributed to ingestion of the dose

solution washed from the eye. In contrast, rabbit 2 had a low AUC, showing no such peaks following the loss of approximately 59% of the administered dose from the eye. Plasma paraquat profiles of rabbits 1 and 3 were somewhat similar; showing that neutralisation of the dosing solution had no noticeable effect on bioavailability.

Urinary and faecal radioactivity excretion data are presented in Table 11 below. The percentage of the administered dose excreted in the urine by rabbits 1 and 3 over 48 h ranged from 3.8-6.1%. The amounts excreted in the faeces during the same period ranged from 1.7-13.5%. According to the study authors, the high level of urinary radioactivity seen in rabbit 2 at 3 h post treatment was due to loss of part of the administered dose from the eye, which fell directly into the cage and contaminated the urine sample of that animal. However, it was unclear whether this loss occurred during administration or later, or was caused by the anaesthetic solution. The total amount of radioactivity excreted in the faeces by this animal accounted for approximately 21% of the administered dose.

Table 11: Urinary and faecal excretion* of paraquat after ocular instillation

Sampling time (h)	Rabbit 1 (dose = 20.8 mg)		Rabbit 2 (dose = 9.4 mg)		Rabbit 3 (dose = 12.2 mg)	
	Paraquat concentration	% of dose	Paraquat concentration	% of dose	Paraquat concentration	% of dose
Urinary excretion						
3	384.7	1.85	4318.5	45.7	45.1	0.37
7	120.9	0.58	118.3	1.25	241.3	1.98
12	120.0	0.58	31.0	0.33	254.7	2.09
24	95.9	0.46	11.0	0.12	119.8	0.98
48	85.8	0.41	118.8	1.26	79.8	0.65
Faecal excretion						
12	783.1	3.76	1412.7	14.97	119.0	0.97
24	674.7	3.24	265.5	2.81	9.85	0.08
48	1349.3	6.48	310.9	3.29	87.24	0.71

*Expressed as total µg equivalents of paraquat ion

The tissue concentrations of paraquat following ocular administration were relatively low being approximately 0.14-0.18, 0.11-0.32 and 0.17-0.18 µg of paraquat/g of wet tissue for the kidney, liver and lung, respectively. Histopathology did not reveal any paraquat-induced tissue damage.

Conclusions: In rabbits given paraquat ocularly at doses up to approximately 20.8 mg/animal, no signs of systemic toxicity were observed. The percentage of the administered dose excreted in the urine and faeces by 2 rabbits over 48 h ranged from 3.8-6.1%, and 1.7-13.5%, respectively. Tissue paraquat levels were relatively low and no paraquat-related tissue damage was seen following histopathological examination.

4.2.5 Monkeys

Murray RE & Gibson J (1974) Paraquat disposition in rats, guinea pigs and monkeys. Toxicol Appl Pharmacol 27: 283-291.

Study & observations: A single po dose of ^{14}C-Paraquat (specific activity: 0.4 µCi/mg, source: unspecified) was administered to 3 monkeys (*Macaca fascicularis,* bw: 1-2 kg, Centre for Laboratory Animal Resources, Michigan State University) at 50 mg/kg bw (equivalent to LD_{50}). After dosing, animals were placed in metabolism cages, and urine and faeces collected.

Blood samples were collected from the inguinal sinus at 0.5, 1, 2, 4, 8, 16 and 32 h, and daily thereafter for 21 days to determine serum paraquat concentrations. Urine and faeces were collected daily, and were frozen for radioactivity analysis. Animals were sacrificed 21 days after dosing.

Findings: One animal died on day 8 after dosing. It was reported that maximum serum concentrations were noted at one hour after dosing, which then declined rapidly during the first day following dosing. Serum paraquat levels remained relatively constant 1-7 days after dosing (~0.2 µg/mL), with a total of 21% of the administered dose being excreted within a day (see Table 12 below). Urinary and faecal excretion slowly declined with time, and was measurable at 21 days post dosing. Twelve days after treatment, the elimination in the urine and faeces appeared somewhat similar.

Table 12: Urinary and faecal excretion of paraquat in monkeys following a single oral dose

Days after dosing	% of administered dose excreted		
	Urine	Faeces	Total
1	8.9 ± 4.2	12.6 ± 12.6	21.5
2	3.0 ± 0.7	10.3 ± 7.7	13.3
3	1.4 ± 0.1	6.9 ± 1.4	8.3
4	0.7 ± 0.5	5.1 ± 3.0	5.8
5	0.2 ± 0.1	3.1 ± 2.1	3.3
6	0.2 ± 0.1	6.6 ± 4.7	6.8
7	0.1 ± 0.0	3.9 ± 2.0	4.0
8	0.2 ± 0.1	0.4 ± 0.2	0.6
9	0.2 ± 0.1	0.6 ± 0.4	0.8
10	0.3 ± 0.2	0.7 ± 0.3	1.0
11	0.3 ± 0.1	0.9 ± 0.4	1.2
12	0.2 ± 0.1	0.4 ± 0.4	0.6
13	0.1 ± 0.1	0.4 ± 0.2	0.5
14	0.3 ± 0.0	0.3 ± 0.0	0.6
15	0.2 ± 0.0	0	0.2
16	0.2 ± 0.0	0.2 ± 0.1	0.4
17	0.2 ± 0.1	0.1 ± 0.1	0.3
18	0.1 ± 0.1	0.1 ± 0.1	0.2
19	0.1 ± 0.1	0.7 ± 0.7	0.8
20	0.2 ± 0.0	0.1 ± 0.1	0.3
21	0.3 ± 0.1	0.2 ± 0.2	0.5

Conclusions: Maximum plasma paraquat levels were seen in monkeys one hour after dosing. Approximately 22% of the administered dose was excreted in the urine and faeces within the first 24 hours, however low levels were still measurable 21 days after dosing.

4.2.6 Percutaneous absorption

4.2.6.1 In vitro Studies

Walker M, Dugard PH & Scott RC (date unspecified) Absorption through human and laboratory animal skins: *in vitro* comparisons. Report no. unspecified. Lab & Sponsor: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: unspecified.

Guidelines & GLP: No test guidelines or GLP statement were provided. This study was not quality assured.

Materials & methods: ^{14}C-paraquat (unspecified batch no. & source) was applied as a 1 mg/mL aqueous solution (amount of cation unspecified) to whole human, rat, hairless rat, nude rat, mouse, hairless mouse, rabbit and guinea pig skin mounted in glass diffusion cells. Permeability constants (k_p, $cm.h^{-1} \times 10^5$) for paraquat and tritiated water were calculated from the linear regions of plots of total amount penetrated versus time. No further experimental details were provided.

Results: Paraquat absorption data for the various skin preparations are given in Table 13 below. Paraquat was absorbed slowly by whole human skin. The skin of all laboratory animals tested was significantly (no p values given) more permeable to paraquat than human skin. The magnitude of this difference ranged from 40-fold for rat skin to 1460-fold for hairless mouse skin.

Table 13: Permeability constants for tritiated water and ^{14}C paraquat for human and laboratory animal skin

Species	Water K_p ($cm.h^{-1} \times 10^5$)	Paraquat K_p ($cm.h^{-1} \times 10^5$)
Human	93	0.73
Rat	103	27.2 * (40)
Hairless rat	130	35.3 * (50)
Nude rat	152	35.5 * (50)
Mouse	164	97.2 * (135)
Hairless mouse	254 *	1065 * (1460)
Rabbit	253 *	92.9 * (130)
Guinea pig	442 *	196 * (270)

* Statistically different from human skin; values in parentheses indicate the order of difference between human skin

Conclusions: Whole skin obtained from a variety of laboratory animals was up to 3 orders of magnitude more permeable to paraquat than human skin.

Comments: The following details were unspecified: details of statistical analysis; sample sizes; source and method of skin preparation; assay conditions (temperature, incubation time, whether the skin samples were covered or not, identity of the receptor fluid); the analytical method of paraquat analysis. No raw data were provided. Although the absorption of tritiated water was measured, no specific assessment of the integrity of the skin samples was made. No biocide/antibiotics were used to prevent any possible biological contamination of the receptor fluid.

Dugard PH (1983) Absorption *in vitro* of [^{14}C]-paraquat through human whole skin and epidermis from aqueous solutions of paraquat dichloride and aqueous spray strength dilutions of paraquat dichloride formulations. Report no. CTL/R/710. Lab & sponsor: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: 26 August 1983.

Guidelines & GLP: No test guidelines or GLP statement were provided. This study was not quality assured.

Materials & Methods: Paraquat formulations A, B (Gramoxone S), C and D (Gramoxone UK) containing 20% paraquat dichloride (equivalent to 14.4% paraquat cation, batch no.s unspecified; ICI Plant Protection Division, Alderley Park, Macclesfield, Cheshire, UK) and experimental wetter systems, were diluted 1:40 in distilled water to yield spray strength dilutions of 0.5% paraquat dichloride (equivalent to 0.36% paraquat cation). Radiolabelled spray strength dilutions were prepared by diluting [^{14}C-methyl]-paraquat dichloride (batch no. unspecified; specific activity 30 mCi/mmol; obtained from Amersham, UK as a crystalline solid and then dissolved in distilled water) into each of the spray dilutions to yield a final activity of 6.25 μCi/mL. An aqueous radiolabelled paraquat solution was prepared by dissolving 250 μCi of [^{14}C-methyl]-paraquat dichloride (batch no. unspecified; specific activity 20-40 mCi/mmol; Amersham, UK) into an aqueous 1 mg/mL solution of paraquat dichloride (equivalent to 0.72 mg/mL paraquat cation; batch no. unspecified; ICI, Alderley Park, Macclesfield, Cheshire, UK) to yield a final activity of approximately 8 μCi/mL.

Human whole skin was obtained from the abdomen of post-mortem subjects of varying ages, and epidermal samples prepared by immersing whole skin samples in water at 60°C for 40 seconds. The samples were mounted in glass diffusion cells and their integrity determined by measurement of their permeability to tritiated water. Samples with a permeability coefficient of > 1.5 x 10^{-3} cm.h^{-1} were regarded as damaged and excluded from the experiment. Samples of paraquat were applied to whole skin or epidermis at approximately 5 mL.1.8 cm^{-2} for 54 h at 30°C with stirring (of receptor fluid). There was no indication as to whether samples were covered. At recorded intervals after application, 25 μL samples of the receptor fluid (distilled water or physiological saline containing 1670 U/mL benzyl penicillin) were analysed for paraquat content by scintillation counting.

Results: Absorption of ^{14}C-paraquat following application of spray strength formulations to whole human skin was not detected until 24 h after application of formulations A, B and C, and not until 45 h for formulation D (see Table 14 below). The study author reported that the absorption rate was approximately 0.5 μg.cm^{-2}.h^{-1} over the 24-30 h period and less than 0.1 μg.cm^{-2}.h^{-1} over the 45-54 h period for the majority of samples, however, no details were provided for how these values were calculated. There appeared to be only marginal differences in absorption between the 4 formulations.

Table 14: *In vitro* absorption of ^{14}C-paraquat following application of 4 spray strength formulations to whole human skin

Formulation	Mean amount absorbed (μg.cm^{-2} ± 1 SEM)			
	6 h	12 h	24-30 h	45-54 h
A (n=4)	0	0	0.57 ± 0.097	2.45 ± 1.10
B (n=2)	0	0	0.42 ± 0.07	0.905 ± 0.28
C (n=4)	0	0	0.74 ± 0.37	1.14 ± 0.23
D (n=2)	0	0	0	0.46 ± 0.10

Means and SEMs calculated from raw data by the reviewing toxicologist; limit of detection = 0.3–0.40 μg.cm^{-2}

The absorption of an aqueous solution of ^{14}C-paraquat by human epidermis was approximately twice that by whole skin (see Table 15 below) but according to the study author this difference was not statistically significant.

Table 15: *In vitro* absorption of ^{14}C-paraquat following application of a 1.0 mg/mL aqueous solution to whole human skin and epidermis over 24-48 h

Sample	Mean absorption rate ± 1 SEM (μg.cm^{-2}.h^{-1})	Permeability constant [a] (cm.h^{-1} x 10^{5})
Whole skin	0.0048 ± 0.0011 (n=20)	0.48
Epidermis	0.0081 ± 0.0019 (n=19)	0.81

a: permeability constant = absorption rate (mg/cm/h)/applied concentration (mg/mL)

Conclusions: *In vitro* absorption of radiolabelled paraquat from 4 formulations by whole human skin and epidermis was very low over the first 24 h of contact suggesting that absorption during operational exposure over a comparable time scale would be minimal.

Comments: The following details were unspecified: method of statistical analysis; whether the receptor fluid was maintained at a constant volume following sampling; mean absorption data for the spray strength formulations by whole skin; raw data for the absorption of the aqueous paraquat solution; results of the skin integrity tests (permeability to tritiated water). The amount of unabsorbed ^{14}C-paraquat and the amount of paraquat in the skin were not measured.

Scott RC (1990) Paraquat: the effect of 'Porter' on *in vitro* absorption from 'Gramoxone Export' through human skin. Report no. CTL/L/3432. Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: 7 December 1990.

Guidelines & GLP: No Guidelines or GLP statement were provided. The study was not quality assured.

Materials & Methods: Paraquat (batch no. & source unspecified) was applied to samples (n=6) of whole human skin (unspecified source & method of preparation) in glass diffusion cells as a 2 g/L aqueous solution of paraquat cation, a 1:100 aqueous dilution of a 'Gramoxone Export' formulation (200 g/L paraquat cation) or the latter with 0.1, 0.2 or 0.5% Porter, at 10 μL.cm^{-2} for 55 h at an unspecified temperature. Samples were covered for the duration of the exposure period. The integrity of the skin samples was determined by measurement of their permeability to tritiated water. Samples with permeability coefficients >1.5 x 10^{-3} were discarded. At various intervals over 55 h, 0.5 mL samples of receptor fluid (physiological saline) were taken and analysed for paraquat by HPLC. The receptor fluid was maintained by the addition of 0.5 mL

of fresh physiological saline immediately after removal of each sample. No further experimental details were provided.

Results: Paraquat absorption data are presented in Table 16 below. Absorption of paraquat by human skin over 55 h was low and reached 2.49% of the applied amount for the aqueous solution, 2.36% for the 1:100 Gramoxone dilution, 4.65% in the presence of 0.1% Porter, 2.41% in the presence of 0.2% Porter and 1.82% in the presence of 0.5% porter. The absorption rate over the first 1-9 h was 1.4-2.9-fold greater than that over the 24-55 h period for all samples. Absorption of paraquat from the aqueous solution and the 1:100 dilution of Gramoxone were similar while there was a slight increase in the rate and amount of absorption in the presence of 0.1% Porter. However, this effect was not seen at higher concentrations of Porter where the rate and amount of absorption was similar to the aqueous paraquat solution and the 1:100 dilution of Gramoxone.

Table 16: Effect of Porter on the *in vitro* absorption of paraquat by human skin

Sample	Time Period (h)	Absorption Rate ($\mu g.cm^{-2}.h^{-1} \pm$ SEM)	Amount Absorbed ($\mu g.cm^{-2}$) †	% Absorbed ‡
Paraquat aqueous solution (2.0 g/L) 10 $\mu L.cm^{-2}$ (20 μg ai.cm^{-2})	1-9 24-55	0.015 ± 0.011 0.011 ± 0.005	0.135 0.363	0.675 1.815
1:100 aqueous dilution of Gramoxone Export 10 $\mu L.cm^{-2}$ (20 μg ai.cm^{-2})	1-9 24-55	0.023 ± 0.004 0.008 ± 0.003	0.207 0.264	1.035 1.32
Plus 0.1% Porter	1-9 24-55	0.03 ± 0.002 0.02 ± 0.008	0.27 0.66	1.35 3.3
Plus 0.2% Porter	1-9 24-55	0.017 ± 0.01 0.01 ± 0.001	0.153 0.33	0.765 1.65
Plus 0.5% Porter	1-9 24-55	0.011 ± 0.002 0.008 ± 0.003	0.099 0.264	0.495 1.32

† Amount absorbed = absorption Rate ($\mu g.cm^{-2}.h^{-1}$) x time period (h); ‡ % absorbed = amount absorbed ($\mu g.cm^{-2}$)/applied amount ($\mu g.cm^{-2}$) x 100; † & ‡ calculated by reviewing toxicologist

Conclusions: Paraquat was poorly absorbed by human skin when applied as an aqueous solution (2 g/L cation) or as a 1:100 dilution of 'Gramoxone Export' formulation (2 g/L cation), suggesting that absorption during operational exposure to these formulations would be minimal. Additionally, Porter (a mineral oil) did not affect the *in vitro* absorption of paraquat by human skin suggesting that absorption during operational exposure to any formulation containing Porter would also be low.

Comments: No raw data were provided (ie for individual receptor cells) and no statistical analysis was performed. Formulation details, the identity of Porter and the method for calculating absorption rates were unspecified. The amount of unabsorbed paraquat and the amount of paraquat in the skin were not measured. No biocide/antibiotics were used to prevent any possible biological contamination of the receptor fluid.

Ward RJ & Heylings JR (1993a) Paraquat: *In vitro* absorption from various formulation and mixtures with diquat through rabbit skin. Report no. CTL/L/5261. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca Agrochemicals, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: 14 June 1993.

Guidelines & GLP: No Guidelines or GLP statement were provided. The study was not quality assured.

Materials & Methods: Whole skin, free from subcutaneous fat and muscle, was obtained from male rabbits (approximately 23-weeks old; unspecified strain & source) and mounted in glass diffusion cells. The integrity of the skin samples was determined by measuring their permeability to tritiated water. Samples with a permeability coefficient of > 4 x 10^{-3} $cm.h^{-1}$ were regarded as damaged and thus excluded from the experiment. An aqueous solution of paraquat/diquat (120/30 g/L; unspecified batch no.; Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.), Gramoxone (120 g/L paraquat; batch no. unspecified; Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK) containing 30 g/L diquat, or an Indonesian formulation (see Appendix VI for formulation details) containing paraquat/diquat (120/30 g/L; unspecified batch no.; Zeneca Agrochemicals, Yalding Kent, UK) were applied at 100 $\mu L.cm^{-2}$ at an unspecified temperature.

After 4 h, half of the skin samples were rinsed with 10 x 5 mL of distilled water and then dried by dabbing with a tissue. The experiment was then continued with the skin uncovered. Samples (0.5 mL) of the receptor fluid (distilled water) were taken at various intervals between 2-54 h exposure for the continuously exposed samples and between 2-30 h for the rinsed skin, and analysed for paraquat by HPLC. The receptor fluid was maintained by the addition of 0.5 mL of fresh distilled water immediately after removal of each sample. No further experimental details were provided.

Results: Absorption data are given in Table 17 below. Absorption was low for all test formulations and reached a maximum of approximately 2.5% of the applied amount at 10 h regardless of whether the skin was washed or not. There waslittle difference in the amount absorbed for all 3 formulations over 10 h following continuous exposure. However the absorption of low concentration Gramoxone formulation was 4.5- and 2.6-fold more rapid than the aqueous solution and Indonesian formulation, respectively, over the 10-54 h period. The effect of washing the skin was evident over the 10-30 h period where absorption rates were reduced by 2.4-, 3.5- and 9.6-fold for the aqueous solution, Indonesian formulation and low concentration Gramoxone formulation, respectively, relative to the unwashed 10-54 h values. Additionally, absorption rates were similar over the 10-30 h period for all formulations following washing, however, this did not completely prevent further absorption after 4 h. In the absence of methodological detail and individual cell data, it was unclear why the amount absorbed at 4 and 10 h was greater in the washed samples than the continuously exposed samples.

Table 17: *In vitro* absorption of paraquat following application of various formulations to rabbit skin.

Sample	Time Period (h)	Absorption Rate ($\mu g.cm^{-2}.h^{-1}$ ± SEM)	Amount Absorbed ($\mu g.cm^{-2}$)		% Absorbed	
			4 h	10 h	4 h	10 h
Aqueous Solution Continuous exposure (n=6)	2-10 10-54	12.1 ± 3.17 21.1 ± 4.52	56	132	0.47	1.10
Indonesian formulation Continuous exposure (n=6)	2-10 10-54	19.6 ± 4.28 37.2 ± 3.90	74	202	0.62	1.68
Low concentration Gramoxone + diquat Continuous exposure (n=6)	2-10 10-54	28.7 ± 5.57 95.2 ± 1.79	74	270	0.62	2.25

Sample	Time Period (h)	Absorption Rate ($\mu g.cm^{-2}.h^{-1}$ ± SEM)	Amount Absorbed ($\mu g.cm^{-2}$)		% Absorbed	
			4 h	10 h	4 h	10 h
Aqueous Solution Skin washed at 4 h (n=6)	2-10 10-30	12.6 ± 1.58 8.82 ± 1.03	86	165	0.72	1.37
Indonesian formulation Skin washed at 4 h (n=6)	2-10 10-30	14.8 ± 2.20 10.7 ± 2.31	87	167	0.73	1.39
Low concentration Gramoxone + diquat Skin washed at 4 h (n=6)	2-10 10-30	29.3 ± 4.76 9.89 ± 0.93	120	297	1.0	2.47

Conclusions: Paraquat was poorly absorbed following application of an aqueous solution, an Indonesian formulations and Gramoxone (plus diquat) to isolated rabbit skin. Washing the skin with water after 4 h of exposure appeared to reduce the rate of absorption, however the effect on the amount of absorption was considered equivocal given the lack of reporting detail.

Comments: No raw data were provided including results of the integrity tests (permeability to tritiated water). Statistical analysis was not performed. Formulas for calculating absorption rates were not described. The amount of unabsorbed paraquat, and that remaining on or in the skin was not measured. The use of distilled water as the receptor fluid was not justified and no biocide/antibiotics were used to prevent any possible biological contamination of the receptor fluid.

4.2.6.2 Human Volunteer Studies

Wester RC, Maibach HI & Bucks DAW (1984) *In vivo* percutaneous absorption of paraquat from hand, leg and forearms of humans. J Toxicol Environ Health. 14: 759-762.

[^{14}C-methyl]paraquat dichloride (2.0 mCi/mM; amount of paraquat cation, purity & batch no. unspecified; Chevron Chemical Company, unspecified location) was formulated in distilled water and administered bilaterally to the back of the legs and hands, and ventral forearms of six volunteers (details unspecified) in a crossover design. Paraquat was applied at 4.95 μCi/50 μL over 70 cm^2 of skin surface to yield a dose of 9 $\mu g/cm^2$. Exposure sites were unoccluded, and not washed for 24 h. There was one week of wash-out between applications. Urine samples (unspecified volume) were collected at 4, 8, 12 and 24 h, 2, 3, 4, and 5 days after application. Paraquat was measured in urine samples presumably by scintillation counting although this was not specified.

Percutaneous paraquat absorption was determined by the ratio of ^{14}C urinary excretion following parenteral and topical application according to a method previously reported by the authors. Paraquat (unspecified dose) was administered parenterally to rhesus monkeys (unspecified sex, age, body weight, source & sample size) to correct for the percentage of non-urinary excretion or the amount retained in the body. In the monkey, 58.6 ± 9.4% (n=4) of the parenterally administered dose was excreted in the urine and this value was used as the non-urinary excretion level in humans.

[^{14}C-methyl]paraquat was poorly absorbed following topical application to undamaged skin of the legs, hands and forearms (see Table 18 below), with the mean percentage of applied dose that was absorbed being 0.29, 0.23 and 0.29, respectively. There was no significant

difference in absorption between each application site. The study authors reported an absorption rate of 0.03 μg/cm^2 over 24 h.

Table 18: Percutaneous paraquat absorption (% of applied dose) in human volunteers

Time (h)	Leg	Hand	Arm
0-4	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
4-8	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
8-12	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
12-24	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.01
24-48	0.04 ± 0.04	0.05 ± 0.03	0.04 ± 0.02
48-72	0.06 ± 0.06	0.02 ± 0.01	0.04 ± 0.02
72-96	0.06 ± 0.05	0.02 ± 0.02	0.04 ± 0.01
96-120	0.06 + 0.05	0.02 ± 0.02	0.04 ± 0.01
Total	0.29 ± 0.02	0.23 ± 0.1	0.29 ± 0.1

Results are expressed as the mean ± an unspecified error.

The study methodology/reporting contain a number of limitations. The validity of using a % urinary excretion value in rhesus monkeys after parenteral application was unjustified. The following were unspecified: volunteer details (sex, age, body weight, health status etc); ethics approval; details of statistical analysis; the presence or absence of clinical signs or signs of skin reaction; methodological details of the monkey study. No raw or individual volunteer data were provided. A single dose level only was tested and no rationale was provided for the choice of this dose. Although application sites were not washed for 24 h, the effect of clothing on the removal of paraquat from the skin did not appear to have been accounted for. The amount of unabsorbed paraquat was not determined.

Regardless of these limitations, this study is still considered to be of regulatory value, due to the use of human subjects, and provides further evidence that paraquat is poorly absorbed through intact human skin, with an absorption rate of approximately 0.3%.

4.2.7 Human Poisonings

Beebeejaun AR, Beevers G & Rogers WN (1971) Paraquat poisoning–Prolonged excretion. *Clinical Toxicol 4 (3): 397-407.*

Case history: This study investigated the clinical features, lung, liver and kidney pathology, and urinary excretion of paraquat in a 66-year old female patient, who had ingested approximately 15 g of Weedol (a commercial preparation containing 5% paraquat dichloride; 3.62% paraquat cation) granules in water with suicidal intent. Upon admission to hospital, the patient was treated with alternating iv solutions of 5% dextrose, 1/6 molar lactate, and normal saline with 1 g of potassium chloride (KCl) added to each litre. A diuretic was given to maintain the urine volume. Forty-six days after ingestion, the patient had an episode of collapse with pallor and low blood pressure, and died on the following day, while undergoing a lung biopsy.

Findings: Soon after ingestion, the patient had vomited. A quantity of approximately 15 g of granules of Weedol was seen in the vomitus. On admission, she was immobile and mute, but respiratory signs, pulse and blood pressure were normal. Paraquat was not detectable in the serum at 24 h after ingestion, but was recovered from the urine until sampling was discontinued on the 27th day after ingestion. The total urinary excretion of paraquat over 26 days was 12.89 mg, of which approximately 57% was excreted within the first 3 days. The study authors stated that, based on the observations made in rats by Daniel and Gage (1966), where an average of

5% of orally administered paraquat was found to have excreted in the urine, the estimated paraquat dose absorbed by this patient was approximately 253 mg (equivalent to approximately 5 g of Weedol).

Oedema and redness of the tongue was seen on admission, with severe epigastric tenderness occurring on the following day. An electrocardiogram (ECG) was normal and remained unchanged throughout her illness. Radiological evidence of patchy atelectasis (airlessness of the lungs due to failure of expansion or resorption of the alveoli) started to appear at the left base 4 days after ingestion, and in the right base 2 days later. By the 11th day pulmonary exudates appeared in the right upper lobe, and during the next 10 days fresh areas of patchy exudate and collapse developed in all areas of both lungs. These areas gradually became 'harder', and the overall appearance suggested an organising exfoliative alveolitis. Thereafter, the radiological appearance remained unchanged. Urinary output was 5600 mL within 12 h to midnight on the first day, and 5500, 7000, and 2900 mL in each of the following 24-h periods. The blood urea level of 36 mg/dL on admission rose to 200 mg/dL on the 8th day, and then fell to 40 mg/dL on the 16th day. Proteinuria was observed throughout her illness. A liver biopsy on the 32nd day showed mild fatty change. Arterial oxygen partial pressure (P_aO_2) was decreased from 74 mm Hg on the 3rd day to 53 mm Hg on the 5th day, leading to marked cyanosis, dyspnoea on mild exertion, extensive bilateral pulmonary crepitations and gradual deterioration of respiratory function.

A renal biopsy performed on the 13th day showed severe degenerative changes in the proximal tubules with loss of brush border and thinning of epithelial cells. At necropsy, blood in the pleural cavity, infarction of the left cerebral hemisphere and enlarged liver with soft fatty change were seen. Histopathology revealed moderate to severe thickening of alveolar walls by collagenous tissue of light to medium density, prominent perivascular fibrosis and patches of increased inflammatory cellular exudate, fibrosis, distortion and dilation of terminal bronchioles with epithelial proliferation. The liver showed some centrilobular necrosis, and the kidneys showed widespread tubular necrosis (partly post mortem).

Proudfoot AT, Stewart MS, Levitt T & Widdop B (1979) Paraquat poisoning: Significance of plasma-paraquat concentrations. ***Lancet 2:330-332.***

Study & observations: The significance of plasma paraquat concentrations in 79 patients, for whom the time of paraquat ingestion was known with reasonable certainty were investigated, and the usefulness of such data in predicting the outcome of paraquat intoxicated patients was discussed. The group of patients consisted of 2 children (aged 3 and 7 years) and 75 adults (46 men and 29 women, age: 16-81 yrs, mean: 38). The formulations of paraquat ingested by these patients are given in Table 19 below. However, no record of the amounts of the formulations ingested was provided. Seventy-one of these patients had been seen by a clinician within 35 h of ingestion. Venous blood samples were collected (10 mL) from the patients as soon as possible after admission. Samples which were not analysed immediately, were stored at -20°C. In 54 cases, only one sample was obtained, whilst serial samples were taken from the remainder. Plasma paraquat levels in 9 people were analysed by GC, in 40 cases by RIA and in 30 cases by a colorimetric method. It was reported that these 3 analytical methods gave comparable results.

Table 19: Mortality data following the ingestion of various paraquat formulations

Paraquat formulation		Number of patients	Number of deaths
Trade name	**Paraquat (%)**		
Gramoxone	20	27	21 (71%)
Dextrone	20	1	0
Gramonol	10	1	0
Weedol	5 or 2.5	43	4 (10%)
Pathclear	2.5	3	0
Unknown	-	4	3

Findings: It was reported that the plasma paraquat level of each person was related to the time from ingestion and the patient's outcome (data provided in semilogarithmic graph form). Generally, for any given sampling time, the plasma paraquat concentration in those who died was considerably higher than that in the survivors. Patients whose plasma levels did not exceed 2.0, 0.6, 0.3, 0.16 and 0.1 mg of paraquat/L at 4, 6, 10, 16 and 24 h after ingestion had survived. Four out of 8 patients, whose plasma levels were measured 35 h after ingestion, died. Their plasma paraquat levels were 2.8, 0.39, 1.15, 0.47 mg/L at 45, 52, 57 and 104 h after ingestion, respectively. Four surviving patients had plasma levels of 0.015, 0.04, 0.001 and 0.1 mg/L at 68, 72, 115 and 144 h after ingestion, respectively.

In the majority of patients, whose blood samples were tested serially (some of them underwent therapy to enhance elimination), the peak plasma paraquat concentrations occurred prior to the first blood samples being taken, even when the interval between ingestion and sampling was as short as 2 h. Plasma paraquat levels fell rapidly during the first few hours after ingestion and slowly after 15-20 h.

Conclusions: The measurement of plasma paraquat concentrations appears to be useful in assessing the severity and predicting the outcome of poisoning.

Houzé P, Baud FJ, Mouy R, Bismuth C, Bourdon R & Scherrmann JM (1990) Toxicokinetics of paraquat in humans. *Human & Exp Toxicol 9: 5-12.*

Study & observations: This paper presented toxicokinetic data collected during the course of 18 acute paraquat poisoning incidents. Data for 10 males aged between 20-67 years, 7 females aged between 22-40 years and a 14-month old boy were provided. These patients were admitted to hospital during 1984-1988 following exposure to paraquat by intentional or accidental ingestion. The time between poisoning and hospital admission ranged from 3 to 96 h. The dose ingested by these persons ranged from 0.5 to 6 g, but the details on how these values were calculated were not provided. The quantity of paraquat ingested by the 14-month old boy was unknown. Only patients who had at least 5 blood samples analysed for paraquat following intoxication were chosen for the study. The post-mortem tissue distribution of paraquat was studied in 6 patients. Assuming that first-order processes characterise the paraquat disposition, and that paraquat is eliminated only by the renal route, a composite curve representing the mean paraquat concentrations in patients was determined as follows (only those patients who did not undergo haemodialysis treatment were chosen; time 0 = the time of ingestion; the total duration of plasma paraquat concentration measurement was divided into periods): first, for 4 consecutive periods of 4 h, then 5 consecutive periods of 12 h, then 15 consecutive periods of 24 h, and finally, for a period of 120 h. For each period, the mean concentration relating to the mid-point was calculated. As some patients were monitored until plasma paraquat levels became undetectable, urinary data (of 6 patients) were used for the determination of the amount

of paraquat remaining to be eliminated and urinary half-lives. Plasma, tissue and urinary paraquat concentrations were assayed by RIA. The toxicokinetic data in the 14-month old boy was reported separately, as plasma, urine and cerebrospinal fluid paraquat levels in this patient were measured over a period of 8 months.

Findings:

Clinical status: Upon admission, gastric lavage was performed on all patients. Thereafter, they received either Fuller's earth (kaolin) or activated charcoal orally. Renal function remained normal in one patient, whilst one was anuric and 15 other individuals had non-oliguric acute renal failure. All subjects were treated with the diuretic, furosemide. Haemodialysis was performed in 2 cases. Fourteen patients died from either cardiovascular collapse or pulmonary fibrosis (mean time to death was 2.8 ± 1.5 and 11 ± 5.5 days, respectively).

Toxicokinetics: The time course of the mean plasma concentrations of paraquat for 15 subjects was graphically presented and showed a bi-exponential decline. The half-life of the early phase, which reflected the distribution of paraquat, was 5 h, while the half-life of the late phase was 84 h. Assuming a constant first order rate of distribution and a half-life of 5 h, paraquat distribution to highly perfused organs was achieved within approximately 30-40 h. It was reported that even in patients with normal renal function, the terminal half-life of paraquat was long, suggesting that prolonged elimination depends not only on the renal function but also on the gradual release of paraquat from extravascular tissue into the circulation. The time course of plasma concentrations in the 5 subjects who died from cardiovascular collapse was also similar to the above when analysed separately, with the mean distribution half-life being 7 ± 2 h. The terminal plasma half-life of paraquat in these patients could not be established as they died quickly. For one subject admitted to hospital soon after paraquat ingestion, the maximum serum level of 5600 ng/mL was observed between 3 and 4 h after ingestion.

The toxicokinetics in 2 patients who were treated with haemodialysis, also exhibited bi-exponential decline in plasma paraquat levels. In these patients, the half-lives of the early phase was 3 h, while that of the late phase were 83 and 400 h, respectively.

The amount of paraquat eliminated in the urine until death was determined in 5/6 patients. The mean urinary output of paraquat was 430±270 mg (range: 190-900 mg). The renal clearance of paraquat in 4 patients ranged from 7.4 to 21 mL/min. The renal clearance of paraquat was always lower than the estimated renal clearance of creatinine (see Table 20 below). A statistically significant correlation was observed between urinary paraquat and plasma half-lives ($r=0.83$, $p\leq0.02$).

Table 20: Paraquat and creatinine renal clearances (mL/minute) in 4 patients*

Subject	Delay between poisoning and admission (h)	Ingested dose (g)	Creatinine clearance	Paraquat clearance
Males				
A (31 years)	30	0.5	19.0 ± 9.0	8.4 ± 1.0
B (37 years)	4	6.0	95.0	29.0
Females				
A (30 years)	13	0.8	91.0 ± 24.0	9.9 ± 2.3
B (40 years)	15	4.0	25.0 ± 8.0	7.9 ± 0.7

*Mean ± SEM. Values in parentheses represent the age of the patients.

The apparent volume of distribution of paraquat was estimated only in 3 cases, in which the renal clearance and plasma half-life was measured late during the course of poisoning. In these 3 patients, the volumes of distribution (V_d) were 1.2, 1.4 and 1.6 L/kg.

The peak plasma paraquat level (3189 ng/mL) in the 14-month old boy was observed approximately 4 h after ingestion. Plasma levels showed a rapid decline, with 2 rebounds at 76 and 106 days after ingestion. Corresponding rebounds were also observed in the urine and cerebrospinal fluid of this child. Based on these results, the study authors suggested that paraquat could be recovered from biological fluids up to 3 months after ingestion.

The tissue distribution of paraquat is shown in Table 21 below. Higher levels of paraquat were found in the lung, kidney and heart compared to other tissues. Paraquat was also found in lipophilic organs like the brain and adipose tissue. The study authors claimed that the muscle tissue, pancreas and spleen may represent important reservoirs of paraquat.

Table 21: Paraquat concentrations in different tissues (ng/mg of tissue)

Subject	1	2	3	4	5	6
Days between ingestion and sampling*	19	10	8	6	2	1
Tissue						
Blood	-	75	101	-	150	440
Brain	35	-	71	-	573	-
Lungs	100	62	230	965	3200	7245
Liver	79	36	895	1273	5250	1864
Heart	23	-	160	963	3333	-
Kidneys	742	-	5000	2816	10377	2076
Tongue	40	-	-	-	-	-
Oesophagus	66	-	-	-	-	-
Stomach	-	40	-	-	-	-
Small intestine	-	23	-	790	-	-
Colon	38	-	-	-	-	-
Pancreas	20	58	-	1480	-	-
Spleen	63	-	-	619	3153	-
Skin	20	-	-	-	-	-
Fat	15	-	-	-	-	-
Muscle	120	-	-	2076	-	-
Thyroid	82	-	-	-	-	-
Suprarenal gland	81	23	-	596	-	-
Prostate	61	-	-	-	-	-
Placenta	-	29	-	-	-	-

Based on these data, the study authors suggested that therapeutic techniques such as haemoperfusion or haemodialysis and administration of paraquat antibodies should be employed in treatment during the early distribution phase (ie in the first 36 h) aiding the elimination of paraquat from the body, and thus reducing the risks of cardiovascular collapse.

4.2.8 *In vitro* Studies

Rose MS (1974) Evidence for energy-dependent accumulation of paraquat into rat lung. *Nature, 252: 314-315.*

[Although this report also presented the data on diquat, only observations relating to paraquat are reported here.]

Paraquat was shown to accumulate in rat lung tissue in a linear fashion. After a 2-h incubation period, the maximum tissue concentration achieved was 1 mmol of paraquat/g of wet tissue at 1×10^{-3} M paraquat concentration. KCN plus iodoacetate or rotenone, a specific inhibitor of mitochondrial respiration, inhibited paraquat tissue accumulation. Based on the findings, the study authors concluded that the uptake of paraquat by rat lung tissue slices was energy-dependent. The Michaelis constant (K_m) and maximum velocity (V_{max}) values calculated for the uptake process were 7×10^{-5} M and 300 nmol/g of wet weight/h, respectively.

DeGray JA, Ramakrishna Rao DN & Mason RP (1991) Reduction of paraquat and related bipyridylium compounds to free radical metabolites by rat hepatocytes. *Arch Biochem & Biophys 289 (1): 145-152.*

[Although this paper also presented data on 3 other bipyridylium compounds, only observations relating to paraquat are reported here.]

The *in vitro* generation of the paraquat radical cation was investigated by the addition of paraquat at 1-5 mM concentrations to male CD rat hepatocytes suspended in Fry's buffer of pH 7.4. The electro paramagnetic resonance spectra showed that paraquat was reduced very readily to its radical cation by hepatocytes. The radical cation was identified by its characteristic coupling constants obtained by computer simulation of the experimental spectra. Based on the findings in a series of experiments conducted with 3 other bipyridylium compounds, the study authors concluded that the bipyridyliums generated by rat hepatocytes were largely formed inside the cell and could escape through the plasma membrane.

4.3 Acute Studies

4.3.1 Acute Oral, Dermal and Inhalation Studies

The available acute oral median lethal dose studies are summarised in Table 22 below. Following oral administration of a lethal dose of paraquat, death was frequently delayed by up to 13 days. Hence, median lethal dose calculations using a shorter observation duration may under estimate the lethality of paraquat. Treated animals appeared hypoxic and lethargic for 3-4 days after dosing. Clinical signs observed in rodents were dehydration, hypothermia, irregular breathing, chromodacryorrhea, piloerection, sides pinched in, stains around nose and mouth, upward curvature of the spine and reduced splay reflex. Treatment-induced lung lesions were related to the time after exposure ie after 3-5 days: haemorrhagic and oedematous; 5-7 days,

ruptured alveolar spaces, atelectasis, congestion, and fibrosis; and thereafter more extensive fibrosis, alveolar collapse and congestion. In rabbits, appetite loss with subsequent body weight loss was observed 3 days after dosing. There was no obvious cause of death but histopathological examination showed evidence of renal proximal tubular necrosis leading to the speculation that impaired renal function was a contributing factor in the absence of any lung fibrosis.

The clinical signs and lethal dose of paraquat in Cynomolgus monkeys was reportedly similar to those observed in humans attempting suicide. Early signs involved tachycardia and hyperpnoea followed by convulsions just preceding death which in turn was caused by respiratory failure. Survivors lasting more than 3 days had anorexia, adipsia and diarrhoea that sometimes contained mucous and blood. At necropsy, emphysema, congestion, and haemorrhage of the lung were observed. Histopathological examination revealed mild centrolobular necrosis in the liver and tubular necrosis in the kidneys (Murray & Gibson, 1972). The lesions observed in the lungs were similar to those observed in rodents.

Table 22: Summary of acute oral median lethal dose studies

Species [strain]	Sex	Group Size	Concentration (% w/v)	Doses Tested (mg/kg bw)	Observation period (days)	LD_{50} (mg/kg bw)	Reference
Mouse [NS]	M/F	10/sex	NS	80, 99.8, 126 or 160	14	101 (M) 104 (F)	Fletcher (1967)
Mouse [CD1]	F	5	38	50, 75, 100, 125, 150 175, 200 or 225	10	166 (F)	Heylings & Farnworth (1992)
Mouse [AP]						203 (F)	
Rat [Sherman]	M/F	50/sex	24	NS	15	100 (M) 110 (F)	Kimbrough & Gaines (1970)
Rat [SD]	M	10	21	101, 126, 159, 200 or 252	7	126 (M)	Murray & Gibson (1972)
Rat [NS]	M	5	NS	100 or 200	9	1/5 and 5/5 deaths at 100 mg/kg bw and 200 mg/kg bw respectively.	Keeffe (1982)
Rat [NS]	M	9 or 10	10	50, 75, 100, 125 or 150	15	141 (M)	Barber (1983)
Rat [Alpk:APfSD]	M/F	5/sex	29	72, 145, 290 (M/F) or 434 (M)	15	249 (M) 205 (F)	Duerden (1994b) [GLP]
Guinea pig [NS]	M/F	6	21	20, 21.2, 22.4, 23.7 or 25	7	22 (M & F)	Murray & Gibson (1972)
Rabbit [NZW]	F	2 but 4 at 40 mg/kg bw	29	4, 8, 12, 16, 20, 24, 30, 40 or 50	10	40-50 (F)	Farnworth, *et al* (1993a)
Monkey (Cynomolgus)	M/F	1 except for 1/sex at 40 and 50 mg/kg bw	21	35, 40, 45, 50, 53, 63, 76 or 126	7	50 (M & F)	Murray & Gibson (1972)

(NS = not stated)
M – males
F - females

The available acute dermal median lethal dose studies are summarised in Table 23 below. In the study which reported no deaths following dermal administration at 1448 mg/kg bw (Duerden, 1994c), only slight to moderate signs of irritation were observed at the application site. The studies of Kimbrough & Gaines (1970) and Duerden (1994c) indicate a marked disparity in apparent lethality following dermal administration ie LD_{50} of 80-90 mg/kg bw *vs* >1448 mg/kg bw. Since percutaneous absorption of paraquat is poor (see Section 3.2.6), a plausible explanation may be that oral ingestion by the rats of some of the applied material occurred in the Kimbrough & Gaines study, as according to the methodology of Gaines (1960) referenced in this study, it is most likely that the test sites were not covered or the animals restrained such that they could not groom the treated area The difference could also have been

due to the presence of the highly toxic impurity 2,2': 6',2"-terpyridine, which has po and dermal LD_{50} values of 2.17 and 4.31 mg/kg bw, respectively, in rats (see Table 25 below).

Table 23: Summary of acute dermal median lethal dose studies

Species [strain]	Sex	Group Size	Concentration (% w/v)	Doses Tested (mg/kg bw)	Observation period (days)	LD_{50} (mg/kg bw)	Reference
Rat [Sherman]	M/F	50 M/40 F	24	NS	15	80 (M) 90 (F)	Kimbrough & Gaines (1970)
Rat [Alpk:APfSD]	M/F	5/sex	29	1448 (undiluted, 24 h, occluded)	15	>1448 (M & F; no deaths)	Duerden (1994c) [GLP]

(NS = not stated)

The available acute inhalational median lethal dose studies are summarised in Table 24 below. Rats exposed to lethal paraquat aerosols displayed progressive deterioration and piloerection some days after exposure together with slow and shallow noisy respiration suggestive of respiratory tract irritation (especially when exposed to small particle aerosols). Necropsy revealed congested lungs with petechial haemorrhage and pale kidneys.

Table 24: Summary of acute inhalational median lethal dose studies

Species [strain]	Sex	Group Size	Concentration (%)	Concentrations Tested (mg/m^3)	Observation duration (days)	LC_{50} (mg/m^3)	Reference
Rat [AP]	M/F	4/sex	NS	0.29, 0.54, 0.94, 1.09, 1.88, 3.47, 9.92 or 23.5 (Whole-body exposure for 6 h; 90% of aerosol ≤2.5μm)	20	0.7	Gage & Manley (1966)
Rat [SD]		3/sex	29.2	0.16, 0.49 or 0.95 (Whole-body exposure for 4 h; Droplet size of aerosol not measured but anticipated to be in the range of 0.5-3 μm)	14	0.5	Hathaway (1966)
		5/sex	1#	14.5, 23.2, 34.7, 52.8, 67.3, 108.6 or 211.4 (Whole-body exposure for 4 h; Droplet size of aerosol not measured but anticipated to be in the range of 0.5-3μm)		46	Palazzolo (1964)
Rat [Alpk:APfSD]			21.5	0.2, 0.6 or 1.4 (Nose-only exposure for 4 h; >90% of aerosol ≤0.3 μm)		<1.4 (No deaths at 0.6 mg/m^3 but all dead at 1.4 mg/m^3)	McLean Head, *et al* (1985a)
Rat [Alpk:APfSD]			21.5	0.93 or 3.5 (Nose-only exposure for 4 h; 0.12-0.25% of aerosol <2.5 μm)		≥3.5* (5/10 deaths)	McLean Head, *et al* (1985b)

AP=Alderley Park SPF strain; Alpk:APfSD=Wistar-derived; #=dilute solution; *=coarse aerosol; M male; F female

Impurities:

In addition, acute median lethal studies for three impurities are presented in Table 25 – Table 27.

2,2':6',2"-terpyridine (terpyridine)

Table 25: Median lethal studies for the impurity 2,2':6',2"-terpyridine (terpyridine)

Species [strain]	Sex	Group size	Vehicle	Purity (%)	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral administration							
Rat [SD]	M/F	5/sex	Propylene glycol	99.6	1.0, 2.25, 4.0, 15, 40 (M) 0.5, 1.0, 4.0, 15, 40 (F)	2.61 (M) 2.17 (F)	Kuhn (2002a) [GLP]
Dermal							
Rat [NS]	M/F	5/sex	Propylene glycol	99.6	4.0, 5.5, 7.0, 15 (M) 2.0, 4.0, 5.5, 7.0, 15 (F)	5.04 (M) 4.31 (F)	Kuhn (2002b) [GLP]

2,2'-bipyridyl

Table 26: Median lethal studies for the impurity 2,2'-bipyridyl

Species [strain]	Sex	Group size	Vehicle	Purity (%)	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral administration							
Rat [Sherman]	M/F	10/sex	20% ethanol	-	75, 100, 120, 150	100 (M) 107 (F)	Groce & Kimbrough (1982)

4,4'-bipyridyl

Table 27: Median lethal studies for the impurity 4,4'-bipyridyl

Species [strain]	Sex	Group size	Vehicle	Purity (%)	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral administration							
Rat [Sherman]	M/F	10/sex	20% ethanol	-	100, 150, 160, 200	175 (M) 172 (F)	Groce & Kimbrough (1982)

4.3.2 Eye and Skin Irritation and Skin Sensitisation Studies

Acute toxicity testing on paraquat reported slight and moderate to severe eye irritation in rabbits and that it is not a skin sensitiser in guinea pigs, as summarised in Table 28 below.

Table 28: Summary of the findings of eye/skin irritation and skin sensitisation studies

Study	Species	Sex	Group size	Method	Findings	Reference
Paraquat manufacturing concentrate (34% w/w paraquat cation)						
Skin sensitisation	Guinea pigs, [Hartley]	M	45	0.3 mL of 1% solution, induction & challenge exposures	Non-sensitiser	Thompson *et al* (1985)§
Paraquat manufacturing concentrate (33% w/w paraquat cation)						
Eye irritation	Rabbit [NZW]	F	3	0.1 mL, conjunctival sac, unwashed	Severe irritant	Bugg & Duerden (1994)§¶GLP
Skin irritation	Rabbit [NZW]	F	3	0.5 mL, intact, 4 h, occluded, washed	Slight irritant	Duerden (1994a)¶GLP
Paraquat manufacturing concentrate (28.6% w/w paraquat cation)						
Skin irritation	Rabbit [NZW]	F	11	0.25 mL (various dilutions), intact, 4 h, occluded, washed	Moderate-severe irritant	Bullock (1983)

NZW = New Zealand White; M = male; F = Female; ¶ = US EPA and OECD Guidelines; § = US EPA guideline

Paraquat dichloride manufacturing concentrate

4.3.2.1 Eye irritation

Bugg L & Duerden L (1994) Paraquat dichloride technical concentrate: Eye irritation to the rabbit. Study no: FB5012. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca Agrochemicals. Study duration: August-October, 1994, Report no: CTL/P/4566, Report date: December 23, 1994.

Quality assured, GLP (US EPA, UK, OECD & Japanese-MAFF) study. No test guidelines were cited.

Species/strain: Rabbit/NZW (Charles River UK Ltd)

Number/sex of animals: 3/Female (young adult, bw range: 2.6-3.6 kg).

Observation period: The eyes were examined for signs of irritation at 1 h, 1, 2, 3, 4, 7, 8/9, 10/11, 14, 17, 21 and 22/28 d after instillation, and scored according to the Draize scale (1959). The modified method of Kay and Calandra (1962) was used to interpret and classify the numerical scores.

Method of administration: Fluorescein staining was conducted within 24 h prior to instillation, to aid in the visual assessment of the eyes of each rabbit. The undiluted test material (0.1 mL, paraquat content: 33%, w/w, Ref. no: YF6219) was instilled into the conjunctival sac of the left eye of each rabbit. The lids were then gently held together for 1-2 sec. The right eye served as the control.

Results: Application of the test substance caused practically no (1/3) or slight (2/3) (score 1-2 on a scale of 0-5) initial pain in the test animals. Slight (score 1) to mild (score 2) corneal opacity covering an area of approximately 25% (score 1-2) of the corneal surface was seen in 2/3 rabbits commencing at 1 h post treatment, persisting for 4 d in one animal, and for 14 d in the other. In the third rabbit, slight (score 1) corneal opacity covering an area of approximately

25% (score 1) of the corneal surface was observed from d 7 through 9 after treatment. All corneal effects disappeared by d 17. No iridial effects were seen during the study. Slight to (score 1) severe (score 3) conjunctival erythema was observed in all animals commencing from d 1 after treatment and persisting in the three animals for 14, 17 or 21 days. All signs of erythema disappeared by d 28. Slight (score 1) or mild (score 2) chemosis was observed in all 3 animals at 1 h, and persisted for 4, 8 or 10 d. Chemosis regressed in all animals by d 14. Slight (score 1) to mild (score 2) ocular discharge was seen in 2/3 rabbits at 1 h post treatment, progressing to a severe (score 3) reaction in one animal by d 1 in one animal and by d 4 in the other. Slight or moderate discharge persisted in 2/3 animals until d 28.

Table 29: Mean scores

Lesion	Time after decontamination					
	1 h	1 d	2 d	3 d	4 d	7 d
	Mean Score					
Cornea						
Opacity	0.7	0.7	0.7	0.7	0.7	1
Area	0.7	0.7	0.7	0.7	0.7	1
Conjunctiva						
Erythema	1.3	2	2	2.7	2.3	2.3
Chemosis	1.3	1.7	1.7	1.3	1	0.7
Discharge	1	1.7	1.7	2	2.3	1.3

Conclusions: According to OCS guidelines, the observation of corneal opacity, not reversible in 7 days, paraquat dichloride 33% (w/w) manufacturing concentrate, YF 6219 was a severe eye irritant in rabbits.

4.3.2.2 Skin irritation

Bullock CH (1983) The comparative four-hour skin irritation potential of aqueous dilutions of paraquat CL (SX-1420). Study no: C2.2/05. Lab: Chevron Environmental Health Center, Richmond, CA, USA. Sponsor: Chevron Chemical Co., Study duration: October 26-November 2, 1982, Report no: SOCAL 2035, Report date: September 9, 1983.

Quality assured, GLP (US EPA) study. No test guidelines were cited.

Species/strain: Rabbit/ NZW (Nitabell Rabbitry, Hayward, CA, USA))

Number/sex of animals: 11/female (4/formulation; 10/11 had 3 application sites; 1/11 had 2 application sites) (age: 10-12 weeks, bw: unstated)

Observation period: The skin was examined for erythema and oedema 1 h, 1, 2, 3, 4, 10, 13 and 14 d after removal of the test material. The Draize scale (1959) was used to assess the degree of erythema and oedema.

Method of administration: The test material or its various aqueous dilutions (1:2, 1:5, 1:10, 1:25, 1:50, 1:100 or 1:200, 0.25 mL, 28.6% paraquat, Ref. no: SX-1420) were applied to the clipped intact skin of the dorsum of each rabbit under occluded conditions. After 4 h, the dressings were removed, and the application site cleansed with warm water to remove the residual test material, and then dried with tissue paper.

Results: Neat formulation: Well defined (score 2) erythema was seen in all 4 animals at 1 h. A moderate to severe (score 3) reaction was observed in 4/4 animals by d 3, and persisted until d 7, which was the last observation day. Slight (score 2) oedema was seen in 4/4 animals at 1 h. Slight (score 1) to moderate (score 2) oedema was observed in all animals until d 7.

1:2 dilution: Well defined (score 2) erythema was observed in 4/4 animals at 1 h, and progressed to a well-defined (score 2) to moderate (score 3) reaction by d 3. Moderate erythema was seen in all animals on the last observation day (d 7). Slight (score 1, 1/4) to well-defined (score 2) oedema were observed (2/4) at 1 h after decontamination. Slight (score 1) to moderate oedema was observed in all 4 animals until d 7.

1:5 dilution: Well-defined (score 2) erythema was seen in 4/4 animals at 1 h. Slight (score 1) or moderate (score 3) reaction was observed in all animals on d 3. A slight (score 1) or severe (score 4) erythema response was seen in all test animals on d 7. Slight (score 1) oedema in 2/4 animals, and a well-defined (score 2) oedema in 1/4 animals were observed at 1 h post patch removal, and persisted until d 3. The signs of oedema (slight or well-defined) persisted in 3 animals for 7 d.

1:10 dilution: Well defined (score 2) erythema was seen in all 4 animals at 1 h, and on d 1. Well-defined erythema in 2/4 animals and a moderate (score 3) response in 2/4 animals were observed on d 2 and 3, and persisted until d 7. Very slight (score 1) oedema in 2/4 animals, and well-defined (score 2) oedema in 1/4 animals were seen at 1 h after decontamination, and persisted until the last observation day (d 7).

1:25 dilution: Slight (score 1) or well-defined (score 2) erythema was observed in all 4 animals on d 1, progressing to well-defined or moderate (score 3) response by d 3 and a slight or severe (score 4, 1/4) response by d 7. Very slight (score 1) oedema was observed in 2/4 animals at 1 h, with a slight (score 2) reaction in 1/4 animals on d 2 and 3. Slight oedema was seen in 2/4 rabbits until d 7.

1:50 dilution: Slight (score 1) or well-defined (score 2) erythema was observed in 3/4 animals at 1 h. Slight or moderate (score 3) erythema in 2/4 animals on d 3, and moderate oedema in one animal was observed on d 7. Well-defined (score 2) oedema was present in one animal on d 2, and persisted until d 7. One rabbit showed slight oedema on d 3 after decontamination, and not thereafter.

1:100 dilution: Slight (score 1) or well defined (score 2) erythema and slight oedema (score 1) were observed in one animal throughout the post-treatment observation period. Slight erythema response seen in another rabbit at 1 h after decontamination persisted for 2 d.

1:200 dilution: Slight (score 1) erythema was observed in 2/4 animals at 1 h, persisting in one animal for 3 d, and not thereafter. No signs of oedema were seen.

Table 30: Mean erythema and oedema scores*

Dilution (v/v)	Time after decontamination									
	Mean Score									
	1 h		1 d		2 d		3 d		7 d	
	E	O	E	O	E	O	E	O	E	O
Undiluted	2	2	2	1	2.5	2	3	1.75	3	2.25
1:2	2	1.25	2	0.75	2	1.75	2.75	2	3	2
1:5	2	1	1.75	0.75	2.25	1.25	2.25	1	2.5	1
1:10	2	1	2	0.25	2.5	1.5	2.5	1.25	2.25	2
1:25	1.75	0.5	2	0.25	2	1	2.25	1	2.5	0.5
1:50	1.25	0	0.75	0	1	0.5	1	0.5	0.75	0.5
1:10	1	0.25	0.5	0	0.75	0.25	0.5	0.24	0.5	0.25
1:20	0.5	0	0.25	0	0.25	0	0.25	0	0	0

*E = erythema, O = oedema

Conclusions: The undiluted test substance, 1:2, 1:10 and 1:25 (v/v) dilutions were moderate to severe irritants to the rabbit skin following a single 4-h application. 1:50, 1:100 and 1:200 (v/v) aqueous dilutions of paraquat were slight skin irritants in rabbits following a single 4-h application.

Duerden L (1994a) Paraquat dichloride technical concentrate: Skin irritation to the rabbit. Study no: EB4279. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Zeneca Agrochemicals, Study duration: March-April, 1994, Report no: CTL/P/4411, 30, Report date: September 1994.

Quality assured, GLP (compatible with the US EPA, UK, OECD and Japanese-MAFF guidelines) study. No test guidelines were cited.

Species/strain: Rabbit/NZW (Charles River UK, Kent, UK)

Number/sex of animals: 3/Females (bw range: 2.9-3.5 kg)

Observation period: The skin was examined for erythema and oedema at 30-60 min, 1, 2, 3, 4, 7, 14, 17, 20, 21 and 23 days after removal of the test substance. The Draize scale (1959) was used to assess the degree of erythema and oedema.

Method of administration: The undiluted test material (0.5 mL, purity: 33% w/w) was applied to the shorn intact skin of the left flank of each rabbit under occluded conditions. After 4 h, the dressings were removed and the skin was cleaned with warm water to remove any residual test substance, and dried with tissue paper.

Results: Very slight erythema (score 1) was observed in all 3 animals until d 2 after decontamination, persisting in 2/3 animals for 3 d, and in one rabbit for 23 d. Very slight oedema (score 1) was seen in 2/3 rabbits at 30-60 min after decontamination. This response was resolved in one rabbit by the next day, but persisted in the other animal until d 4. Additional signs of irritation were confined to one rabbit, and included desquamation, thickening, scabbing of the skin at the application site and the appearance of new skin.

Table 31: Mean erythema and oedema scores

	Time after decontamination					
	30-60 min	**1 d**	**2 d**	**3 d**	**4 d**	**7 d**
Mean score	**Erythema**					
	0.7	1	1	0.7	0.3	0.3
	Oedema					
	0.7	0.3	0.3	0.3	0.3	0

Conclusions: According to OCS guidelines, the observation of slight irritation at 72 hours indicates paraquat is a slight skin irritant in rabbits following a single 4-h application.

4.3.2.3 Skin sensitisation

Thompson JD, Cushman JR & Wong ZA (1985) Modified Buehler test for the skin sensitisation potential of paraquat concentrate (SX-1465). Study no: SOCAL 2355, Lab: Chevron Environmental Health Centre, Richmond, CA USA, Sponsor: Chevron Chemical Co. Richmond CA USA. Study duration: June 5-July 15, 1985. Report no: R2/34, Report date: October 3, 1985.

Quality assured GLP (US EPA FIFRA & TSCA) study. A modification of the test described by Buehler (1965) was used.

Study & observations: A group of 45 male Hartley guinea pigs (Charles River, MA, USA) weighing 369-528 g were used in the study. Of these, 15 guinea pigs each received 10 occluded, epicutaneous induction applications of 0.3 mL of 1% solution of paraquat 3 concentrate [(w/w), SX-1465, paraquat content: 34%, brown liquid, Chevron Chemical Co, CA, USA) in distilled water on the shorn right flank for 6 h/d, on alternate days for 22 days. The first induction application was made on a Hill Top Chamber, whereas for the 9 remaining applications, 2.5 cm^2 gauze pads were used. A group of 10 guinea pigs treated similarly, but with distilled water only served as controls. The dose level used in the study was based on the results of a preliminary screening study using 10% or 1% solutions of the test formulation. After 6-h, the patches were removed and the application sites were cleaned. After the first induction application, the irritation responses were graded at 24 or 48 h post patch removal, whilst thereafter, irritation grading was done only at 24 h after the 5^{th} and 10^{th} applications. Skin irritation was evaluated using a modification of the Draize scoring system (1959).

Fourteen days after the last induction application, the animals were challenged with 0.3 mL of a 1% solution of the test formulation, applied on the skin of the shorn left flank of each animal on a Hill Top Chamber for 6 h as previously described. Approximately 24 h after the challenge dose, skin patches were removed and irritation responses graded.

An additional group of 10 guinea pigs each received a solution of 0.1% 1-chloro-2, 4 dinitrobenzene (DNCB) in 80% ethanol and acetone (w/w) for induction and challenge, respectively, and served as positive controls (data provided). A group of 10 animals treated likewise with 80% ethanol or acetone served as concurrent positive controls. During the study, the test animals were individually housed under standard laboratory conditions and provided with food (RGP Diet, Labsure, UK) and water *ad libitum.* Prior to initiation of the study, samples of 1, 5 and 10% solutions of the test formulation were collected to analyse the concentration of the test substance in the respective solutions.

Findings: Analysis of the test solution indicated that the paraquat concentrations in the dosing solutions were within ± 10% range of the nominal concentrations. Slight erythema (score 1) was seen in 1/15 at 24 and 48 h after the first induction exposure. Twenty-four hours after the 5th induction exposure, 6/15 animals had irritation scores ranging from slight (score 1) to severe (score 4) erythema together with no (score 0) to moderate (score 3) oedema. Severe erythema was seen in one animal by the 10th application, with no irritation responses being observed in the remaining test animals and corresponding controls. Following challenge, 4 test animals showed slight erythema but no oedema, with an equal number of animals in the control group responding similarly. As expected, the animals in the DNCB group showed skin responses such as well defined (score 2) to severe erythema and no to well defined (score 2) oedema following challenge.

Conclusions: Based on these results, the 1% preparation of the paraquat 3 concentrate (SX-14650) formulation in distilled water was not a skin sensitiser in guinea pigs. The number of animals included in the test substance group in this study was low (15), compared to the minimum of 20 test animals that has been specified in the current OECD guideline for skin sensitisation studies using the Buehler method however, it was consistent with the OECD guidelines of the time.

4.4 Short-Term Repeat dose Studies

4.4.1 Oral Administration

4.4.1.1 Mice

Sotheran MF, Clapp MJL, Banham PB, Doss A & Taylor K (1979a) Paraquat: 28-day preliminary feeding study in the mouse. Report no. CTL/P/426. Lab/Sponsor: ICI Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: 16 May 1977 – 11 July 1979. Report date: 11 July 1979.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Crystalline paraquat dichloride (100% purity; batch no. ADY M 76G; ICI Ltd., Plant Protection Division, Yalding, Kent, UK) was diluted in water (unspecified source) to yield a 1% solution of paraquat cation. Experimental diets were prepared by mixing 10 kg Porton Mouse Diet (Oaks Ltd., Congleton, Cheshire, UK) with 10% w/v water containing the appropriate volume of 1% paraquat solution to yield concentrations of 0 (control), 25, 50, 75, 100, 125 and 200 ppm. Random samples of each batch of diet were analysed for paraquat content and stability by passing aqueous extracts through a cation-exchange column, and measuring the amount of eluted paraquat cation via a colourimetric assay.

Four-hundred, 19-day old, Swiss-derived SPF mice (200/sex, ICI Ltd., Alderley Park, Cheshire, UK) were acclimatised for a period of 7 days prior to commencement of the study. Initial body weights ranged from 5-15 g. Mice were randomly placed (20/sex) into the following 8 groups: Group 1 (0 ppm – control), Group 2 (25 ppm), Group 3 (50 ppm), Group 4 (75 ppm), Group 5 (100 ppm), Group 6 (125 ppm), Group 7 (200 ppm) and Group 8 (0 ppm, microbiological sentinels). The nominal dose levels of paraquat cation received by mice in Groups 2-7 were equivalent to 3.75, 7.5, 11.25, 15, 18.75 and 30 mg/kg bw/d, respectively.

Five mice were housed per stainless steel cage (ie there were 4 randomly distributed replicates/group) with each cage suspended over collecting trays lined with absorbent paper. One male and one female replicate were started on the test diet each day for 4 days with the actual starting age of the mice ranging from 26-30 days. Animals were supplied with the prepared diets and water (unspecified source) *ad libitum* during the 28-day dietary study, and were housed under standard laboratory conditions.

Urine was collected from replicates from Groups 3, 5 and 7 at week 2 and 4 (2/sex/group). These mice were housed in metabolism cages for 18 h with food and water available *ad libitum*. At termination, lungs and kidneys were removed, and blood collected by cardiac puncture from up to 5 mice/group. Urinary and tissue paraquat content was determined by passing sample extracts through a cation-exchange column and measuring the amount of eluted paraquat cation via a colourimetric assay. The paraquat content of plasma was determined by gas chromatography.

All animals were observed daily for any abnormal clinical or behavioural signs. All animals were observed twice daily and sacrificed if necessary. Body weights were recorded initially and then weekly throughout the study. Food consumption per cage was recorded daily for the first week to assess the palatability of the diet, and thereafter weekly. Terminally ill mice were sacrificed by halothane overdose, with mice designated for organ weight determinations exsanguinated by cardiac puncture. At necropsy, tissues and organs from all mice were examined for any gross abnormalities. The liver, kidneys, spleen, testes, lungs, heart and brain from up to 10 mice/sex from Groups 3, 5 and 7 were weighed.

At weekly intervals, 10 Group 8 mice (5/sex) were randomly selected, sacrificed by CO_2 inhalation and blood samples collected by cardiac puncture. Serum was analysed for antibodies to Sendai virus at weeks 0, 1, 2, and 3, and Mouse Pneumonia virus, Minute virus of mice, Toolans H-I virus, Reovirus type III and Polyoma virus at weeks 0, 2 and 3.

Statistical analysis: Body weights, body weight gain, food consumption, food utilisation and organ weight data were analysed separately for males and females using a Student's t-test.

Results

Dietary analysis: Analysis of paraquat in pelleted diet samples indicated that the mean analytical levels of 27 (n=3), 46 (n=3), 100 (n=20), 125 (n=2) and 205 ppm (n=2) complied with nominal levels of 25, 50, 100, 125 and 200 ppm, respectively. The nominal 75 ppm diet was determined to contain 38 ppm (n=3) which the authors attributed to a weighing error. Consequently, Group 4 animals were fed this diet for 3 weeks, before a replacement diet containing the correct concentration was given. Paraquat was shown to be stable over 35 days at an unspecified temperature as analytical concentrations of 105 and 220 ppm complied with nominal concentrations of 100 and 200 ppm, respectively.

Mortalities & clinical signs: Deaths were confined almost entirely to the highest treatment group, with more Group 7 females dying during the study than males (see Table 32 below). Single deaths occurred in Group 1 males, Group 3 females and Group 6 males. Animals that died during the study showed no clinical signs prior to death. At the termination of the study, 5 female mice were found to be incorrectly sexed and were subsequently excluded from the analysis. A failure in the automatic watering system caused a total of 16 replicates from various

groups to become dehydrated but, according to the study authors, most recovered within a few days following correction of the fault. These replicates were excluded from the analysis.

Body weight & food consumption: Paraquat-related effects on body weight gain were confined to the highest dose group, where males had a significantly lower body weight gain over 4 weeks ($p<0.01$) than the control group. There was a trend of higher food consumption in paraquat-treated mice (ie food was used less efficiently) compared to controls which was statistically significant ($p<0.01$-0.05) at 125 and 200 ppm. There was also a trend of higher food utilisation in paraquat-treated mice. At 125 ppm, food utilisation was statistically higher ($p<0.05$) than the control groups but appeared similar to that seen in the control and other groups. Food utilisation was markedly affected in males at 300 ppm and reflects the significant decrease in body weight gain and increases in food consumption. Although this food utilisation value was not statistically significant compared to controls, it appears as if this finding was treatment-related.

A dose-response relationship was evident between the dietary intake of paraquat and urinary paraquat levels. No paraquat was detected in any of the pooled lung or kidney samples. Virtually no paraquat was detected in plasma samples with only one Group 3 male (0.04 ppm), and two Group 7 males (0.02 ppm) showing levels that were close to the limit of detection of the GC method (0.01 ppm).

A paraquat-related increase in lung weight was evident in high-dose males. No other effects were seen with regard to organ weight differences between paraquat-treated and control animals. No organs were weighed from Group 7 females because of the high mortality in this group. There was no difference in organ:body weight ratios between paraquat-treated and control mice with the exception of the lung:body weight ratio in Group 7 males which was significantly higher ($p<0.05$) than controls. A higher brain:body weight ratio ($p<0.05$) was seen in Group 3 females, but this was considered to be incidental.

Table 32: Effects of dietary-administered paraquat on mice over 28 days (excluding results from animals affected by water deprivation)

Group	1 0 ppm	2 25 ppm	3 50 ppm	4 75 ppm	5 100 ppm	6 125 ppm	7 200 ppm
Deaths							
♂	1/18	0/19	0/20	0/20	0/18	1/20	5/20
♀	0/20	0/20	1/20	0/20	0/20	0/20	14/20
Body weight gain (g)							
♂	22.8±3.7 n=12	22.2±2.8 n=15	21.3±3.6 n=15	21.5±2.5 n=10	21.4±2.7 n=9	22.4±3.1 n=19	17.7±4.8** n=10
♀	13.1±2.8 n=15	11.8±2.2 n=20	12.5±2.6 n=14	12.2±4.2 n=15	12.2±0.8 n=5	12.5±1.6 n=15	14.0±3.4 n=4
Food Consumption (g/mouse)							
♂	174±6.3 n=14	194±15.6 n=15	182±12.6 n=15	177±36 n=15	181±13.7 n=10	207±4.6** n=20	264±53* n=15
♀	138±7.2 n=15	148±19 n=20	169±18.7 n=14	175±22 n=15	165±0 n=15	173±14* n=15	186±14* n=6
Food Utilisation ⇊							
♂	7.9±0.7 n=14	8.7±0.5 n=15	8.5±0.4 n=15	9.1±1.4 n=10	8.7±1.8 n=10	9.2±0.4* n=19	13.1±3.2 n=15
♀	10.7±1.4 n=15	12.6±0.5 n=15	13.6±1.5 n=15	15.1±4.7 n=15	13.5±0 n=15	14±1.4* n=15	9.5±1.8 n=6
Urinary PQ cation (µg/mL)							
♂							
d 14-17	-	-	5.1	-	14.2	-	16
d 22-23	-	-	3.4	-	6.7	-	20.1
♀							
d 11-15	-	-	4.6	-	14.1	-	17.2
d 21-22	-	-	9.3	-	12.9	-	29.2
Lung Wts (g)							
♂	0.22±0.5 n=10	-	0.24±0.04 n=9	-	0.26±0.04 n=9	-	0.33±0.09* n=7
♀	0.23±0.2 n=10	-	0.22±0.02 n=10	-	0.28±0.12 n=9	-	-
Lung: bw							
♂	0.57±0.096 n=10	-	0.64±0.081 n=9	-	0.63±0.067 n=9	-	1.09±0.61* n=7
♀	0.71±0.069 n=10	-	0.73±0.041 n=10	-	0.88±0.439 n=9	-	-
Microscopic Lung Effects							
Congestion							
♂	0/10	-	2/11	-	0/9	8/10	4/10
♀	0/10	-	0/9	-	2/9	7/10	-
Collapse							
♂	0/10	-	0/11	-	0/9	4/10	7/10
♀	0/10	-	0/9	-	0/9	6/10	-

Data shown are for mice surviving the duration of the experiment; data are expressed as mean ± 1 SD unless specified; ** $p<0.01$; * $p<0.05$; PQ = paraquat; ⇊ = food intake/g bw gained.

Pathology (animals that died during the study): The lungs of all mice that died during the study (with the exception of a single male control) appeared dark red or black, and upon microscopic examination, these were found to be congested. Histology revealed that the lungs of the majority of Group 7 mice had oedema (3/5 male, 8/14 female) with collapsed lungs in 7/14 of females. The lungs of two Group 7 females and one male showed evidence of haemorrhage. Macroscopic evidence of kidney abnormalities were confined to a single Group 6 male which had enlarged kidneys. There was some histological evidence of kidney abnormalities in

paraquat-treated mice with the same Group 6 male and 6/14 Group 7 females showing congestion. A further two Group 7 females showed tubular degeneration or slight hydronephrosis.

Pathology (animals autopsied at week 4): There were no observable macroscopic abnormalities in paraquat-treated animals that were sacrificed at the end of the study with the exception of 4/14 Group 7 males that had dark or patchy lungs. Pale kidneys were observed in 3/20 Group 2 females only.

Histology revealed hydronephrosis in 9/10 control males which was more than double the level seen in the treatment groups. The only microscopic evidence of a paraquat-related effect was lung congestion in 4/7 Group 7 males. Paraquat-treated mice showed signs of lung congestion and collapse (see Table 32 above), which was consistent with observations made on animals that died during the study. Although data on all treatment groups was not acquired, a dose-response trend was evident between the dietary intake of paraquat and the incidence of lung collapse in males and lung congestion in females.

Microbiology: The study authors stated that there was no evidence of antibodies to any of the virus types tested.

Conclusions: The NOEL in Swiss-derived mice fed paraquat for 28 days was 100 ppm (equivalent to 15 mg/kg bw/d paraquat cation), based on microscopic lung abnormalities (congestion and collapse) at and above 125 ppm (equivalent to 18.75 mg/kg bw/d paraquat cation).

Comments: Although this experiment was commensurate with a dose range-finding study, it was considered to be unsuitable for regulatory purposes due to the following issues: the failure of the automatic watering system affected at least one replicate from each group with the sample size of Group 5 and 7 females reduced from 20 to 5 and 4, respectively; Group 4, 5 and 7 males had their sample sizes halved; Group 4 animals were fed an incorrect diet for 3 weeks; 5 females were incorrectly sexed; no control urine, serum or tissue samples were analysed; no individual animal data were provided; organ weights were not measured for all dose groups; no organs were examined from high-dose (200 ppm) females.

Sotheran MF, Clapp MJL, Banham PB & Woollen BH (1979b) Paraquat: Additional 28-day preliminary feeding study in the mouse. Report no. CTL/P/433. Lab/Sponsor: ICI Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: 27 September 1977 – 11 July 1979. Report date: 11 July 1979.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Crystalline paraquat dichloride (100% purity; batch no. ADYM76/G; ICI Ltd., Plant Protection Division, Yalding, Kent, UK) was diluted in water (unspecified source) to yield a 0.05% solution of paraquat cation. All experimental diets were prepared by mixing 20 kg Porton Rat Diet plus Vitamin E (BP Nutrition Ltd., Stepfield, Witham, Essex, UK) and 17.5% w/v water containing the appropriate volume of 0.05% paraquat cation solution to yield concentrations of 0, 12.5, 25 and 50 ppm. Random samples of each batch of diet were analysed for paraquat

content by passing aqueous extracts through a cation-exchange column and measuring the amount of eluted paraquat cation via a colourimetric assay.

One-hundred and five male and 103 female, 19 day old, Swiss-derived SPF mice (ICI Ltd., Alderley Park, Cheshire, UK) were acclimatised for a period of 10 days prior to commencement of the study. At the start of the experiment the mean body weight of males and females was approximately 31 and 27 g, respectively. Twenty males and twenty females were randomly divided into the following 4 groups: Group 1 (0 ppm- control), Group 2 (12.5 ppm), Group 3 (25 ppm) and Group 4 (50 ppm). The nominal dose levels of paraquat cation received by mice in Groups 2-4 were equivalent to 1.88, 3.75 and 7.50 mg/kg bw/d, respectively. Five mice were housed per stainless steel cage (ie there were 4 randomly distributed replicates/group) with each cage suspended over collecting trays lined with absorbent paper. One male and one female replicate were started on the appropriate test diet each day for 4 days with the actual starting age of the mice ranging from 32 to 36 days. Animals were supplied with the test diets and water (unspecified source) *ad libitum* for 28 days, and were housed under standard laboratory conditions.

Urine was collected from one replicate of each group during the last week of the study. These mice were housed in metabolism cages for 18 h with food and water available *ad libitum*. At the termination of the study, blood was collected by cardiac puncture from one mouse/sex/group. Paraquat levels in urine and plasma were determined by RIA.

All animals were observed daily for any abnormal clinical or behavioural signs. Body weights were recorded initially and then weekly. Food consumption per cage was recorded weekly. At the end of the treatment period, all mice were sacrificed by an overdose of halothane and autopsied as soon as possible. All mice were exsanguinated by cardiac puncture and their lungs weighed. Tissues and organs (unspecified) of all mice were examined for gross abnormalities. No histopathology was performed.

Statistical analysis: Body weights, body weight gain, food consumption, food utilisation and lung weight data were analysed separately for males and females by comparing each treatment group against the control group using a Student's t-test.

Results

Dietary analysis: Analysis of the paraquat cation content of pelleted diet samples indicated that the analytical concentrations of 13.5 (n=2) and 26 ppm (n=2) complied with the nominal concentrations of 12.5 and 25 ppm, respectively. The analytical level of 42.5 ppm (n=2) did not comply with the nominal level of 50 ppm as this result fell below the ± 10% acceptability limit. Regardless of this non-compliance, the later diet was fed to Group 4 mice for the duration of the study.

Mortalities & clinical signs: There were no deaths or any abnormal behavioural or clinical signs observed.

Body weight & food consumption: There was no treatment-related effect on body weight gain. Food consumption was marginally higher in paraquat-treated mice compared to the controls with the effect being statistically significant ($p<0.05$) only in males at 25 and 50 ppm. However, in the absence of a dose-response effect and that the level of consumption was similar in both

sexes at 25 and 50 ppm, this finding was not attributed to paraquat. Food consumption data for females were not statistically analysed due to failure in the watering system during week one which caused water to leak over the food of an unspecified number of female mice. Additionally, there was a problem of waste food getting damp in 4 cages of females during the last week of the study. Consequently, intergroup comparisons of total food consumption in females could not be statistically analysed as animals affected by the water leak and the damp waste food were excluded from the analysis.

In females there was a trend of poorer food utilisation (food intake to gain one gram of body weight) in paraquat-treated mice compared to controls (see Table 33 below) but the data were not statistically analysed. In the absence of statistical analysis and due to the very shallow dose-response relationship, this result was considered to be equivocal. Although there was a statistically significant elevation in food utilisation ($p<0.05$) at 50 ppm in males, this effect was not considered to be treatment-related due to the lack of a dose-response relationship.

A dose-response relationship was evident between dietary and urinary paraquat levels with urinary paraquat concentrations relatively consistent between males and females. Paraquat was detected only in plasma from a single male and female from Group 4 (0.01 and 0.012 μg/mL, respectively).

Table 33: Effects of dietary-administered paraquat on mice over 28 days

Group	1 Control		2 12.5 ppm		3 25 ppm		4 50 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Total Food Consumption (g)								
Mean	146	117	152	129	161*	151	161*	152
SD	6.7	-	6.1	-	7.2	-	8.4	-
Food Utilisation (food intake to gain 1 g bw)								
Mean	11.1	15.1	12.7	15.8	11.3	18.4	13.1*	20
SD	0.8	-	1.2	-	1	-	1.4	-
Urinary PQ cation levels								
(μg/mL)	0	0	0.37	0.34	1.20	0.48	1.50	1.50
(μg/mouse)	0	0	2.96	1.36	4.80	4.03	6.00	10.2
Lung: bw ratio								
Mean	0.557	0.711	0.549	0.681	0.544	0.635*	0.549	0.645*
SD	0.045	0.120	0.035	0.243	0.054	0.057	0.042	0.070

* $p<0.05$; PQ = paraquat; SD = standard deviation

Gross pathology: Although a significant decrease ($p<0.05$) in relative lung weight occurred at 25 and 50 ppm in females, this was not considered to be treatment-related due to the absence of a dose-response relationship and also that a similar effect was not seen in males (see Table 33 above). Additionally, paraquat intoxication is typified by an increase in lung weight. There were no other macroscopic abnormalities that could be attributed to paraquat administration.

Conclusions: The NOEL in Swiss-derived mice following administration of paraquat in the diet for 28 days was 50 ppm (equivalent to 7.5 mg/kg bw/d paraquat cation). Significant effects on food consumption, food utilisation and relative lung weight were not considered to be treatment related as the effects were weakly significant and did not follow a dose-response relationship.

Comments: The experimental design and report were commensurate with a dose range-finding study. The main limitations of this study were the absence of individual animal data and the lack of statistical analysis of food consumption data in female mice due to flooding from the automatic watering system. Additionally, no histology was performed on any organs/tissues. This study is however acceptable as a dose-ranging study.

Sotheran MF, Banham PB, Doss A & Weight TM (1979c) Paraquat: A study to compare the toxicity of pure and technical paraquat in mice at two starting ages. Report no. CTL/P/415. Lab/Sponsor: ICI Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: 31 January 1978 – 26 October 1979. Report date: 26 October 1979.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Crystalline paraquat dichloride (100% purity; batch no. ADY M 76G; ICI Ltd., Plant Protection Division, Yalding, Kent, UK) and technical paraquat liquor (32.7% paraquat cation; batch no. S358.1. ICI Ltd., Mond Division, Widnes, Cheshire, UK) were diluted in water (unspecified source) to yield a 1% solution of paraquat cation. Each batch of diet consisted of 25 kg Porton Rat Diet with Vitamin E supplement (BP Nutrition, Stepfield, Witham, Essex, UK) and 17.5% w/v water containing the appropriate volume of 1% paraquat cation solution to yield dietary concentrations of 0, 100, 125 or 150 ppm. Random samples of each batch of diet (except for one batch of control diet) were analysed for paraquat content by passing aqueous extracts through a cation-exchange column and measuring the amount of eluted paraquat cation via a colourimetric assay.

Four-hundred and twenty, 19-day old, SPF mice of unspecified strain (210/sex, ICI Ltd., Alderley Park, Cheshire, UK) were quarantined for a period of 10 days prior to commencement of the study. Fifteen males and thirty females were randomly placed into the following 7 groups: Group 1 (0 ppm – control), Group 2 (100 ppm pure paraquat), Group 3 (125 ppm pure paraquat), Group 4 (150 ppm pure paraquat), Group 5 (100 ppm technical paraquat), Group 6 (125 ppm technical paraquat) and Group 7 (150 ppm technical paraquat). Nominal dose levels of paraquat cation were equivalent to 15, 18 and 22.5 mg/kg bw/d for Groups 2 and 5, 3 and 6, and 4 and 7, respectively. Five mice were housed per stainless steel cage (ie there were 6 randomly distributed replicates/group), with each cage suspended over collecting trays lined with absorbent paper. One replicate of animals was started on the test diet each day for 3 days with the process repeated for the remaining replicates the following week. In this manner 3 replicates were commenced on their diet when 4 weeks of age and the other 3 replicates when 5 weeks of age. The initial body weight of 4-wk old male and female mice was 18–19 g and 16–17 g, respectively, while the initial body weight of 5-wk old male and female mice was 26–27 g and 24–25 g, respectively. Animals were supplied with diet and water *ad libitum* for 28 days and were housed under standard laboratory conditions. At the completion of the study all surviving animals were sacrificed with an overdose of halothane.

Animals were observed daily for abnormal clinical or behavioural signs, while any ill animal was observed twice daily. Body weights and food consumption were measured weekly. Macroscopic examination of all mice was performed as soon as possible after death. Mice designated for lung weight determinations were exsanguinated by cardiac puncture. The lungs

of all mice that died during the study, and a randomly chosen sample of 10 mice/sex/group at termination, were examined histologically.

Statistical analysis: Body weight gain, food consumption, food utilisation and lung weights were analysed by ANOVA separately for males and females of both starting ages. Adjusted treatment group means were compared to control using a Student's t-test. Unspecified 'formal tests' were utilised to detect differences between groups of treated animals and controls, and between mice fed pure and technical paraquat. A combined analysis of the two starting ages was carried out with regard to body weight gain, food consumption and food utilisation data from the 3 week period that was common to both starting ages (week 2-4 for 4-wk old and week 1–3 for 5-wk old mice). Differences in mortalities between mice fed pure and technical paraquat, and mice started on their diet at 4 and 5 weeks of age were analysed using Cochrans Method (1954) for combining evidence from 4-fold tables.

Results

Dietary analysis: Analysis of paraquat cation in pelleted diet samples indicated that analytical concentrations were between 1–22% of nominal concentrations (see Table 34 below). The study authors indicated that the first batches of diet containing pure paraquat were below the acceptable limit of ± 10% of target levels and consequently replacement diets were prepared to meet this acceptable limit.

Table 34: Mean analytical levels of paraquat cation in each batch of diet

Group	Control	Pure paraquat			Technical paraquat		
	1	2	3	4	5	6	7
Nominal levels (ppm)							
	0	100	125	150	100	125	150
Analytical levels (ppm)							
Batch 1	0 (n=1)	88 (n=3)	97 (n=1)	134 (n=3)	98 (n=3)	128 (n=3)	147 (n=3)
Batch 2	-	107 (n=2)	124 (n=2)	145 (n=2)			166 (n=3)
Batch 3							167 (n=3)

Mortalities & clinical signs: No control mice died but 17 deaths occurred in paraquat-treated mice, the majority occurring in females at 150 ppm (see Table 35 below). These deaths comprised 5 animals that were found dead and 12 that were sacrificed as they exhibited some of the following signs: thin, hunched, subdued, piloerection, laboured or rapid breathing, and suspected cyanosis. The general condition of all other animals was described by the study authors as 'good'. Mortalities in 4-week old mice were significantly higher (no p value specified) than 5-week old mice. Additionally, the incidence of death in mice that were fed technical paraquat was significantly higher than animals fed pure paraquat (no p value specified).

Body weight & food consumption: There was no significant treatment-related effect on body weight gain during treatment in 4-wk old mice. Although the body weight gain of 5-wk old mice treated with paraquat was significantly lower than the control at some doses, in the absence of a dose-response relationship this effect was not considered to be treatment-related. Paraquat-treated mice generally consumed less food than controls. Total food consumption in 4-wk old mice treated with 100 ppm pure and technical paraquat, and in males treated with 150 ppm technical paraquat, was significantly lower than the control ($p<0.01$-0.05), but in the absence of a dose-response relationship this result was not considered to be treatment-related. In 5-wk old paraquat-treated mice, all treatment groups except Group 7 consumed significantly

less food ($p<0.01$) than the controls, but in the absence of a dose-response relationship this result was also not considered to be treatment-related. Food utilisation was significantly elevated ($p<0.05$) only in females at the highest dose. In the absence of an effect in males this result was considered equivocal.

The achieved dose of paraquat cation fell throughout the study by 32–41% in 4-wk old males, 20–30% in 5-wk old males, 26–37% in 4-wk old females and 15–25% in 5-wk old females (calculated by reviewing toxicologist). Generally, mice that were dosed from 4 weeks of age had a greater intake of paraquat compared with those dosed from 5 weeks of age.

Gross pathology: The relative lung weights of paraquat-treated mice did not differ significantly from control animals, with the exception of 4 and 5-wk old Group 4 females, and 5-wk old Group 6 females, which were significantly higher than the controls ($p<0.05$). As the magnitude of these effects was small and in the absence of a dose-response trend relationship, relationship with treatment was unclear. The relative lung weight of mice fed pure or technical paraquat was unaffected by treatment.

Gross lung abnormalities, such as dark red or congested appearance were seen in 15/17 mice that died during the study. There was a relatively low incidence of the same gross lung abnormalities in mice that were terminated at the end of the study.

Table 35: Effects of dietary-administered pure and technical paraquat on 4 and 5-week old mice over 28 days

Group	1		2		3		4		5		6		7	
	Control		Pure paraquat						Technical paraquat					
			100 ppm		125 ppm		150 ppm		100 ppm		125 ppm		150 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality														
4-wk old	0	0	0	0	0	0	2	1	0	0	0	1	3	6
5-wk old	0	0	0	0	0	0	0	0	0	1	1	0	1	1
Bw gain to wk 4 (g)†														
4-wk old	22.9	16.1	22.2	14.8	21.7	15.8	21.3	15.4	23.1	15.1	22.3	14.9	21.3	15.1
5-wk old	14.7	8.53	12.7	7.6	13.1	7.7	12.7	***6.5***	13.7	***6.5***	13.8	7.5	13.4	***6.7***
Total food consump. per mouse (g) †														
4-wk old	152	138	**142**	***121***	143	136	**143**	128	**140**	***120***	141	130	146	132
5-wk old	160	130	***140***	***113***	***142***	***109***	***143***	**119**	***144***	***111***	***146***	***125***	154	126
Achieved dose (mg/kg bw/d)†														
Wk 1														
4-week old	-	-	20.4	20.2	26.8	27.0	30.2	30.2	19.2	21.2	24.7	25.5	33.1	35.1
5-week old	-	-	16.2	15.5	21.3	19.3	25.0	25.2	16.7	15.6	20.4	21.9	28.5	27.1
Wk 4														.
4-wk old	-	-	13.0	14.3	15.6	19.0	19.9	22.2	13.0	13.3	16.0	17.8	22.5	25.6
5-wk old	-	-	12.0	13.2	16.0	14.4	18.0	20.8	12.4	13.0	16.3	16.9	20.0	21.6
Food intake per g bw gained†														
4-wk old	6.7	8.7	6.4	8.2	6.6	8.6	6.7	8.4	6.1	8.1	6.3	8.7	6.9	8.8
5-wk old	11.0	15.3	11.2	15.0	10.9	14.4	11.4	**18.5**	10.8	17.3	10.7	16.8	11.5	**18.9**
Relative Lung wt (g) (n=10)†														
4-wk old	0.22	0.23	0.24	0.24	0.25	0.24	0.26	**0.26**	0.24	0.23	0.25	0.24	0.25	0.25
5-wk old	0.26	0.20	0.24	0.21	0.25	0.21	0.27	**0.22**	0.26	0.21	0.26	**0.22**	0.25	0.22
Gross lung lesions														
4-wk old	1	0	0	0	0	0	0$^{\overline{\top}}$	1	0	0	0	1‡	1$^{\overline{\top}}$	2$^{\perp}$
5-wk old	0	0	0	0	0	1	1	1‡	0	1	0‡	0	2‡	1‡
Histopathological lung lesions (n=10)														
4-wk old	0	0	2	3	5	6	6	4$^{\perp}$	2	1	3	4$^{\perp}$	6	3$^{\perp}$
5-wk old	0	0	2	0	4	2	3	6	1	1	4$^{\perp}$	4	7$^{\perp}$	8

n=15 unless indicated; bold values were statistically different to control values at p<0.05; bold and italicised values were statistically different to the control at p<0.01; † = group mean values; ‡ n=14; $^{\overline{\top}}$ n=13; $^{\overline{\top}}$ n=12; $^{\perp}$ n=9; $^{\perp}$ n=5

Histopathology: Observations of alveolar wall thickening, congestion and oedema, were detected in all 15 mice that showed macroscopic lung abnormalities. In mice that were terminated at the end of the study, there was a clear paraquat-related effect on the incidence of these abnormalities across all treatment groups (see Table 35 above). A dose-related increase in the incidence of these abnormalities was seen in 4-wk old males, 5-wk old males fed technical paraquat, and 5-wk old females. The study authors stated that there was no apparent difference in the type or degree of lung lesions between 4- or 5-wk old mice or between mice treated with pure or technical paraquat.

Conclusions: The LOEL following dietary administration of paraquat to 4 and 5-wk old mice for 28 days was 100 ppm (equivalent to 15 mg/kg bw/d paraquat cation), the lowest dose tested, based on the occurrence of histopathological lung abnormalities such as alveolar wall thickening, congestion and oedema. Besides mortalities which were higher in mice treated with technical paraquat compared to mice treated with pure paraquat, there was no evidence to suggest a difference in effect between pure and technical paraquat, or between mice of 4- or 5-wk starting age.

Comments: The following limitations were noted: no individual animal data were provided with the majority of results presented as group means; the absence of standard deviations or errors; replicates within each group were not started on their respective diet at the same time (one replicate was started on the experimental diet per day over 3 consecutive days – if one considers that only a week separated the two age groups of mice this lack of synchrony could have impacted on the results); mice in Groups 2-4 were underdosed for 4 days prior to receiving replacement diets which complied with nominal paraquat levels. Although the dose range was narrow, this was justified by the fact that the experiment was designed to confirm a previous study. While these factors lessen the regulatory value of the study the results support those of the other Sotheran *et al.* studies and it is therefore considered to be of regulatory value.

4.4.1.2 Rats

Hodge MCE, Banham PB, Davies S, Doss A & Taylor K (1980) 28-day study to determine the toxicity of technical paraquat liquor in the Charles River CD rat. Report no. CTL/P/435. Lab/Sponsor: ICI Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: 8 May 1978 – 8 January 1980. Report date: 8 January 1980.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Technical grade paraquat dichloride liquor (32.7% paraquat cation; batch no. S358/2. CTL Ref No. Y00061/009/001; ICI Ltd., Mond Division, Widnes, Cheshire, UK) was diluted in water to yield a 1% stock solution. Each batch of diet consisted of 25 kg Porton Rat Diet with Vitamin E (BP Nutrition, Witham, Essex, UK) and 17.5% w/v water containing the appropriate volume of 1% paraquat stock solution to yield dose levels of 0, 150, 175 or 200 ppm paraquat. Prior to feeding, samples of the prepared diet were analysed for paraquat content by passing aqueous extracts through a cation-exchange column and measuring the amount of eluted paraquat via a colourimetric assay.

Eighty-eight Charles River CD rats (ICI Ltd., Alderley Park, Cheshire, UK), approximately 21-days old, were quarantined for 10 days and then acclimatised for 4 days prior to commencement of the study. Twenty rats were randomly distributed (10/sex) by litter into the following groups: Group 1 (control, 0 ppm), Group 2 (150 ppm), Group 3 (175 ppm) and Group 4 (200 ppm). The nominal doses received by Groups 2-4 were equivalent 15, 17.5 and 20 mg/kg bw/d paraquat ion, respectively. Any litters that had unhealthy pups, extremes of weight or large weight variations were discarded. Initial mean body weight of males and females was approximately 54 and 48 g, respectively. Two rats/sex were housed in multiple racks which contained 20 randomly positioned stainless steel cages. During the first 2 weeks of the study, groups were arranged on the racks in 10 replicates (5 males and 5 females). The number of male replicates was doubled during week 3 and 4 to give replicates of one male per group (ie during week 3 and 4 of the study males were housed individually). Animals were supplied with the test diet and water *ad libitum* for 28 days and were housed under standard laboratory conditions. Eight microbiological sentinel animals were housed in the same room as the experimental animals, 4 receiving 0 ppm (Control) and 4 receiving 200 ppm paraquat cation in their diet. At the completion of the study all surviving animals were sacrificed with an overdose of halothane.

Rats were observed daily for abnormal behavioural or clinical signs, with a detailed examination performed weekly. Body weights were measured weekly. Food consumption and wastage per cage were measured weekly and daily, respectively. Sentinels and test animals that showed any signs of infection were analysed for bacterial and viral pathogens. Animals found dead or moribund were examined macroscopically and their lungs and abnormal organs histologically examined. The lungs of 5 rats/sex/group were weighed and histologically examined. The lungs of all the remaining animals were histologically examined at the end of the study.

Statistical analysis: Body weights, body weight gain, food consumption and organ weights of paraquat-treated animals were compared statistically to control animals using a two-sided Student's t-test.

*Result*s

Dietary analysis: Analysis of paraquat in pelleted diet samples indicated that the average analytical levels of 0, 157, 174 and 201 ppm complied with the nominal levels of 0, 150, 175 and 200 ppm, respectively.

Mortalities & clinical signs: There were no deaths among controls, while a dose-related increase in mortality was evident in paraquat-treated rats (see Table 36 below). Coughing and sneezing noises were heard from an unspecified number of animals from all groups throughout the study. A brown, stained, encrusted mucus was also observed around the nose of an unspecified number of animals. These observations suggested the presence of a respiratory tract infection.

Body weight & food consumption: A significant ($p<0.01$) dose-related decrease in body weight was observed in males at and above 150 ppm. In females, week 4 body weights were statistically lower ($p<0.01$-0.05) than the controls at and above 175 ppm. A dose-response relationship was evident between the dietary intake of paraquat and total food consumption in males with all paraquat-treated animals showing significantly lower total food consumption ($p<0.01$) than control animals. This lower food consumption was evident at, and persisted from, week 3. Females also showed a paraquat-related decrease in total food consumption with Group 2 and 3 animals having significantly ($p<0.01$-0.05) lower total food consumption than control animals. Total food consumption in Group 4 females was not statistically different to control animals, however, weekly food consumption data indicated that Group 4 females had consumed significantly less ($p<0.01$) than control animals at week 3. The low sample size of Group 4 females ($n=4$) may have contributed to this apparent lack of a statistical difference with control animals. A dose-related increase in food conversion efficiency was apparent in both males and females, but the statistical significance of this observation was not indicated by the study authors.

Table 36: Effect of dietary-administered paraquat on Charles River CD rats over 21 days

Study Parameter	Group 1 Control		Group 2 150 ppm		Group 3 175 ppm		Group 4 200 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Mortalities	0	0	0	1	1	1	4	4
Body weight (g)								
Pre-experimental								
Mean	55	49	54	49	54	49	55	49
SD	5.7	4.3	4.7	3.2	4.2	3.3	4.1	3.5
Week 4								
Mean	256	172	224**	161	204**†	151**†	195**‡	154*‡
SD	18.9	19.2	21.1	9.9	17.5	10.2	23.2	20.8
Total food consumption/week (g)								
Mean	573	448	519**	413*	481**	399**	474**	419
SD	15.7	23.2	9.5	21.9	21	20.4	33.9	15.8
Food Conversion Efficiency ⧺	2.9	3.6	3.1	3.7	3.2	3.9	3.4	4.0
Lung weight (g) (n=5)								
Mean	1.28	0.96	1.16	0.97	1.14	0.96	1.47	1.10
SD	0.15	0.78	0.03	0.15	0.18	0.18	0.35	0.30
Lung:bw								
Mean	0.52	0.58	0.54	0.62	0.59	0.66	0.75	0.74
SD	0.04	0.04	0.05	0.08	0.11	0.13	0.18	0.25
Incidence of gross lung abnormalities in dead or moribund rats								
	0/0	0/0	0/0	1/1	1/1	1/1	4/4	6/6
Histopathology at termination								
Alveolar thickening, oedema, congestion &/or macrophages in lumen	1/5	0/5	2/5	3/5	3/5	3/5	4/5	4/5
Lymphocytic infiltration	4/5	5/5	5/5	4/5	4/5	5/5	5/5	5/5

n=10 unless indicated otherwise; SD = standard deviation; † = n=9; ‡ = n=6; ⧺ = food intake per gram of body weight gained; * $p<0.05$; ** $p<0.01$.

Gross Pathology: There was no significant difference between the lung weights of paraquat-treated and control rats although the lung weights of high-dose animals were higher than those from all other groups (see Table 36 above). There was a statistically insignificant trend of increased relative lung weight in paraquat-treated rats, with more variability found in high-dose animals. Postmortem examination of rats that were found dead or moribund during the study indicated that all displayed gross lung abnormalities such as red or white spots/patches and congestion.

Microbiology: *E. coli* was isolated from one experimental female, while no unusual pathogenic bacteria were detected in any of the other samples. A haem-absorbing virus of the myxovirus group, which according to the authors was likely to be Sendai virus, was isolated from one female sentinel, 4 experimental animals (2 male and 2 female) and 2 stock animals. The same virus was also isolated from lung preparations of 4 stock animals. These findings indicated the presence of a viral pathogen in the study and stock population.

Histopathology: Of the 11 animals that died during the experiment, 10 had alveolar wall thickening, oedema, congestion and/or macrophages in the alveolar lumen with 8 also having peribronchial and/or perivascular lymphocytic infiltration. These results are consistent with gross pathological observations of lung abnormalities in the same rats. Histopathological examination of animals that survived the duration of the experiment indicated a dose-related

increase in the incidence of alveolar wall thickening, oedema, congestion and/or the presence of macrophages in the alveolar lumen. The majority of animals that were sampled from all groups were found to have peribronchial and/or perivascular lymphocytic infiltration, possibly due to a respiratory tract infection.

Conclusions: This 28-day dietary rat study demonstrated effects at all doses tested, with a LOEL of 150 ppm (equivalent to 15 mg/kg bw/d paraquat cation) based on decreased body weight gain in males, decreased food consumption and histopathological lung abnormalities (alveolar wall thickening, oedema and congestion). The results are confounded by the concurrent presence of a respiratory tract infection in rats across all groups. This is particularly pertinent to paraquat toxicity which is known to target the lungs in particular. Despite these confounding factors the results are consistent with other studies and are considered of regulatory value.

Comments: Limitations noted in this study included: the absence of individual animal data; the presence of a respiratory tract infection in animals from all dose levels which may have increased animals' susceptibility to paraquat poisoning.

Guttman E, Banham PB & Lindsay S (1981) 21-day feeding study in the rat to determine the toxicity of 150 ppm paraquat. Report no. CTL/P/566. Lab/Sponsor: ICI Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: 13 Nov - 4 Dec 1979. Report date: 14 January 1981.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods: Technical grade paraquat liquor (32.7% paraquat cation; batch no. Y00061/009/002; ICI Ltd. Mond Division, Widnes, Lancs, UK) was incorporated into Porton Combined Diet (PCD) (BP Nutrition Ltd., Essex, UK) to yield a paraquat concentration of 150 ppm. Dietary paraquat levels were analysed in random samples by an unspecified method. Eighty female and forty male, 21-day old, SPF Wistar rats (Animal Breeding Unit, ICI Ltd., Alderley Park, Cheshire, UK) were acclimatised for 6-7 days, weighed and randomly distributed among control and treatment groups using a randomised block design made up of replicates. The initial body weights of the animals were unspecified. Control animals (30 females and 15 males) received the untreated diet for 21 days while paraquat-treated animals (30 females and 15 males) received diets containing 150 ppm (equivalent to 15 mg/kg bw/d) paraquat for 21 days. Two females were housed per cage while males were housed individually. Animals were housed under standard laboratory conditions.

Rats were examined daily for clinical signs. A macroscopic examination was performed on any animal found dead or moribund. All other animals were sacrificed by cervical dislocation and discarded after the completion of the study. No further experimental details were provided.

Results: Dietary analysis indicated that the paraquat concentration in the feed was 162 ppm compared to the nominal concentration of 150 ppm. No treatment-related effects were observed in any animal. One control female was found dead and partially cannibalised on day 2. No further findings were presented.

Conclusions: This study indicated that 4-week old Alderley Park rats could tolerate the level of 150 ppm paraquat (equivalent to 15 mg/kg bw/d) in their diet over 21 days. The study authors

concluded that a dietary level of 150 ppm paraquat cation (equivalent to 15 mg/kg bw/d) could be used as a maximum tolerated dose (MTD) in a future multi-generation study.

Comment: As this was a dose-ranging study, the standard range of parameters were not examined.

Farnworth MJ, Heylings JR & Wheeldon EB (1994) Paraquat: the effect of multiple oral doses in the rat. Report no. CTL/R/1178. Lab/Sponsor: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: 31 January 1994.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Paraquat dichloride (33.0% paraquat cation; batch no. YF6219; ICI Chemicals and Polymers, Widnes, Cheshire, UK) was diluted in deionised water (Y04517/015) to yield stock solutions of 12, 18, 24, 30, 36 and 42 mg/mL paraquat. The study authors reported that HPLC analysis of the 12 mg/mL stock solution revealed a concentration of 11.5 mg/mL.

Female Alderley Park Rats of approximately 200 g body weight and of unspecified age (BABU; location unspecified) were acclimatised for 4 days prior to commencement of the study. Housing conditions were unspecified. All rats received pelleted Porton Controlled diet (Special Diet Services, Witham, Essex, UK) and water (unspecified source) *ad libitum*. Animals were housed under standard laboratory conditions.

In a preliminary study, 10 rats/group were dosed daily with deionised water or 12 mg/kg bw paraquat cation at a dose volume of 1 mL/kg. In the subsequent dose-response study, 5 rats/group were dosed daily with 18, 24, 30, 36 and 42 mg/mL paraquat cation at a dose volume of 1 mL/kg for 3 days. Rats were terminated at 72 h with an overdose of halothane. Each animal was observed throughout the study for clinical signs. Body weights were recorded prior to dosing on each day and then prior to termination. Blood samples (0.25 mL) were collected (5/group) during the preliminary study from the peripheral tail vein daily before dosing, and by cardiac puncture at termination. The remaining animals (5/group) in the preliminary study had 0.25 mL blood samples taken only before the initial dose and on termination. All rats from the dose-response study had 0.25 mL blood samples taken at termination by cardiac puncture. Plasma samples were stored at -4°C prior to analysis.

Plasma paraquat concentrations were determined by RIA. The following clinical chemistry parameters were measured: urea, creatinine, AST and ALT. AST and ALT activities were not determined in the dose-response samples. Hct was the only haematological parameter measured. After termination, all rats were macroscopically examined with particular attention given to the gastric mucosa. The pH of the stomach contents was recorded. The stomach, lungs, liver and kidneys were weighed, and histopathologically examined. No further experimental details were provided.

Results

Preliminary study: Data generated from rats that were administered 12 mg/kg bw/d paraquat cation and bled daily were considered by the study authors to be invalid due to a progressive reduction in Hct in both control and treated rats. In rats that were bled pre-dose and then at termination, no deaths, clinical signs, effects on plasma urea, creatinine AST, ALT, Hct or gastric pH occurred. No gastric lesions, macroscopic or histopathological abnormalities were observed. Plasma paraquat concentrations were below the limit of detection.

Dose-response study: Mean (± SEM) plasma paraquat cation concentrations were 0.00, 0.11 ± 0.07, 0.18 ± 0.09, 0.23 ± 0.07 and 0.36 ± 0.05 μg/mL at dose levels of 18, 24, 30, 36 and 42 mg/mL, respectively (r=0.97).

A single death was reported at 24 mg/kg bw/d which was suggested by the study authors to be possibly due to mis-dosing or inhalation of the dose solution. Respiratory sounds similar to wheezing were heard in 4/5 rats at 42 mg/kg bw/d. The study authors indicated that an unspecified number of rats from the control and other treatment groups displayed some clinical signs including hunching and respiratory sounds.

There was no treatment-related effect on plasma urea, creatinine or Hct, however, plasma urea levels appeared to be lower at 42 mg/kg bw/d compared to 18 and 24 mg/kg bw/d (see Table 37 below). In the absence of a dose-response relationship and control data for plasma creatinine, urea and Hct, these findings were considered to be equivocal.

Table 37: Effect of paraquat on plasma urea levels and gastric pH in female rats

Dose (mg/kg bw/d)	**0 (control)**	**18**	**24**	**30**	**36**	**42**
Plasma urea (mg/dL)						
Mean	NS	42.20	36.5	32.00	35.00	28.80
SEM	NS	2.33	1.32	3.16	1.64	1.11
Gastric pH						
Mean	NS	2.50	2.06	1.87	2.24	1.85
Range	NS	1.85–4.05	1.79–.43	1.52–2.43	1.56–4.1	1.55–2.63

NS = not specified

Gastric pH was similar across all treatment groups (see Table 37 above) but in the absence of control data this finding was considered to be equivocal. The study authors stated that the pH values for paraquat-treated rats in the dose-response study were significantly lower (p value unspecified) than the controls (pH 3.5-4.0) from the preliminary study.

Macroscopic stomach abnormalities included fluid engorgement in one rat treated at 18 mg/kg bw/d, and clear mucosal irritation/erosion in 1/5 and 3/5 rats at 24 and 42 mg/kg bw/d, respectively. Similar effects were not seen at intervening doses. Additional macroscopic abnormalities were observed in rats at 42 mg/kg bw/d and included gas distended caeca (4/5), pale red areas on the lungs and red areas on the thymus (2/5). Histopathological examination revealed an unspecified incidence of erosions in the mucosa of the glandular stomach at 36 and 42 mg/kg bw/d. Lung abnormalities indicative of early alveolitis were detected at and above 30 mg/kg bw/d. The study authors reported a dose-related increase in mild hydropic abnormalities in the kidneys at 36 and 42 mg/kg bw/d.

Conclusions: Female Alderley Park Rats tolerated 3 daily oral doses of 12 mg/kg bw/d paraquat cation. This dose was selected as being equivalent 1/8th of the single oral maximum lethal dose (MLD). The absence of any detectable toxicological effect at this dose probably reflected the absence of paraquat cation in the plasma (ie low bioavailability at this dose). The NOEL in the dose-response experiment was 24 mg/kg bw/d paraquat cation based on histopathological signs of alveolitis in the lungs at and above 30 mg/kg bw/d, and erosions in the glandular stomach and hydropic changes in the kidneys at and above 36 mg/kg bw/d.

Comments: Both studies had a number of limitations which made them inadequate for regulatory purposes. The following were unreported: control data for creatinine, urea, Hct and gastric pH; AST, ALT and body weight data. No historical control data were provided and no statistical analyses were performed. Housing conditions and the exact method of oral administration were unspecified, and only a few haematology and clinical chemistry parameters were analysed.

4.4.1.3 Rabbits

Horner SA (1992) Paraquat: Investigation of the influence of dosing concentration, volume and gastric irritation on oral toxicity in the rabbit. Report no. CTL/T/2786. Lab/Sponsor: ICI Central Toxicology Laboratory Alderley Park, Macclesfield, Cheshire, UK. Study duration: December 1991 – January 1992. Report date: 31 March 1992.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Paraquat liquor (33.42% w/w paraquat cation; batch no. YF6219; ICI Chemicals and Polymers, Widnes, Cheshire, UK) was diluted in deionised water (CTL ref no. Y04517/012) to yield dosing solutions of 0.33, 0.67, 1.00, 1.33, 2.00, 4.00 and 6.00 mg paraquat/mL. Samples of each bulk preparation were analysed for paraquat concentration and stability by HPLC.

Thirteen female NZW rabbits (Interfauna UK Ltd., Huntington, Cambridgeshire, UK), of unspecified age and body weight, were acclimatised for at least 2 weeks prior to commencement of the study. Rabbits were housed individually in mobile rabbit units (unspecified source) with CRB pellets (Labsure Animal Diets, Lavender Mill, Manea, Cambridgeshire, UK) and tap water available *ad libitum* throughout the study. Animals were housed under standard laboratory conditions.

In phase 1 of the study, 2 rabbits/group received a single dose of 2 mg/kg bw paraquat cation at dose volumes of 1, 3 or 6 mL/kg from stock solutions of 2.00, 0.67 and 0.33 mg/mL, respectively. The control group received deionised water at a dose volume of 6 mL/kg. In phase 2 of the study, one rabbit/group was administered 6 mL/kg of a 1.33 mg/mL stock to yield a dose level of 8.0 mg/kg bw/d, 6 and 1mL/kg of 0.67 and 4.00 mg/mL stocks, respectively, to yield a dose level of 4.0 mg/kg bw/d, or 6 and 1mL/kg of 1.00 and 6.00 mg/mL stocks, respectively, to yield a dose level of 6.0 mg/kg bw/d. All animals were dosed once daily for up to 10 consecutive days using a rubber dosing and a disposable syringe.

All animals were examined twice daily for abnormal clinical signs. Body weights were recorded daily. Food consumption of phase 1 animals was measured at 3-4 day intervals, while

for phase 2 animals it was measured daily. Prior to dosing, 0.25 mL blood samples were taken from an ear vein or artery of phase 1 rabbits on day 1 and 10, and at 0.25, 0.5, 1, 2, 4, 7 and 24 h after dosing. Blood samples from phase 2 rabbits were taken immediately before dosing on day 1, and at 0.25, 0.5, 1, 2, 4, 7 and 24 h after dosing. Additional blood samples were taken from phase 2 animals 48 h after the first dose of 4.0 and 6.0 mg/kg bw/d, and from the rabbit dosed at 8.0 mg/kg bw/d at 72 h after the first dose.

Plasma urea and creatinine were measured in blood samples taken from the rabbit treated with 8.0 mg/kg bw/d following its termination on day 4. Plasma urea and creatinine were also measured in blood samples from all remaining phase 2 animals at 0, 24, 48, 72 h after the first dose or prior to termination. All animals were terminated on day 10 or sacrificed in a moribund condition by an iv injection of pentobarbitone sodium solution. Plasma paraquat concentrations were measured by RIA. A macroscopic post mortem examination was performed on all animals including an assessment of the stomach, small intestine and contents. No histopathological examinations were performed.

Results

Phase 1: The mean analytical paraquat cation concentration in the dose solutions were 0.33, 0.67 and 1.88 mg/mL, which were within 6% of the nominal concentrations of 0.33, 0.67 and 2.0 mg/mL, respectively. A 0.33 mg/mL solution of paraquat cation was determined to be stable for up to 19 days at an unspecified temperature.

One animal that received 3 mL/kg of the 0.67 mg/mL stock solution was sacrificed in a moribund condition on day 9, but in the absence of any deaths at the highest or lowest dose volume, this finding was not considered to be treatment-related. There were no clinical signs that could be attributed to paraquat and no treatment-related effect on body weight or food consumption.

There was a trend of increased plasma paraquat levels with increased dose volume at day 1, but this was not evident at day 10. At day 1, peak plasma paraquat concentrations were observed at 0.5-2 h after dosing, with 6 mL/kg resulting in higher concentrations (up to 1.603 μg/mL) than the 3 and 1 mL/kg dose volumes (up to 1.14 and 0.85 μg/mL, respectively). At day 10, peak plasma paraquat concentrations were observed at 0.5-4 h after dosing and were generally comparable across all treatment groups (up to 0.74 μg/mL at 6 mL/kg, up to 0.48 μg/mL at 3 mL/kg and up to 0.68 μg/mL at 1 mL/kg).

Although no macroscopic abnormalities were reported, both animals treated with 6 mL/kg had prominent Peyer's patches on the ileum or jejunum which were not evident in any other animals.

Phase 2: The mean analytical concentrations of paraquat cation in the dose solutions were 0.68, 1.03, 3.76 and 5.8 mg/mL which were within 6% of the nominal concentrations of 0.67, 1.0, 4.0 and 6.0 mg/mL, respectively.

The rabbit dosed at 6.0 mg/kg bw/d in a dose volume of 1mL/kg was found dead on day 4 while all other animals were sacrificed in a moribund condition on day 4. All animals showed inappetance and body weight loss prior to death or sacrifice. Additionally, all animals passed few or no faeces.

Rabbits administered 4.0 mg/kg bw/d at 6 or 1 mL/kg had peak plasma levels of 1.29 and 1.30 μg/mL, respectively at 1-2 h after the first dosing. Rabbits administered 6.0 mg/kg bw/d at 6 or 1 mL/kg had peak plasma levels of 2.19 and 1.81 μg/mL, respectively at 1 h after the first dosing. The rabbit administered 8.0 mg/kg bw/d at 6 mL/kg showed peak plasma levels at 0.25-1 h post-treatment with progressively lower levels detected up to 24 h. At 72 h and after 3 doses, the plasma paraquat concentration was 5.55 μg/mL which was markedly higher than that measured at 24 h and after a single dose (0.33 μg/mL).

Plasma urea levels were elevated in all rabbits at 48-72 h after the first dose, excluding the rabbit administered 4.0 mg/kg bw/d at 1 mL/kg (see Table 38 below). These data also suggested a trend of increased plasma urea with increased dose or dose volume. Plasma creatinine levels were elevated at 72 h in rabbits administered 8.0 mg/kg bw/d at 6 mL/kg or 6.0 mg/kg bw/d at 1 mL/kg. In the absence of control data and due to the low sample size, these findings were considered to be equivocal.

All rabbits exhibited macroscopic GIT abnormalities but there was no clear evidence of an effect of dose volume. At 4.0 mg/kg bw/d, fluid stomach contents and/or glandular depressed areas, and gassed-filled areas in the small or large intestine, were observed. At 6.0 mg/kg bw/d, rabbits showed marked irritation of the stomach (oedema, reddening of the glandular region, sloughing off of mucosa), and some distension of the small intestine which also had fluid-filled contents. The rabbit administered 8.0 mg/kg bw/d showed more severe irritation of the stomach and distension of the small intestine. At 4.0 mg/kg bw/d and a dose volume of 1 mL/kg, slight pelvic dilation of the right kidney was observed, while pale kidneys were seen at 6.0 and 8.0 mg/kg bw/d, with multiple pale depressed areas also observed at 8.0 mg/kg bw/d. All rabbits showed macroscopic lung abnormalities including dark areas at 4.0 mg/kg bw/d, red areas/spots at 6.0 mg/kg bw/d and multiple red areas at 8.0 mg/kg bw/d.

Table 38: Effect of paraquat dose volume on plasma urea and creatinine levels in rabbits

Group	4.0 mg/kg bw/d (at 6 mL/kg)	4.0 mg/kg bw/d (at 1 mL/kg)	6.0 mg/kg bw/d (at 6 mL/kg)	6.0 mg/kg bw/d (at 1 mL/kg)	8.0 mg/kg bw/d (at 6 mL/kg)
Urea (mg/100 mL)					
0 h	37	41	49	43	45
24 h	34	33	62	35	34
48 h	39	35	182	40	not recorded
72 h	81	44	dead	166	215
Creatinine (mg/100 mL)					
0 h	1.2	1.2	1.2	1.0	1.0
24 h	1.2	1.2	1.2	1.1	1.1
48 h	1.2	1.3	1.6	1.2	not recorded
72 h	1.5	1.2	dead	4.2	9.9

Conclusions: No evidence of toxicity was observed in rabbits administered a repeat oral dose of 2 mg/kg bw/d paraquat cation at dose volumes of 1, 3 and 6 mL/kg for up to 10 days. There appeared to be an initial dose-volume-related increase in plasma paraquat concentrations, however this was not evident by the end of the study. Toxicity was observed in all rabbits administered 4.0, 6.0 and 8.0 mg/kg bw/d paraquat cation at dose volumes of 1 or 6 mL/kg and included mortality, constipation, inappetance and body weight loss, increased plasma urea and macroscopic signs of GIT irritation, lung and kidney abnormalities. At the highest dose (8.0 mg/kg bw/d) an elevation in plasma creatinine also occurred. Based on these 2 studies, the

NOEL was 2 mg/kg bw/d paraquat. There was no clear evidence of an effect of dose volume on paraquat toxicity.

Comments: This study was commensurate with a preliminary or dose range-finding study only.

Farnworth MJ, Heylings JR & Wheeldon EB (1993b) Paraquat: Effect of multiple oral doses in the rabbit. Report no. CTL/R/1170. Lab/Sponsor: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: 16 December 1993.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Paraquat dichloride (33.0% w/w paraquat cation; batch no. YF6219, CTL reference No Y00061/160; ICI Chemicals and Polymers, Widnes, Cheshire, UK) was diluted in deionised water to formulate a stock solution of 6.5 mg/mL paraquat cation. Female NZW rabbits (Frozfields, UK) were acclimatised for at least two weeks prior to the study. The initial body weight of the animals ranged from 4421-5252 g (age unspecified). All animals received STAN RAB SQC diet (Special Diet Services, Stepfield, Essex, UK) and water *ad libitum*. Animals were housed under standard laboratory conditions.

Prior to dosing, all rabbits were bled via a peripheral ear vein, and weighed. Rabbits were divided into two groups and then dosed orally (method unspecified): Group 1 (n=4) was dosed with 1 mL/kg of deionised water (vehicle control) and Group 2 (n=8) was dosed with 1 mL/kg of the 6.5 mg/mL paraquat solution per day for 72 hours. Each animal was examined during the study for clinical signs. Food consumption was recorded daily. Body weights were recorded, and blood samples (0.75 mL) taken from peripheral ear veins, prior to dosing on all days. All blood samples were stored at -20°C until analysis. All animals were sacrificed at 72 h with an overdose of Euthatal.

Plasma paraquat concentrations were determined by RIA. Plasma creatinine, urea, AST, ALT and Hct were measured. Gross necropsy was performed on all animals with particular attention given to the gastric mucosa. The stomach, lungs, liver, kidneys and duodenum were weighed and examined histopathologically. The pH of the stomach contents was also measured using a glass electrode. No details of statistical analyses were provided.

Results

Mortalities & clinical signs: One paraquat-treated rabbit died at 72 h, with 4 others described as being slightly subdued. An unspecified number of animals showed reduced faecal output. There were no deaths or clinical signs in the control group.

Plasma paraquat levels: Paraquat was detected in the plasma of 7/8 treated rabbits at 24 h and in the plasma of all treated rabbits at 48 and 72 h. Plasma paraquat levels rose sharply over 72 h (see Table 39 below).

Body weight & food consumption: Paraquat-treated rabbits were reported to consume little or no food at 48 and 72 h. Paraquat-treated rabbits showed a 5.19 ± 0.98% decrease in body weight

over 72 h, but this result was not statistically analysed. Although the magnitude of this effect was small, the fact that it occurred over a relatively short period of time suggests that it was treatment-related.

Clinical chemistry & haematology: There was no treatment-related effect on Hct or on AST or ALT activities. There was a significant treatment-related increase ($p<0.005$-0.01; statistical test unspecified) in plasma creatinine and urea compared to controls. Positive correlations were established between serum paraquat concentrations and plasma urea ($r=0.728$) and creatinine ($r=0.966$).

Gross pathology: The study authors stated that there were no differences in organ weights between paraquat-treated and control rabbits, although no supporting data were presented. The majority (5/7) of paraquat-treated rabbits exhibited macroscopic changes in the glandular area of the stomach. Three paraquat-treated animals were classified as having mild changes (fluid engorgement), with one individual also exhibiting reddening and ulceration. Two paraquat-treated animals had mucosal erosion. The stomach contents of most paraquat-treated rabbits (unspecified number) showed various levels of turbidity. The pH of the gastric contents of paraquat-treated animals was marginally lower than controls, but the statistical significance of this finding was not determined.

Table 39: Effects of daily oral administration of paraquat on female rabbits

Study parameter	**Group 1 (control)** (n=4)	**Group 2 (6 mg/kg)** (n=8)
Serum paraquat concentration		
24 h	0.000 µg/mL	0.12 ± 0.04 µg/mL
48 h	0.000 µg/mL	0.84 ± 0.14 µg/mL
72 h	0.000 µg/mL	1.23 ± 0.34 µg/mL
Start body weight	4858 ± 159 g	4890 ± 85.96 g
Finish body weight	4811 ± 174 g	4603 ± 110.04 g (n=7)
72h Hct	0.41 ± 0.01	0.44 ± 0.02 (n=7)
72h Creatinine	1.08 ± 0.05	3.6 ± 0.85* (n=7)
72h Urea	45.50 ± 2.18	129.30 ± 19.45** (n=7)
pH gastric contents	1.28 (log scale)	1.05 (log scale) (n=7)

Results expressed as mean ± SEM; *p<0.05; **p<0.01

The majority of paraquat-treated rabbits exhibited macroscopic liver and kidney abnormalities. No abnormalities were seen in the lungs or duodenum. Four rabbits exhibited abnormalities in the liver and kidneys (pale livers and accentuated lobular patterns on the liver surface; pale areas of the kidney, streaking of the cortex and reddened areas), while one rabbit had abnormalities only in the liver. Of the 4/7 rabbits with liver and kidney effects, one also had enlarged kidneys and another a distended bladder with creamy coloured urine.

Histopathology: Gastric lesions/abnormalities noted macroscopically could not be confirmed histopathologically. Additionally, macroscopic liver changes were histologically confirmed in only one individual. The kidneys of all paraquat-treated rabbits exhibited hydropic changes that extended beyond regional glomeruli and into the subcapsular region of the cortex. Protein casts were detected in the S2 proximal tubules of all treated rabbits. Low level interstitial nephritis and periportal inflammation in the liver, were detected in both control and paraquat-treated animals and were considered to be background findings (possibly due to an infection). There were no treatment-related histopathological effects on either the lung or duodenum.

Conclusions: The LOEL in NZW rabbits following oral administration of paraquat cation for 3 days was 6 mg/kg bw/d, the only dose tested, based on significant elevations in serum urea and creatinine levels, and macroscopic and histopathological kidney abnormalities at this dose. Macroscopic stomach and liver abnormalities were also observed, but these findings were not confirmed by histopathology.

Comments: This study did not conform to current OECD test guidelines in its design or level of reporting detail but was commensurate with a pilot investigation. The following details were unspecified: statistical analyses; organ weights; food consumption data. Other limitations included inconsistencies in the reporting of experimental parameters and results and the deceased animal did not appear to have been examined. The study authors' conclusion that there was a clear association between elevated plasma paraquat, renal damage and macroscopic changes to the stomach, was made difficult considering the low sample size, the use of only one dose level and that there was no trend evident between blood paraquat and gastric pH. There was however a clear correlation between elevated serum paraquat levels and renal damage.

4.4.1.4 Dogs

Sheppard DB (1980) Paraquat 6 week oral (capsule administration) dose range finding study in beagle dogs. Report no. CTL/C/973. Lab: Hazelton Laboratories Europe Ltd., Harrogate, England. Sponsor: ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Study duration: 10 October – 12 December 1979. Report date: 1 April 1980.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Technical grade paraquat liquor (32.2% paraquat cation; batch no. Y00061/009/004; ICI Ltd., Mond Division, Runcorn, Cheshire, UK) was diluted in distilled water to produce stock solutions of 5, 15, 30 and 60 mg/mL paraquat. The paraquat concentration of each stock solution was spectrophotometrically analysed at various times during the study. Four groups of pure bred Beagle dogs (1/sex/dose, ICI Ltd., Alderley Park, Macclesfield, UK) received paraquat in gelatin capsules at 0 (distilled water), 0.25, 0.75, 1.5 or 3.0 mg/kg bw/d for 6 weeks. The study was initiated with the two highest doses, but due to overt toxicity, two lower doses were introduced after 3 weeks. Control dogs were treated for a maximum of 9 consecutive weeks.

Prior to delivery, dogs were treated for parasites, and vaccinated against distemper, infectious canine hepatitis, *Leptospira canicola* and *L.icterohaemorrhagiae*. On arrival dogs were 4-6 months old and weighed 10.5-12.9 kg. Dogs were acclimatised for 5 weeks and were housed individually in rooms under standard conditions. They were offered 400 g of Laboratory diet A (BP Nutrition UK Ltd., Stepfield, Witham, Essex, UK) after dosing in the morning, with any remaining diet removed at the end of the day. Water was available *ad libitum*.
All dogs were observed periodically for clinical signs during the day. A detailed clinical examination was performed prior to treatment and again pre-terminally. Body weight and food consumption were recorded weekly and daily, respectively.

Fasted blood samples (unspecified volume) were collected from the jugular vein prior to treatment and then after 2, 4 and 6 weeks. Additional blood samples were collected from both dogs at 3.0 mg/kg bw/d to confirm any treatment-related effects, and then prior to sacrifice from the 1.5 mg/kg bw/d group. The following haematology parameters were measured: Hb, PCV, RBC, MCH, MCV, MCHC, WBC, platelet count, reticulocyte count, PT and APTT. The following clinical chemistry parameters were measured: glucose, cholesterol, BUN, total protein, ALP, ALT, AST, Na, K, Cl, Ca, Mg, phosphate and triglycerides.

Twenty-four hour urine samples were collected from water-deprived dogs, at pre-dose and then weekly throughout the study, by direct catheterisation. Additional fresh urine samples were collected from both dogs in the 3.0 mg/kg bw/d group during week 2 and 3 to confirm suspected treatment-related findings. The following urinary parameters were measured: volume, specific gravity, pH, protein, ketones, glucose, blood, bilirubin, reducing agents, urobilinogen and microscopy of spun deposits. Ophthalmoscopy was performed on all dogs, once prior to treatment and again pre-terminally. Eyes were treated with 1% tropicamide approximately 15 min before examination. Auscultation was performed on all dogs pre-dose and pre-terminally.

All dogs were sacrificed by an iv overdose of thiopentone sodium, exsanguinated immediately by severance of the major neck vessels and necropsied. Lung and kidney weights were recorded. Histopathology was performed on the following tissues/organs: adrenals, aorta, bone marrow, brain, caecum, colon, duodenum, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, rib and bone marrow, sciatic nerve, skin and mammary gland, spinal cord, spleen, stomach, salivary gland (submaxillary), testes, thymus, thyroid, tongue, trachea, skeletal muscle, urinary bladder, uterus, and all unusual lesions.

Results

Paraquat dose analysis: Spectrophotometric analysis of paraquat in the 5, 15, 30 and 60 mg/mL stock solutions revealed concentrations of 5.54-5.76, 16.6-16.9, 33.9-40.0 and 70.8-78.7 mg/mL, respectively.

Mortalities & clinical signs: Males dosed at 1.5 and 3.0 mg/kg bw/d were sacrificed in a moribund condition on day 17 and 9, respectively. The female treated at 3.0 mg/kg bw/d was found dead on day 28, while the female at 1.5 mg/kg bw/d was sacrificed in a moribund condition on day 21. The study author reported that all these deaths were preceded by marked loss of appetite, respiratory distress, subdued behaviour and lethargy. No deaths or clinical signs were reported in any other dogs.

Body weight & food consumption: At 1.5 and 3.0 mg/kg bw/d, a slight reduction in body weight was noted at the time of death/sacrifice. In the 2 males, this effect was at or below 2% of pre-treatment body weight values. The 2 females at 1.5 and 3 mg/kg bw/d, exhibited a 2.4 and 13% reduction in body weight, respectively. As there was only 1 dog/sex/group, these results were considered to be equivocal. Incidental variations in body weight occurred in the other treated dogs, while the body weights of the male and female controls increased by 12 and 2%, respectively over the treatment period.

At the time of death/sacrifice, the 2 males at 1.5 or 3 mg/kg bw/d showed a 19.3 and 46% reduction in weekly food consumption, respectively. At the same doses in females, reductions

of 6.4 and 57%, respectively were noted. These results suggested an effect of treatment on food consumption but as there was only one dog/sex/group, no definitive conclusion could be made. The food consumption of dogs at 0.75 or 0.25 mg/kg bw/d was relatively stable over the experimental period. The control male showed a 39% increase in food consumption over the experimental period, while the control female maintained a relatively stable level of consumption.

Haematology & clinical chemistry: There was a trend of increased Hb in male and female dogs dosed at 1.5 and 3.0 mg/kg bw/d compared to their pre-dose value (see Table 40 below) while this was not evident in the control or other treatment groups. A similar pattern of increase was observed for RBC in males at and above 1.5 mg/kg bw/d and in females at 3.0 mg/kg bw/d. Although these results suggested a treatment-related effect, in the absence of sufficient samples they were considered to be equivocal. There was no treatment-related effect on any of the clinical chemistry parameters tested.

Urinalysis: At 3.0 mg/kg bw/d, protein was detected in urine from the male dog at week 1 and 2, and in the female at week 2–4 prior to death. There was an increase in urinary protein at week 3 in dogs at 1.5 mg/kg bw/d paraquat. No perturbation was evident in any other urinary parameter.

Table 40: Effect of administration of paraquat capsules on beagle dogs

Study Parameter	Control		0.25 mg/kg bw/d		0.75 mg/kg bw/d		1.5 mg/kg bw/d		3.0 mg/kg bw/d	
Hb (g/dL)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pre-dose	15.2	15.9	17.3	16.1	16.6	17.1	15.3	15.5	16.5	15.2
Wk 2	15.0	15.6	17.3	16.9	17.1	16.8	16.0	19.0	17.2†	18.4
Wk 3	-	-	-	-	-	-	17.8†	18.3†		-
Wk 4	15.0	15.0	17.6	15.9	16.1	15.8				20.3†
Wk 6	15.0	16.2	17.0	17.3	16.3	18.2				
RBC (g/dL)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pre-dose	6.74	6.96	8.0	6.85	7.56	7.69	6.76	7.09	7.63	6.77
Wk 2	6.59	6.86	8.3	7.42	7.81	8.02	6.93	8.19	7.73†	7.48
Wk 3	-	-	-	-	-	-	7.8†	7.66†		-
Wk 4	6.69	6.68	7.96	6.86	7.13	7.06				8.79†
Wk 6	6.79	7.15	7.88	7.34	7.38	8.43				

Wk = week; † = terminal blood sample

Ophthalmoscopy & stethoscopy: The study author reported that both dogs at 1.5 mg/kg bw/d, showed marked retinal vascular engorgement prior to death, while evidence of marked pulmonary congestion was observed in dogs at 1.5 and 3.0 mg/kg bw/d.

Pathology: There was no perturbation in relative kidney weight, while males at 1.5 or 3.0 mg/kg bw/d had a higher relative lung weight than the control (1.61 and 1.77, respectively versus the control value of 1.02). An elevated relative lung weight was detected in the female treated with 1.5 mg/kg bw/d (2.16 versus 0.96 for the control), however no effect was seen at 3.0 mg/kg bw/d (1.06). Macroscopic examination revealed the presence of lung lesions in dogs at and above 0.75 mg/kg bw/d. These lesions were pink to light purple, pale or greyish, raised, up to 50 mm in diameter and affected most of the lobes. Histopathological examination confirmed the lesions and also revealed kidney degeneration and/or lesions at and above 1.5 mg/kg bw/d.

Conclusions: The NOEL following capsular administration of paraquat cation for up to 6 weeks in beagle dogs was 0.25 mg/kg bw/d based on the presence of lung lesions at 0.75 mg/kg bw/d. There was evidence of a treatment-related effect on food consumption at and above 1.5 mg/kg bw/d, and on body weight at 3 mg/kg bw/d, but due to the small sample size all findings (including the NOEL) were considered to be equivocal.

Comments: This study was commensurate with a dose range-finding study but was not considered suitable for regulatory purposes due to the low sample size (1/sex/dose).

Sheppard DB (1981a) Paraquat 6 week oral (capsule and dietary administration) toxicity in beagles. Report no. CTL/C/1073. Lab: Hazelton Laboratories Europe Ltd., Harrogate, England. Sponsor: ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Study duration: April – June 1980. Report date: 1 June 1981.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Paraquat was supplied as a technical grade liquor (32.2% paraquat cation; batch no. Y00061/009/004; ICI Ltd., Mond Division, Runcorn, Cheshire, UK) and administered to three randomly-assigned groups of Beagle dogs (3/sex/group) (ICI Ltd., Alderley Park, Macclesfield, Cheshire, UK) for 6 consecutive weeks at dietary levels of 35 or 90 ppm (equivalent to 0.875 and 2.25 mg/kg bw/d paraquat, respectively), or in gelatin capsules at 0.75 mg/kg bw/d paraquat. The diet was prepared by adding a suitable volume of paraquat stock solution to a small amount of expanded and reground Laboratory diet A (B.P. Nutrition UK Ltd., Stepfield Witham, Essex, UK) in a pestle and mortar to form a pre-mix. This pre-mix was diluted with untreated diet to produce a 20 kg batch. The stability and concentration of the paraquat stock solution was analysed using a spectrophotometric method. The paraquat levels in the diets were analysed (during weeks 1, 3 and 5) using a colorimetric assay.

Prior to delivery, dogs were treated for parasites, and vaccinated against distemper, infectious canine hepatitis, *Leptospira canicola* and *L. icterohaemorrhagiae*. On arrival dogs were 5–6 months old and of 7.3–18.2 kg bw, while at the start of treatment dogs were 7–9 months old. Dogs were offered 400 g of the test or control diet once daily and those that received the capsules were dosed once in the morning immediately before feeding. These dogs also received 400 g of untreated diet. Water was available *ad libitum* throughout the study. Dogs were individually housed under standard conditions.

All dogs were observed periodically for clinical signs during the test period. A detailed clinical examination was performed prior to treatment and again pre-terminally. Body weight and food consumption were recorded weekly and daily, respectively. Fasted blood samples (unspecified volume) were collected by jugular venipuncture prior to treatment and then after 3 and 5 weeks. The following haematology parameters were measured: Hb, MCV, RBC, MCH, PCV, MCHC, WBC. The following clinical chemistry parameters were measured: glucose, BUN, ALT and AST. Urine samples were collected from water-deprived dogs at unspecified times using a catheter. The following urinary parameters were measured: specific gravity, pH, protein, ketones, glucose, blood, bilirubin, reducing agents, urobilinogen and microscopy of spun deposits. Ophthalmoscopy was performed on all dogs using a hand held Keeler direct ophthalmoscope, once prior to treatment and again pre-terminally. Eyes were treated with 1%

tropicamide approximately 15 minutes before examination. Auscultation was performed on all dogs prior to treatment and pre-terminally.

Dogs were sacrificed by an iv overdose of thiopentone sodium and exsanguinated immediately by severance of the major neck vessels. A full internal and external post-mortem examination was performed. Lung and kidney weights were recorded. Histology was performed on the following tissues/organs: adrenals, aorta, bone marrow, brain, caecum, colon, duodenum, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, rib and bone marrow, sciatic nerve, skin and mammary gland, spinal cord, spleen, stomach, salivary gland (submaxillary), testes, trachea, thymus, thyroid, tongue, skeletal muscle, urinary bladder, uterus, and all unusual lesions.

Statistical analysis: Group data were compared to a concurrent control group from a 13-wk feeding study. Body weight data were analysed using an ANOVA followed by a Students t-test.

Results

Dietary analysis: Analysis of various batches of paraquat stock solution indicated that they were within 3.0% of the nominal concentration, and were stable for up to 23 days. Dietary analysis revealed that the nominal paraquat concentrations of 35 and 90 ppm complied with the analytical concentrations of 32-35 and 96-99 ppm, respectively, at week 1, 37.3 and 94 ppm, respectively at week 3, and 38.4 and 95.5 ppm, respectively, at week 5. These data also indicated that the dietary concentrations of paraquat were stable for up to 5 weeks. The homogeneity of the diet was not tested.

Mortalities, clinical signs, body weight & food consumption: There were no deaths or clinical signs reported during the study. There was a statistically insignificant reduction in group body weight gain in males at 35 and 90 ppm paraquat compared to a concurrent 13-wk control (see Table 41 below). The reliability of these observations is doubtful due to the low body weight gain of the control group. Females at 0.75 mg/kg bw/d (capsules), 35 or 90 ppm paraquat showed a statistically lower ($p<0.05$–0.01) body weight gain than the concurrent 13 week control, with the 90 ppm group actually losing body weight during the 6-wk treatment period. Females at 90 ppm showed a trend of reduced food consumption from week 4–6, however, no statistical analysis was performed on these data.

Table 41: Effect of dietary or capsular administration of paraquat on beagle dogs (n=3)

Study parameter	Control†	20 ppm†	Capsular (0.75mg kg bw/d)	35 ppm	90 ppm
Group bw gain ♂ Wk –1 – 6 (g)	50	-170	170	-200	-600
Group bw gain ♀ Wk –1 – 6 (g)	750	100	170**	370*	-400*
Lung:bw ratio (mean ± 1 SD)					
♂ ♀	0.90 ± 0.087 0.83 ± 0.097	0.84 ± 0.118 0.94 ± 0.013	1.11 ± 0.174 1.19 ± 0.027	0.92 ± 0.103 1.0 ± 0.113	1.08 ± 0.184 1.5 ± 0.323

*p<0.05; **p<0.01; † = data taken from a concurrent 13-wk dietary study and used for comparison

Ophthalmoscopy & auscultation: At the pre-terminal examination, two females fed 90 ppm paraquat displayed injected retinal vessels in both eyes, while a single female at 35 ppm exhibited pronounced retinal vessels. A female, dosed at 0.75 mg/kg bw/d (capsular) had a small area of pigment missing on the tapetum negrum. No other ophthalmoscopic anomalies were observed. Auscultation at week 6 revealed that all high-dose dogs (90 ppm) had a low to marked increase in respiratory sounds, while 2/3 control males exhibited slight or moderate respiratory sounds, and only one female had low sounds. Two females dosed at 35 ppm also had a dull or irregular heartbeat, which according to the study author, is typical of beagle dogs of this age.

Haematology, clinical chemistry & urinalysis: There were no treatment-related effects on any clinical chemistry or urinary parameters. Although the study author drew attention to a slight, progressive decrease in red blood cell parameters (Hb, RBC and PCV), this was not considered to be treatment-related as the result was statistically insignificant, no age-matched historical control data was provided for comparison, the data was variable (overlapping standard deviations), and no such result was observed in females (which showed other toxicological signs such as decreased food consumption and body weight gain, increased respiratory sounds and macro/microscopic lung lesions).

Pathology: There was no treatment-related effect on relative kidney weight. The relative lung weight of high-dose females (90 ppm) appeared to be higher than all other groups (see Table 41 above), but in the absence of any statistical analysis the significance of this result could not be determined. At necropsy, all female dogs treated with 0.75 mg/kg bw/d (capsules) or 90 ppm paraquat exhibited gross lung lesions which were described as purple, red/grey, pink/grey or red/brown depressed areas that ranged in size from a few mm to involvement of the majority of the lobe. Only a single male from the 0.75 mg/kg bw/d group and 2 from the 90 ppm group displayed gross lung lesions. Following histopathological examination, alveolitis was detected in all dogs administered paraquat capsules (0.75 mg/kg bw/d) and 5/6 dogs fed 90 ppm paraquat. A single male from the 35 ppm group showed signs of alveolitis. Although these observations would appear to be treatment-related, in the absence of control data the relationship with treatment is unclear.

Conclusions: The LOEL for dietary administration of paraquat cation to beagle dogs over 6 weeks was 35 ppm (equivalent to 0.875 mg/kg bw/d). At 35 ppm, female dogs had a significantly lower body weight gain ($p<0.05$) than a concurrent 13-wk control group. At 90 ppm (equivalent to 2.25 mg/kg bw/d), decreased body weight gain, clinical signs (increased respiratory sounds), ophthalmoscopic abnormalities (injected retinal vessels – females only), macroscopic lung lesions and alveolitis were observed in both males and females. Dogs that received a daily capsular dose of 0.75 mg/kg bw/d paraquat cation exhibited similar gross and histopathological lung abnormalities as those fed 90 ppm paraquat.

Comments: Generally, this study was poorly designed due to the absence of a conventional control group, the use of only 2 dose levels and the low group sizes (n=3). The use of a concurrent control from a parallel 13-wk dietary study was not ideal as this experiment was started 7 weeks prior to the current study. No standard deviations were given for the mean body weight or food consumption data. Details of statistical analyses were unspecified. No gross pathological or histopathological results for the concurrent 13-wk control were provided. No historical control data was provided. The results for the dogs administered paraquat via a

gelatin capsule were considered to be preliminary as no vehicle control was tested. Overall this study was not considered suitable for regulatory purposes.

4.4.2 Inhalational Administration

4.4.2.1 Rats

Anonymous (1965) Subacute aerosol inhalation toxicity of ortho paraquat (emulsifiable concentrate of paraquat dichloride). Report no. CTL/P/194. Lab: unspecified. Sponsor: ICI Ltd., (unspecified location). Study duration: unspecified. Report date: 25 June 1965.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Ortho paraquat (21.2% paraquat cation; batch no. unspecified; ICI Ltd., unspecified location) in water was administered to Sprague-Dawley albino rats (5/sex, unspecified source) as an aerosol of 1% (v/v) (0.24 mg paraquat cation/L of air; equivalent to 240 mg/m^3) for 6 h/d for 5 d/wk for 3 weeks (whole body). The control rats (5/sex) received aerosols of water. The age of the rats was unspecified, while the mean initial body weights were 73 and 89 g for control and paraquat-treated males, and 92 and 86 g for control and paraquat-treated females, respectively. Rats were housed individually in stock cages (unspecified source) with food (Wayne Lab-Blox, Allied Mills, Chicago, IL, USA) and water available *ad libitum* throughout the study. No further details of housing and feeding conditions were provided.

All rats were exposed to aerosols of paraquat in a Rochester Inhalation Chamber, which had a capacity of 1.3 m^3. An Ohio Nebuliser was used to introduce the paraquat aerosols into the chamber. The total metered air flow through the chamber was 800 L/min. The mean concentration of paraquat in the chamber was determined to be 0.11 mg/L for the 15 exposures.

Mortalities and clinical signs were observed daily. Body weight was recorded at the commencement of the study and thereafter weekly. Blood samples (unspecified volume) were collected from 3 rats/sex/group at the end of the study by an unspecified means. The following haematology parameters were measured: Hb, Hct, RBC, WBC and WBC-DC. ALP and BUN were the two clinical chemistry parameters measured. At the end of the study all animals were sacrificed by an unspecified means and a gross pathological examination performed. The lungs, liver, kidneys and trachea were histopathologically examined. No further experimental details were given.

Results

Mortalities, clinical signs & body weight: No mortalities or clinical signs were observed during the study. The total mean body weight gain of paraquat-treated males was approximately 28% (89 g) lower than the control group (124 g). In the absence of statistical analyses, a similar effect in females or individual animal data, this result was considered to be equivocal.

Haematology: Paraquat-treated rats showed a slight elevation in Hb, leukocytes and neutrophils compared to the controls (see Table 42 below), but in the absence of pre-dose baseline values,

statistical analysis or historical control data, this was not considered to be a treatment-related effect. There was no treatment-related effect on any other haematology parameter.

Table 42: Effect of repeat exposure to paraquat aerosols on haematology and clinical chemistry parameters

Study parameter	Control		0.24 mg paraquat cation/L	
	♂	♀	♂	♀
Hb (g/100 mL)	13.5 ± 0.53	13.83 ± 0.45	14.67 ± 0.15	15.10 ± 0.66
Leukocytes (thousands/mm^3)	21.30 ± 2.72	14.33 ± 1.70	24.50 ± 4.10	19.10 ± 3.20
Neutrophils (no cells/100)	10.67 ± 6.03	14.33 ± 4.62	21.67 ± 8.62	22.00 ± 8.72
ALP activity (Bessey-Lowry Units)	8.49 ± 2.57	8.31 ± 1.24	7.81 ± 1.09	5.71 ± 1.15
BUN (mg urea N/100 mL)	20.60 ± 2.16	23.40 ± 4.33	16.83 ± 1.19	21.13 ± 2.80

Data are expressed as the mean ± SD. Calculated from individual animal data by the reviewing toxicologist.

Clinical chemistry: The data are given in Table 42 above. Paraquat-treated females showed reduced ALP activity relative to the control group, but no such effect was observed in males. Paraquat-treated males showed a depression in BUN relative to the control group but no effect was seen in females. In the absence of pre-dose baseline values, statistical analysis or historical control data, it was uncertain whether these observations were treatment-related.

Pathology: No treatment related gross or histopathological abnormalities were observed.

Conclusions: There was no clear evidence that aerosols of paraquat administered at a concentration of 0.24 mg/L (equivalent to 240 mg/m^3) for 6 h per day, on 15 occasions over 3 weeks, were toxic to rats.

Comments: This study was considered inadequate for regulatory purposes. The following details were unspecified: age of the rats; laboratory/cage temperature and humidity; equilibration of the exposure chamber; method of blood sampling; termination method. Only a single dose-level was tested. No statistical analysis was performed. No standard deviations/errors were included with the mean body weight data. No individual body weight data were provided. No historical control data was provided. No pre-dose haematology or clinical chemistry measurements were taken. No particle size or stability analysis was performed. Few haematology and only two clinical chemistry parameters were measured. Additionally, only a few organs/tissues were histopathologically examined.

Hardy CJ, Grimshaw P, Cobb LM, Lewis DJ & Prentice DE (1979) Three week inhalation study in rats exposed to an aerosol of paraquat. Report no. ICI 254/7949. Lab: Huntingdon Research Centre, Huntingdon, Cambridgeshire, UK. Sponsor: ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Study duration: 9 August 1978 – 13 October 1978. Report date: 8 June 1979.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Sprague Dawley CD rats (150/sex; Charles River UK Ltd., Manston, Kent, England) were exposed (whole body) to aerosols of paraquat (40% paraquat cation; batch no. Y 0061/009/002; ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK) in distilled water at 0 (n=32), 0.01 (n=36), 0.1 (n=36) or 1.0 (n=36) µg/L for 6 h/d, on 15 occasions over

3 weeks (equivalent to 0, 0.01, 0.10 and 1.00 mg/m^3, respectively). Rats were randomly assigned to these groups and the highest dose was reduced to 0.5 μg/L (n=16; equivalent to 0.5 mg/m^3) following mortalities after a single exposure.

At the commencement of the study the age and weight of male rats was 26–30 days and 72–96 g, respectively, while females were 28-32 days old and 64-98 g, respectively. Four rats/sex were housed in cages constructed of polypropylene and stainless steel (Type RCI, North Kent Plastic Cages Ltd., Home Gardens, Dartford, Kent, England) under standard laboratory conditions, and were provided with food and water *ad libitum* at all times except during exposure.

Aerosols of paraquat in distilled water were generated using a DeVilbiss ultrasonic nebuliser (Model 65) fitted with a 3 L reservoir and constant feed assembly (DeVilbiss Company, Somerset, Pennsylvania, USA). Exposure was performed in chambers constructed of stainless steel and glass and having an internal volume of approximately 6 m^3. The beginning of the 6 h exposure was measured from the time at which the chamber was estimated to have reached 90% of the final atmospheric concentration (approximately 10 minutes). Rats were placed individually into one of four compartments of equal size in stainless steel mesh cages which were placed in the exposure chamber. Rats were left in the exposure chamber for 15 minutes at the end of the exposure session. The distribution of aerosols in the chamber was determined in preliminary experiments using aerosols of Evan's blue dye and paraquat.

The paraquat concentration in the chamber was determined at least twice during the course of each exposure by drawing chamber air through a glass microfibre filter and then analysing for paraquat using a spectrophotometric method. Particle size estimates were determined once during exposure using a May multistage liquid impinger. Wet and dry bulb temperatures were measured 4 times during each exposure for determination of the relative chamber humidity. Chamber airflow was set at the beginning of each day. Chamber negative pressure was monitored continuously with an inclined manometer and recorded 4 times during each exposure session.
Rats were weighed prior to each exposure and then twice weekly. Food and water consumption per cage were recorded weekly. Clinical signs were examined before and after each exposure session with a complete physical examination (eyes, ears, snout, jaw and feet, palpation of abdomen and respiration) performed at least once a week. On the remaining days, the animals were examined in their holding cages in the morning and afternoon.

Following the 5th and 15th exposures, and on each of the following 3 days, 4 rats/sex/group were sacrificed by an ip injection of Expiral (Abbott Laboratories, Queensborough, Kent, England) and exsanguinated by incision of the jugular and carotid veins. The larynx, trachea, lungs and heart were removed and stored at -20°C for paraquat analysis.

Sixteen rats from the control group and 8 rats from the 0.01, 0.10 and 0.50 μg/L groups were killed by exsanguination under pentobarbitone anaesthesia, and macro/microscopically examined immediately after 3 weeks of exposure or following a 2 week recovery period. The entire respiratory tract was examined for gross abnormalities. No other organs were macroscopically examined. Histopathology was performed on the nasal passages, larynx, pharynx, tongue, trachea and lung.

Statistical analysis: A William's test for contrasting increased dose levels of a substance with a control was used to compare the treatment group means with the control.

Results

Chamber paraquat concentration & particle size analysis: Analytical (mean ± SD) chamber paraquat concentrations of 0.012 ± 0.004, 0.112 ± 0.021 and 0.487 ± 0.100 μg/L complied with the nominal concentrations of 0.01, 0.10 and 0.50 μg/L, respectively. The chamber paraquat concentration at the initial high dose was determined to be 1.17 and 1.38 μg/L, which complied with the nominal concentration of 1.0 μg/L. All particles were determined to be less than approximately 2 μm in aerodynamic diameter and were thus respirable. The study authors indicated that there may have been a slightly greater proportion of particles < 0.5 μm in the 0.01 and 0.10 μg/L chambers compared to the 0.5 and 1.0 μg/L chambers.

Mortalities & clinical signs: The high dose (1.0 μg/L paraquat) was aborted after a single 6 hour exposure, with deaths observed in 28/36 males and 29/36 females from 2-9 days after exposure. Signs of rapid respiration and general malaise were observed 24–48 h before death. Death generally occurred earlier in males than in females with 17/36 males and 9/36 females found dead after 2 days. There were no further deaths. The incidence of a brown nasal discharge following exposure to paraquat appeared to increase with dose (see Table 43 below). It was unclear whether this finding was treatment-related as 2 females from the 0.01 μg/L group exhibited a nasal discharge at pre-dose as well as during the treatment period.

Body weight & food consumption: The mean body weight of males treated with 0.01 and 0.10 μg/L paraquat cation was significantly lower than control animals at day 7 and 21 ($p<0.01$-0.05) but the magnitude of this effect was only 2% and therefore this was not considered to be treatment-related. In females, only the mean body weight of rats treated with 0.10 μg/L was significantly lower ($p<0.05$) than the control group at day 7, but again the magnitude of this difference was only 2%. No body weight data were provided for high-dose (0.5 μg/L) animals. Males treated at 0.01 and 0.1 μg/L consumed statistically less food ($p<0.01$–0.05) than the control group at week 2 and 3 but no effect on food consumption was observed in females. There was no treatment-related effect on water consumption. The lack of body weight and food consumption data at 0.5 μg/L makes a relationship with dose difficult to establish.

Table 43: Effects on Sprague Dawley CD rats following inhalation of paraquat cation for 6 h/d on 15 occasions over 3 weeks

Study Parameter	0 µg/L		0.01 µg/L		0.10 µg/L		0.5 µg/L	
	♂	♀	♂	♀	♂	♀	♂	♀
n	32	32	36	36	36	36	16	16
Mean bw† (g)								
pre-dose	258	171	258	177	259	176	-	-
d 7	311	197	305*	195	301**	193*	-	-
d 21	392	232	378**	232	375**	231	-	-
d 35	429	247	437	253	440*	250	-	-
Mean food consumption (g)								
Wk 2	192	129	176*	130	177**	130	-	-
Wk 3	193	132	178**	135	181*	135	-	-
Clinical signs	(n=32)	(n=32)	(n=16)	(n=16)	(n=16)	(n=16)	(n=16)	(n=16)
Nasal discharge	0	0	0	2‡	3	2	4	9
Histopathology # (3-wk exposure)	(n=16)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
Larynx								
Hyperplasia	0	2	0	0	8	6	6	5
Squamous metaplasia	0	0	0	0	7	6	8	8
Keratinisation	0	2	0	0	7	7	8	8
Ulceration/necrosis	0	0	0	0	1	0	8	8
Lung								
Macrophages, debris, mucus	0	0	0	0	0	0	8	8
Alveolar thickening	0	0	0	0	0	0	4	4
Loss of cilia and Clara cells	0	0	0	0	0	0	6	5
Histopathology # (3-wk exposure and 2-wk recovery)	(n=16)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
Larynx								
Hyperplasia	0	0	1	0	6	1	8	7
Squamous metaplasia	0	0	0	0	7	3	8	7
Keratinisation	0	0	0	0	1	1	8	7
Ulceration/necrosis	0	0	0	0	0	0	0	0
Lung								
Macrophages, debris, mucus	0	0	0	0	0	0	5	5
Alveolar thickening	0	0	0	0	0	0	1	2
Loss of cilia and Clara cells	0	0	0	0	0	0	6	5

*p<0.05; **p<0.01; † = adjusted for pre-exposure bw; ‡ = clinical signs were observed pre-dose and during the study; # = summarised from individual animal data by the reviewing toxicologist

Pathology & histopathology: There were no treatment-related macroscopic abnormalities detected in any rats. Treatment-related histopathological effects were clearly identified in the larynx and lung (see Table 43 above). In rats that were sacrificed and examined immediately after the 3 week exposure period, squamous keratinising metaplasia and/or hyperplasia of the base of the epiglottis and arytenoid projections were observed at 0.1 and 0.5 µg/L in the majority of male and female rats. All rats from the high-dose group (0.5 µg/L) also showed ulceration and/or necrosis which was not detected in rats that were allowed to recover for 2 weeks prior to sacrifice. Keratinisation was also reduced following the 2 week recovery period in rats treated with 0.10 µg paraquat/L, but not 0.5 µg/L. Treatment-related histopathological

effects on the lungs were observed only at the highest dose (0.5 μg/L) and included the presence of macrophages/debris/mucus, thickening of the alveolar wall and the loss of cilia and Clara cells. There was a slight reduction in the number of rats with macrophages/debris/mucus and alveolar wall thickening following the 2 week recovery period.

Conclusions: The NOEL in rats exposed to aerosols of paraquat cation for 6 h per day, on 15 occasions over 3 weeks was 0.01 μg/L (equivalent to 0.01 mg/m^3) based on the occurrence of clinical signs (nasal discharge) and histopathological abnormalities in the larynx (keratinising metaplasia and/or hyperplasia of the epiglottis and arytenoid projections) at 0.10 μg/L (equivalent to 0.10 mg/m^3). These findings also occurred at 0.5 μg/L (equivalent to 0.5 mg/m^3) with the inclusion of ulceration/necrosis in the larynx and histopathological lung abnormalities (macrophages/debris/mucus, alveolar wall thickening and loss of cilia and Clara cells). There was evidence that some of the histopathological anomalies were reversible as rats allowed to recover for 2 weeks showed no signs of lung ulceration/necrosis and a reduction in alveolar wall thickening and the presence of macrophages/debris/mucus.

Comments: Limitations noted in this study include: uneven sample sizes for the chamber control (n=32) and high dose replacement group (n=16) compared to all other groups (n=36); no organs were weighed; no haematology or clinical chemistry parameters were measured. The following details were unspecified: statistical analyses; body weight data for high-dose rats; standard deviations for the group mean body weight and food consumption data; statistical analyses of the high-dose replacement group (there was no comparable control group); results of lung paraquat analysis; historical histopathological control data; and statistical analysis of the histopathology data.

Grimshaw P, Hardy CJ, Cobb LM, Lewis DJ & Prentice DE (1979) Three week inhalation study in rats exposed to an aerosol of paraquat (repeat study). Report no. CTL/C/810. Lab: Lab: Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Sponsor: ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Study duration: 25 October – 21 December 1978. Report date: 6 December 1979.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Thirty-six male and 32 female Sprague Dawley CD rats (Charles River UK Ltd., Manston, Kent, England) per group were exposed (whole body) to aerosols of paraquat technical liquor (approximately 40% paraquat cation; batch no. Y 0061/009/002; ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England) in distilled water at 0 (no aerosol), 0 (saline aerosol), 0.01 and 0.1 μg/L paraquat for 6 h/d, on 15 occasions over 3 weeks (equivalent to 0, 0.01 and 0.1 mg/m^3, respectively). Rats were randomly assigned to these groups.

At the commencement of the study, the age and weight of male rats was 33–36 days and 112–138 g, respectively, while females were 36-39 days old and 106-126 g, respectively. Four rats/sex were housed in cages constructed of polypropylene and stainless steel under standard conditions and provided with food (Spratt's Patent Ltd., Central House, Barking, Essex, England) and water *ad libitum* at all times except during exposure.

Aerosols of paraquat in distilled water were generated using a DeVilbiss ultrasonic nebuliser (Model 65) fitted with a 3 L reservoir and constant feed assembly (DeVilbiss Company, Somerset, Pennsylvania, USA). The concentrations of paraquat required to produce 0.01 and 0.1 paraquat cation/L were 3 and 30 μg/mL paraquat cation, respectively, using a chamber airflow of 1500 L/min. The saline aerosol control was generated in the same manner using a solution of 45 μg/mL sodium chloride in distilled water. Exposure was performed in chambers constructed of stainless steel and glass and having an internal volume of approximately 6 m^3. The beginning of the 6 h exposure was measured from the time at which the chamber was estimated to have reached 90% of the final atmospheric concentration (approximately 10 minutes). Rats were placed individually into one of four compartments of equal size in stainless steel mesh cages which were then placed in the exposure chamber. Animals were left in the exposure chamber for 15 minutes at the end of the exposure session. The distribution of aerosols in the chamber was determined in preliminary experiments using aerosols of Evan's blue dye and paraquat.

The paraquat concentration in the air of each chamber was determined at least twice during each exposure by drawing chamber air through a glass microfibre filter and then analysing for paraquat spectrophotometrically. Particle size estimates were determined once during exposure using a cascade impactor, which consisted of a system of 4 air jets which impinged on a series of 4 filter discs with specific particle retention characteristics. The amount of paraquat cation retained on the filter discs was then measured using a spectrophotometric assay. Wet and dry bulb temperatures were measured 4 times during each exposure for determination of the relative chamber humidity. Chamber airflow was set at the beginning of each day. Chamber negative pressure was monitored continuously with an inclined manometer and recorded 4 times during each exposure session.

Rats were weighed daily prior to each exposure for the first week and then twice weekly. Food consumption per cage was recorded weekly. Water consumption was determined daily over a 5-day period each week. Rats were examined for clinical signs before and after each exposure session with a complete physical examination (eyes, ears, snout, jaw and feet, palpation of abdomen and respiration) performed at least weekly.

Four rats/sex/group were sacrificed by exsanguination under pentobarbitone anaesthesia three days after the first exposure and one day after the third exposure, and the lungs examined for gross abnormalities. A histopathological examination was performed on the nasal passages, larynx, pharynx, tongue, trachea and lungs of these rats. Additionally, the turbinates and nasal passages of rats that were sacrificed one day after the third exposure were macroscopically examined. All remaining rats were sacrificed either at the end of the 3 week treatment period, or following a 2 week recovery period, and a macroscopic examination performed on the nasal passages, larynx, pharynx, tongue, trachea and lungs. No histopathological examination was performed on these later rats.

Statistical analysis: An ANOVA followed by a Student's t-test were used to determine any statistical differences between control and treatment groups.

Results

Chamber paraquat concentration & particle size analysis: Analytical (mean ±1 SD) chamber paraquat concentrations of 0.012 ± 0.004 (n=30) and 0.110 ± 0.011 μg/L (n=30) complied with the nominal concentrations of 0.01 and 0.10 μg/L. All particles were retained in filter disc 4 which indicated that they were 0.5–0.7 μm in aerodynamic diameter and thus respirable.

There were no deaths, treatment-related clinical signs, effects on body weight, food or water consumption. No treatment-related macroscopic abnormalities were detected in the nasal passages, larynx, pharynx, tongue, trachea and lungs. In contrast, treatment-related histopathological effects were observed in the larynxes of interim-sacrificed rats treated with 0.1 μg/L paraquat. Squamous meta/hyperplasia of the epiglottis or some focal metaplasia of the respiratory epithelium was observed in all 8 rats that were sacrificed 3 days after the first exposure. All 8 rats sacrificed one day after the third exposure exhibited areas of ulceration/necrosis, acute inflammatory cell infiltration or squamous meta/hyperplasia in the epithelia, particularly at the base of epiglottis and arytenoid projections.

Conclusions: The NOEL in Sprague Dawley CD rats exposed to aerosols of paraquat for 6 h per day (whole body), on 15 occasions over 3 weeks was 0.01 μg/L (equivalent to 0.01 mg/m^3), based on histopathological evidence in the larynx of metaplasia and/or hyperplasia, ulceration/necrosis and acute inflammatory cell infiltration at 0.10 μg/L (equivalent to 0.10 mg/m^3).

Comments: Limitations noted in this study were that no histopathology was performed on rats sacrificed at the end of the study, no organs were weighed, and no haematology, clinical chemistry or urinary parameters were measured.

4.4.2.2 Guinea Pigs

Anonymous (1965) Subacute aerosol inhalation toxicity of ortho paraquat (emulsifiable concentrate of paraquat dichloride). Report no. CTL/P/194. Lab: unspecified. Sponsor: ICI Ltd. (unspecified location). Study duration: unspecified. Report date: 25 June 1965.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods

Ortho paraquat (21.2% paraquat cation; batch no. unspecified; ICI Ltd., unspecified location) was administered to 5 male and 5 female English strain guinea pigs (unspecified source) as an aerosol of 1% (v/v) in water (0.24 mg/L of air; equivalent to 240 mg/m^3) for 6 h/d, 5 d/wk for 3 weeks. The control group of 5 males and 5 females received aerosols of water. The age of the guinea pigs was unspecified while the mean starting body weights were 295 and 266 g for control and paraquat-treated males, respectively, and 285 and 286 g for control and paraquat-treated females, respectively. Animals were housed individually in stock cages (unspecified source) with food (Wayne Guinea Pig Diet, Allied Mills, Chicago, IL, USA) and water available *ad libitum* throughout the study. No further details of housing and feeding conditions were provided.

All animals were exposed to aerosols of paraquat together in a Rochester Inhalation Chamber (unspecified source) which had a capacity of 1.3 m^3. An Ohio Nebuliser (unspecified source) was used to introduce the paraquat aerosols into the chamber. The total metered air flow through the chamber was 800 L/min. The mean concentration of paraquat in the chamber was determined to be 0.11 mg/L for the 15 exposures.

Mortalities and clinical signs were recorded daily. Body weight was recorded initially and then weekly throughout the study. Blood samples (unspecified volume) were collected from 3 animals/sex/group at the end of the study by an unspecified means. The following haematology parameters were measured: Hb, Hct, RBC, WBC and differential WBC. At the end of the study all animals were sacrificed by an unspecified means and necropsied. The lungs, liver, kidneys and trachea were histopathologically examined. No further experimental details were given.

Results

Mortalities, clinical signs & body weight: No mortalities or clinical signs were observed during the study. Paraquat-treated guinea pigs had an approximately 50% lower body weight gain over the 3 week study period (82 and 55 g for males and females, respectively) compared to the control group (160 and 133 g for males and females, respectively). This result is suggestive of a treatment-related effect on body weight however no statistical analysis was performed.

Haematology & pathology: There was no treatment-related effect on any of the haematology parameters tested and no gross or histopathological abnormalities were observed.

Conclusions: The LOEL in English strain guinea pigs following exposure to aerosols of paraquat for 6 h per day, on 15 occasions over 3 weeks, was 0.24 mg/L (equivalent to 240 mg/m^3) based on an apparent reduction in body weight gain at this dose.

Comments: This study was considered inadequate for regulatory purposes. Only a single dose-level was tested. The following details were unspecified: age of the guinea pigs; laboratory/cage temperature and humidity; equilibration of the exposure chamber; method of blood sampling; termination method. No statistical analysis was performed. No standard deviations/errors were included with the mean body weight data. No individual body weight data was provided. No historical control data was provided. No clinical chemistry parameters were measured. No pre-dose haematology measurements were taken. No particle size or stability analysis was performed. A small number of haematology parameters were measured. Additionally, only a few organs/tissues were histopathologically examined (lungs, liver, kidneys and trachea).

4.4.2.3 Dogs

Anonymous (1965) Subacute aerosol inhalation toxicity of ortho paraquat (emulsifiable concentrate of paraquat dichloride). Report no. CTL/P/194. Lab: unspecified. Sponsor: ICI Ltd. (unspecified location). Study duration: unspecified. Report date: 25 June 1965.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods: Ortho paraquat (21.2% paraquat cation; batch no. unspecified; ICI Ltd., unspecified location) was administered to one male and one female Beagle dog (unspecified

source) as an aerosol of 1% (v/v) in water (0.24 mg paraquat cation/L of air; equivalent to 240 mg/m^3) for 6 h/d, 5 d/wk for 3 weeks. No control was used. The age of the dogs was unspecified while the mean starting body weights were 7.1 and 5.0 kg for the male and female dog, respectively. Dogs were housed individually in stock cages (unspecified source) with food (Purina Dog Chow, Ralston-Purina Co, St Louis, MO, USA) and water available *ad libitum* throughout the study. No details of housing and feeding conditions were provided.

Both dogs were exposed to aerosols of paraquat together in a Rochester Inhalation Chamber which had a capacity of 1.3 m^3. An Ohio Nebuliser was used to introduce the paraquat aerosols into the chamber. The total metered air flow through the chamber was 800 L/min. The mean concentration of paraquat in the chamber was determined to be 0.11 mg/L for the 15 exposures.

Mortalities and clinical signs were observed daily. Body weight was recorded initially and then weekly throughout the study. Blood samples (unspecified volume) were collected from each dog at the end of the study by an unspecified means. The following haematology parameters were measured: Hb, Hct, RBC, WBC and differential WBC. At the end of the study both animals were sacrificed by an unspecified means and a gross pathological examination performed. The lungs, liver, kidneys and trachea were histopathologically examined. No further experimental details were given.

Results & Conclusions: No mortalities, clinical signs, macroscopic or histopathological abnormalities were observed. The data was not useful for regulatory purposes due to the absence of a control group and the low sample size. This experiment did not adequately determine the short-term inhalational toxicity of paraquat aerosols.

Comments: This study was considered inadequate for regulatory purposes. Besides the lack of a control and the low sample size, this study had numerous other limitations. Only a single dose-level was tested. The following details were unspecified: age of the dogs; laboratory/cage temperature and humidity; equilibration of the exposure chamber; method of blood sampling; termination method. No clinical chemistry parameters were measured. No pre-dose haematology measurements were taken. No particle size or stability analysis was performed. A small number of haematology parameters were measured. Additionally, only a few organs/tissues were histopathologically examined (lungs, liver, kidneys and trachea).

4.4.3 Dermal Administration

4.4.3.1 Rats

Levin PJ, Klaff LJ, Rose AG & Ferguson (1979) Pulmonary effects of contact exposure to paraquat: a clinical and experimental study. *Thorax. 34: 150-160.*

Materials & Methods: Eight mg of paraquat (unspecified purity batch no. and source) was painted onto the back of the shaved necks of 18 male Long Evans rats (~250 g body weight; age and source unspecified) in a one mL solution (vehicle unspecified) at weekly intervals for 8 weeks. The site was unoccluded, however the study authors specified the site was an area unable to be licked by rats. The dose was increased to 28.5 mg/mL for the last 2 weeks as the rats appeared to tolerate the initial dose. A control group (n=7) had only their necks shaved. The area of application and details of housing or feeding conditions were unspecified. From the 4th week, 2 rats from the treatment group and one from the control group were sacrificed

by an unspecified means at weekly intervals. Lungs, kidneys, liver and affected skin were removed, fixed, sectioned and stained with haematoxylin and eosin. Morphometric measurements were made on transversely-sectioned vessels from each rat including measurement of the medial thickness of the first 16 muscular arteries encountered randomly, and the medial thickness of 8 large (~100-300 μM) and 8 small (~100 μM) pulmonary arteries. The single medial thickness of each artery was expressed as a percentage of the external diameter of each vessel. Alveolar capillaries were examined by electron microscopy in 3 rats/group.

Results

Mortalities & clinical signs: All control rats survived the duration of the study while 2 paraquat-treated rats died at unspecified times. No clinical signs were reported.

Pathology: Nine paraquat-treated rats exhibited foci of recent intra-alveolar haemorrhage, with 3 also having small foci of haemosiderin in the lung, suggestive of previous haemorrhage. The lungs of one of the dead paraquat-treated rats showed foci of infarction and sub-infarction and several occlusive thrombi in muscular pulmonary arteries, but pulmonary veins appeared normal. The other deceased paraquat-treated rat was not examined due to autolysis. The livers and kidneys of paraquat-treated rats appeared normal, while electron microscopy revealed no abnormalities in the pulmonary alveolar capillaries. Control rats exhibited no lung, kidney or liver abnormalities. Electron microscopy also revealed that the capillaries of control rats were normal. The medial thickness of randomly encountered arteries, and of large and small arteries was significantly higher ($p<0.05$-0.001) in paraquat-treated rats compared to the controls (see Table 44 below).

Table 44: Medial thickness of pulmonary arteries in rats dermally-treated with paraquat

	Control	Paraquat-treated
Randomly encountered arteries	11.9 ± 0.41%	25.28 ± 0.94% **
Large arteries	13.6 ± 0.49%	25.9 + 1.44% **
Small arteries	9.0 + 0.73%	14.2 ± 1.35% *

Results are expressed as the mean % medial thickness ± 1 SEM; * $p<0.05$; ** $p<0.001$.

Conclusions: Weekly dermal application of 8 mg paraquat for 8 weeks, followed by 28.5 mg for 2 weeks to male Long Evans rats resulted in deaths, intra-alveolar haemorrhage and significant thickening ($p<0.05$) of the pulmonary arteries. These results suggested that toxicologically significant levels of dermal absorption occur in Long Evans rats.

Comments: This study was considered to have limited regulatory value as it lacked methodological and reporting details. Although the study design was based on a possible occupational exposure scenario, there were several limitations in design and reporting: only a single dose-level was tested; rabbits or guinea pigs should have been used as the test animal, with the use of the rat in this study unjustified; no clinical chemistry, haematology or urinary parameters were measured; no food consumption or body weight data were collected; exposure occurred only once per week.

Luty S, Latuszynska J, Halliop J, Tochman A, Obuchowska D, Korczak B, Przylepa E & Bychawski E (1997) Dermal toxicity of paraquat. ***Ann. Agri. Environ. Med. 4: 217-227.***

Materials & Methods: Paraquat (purity unspecified, batch no. D-8024; Sigma, unspecified location) in a solution of water and alcohol (4:1) was applied dermally to 3-month old female Wistar rats (220-250 g bw; unspecified source) at 0 (control), one-half and one-tenth the LD_{50} (n=10) with actual administered doses not specified. However, as it was stated that the acute dermal LD_{50} for female rats is 90 mg/kg bw, it is assumed that the rats in this study received 45 or 9 mg/kg bw, as doses that are one-half and one-tenth of the LD_{50}, respectively. Each rat was dosed for 4 h/d, 5 days/week for 4 weeks, with the sample applied to a band of absorptive fabric FPP-15 which was then attached at an unspecified site and isolated with aluminium foil. Housing conditions were unspecified while food (standard grain-based fodder, unspecified source) and water were available *ad libitum*. Behavioural neurotoxicity was evaluated in the control and high-dose groups before exposure, and at 2 and 4 weeks using the open field method (Rump & Kleinrok 1982). Rats were anaesthetised (unspecified method) after 28 days and exsanguinated by cardiac puncture. Neutrophil activity was determined using the phagocytosis assay with Bacto-Latex (Difco USA) and the microbicidal functioning of neutrophils examined using the method of Park (1968). One hundred cells were analysed in both tests. The following organs were histopathologically examined: brain, heart, kidney, liver, lung, lymph nodes, spleen and thymus. The heart, kidneys, liver and lungs were ultrastructurally examined by electron microscopy. Statistical differences between groups were evaluated using a Student's t-test.

Results

Mortalities, clinical signs & body weight effects: No mortalities or clinical signs were reported. Both paraquat-treated groups of rats were reported to show no increase in body weight over the 4 week study period, however no data were provided to substantiate this finding. No control body weight data were provided for comparison.

Histopathology: The study authors described a variety of lung (cellular infiltration, alveolar exudate, collagen fibres), heart (focal hypertrophy of the interstitial tissue, mononuclear cell infiltration, collagen fibres), kidney (lymphatic infiltrations in the cortex and medulla, spaces between the tubuli contortus filled with interstitial elements), liver (lymphocytic infiltration, accumulation of hepatocyte nuclei in the subcapsular layer), brain (abnormal CA2 pyrimidal cells, cells from the granular layer of the hippocampal gyrus, purkinje cells in the ganglionic layer of the cerebellar cortex, pyknosis in neurocytes) and lymph node (widening of subcapsular sinuses, blood extravasations, macrophages) abnormalities in paraquat-treated rats which tended to be more pronounced in the high-dose group. Additionally, abnormalities were reported to be 'most severe' in the lungs followed by the heart and then the remaining organs. No abnormalities were reported in the spleen or thymus. Although a variety of photographs were provided to illustrate these findings, no attempt was made to determine the number of rats affected or the severity of the effects. The value of these observations was also weakened by the absence of control data and therefore the reviewing toxicologist considered that the findings had limited regulatory value.

Electron microscopy: Ultrastructural findings were generally consistent with histopathological observations, but due to the absence of control data, and quantitative data, they were considered to have limited regulatory value. The lungs exhibited evidence of oedema, collagen fibres,

cellular infiltration and perturbations to the endothelium. Swelling of the endothelium, bunches of collagen fibres and numerous intracellular abnormalities were observed in the heart. Injuries to the proximal tubuli were evident in the kidneys in addition to mitochondrial abnormalities (swelling, loss of crista), a reduction in endoplasmic reticulum, widening of the channels of the smooth endoplasmic reticulum (ER) and an increase in the number of peroxisomes. The liver of paraquat-treated rats exhibited ultrastructural abnormalities similar to those seen in the kidneys.

Immunotoxicity: At both doses of paraquat there was a statistically significant increase ($p<0.01$) in the index of the nitroblue-tetrazolium test (NBT) suggesting that paraquat had a stimulatory effect on neutrophil function (see Table 45 below). There was an incidental depression ($p<0.05$) in the index of the phagocytosis latex test in the low-dose group, but in the absence of an effect at the high-dose this finding was not considered to be treatment-related.

Table 45: Effect of dermally applied paraquat on neutrophil activity

Parameter	Control	1/10 LD_{50} PQ	1/2 LD_{50} PQ
NBT index	6 ± 4	15 ± 7**	17 ± 10**
Phagocytosis latex test index	53 ± 12	41 ± 12*	54 ± 11

Results are expressed as means ± 1 SD; * $p<0.05$; ** $p<0.01$; PQ = paraquat; NBT = nitroblue-tetrazolium

Behavioural neurotoxicity: Paraquat-treated rats exhibited a transient significant reduction in climbing (8.50 ± 1.95, $p\leq0.01$), interest in blocks (3.70 ± 1.92, $p\leq0.005$) and defecations (0.10 ± 0.10, $p\leq0.01$) relative to the control (17.20 ± 2.10, 10.90 ± 2.03 and 2.10 ± 0.64, respectively) after 2 weeks of exposure. The evaluating toxicologist did not consider that the effect on defecation was treatment-related as the treatment group exhibited a significantly lower ($p\leq0.01$) level of defecations than the control group prior to dosing. No significant behavioural effects occurred after 4 weeks of exposure. The study authors interpreted this as an indication of adaption to the test compound.

Conclusions: The LOEL following dermal application of paraquat to female Wistar rats was $1/10^{th}$ LD_{50} (assumed to be 9 mg/kg bw). At both $1/10^{th}$ and 1/2 the LD_{50}, histopathological lung abnormalities predominated while a range of ultrastructural effects on the heart, liver, kidneys and brain were described. Evidence of immunotoxicity was observed at both doses as shown by the significant increase ($p\leq0.01$) in neutrophil activity. A significant transient depression ($p\leq0.005$-0.01) in behavioural activities (climbing, interest in blocks) occurred following 2 weeks of dosing with 1/2 the LD_{50} but normal behaviour had been re-established by 4 weeks of exposure.

Comments: The study was considered to be of limited regulatory value for the following reasons: no body weight data were provided; no quantitative histopathological data were collected; no histopathological control data were provided; the apparent absence of deaths or clinical signs at the high-dose; the stability of paraquat in the vehicle was not determined; only 2 dose levels were tested; the use of the test species was unjustified; no clinical chemistry, haematology or urinary parameters were measured; exposure was for 4 h per day while the OECD guideline recommends exposure for at least 6 h per day. Additionally the following details were unspecified: purity of paraquat; housing conditions; application site; doses tested; rationale for these doses; anaesthetic; units of behavioural measurements.

4.4.3.2 Rabbits

Cox RH, Serabian MA & Quander R (1986) Twenty-one day dermal toxicity study in albino rabbits with paraquat technical (SX-1465). Project no. 2107-132. Hazleton Laboratories America Inc., Vienna, Virginia USA. CEHC project No. S-2718. Sponsor: Chevron Environmental Health Center Inc., Richmond, California, USA. Study duration: 10 Sep – 2 Oct 1985. Report date: 21 January 1986

*Guidelines & GLP***:** US EPA/FIFRA GLP regulations 40 CFR Part 160, May 2, 1984. This study was quality assured.

Materials & Methods

Paraquat technical (33.5%; batch no. SX-1465; Chevron Environmental Health Centre Inc. Vienna, Virginia USA) was diluted in Polar® distilled water (lot no. 51267, Polar Water Company, Beltsville, Maryland, USA) to formulate stock solutions of 0, 1.5, 3.4, 7.8 and 17.9 mg/mL paraquat technical (0, 0.5, 1.15, 2.6 and 6 mg/mL paraquat cation). Thirty male and thirty female NZW rabbits (Hazleton Research Products Inc., Denver, Pennsylvania, USA), of approximately 8-10 weeks of age, were randomly divided into 5 groups of 6 based on body weight. The initial body weights of males and females ranged from 1974-2423 and 1945–2451 g, respectively. Each rabbit received 1 mL/kg bw/d of the appropriate stock solution to yield the following doses: Group 1 (vehicle control); Group 2 - 1.5 mg/kg bw/d paraquat (0.5 mg/kg bw/d paraquat cation); Group 3 - 3.4 mg/kg bw/d paraquat (1.15 mg/kg bw/d paraquat cation); Group 4 - 7.8 mg/kg bw/d paraquat (2.6 mg/kg bw/d paraquat cation); Group 5 – 17.9 mg/kg bw/d paraquat (6 mg/kg bw/d paraquat cation).

Animals were housed individually in elevated wire-mesh cages under standard laboratory conditions. Commercial rabbit feed (Purina Certified Laboratory Rabbit Chow® #5322) and tap water (via an automated system) was available *ad libitum*. Water was routinely retrospectively analysed for specific microorganisms, pesticides, heavy metals, alkalinity and halogens.

Paraquat was administered daily to the shaved dorsal area of each rabbit for approximately 6 h under an occlusive dressing (details unspecified). Each animal was observed twice daily for any visible signs of toxicity. Thorough clinical evaluations were made at day 1, 2, 4, 8, 11, 15, 18 and 21. Body weights were recorded on day -7, -4, 1, 4, 8, 11, 15, 18 and 21 and food consumption recorded weekly. All animals were observed for signs of dermal irritation prior to dosing on day 1, 2, 4, 8, 11, 15, 18 and 21 according to the method of Draize *et al* (1959). Baseline fasted blood samples were collected from the medial ear artery of 35 males and 35 females at day -5 and pooled. At day 22 (Group 1-3) or 23 (Group 4–5) animals were weighed, anaesthetised with sodium pentobarbital and exsanguinated after collection of a 20 mL blood sample. When present, urine was collected and shipped to the sponsor for analysis of the test material and/or metabolites but no data were included in this study. The following haematology parameters were analysed: RBC, reticulocyte count, Hb, Hct, platelet count, WBC and WBC-DC. The following clinical chemistry parameters were analysed: Ca, Cl, K, Na, P, total protein, albumin, globulin, total bilirubin, BUN, creatinine, BUN/creatinine, glucose, total cholesterol, AST, ALT, ALP, creatine phosphokinase (CPK), LDH, direct bilirubin, triglyceride and uric acid.

Gross necropsy on all animals was performed including an examination of the external body surface, all orifices, brain and cranial cavity, cervical, thoracic and abdominal viscera. The following organs were weighed: brain and entire brainstem, liver with gall bladder, adrenals (right, left and combined), kidneys (right, left and combined), testes (right, left and combined), ovaries (right, left and combined) and lungs. A histopathological examination of the following organs/tissues was performed: lungs, liver, spleen, adrenals, brain and entire brainstem, heart, kidneys, gonads, skin (treated and untreated) and any gross lesions.

Statistical analysis: Initial statistical analyses of absolute body weight (d -7, -4, 1, 4, 8, 11, and 18), total food consumption (wk 1-3), daily food consumption values, clinical pathology values (except RBC morphology) and terminal body and organ weight data began with the Levene's test for homogeneity of variances. Homogeneous data were further analysed using a 1-way ANOVA. If the variances were heterogenous, a series of transformations were performed until either homogeneity was achieved or the entire series of transformations were used. If heterogeneity could not be removed, ANOVA of the rank transformed data was completed. If the ANOVA of the data proved to be significant a Dunnett's t-test was employed. If the ANOVA was not significant then analysis was complete. The Terpstra-Jonckheere non-parametric test for trend was performed on both homo/heterogenous data. If a significant trend was noted, a 1-way analysis of significance was used, otherwise a 2-tailed analysis was used. Differences were determined to be statistically significant when $p \leq 0.05$.

Results

Mortalities & clinical observations: All animals survived the duration of the experiment and no clinical signs were evident.

Body weight & food consumption: There were no treatment related effects on body weight or food consumption.

Dermal irritation: Relevant data are presented in Table 46 below. A clear dose-response relationship was evident between the application of paraquat technical to the skin and dermal irritation. No dermal irritation was observed at or below 3.4 mg/kg bw/d. Dermal irritation became evident in males and females from Group 4 (7.8 mg/kg bw/d) at day 18 and day 15, respectively, with only a small number of animals affected at day 21. Group 5 animals (17.9 mg/kg bw/d) showed an earlier onset of signs (day 11) with all animals affected by day 15. The signs exhibited by Group 5 animals persisted until day 21, with 2 males and one female also showing a progression of slight to well-defined erythema.

Haematology: In Group 3 and 4 males (3.4 and 7.8 mg/kg bw/d, respectively), a statistically significant reduction ($p \leq 0.05$) in terminal RBC was detected. This was not deemed to represent a treatment-related effect because the result was due to a few outlying animals in each group with low RBC counts. Furthermore animals in the highest dose group (Group 5: 17 mg/kg bw/d) exhibited no perturbation in RBC. There were no treatment-related effects on any of the remaining haematology parameters.

Clinical chemistry: There was no treatment-related effect on any clinical chemistry parameter.

Table 46: Incidence of effects of dermally-administered paraquat technical on the skin of male and female rabbits at selected intervals

Group (n=6)	1		2		3		4		5	
Dose (mg/kg bw/d)	0		1.5		3.4		7.8		17.9	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Day 15										
Normal	6	6	6	6	6	6	6	5	0	0
Slight erythema	0	0	0	0	0	0	0	0	6	5
Well-defined erythema	0	0	0	0	0	0	0	0	0	0
Scabbing	0	0	0	0	0	0	0	1	6	6
Day 18										
Normal	6	6	6	6	6	6	4	5	0	0
Slight erythema	0	0	0	0	0	0	0	0	5	4
Well-defined erythema	0	0	0	0	0	0	0	0	1	1
Scabbing	0	0	0	0	0	0	2	1	6	6
Day 21										
Normal	6	6	6	6	6	6	4	5	0	0
Slight erythema	0	0	0	0	0	0	0	0	4	4
Well-defined erythema	0	0	0	0	0	0	0	0	2	1
Scabbing	0	0	0	0	0	0	2	1	6	6
Gross Pathology										
Scabbing	0	0	0	0	0	0	2	1	6	5
Redness	0	0	0	0	0	0	0	0	1	0
Thickened epidermis	0	0	0	0	0	0	0	0	2	1
Swollen sc vessels	0	0	0	0	0	0	0	0	1	1
Histopathology										
Chronic active inflammation	3	4	3	2	3	3	4	5	6	5
Erosion/ulceration	0	1	0	0	0	0	2	0	5	4
Surface exudate	0	1	0	0	0	0	2	0	5	4
Acanthosis	0	1	1	0	2	0	2	3	4	2
Hyperkeratosis	0	0	0	0	0	1	0	0	0	3

Gross pathology: At necropsy, the dermal features noted during the study in both sexes from Groups 4 and 5 were confirmed and additional observations of redness, thickened skin and swollen sc vessels were made in a few animals at 17.9 mg/kg/d (see Table 46 above). The incidence of scabbing in Group 5 females was lower (5/6) than that previously scored (6/6) at day 21. This inconsistency is likely due to the extra day that Group 5 (and 4) animals had to recover following paraquat administration. Ovarian cysts, oviduct cysts or H-cysts (undefined) were detected in individuals from all groups (3/6, 4/6, 3/6, 4/6 and 2/6 for Groups 1-5, respectively) but these were not treatment-related and were concluded to have arisen spontaneously. No perturbations in absolute organ weights, organ to terminal body weight ratios or organ to brain weight ratios were detected in any treatment group.

Histopathology: Histopathological examination indicated that most groups contained one individual with nonsuppurative meningoencephalitis. All animals (bar one female in Group 5) had perivascular/peribronchial lymphoid hyperplasia. At least 50% of animals from each group were also positive for pneumonitis. Neither of these two observations were treatment-related and suggested that the animals may have been of poor health at the termination of the study. The incidence of chronic active inflammation on untreated skin in all groups was at least 50% with females showing higher incident levels (up to 100% of animals in some groups). Some individuals also displayed acanthosis. Paraquat-treated skin exhibited a range of features. A dose-related effect was evident in the incidence of erosion/ulceration and the presence of a surface exudate in males. A treatment-related effect was also apparent in the incidence of

acanthosis in Group 5 males. Group 5 females exhibited a range of treatment-related effects such as ulceration, the presence of a surface exudate, acanthosis and hyperkeratosis.

Conclusions: The NOEL in NZW rabbits following daily dermal application of paraquat for 21 days was 3.4 mg/kg bw/d (1.15 mg/kg bw/d paraquat cation) based on clinical, macroscopic and microscopic evidence of dermal irritation at and above 7.8 mg/kg bw/d (2.6 mg/kg bw/d paraquat cation). No systemic toxicological effects were observed during this study.

Comment: A number of limitations were apparent in this study. Not all animals were terminated at the same time: Group 4 and 5 animals had an extra day to recover compared to Group 1, 2 and 3 animals. As a likely consequence, one Group 5 female recovered from scabbing between day 21 (clinical observations) and day 23 (necropsy). Any short-term changes in food consumption could have been missed by the absence of the collection of the first 3 days of food consumption data. A high proportion of incidental histopathological abnormalities was detected in all groups and is suggestive of poor health in the study animals. No results of the urinary paraquat analysis were provided.

4.4.4 Subcutaneous Administration

4.4.4.1 Dogs

Nagata T, Kono I, Masaoka T & Akahori F (1992) Subacute toxicity of paraquat in beagle dogs: clinicopathology and pathologic examinations. *Vet Hum Toxicol* 34(1): 15-20.

Three male and 3 female healthy beagle dogs per group (body weight and age unspecified; Shin Nippon Biomedical Laboratories Ltd., unspecified location) were injected subcutaneously with paraquat (0.1 mg/mL in saline; unspecified purity, amount of cation and source) at 0, 0.055, 0.165 or 0.495 mg/kg bw/d for 4 weeks. The injection site and rationale for the dose selection were unspecified. One male and one female dog per group were allowed to recover for 0, 4 or 8 weeks following the 4 week administration period. Dogs were housed under standard laboratory conditions. Solid food (VE-10, Nippn Pet Food Ltd., unspecified location) and water were available *ad libitum*.

Clinical signs and food consumption were monitored daily. Body weights and water consumption were monitored weekly. Ophthalmological and electrocardiographic (ECG) examinations of surviving dogs were performed at 2, 4, 8 and 12 weeks after dosing commenced using a SL-2 slit-lamp and RC-2 fundus camera and an ECG recorder.

An unspecified volume of urine and blood was collected by unspecified means at 2, 4, 8, and 12 weeks after commencement of dosing. The following urinary parameters were measured: protein, glucose, occult blood, ketones, bilirubin, urobilinogen, colour, pH, specific gravity, Na, K and Cl. The following haematology parameters were measured: RBC, WBC, Hct, Hb, platelets, MCV, MCH, MCHC, reticulocyte count, PT, APTT and ESR. The following clinical chemistry parameters were measured: AST, ALT, ALP, LDH, CPK, LAP (leucine aminopeptidase), total protein, albumin, albumin/globulin (A/G), total cholesterol, triglyceride, phospholipid, glucose, BUN, creatinine, uric acid, inorganic P, Ca, K, Cl and Fe.
Dogs were sacrificed when moribund with pentobarbital sodium, exsanguinated and immediately necropsied. Of the surviving dogs, one animal from each group was sacrificed in

the same manner at 0, 4 and 8 weeks after the dosing period. The following organs were weighed: epididymides, heart, kidneys, liver, lung, ovaries, pituitary, prostate, spleen, submaxillary glands, testes, thymus, thyroids and uterus. Relative organ weights were calculated on day 13 and the day of death.

The following organs were processed for histopathology: adrenals, aorta, bone marrow (femur), brain stem, bronchi, cerebellum, cerebrum, epididymis, eye, gall bladder, heart, ischiatic nerve, kidney, large intestine, liver, lung, mammary gland, mesenteric lymph node, muscle (quadriceps), oesophagus, ovary, pancreas, parathyroid, pituitary, prostate, skin, small intestine, spleen, sternum, stomach, submaxillary lymph node, testes, thyroid, tongue, trachea, urocystis, uterus, vagina and the injection site.

Small portions of the lung, kidney and liver were collected from moribundly sacrificed and surviving dogs at 4, 8 and 12 weeks after commencement of the study. Tissues were fixed with glutaraldehyde and osmium tetroxide, embedded in epoxy resin then sectioned. Sections were stained with uranyl acetate and lead citrate, and then examined with an electron microscope.

Results

Mortalities & clinical signs: Two females and one male were sacrificed in a moribund condition at an unspecified time following treatment with 0.495 mg/kg bw/d paraquat. No other mortalities were reported. Slight vocalisation was heard immediately following injection of paraquat in an unspecified number of dogs at all doses. At 0.495 mg/kg bw/d, a decrease in spontaneous activity and slight undernourishment were observed in a few dogs (number unspecified) during the 4 week administration period. A few (unspecified) dogs in this same group also exhibited induration and ulceration of the injection site. Although the study authors reported decreased appetite or inappetance, water consumption and body weight in a few animals at the highest dose (0.495 mg/kg bw/d) until the end of the administration period, no supporting data were provided to substantiate these findings.

Ophthalmoscopy & ECG: Ophthalmoscopic examination revealed a slight haemorrhage in the nasalis vein of the left fundus in one dog at 0.495 mg/kg bw/d (4 week recovery period). The eyes of all other paraquat-treated dogs appeared normal and therefore this finding was not considered to be treatment-related. There was no treatment-related effect on ECG measurements.

Urinalysis, haematology & clinical chemistry: A number of perturbations in urinary, haematology and clinical chemistry parameters were reported at unspecified times, however no supporting data were provided to substantiate these observations. Increased urinary protein, reticulocyte counts, fibrinogen and plasma phospholipids were reported in an unspecified number of dogs across all treatment groups. Decreased Hct at 0.165 mg/kg bw/d, A/G ratios in a few animals at 0.055 and 0.495 mg/kg bw/d, increased BUN at 0.165 mg/kg bw/d were reported. However, in the absence of detailed data these observations could not be attributed to treatment. At the highest dose (0.495 mg/kg bw/d), increased CPK activity was reported. The study authors concluded that the increase in urinary protein across all treatment groups, and the increased levels of phospholipids, BUN and CPK, and decreased albumin suggested renal injury, however in the absence of detailed data this conclusion is considered to be unsubstantiated.

Pathology: A moderate increase in the absolute and relative lung weight of a single dog at 0.495 mg/kg bw/d paraquat (no recovery period) was reported, however no supporting data were provided. The lungs of moribund sacrificed dogs were reported to show moderate atelectasis. Induration or ulceration was also present at the injection site. Greyish white lung lobes were reported in an unspecified number of dogs at 0.495 mg/kg bw/d following a 4 or 8 week recovery period. No other gross pathological anomalies were reported.

Histopathology: No histopathological abnormalities were detected in any control dogs. Histopathological lung, kidney and liver abnormalities were observed in all treatment groups, however, there was no clear dose-response effect regarding incidence or severity. The majority (16/18, including all dose levels) of paraquat-treated dogs showed a moderate to marked thickening of the alveolar wall with the exception of one female from the 0.055 mg/kg bw/d group (4 week recovery period), and one male from the same group (8 week recovery period) who exhibited only slight thickening. Moderate thickening of the pleura was observed in all but one female from the 0.055 mg/kg bw/d group (4 week recovery period). Marked atelectasis occurred in both dogs that were sacrificed immediately after 4 weeks of treatment at 0.495 mg/kg bw/d. At 0.055 and 0.165 mg/kg bw/d, males showed a moderate level of atelectasis following 0 and 4 weeks recovery, while only one female at 165 mg/kg bw/d (8 week recovery period) showed moderate atelectasis. The high-dose male that was sacrificed in a moribund condition exhibited a moderate fibrosis-like lung. A moderate to marked proliferation of fibroblast-like cells in the lungs was observed across all treatment groups with the exception of one low-dose female (4 week recovery period).

Most paraquat-treated dogs exhibited slight degeneration of the renal tubules, with moderate degeneration generally occurring at 0.495 mg/kg bw/d. Marked liver haemorrhage occurred in all males and one female at 0.495 mg/kg bw/d (no recovery period). Incidental occurrences of moderate liver haemorrhage were observed at 0.055 and 0.165 mg/kg bw/d. Liver congestion was moderate to marked in all dogs at 0.495 mg/kg bw/d, whilst it was low to moderate at 0.055 and 0.165 mg/kg bw/d.

Moderate hepatocyte degeneration occurred in all dogs at 0.055 mg/kg bw/d but this effect was reduced at the higher doses. At 0.055 and 0.165 mg/kg bw/d, all females showed a slight to moderate swelling of hepatocytes while this was not seen at 0.495 mg/kg bw/d. All males at 0.495 mg/kg bw/d had a moderate level of swelling of the hepatocytes, while the magnitude of swelling was less at lower doses. Moderate to marked proliferation of fibres in the lungs occurred across all doses.

Electron microscopy: Electron microscopic examination of the lungs revealed the following: slight to moderate proliferation of myofibroblasts and fibroblasts in all dogs at 0.495 mg/kg bw/d, slight proliferation in one male and one female at 0.165 mg/kg bw/d and no effect was seen at the lowest dose; slight proliferation of type II alveolar cells in all paraquat-treated dogs; slight to moderate degeneration or vacuolation of type I and II alveolar cells in all dogs at 0.495 mg/kg bw/d with some effects seen at 0.055 mg/kg bw/d; slight mitotic figures of type I and II alveolar cells in 5/6 dogs at 0.495 mg/kg bw/d; all treated dogs showed a slight accumulation of lamellar body in type II alveolar cells with 4/6 dogs treated with 0.495 mg/kg bw/d showing a moderate effect; slight increase in mast cells across all treatment groups; at 0.495 mg/kg bw/d, all dogs showed a moderate increase in collagen fibres in the interstitium, while at 0.055 and 0.165 mg/kg bw/d a slight to moderate effect was observed; all dogs treated with 0.495

mg/kg bw/d had a slight to moderate level of lung debris, while at lower doses less than half of dogs showed a slight to moderate effect.

A few incidental liver abnormalities were observed, however none of these were considered to be toxicologically significant. Generally only mild abnormalities were detected in the kidneys and these were found at all dose levels. At the mid and high dose, all dogs exhibited slight proliferation and dilation of smooth endoplasmic reticulum or vacuolation of proximal tubular epithelial cells, in addition to cell debris in the dilated interstitium of renal tubules. At the high dose, all dogs showed slight stratification or thickening of the basement membrane in mesangial matrix, glomerular capillary and proximal tubular epithelial cells. Degeneration of proximal tubular epithelial cells and increased collagen fibres in dilated interstitium were also observed in all animals at the highest dose.

Conclusions: The LOEL following subcutaneous administration of paraquat to beagle dogs for 4 weeks was 0.055 mg/kg bw/d, the lowest dose tested. At and above 0.055 mg/kg bw/d, histopathological lung (thickening of the alveolar wall and pleura, proliferation of fibroblast-like cells, atelectasis, proliferation of fibres, increased collagen fibres), kidney (degeneration of renal tubules) and liver (haemorrhage, congestion, swelling of hepatocytes) abnormalities were observed. Some of these abnormalities tended to be more pronounced at the highest dose (0.495 mg/kg bw/d), for example degeneration of renal tubules and liver haemorrhage, however no clear dose-response effect was evident. The effect of recovery time on the occurrence of these findings was inconclusive. Decreased spontaneous activity, body weight, food and water consumption, and induration and ulceration at the injection site were confined to the high-dose group (0.495 mg/kg bw/d).

Comments: The regulatory value of this study was limited by the lack of methodological and reporting details. No clinical chemistry, haematology, urinalysis or gross pathological data were provided. Clinical signs were not quantified. No statistical analysis was performed. The dose selection and administration route were unjustified. The small sample sizes (ie one male and female per sample) were also a considerable limitation of this study. In addition, this route is not considered relevant to human exposure.

4.5 Subchronic Studies

4.5.1 Oral Administration

4.5.1.1 Mice

Maita K & Saito T (1980) AT-5: subacute toxicity study in mice. Report no. RIC2566. Lab & Sponsor: Toxicology Division, Institute of Environmental Toxicology, Kodaira, Tokyo, Japan. Study duration: unspecified. Report date: 19 December 1980.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods: SPF mice (20/sex/group, Charles River Japan Inc., Japan) received paraquat dichloride (93.3% purity; batch no. and source unspecified) for 13 weeks by admixture in the diet (powdered chow MF; Oriental Yeast Co., location unspecified) at 0, 10, 30, 100 or 300 ppm (equivalent to 0, 2, 6, 20 and 60 mg/kg bw/d, respectively). Formulated diets were prepared fortnightly, and were analysed for paraquat concentration and stability (at unspecified times) using a spectrophotometric assay. On arrival, mice were 4 weeks old and

were acclimatised for one week prior to the study. The mean initial body weight of males and females was approximately 28 and 24 g respectively. Mice were housed under standard laboratory conditions (5/cage) and provided with diets and water *ad libitum.*

All mice were examined daily for clinical signs. Body weights were measured weekly. Food and water consumption were recorded twice weekly. Animals that were found dead were macroscopically and histopathologically examined. At day 91, blood samples (unspecified volume) were collected under light anaesthesia from the posterior vena cava in at least 10 mice/group. The remaining mice were sacrificed at day 92 by dissecting the posterior vena cava and diaphragm. The following haematology parameters were measured: Hb, RBC, platelets, Hct, MCV, MCH, MCHC and WBC-DC. The following clinical chemistry parameters were measured: ALP, BUN, glucose, cholesterol, ALT, AST and Ca. Urine samples were collected from the remaining mice in each group by pressing the lower abdomen. The following urinary parameters were measured: specific gravity, pH, protein, glucose, ketones and occult blood.

The following organ weights were recorded for every rat: brain, pituitary, thyroid, heart, lung, thymus, liver, kidneys, spleen, adrenals, gonads and skeletal muscles (M. triceps surae). The above organs/tissues in addition to the following were histopathologically examined: sciatic nerve, stomach, duodenum, jejunum, ileum, colon, caecum, urinary bladder, urethra, prostate, epididymis, seminal vesicle, coagulating gland, lymph nodes (cervical and mesentery), salivary gland, trachea, eyeballs, Harderian gland, inner lacrymal gland, bone marrow (femur, sternum) and any abnormal lesions.

A Student's t test was used to determine any statistical differences between control and treatment groups.

Results

Dietary analysis: Mean (± SD) analytical paraquat concentrations in the diets of 9.6 $\pm$ 0.4, 29.4 $\pm$ 1.1, 97.2 $\pm$ 3.7 and 289 $\pm$ 10 ppm complied with the nominal levels of 10, 30, 100 and 300 ppm respectively. The level of paraquat in the diet was determined to be within 97–111% of the initial level following storage at 5°C for 4 weeks. The diet was also shown to be homogenous following recovery of 96-98% of incorporated paraquat.

Mortality & clinical signs: Two females from the 300 ppm group died during the study, one of these dying during week 2 and the other during week 11. The study authors reported that both females showed signs of weight loss, rough hair and emaciation 1-2 weeks prior to death. There were no other deaths or clinical signs reported.

Body weight & food consumption: The mean body weight of male mice treated with 300 ppm was consistently lower than the control group over the entire study period and statistically lower ($p<0.05$) than the control group at week 5-8, 11 and 13. Similarly, the 300 ppm female group had a lower body weight than the control group over the entire study period and a statistically lower ($p<0.05$) body weight at week 3, 4, 9, 10 and 12. The body weight of all other groups was comparable to the control group. No treatment-related effect on food or water consumption was observed.

Based on the body weight and food consumption data, the mean paraquat cation intake of males administered 10, 30, 100 and 300 ppm was calculated by the study authors to be 1.18, 3.65, 11.50 and 35.8 mg/kg bw/d, respectively. The mean paraquat cation intake of females administered 10, 30, 100 and 300 ppm was calculated by the study authors to be 1.38, 3.91, 13.8 and 41.9 mg/kg bw/d, respectively. In males, average food efficiency (body weight gain/food intake x 100) was lower in the 300 ppm group (3.6%) compared to the control (4.2%) while no effect was observed at or below 100 ppm. In females, average food efficiency was also lower at 300 ppm (2.9%) compared to the control (3.4%) and no effect was observed at or below 100 ppm.

Haematology: In paraquat-treated mice there were some incidental variations in MCV, MCHC and platelets relative to the control group, but in the absence of a dose-response effect and any pre-dose baseline values for each mouse, none of these observations could be attributed to paraquat.

Clinical chemistry: At 300 ppm, BUN and ALT were significantly lower in males (25 $\pm$ 3 mg/dL and 21 $\pm$ 4 Karmen units respectively; $p<0.05$) compared to the control group (29 $\pm$ 4 mg/dL and 28 $\pm$ 7 Karmen units respectively) while in females, BUN was significantly elevated (25 $\pm$ 5 mg/dL; $p<0.05$) compared to the control group (22 $\pm$ 3 mg/dL). As the clinical relevance of these parameters is unclear and the magnitude of the effects small, these findings were not considered to be toxicologically relevant.

Urinalysis: There was no treatment-related effect on any of the urinary parameters tested.

Pathology: Autopsy findings indicated that the 2 dead females from the 300 ppm group showed consolidation or dark red areas of the lobes of the lung. No other treatment-related macroscopic abnormalities were reported in any other mice.

Variations in absolute and relative organ weights were observed only at the highest dose, (300 ppm). Males exhibited significantly lower heart, liver and muscle weights compared to the control group, while females had significantly elevated pituitary, lung, kidney and spleen weights, and reduced ovary weights (see Table 47 below). As the magnitude of these variations was small, and in the absence of a dose-response relationship, these observations could not be attributed to paraquat. Examination of individual animal data revealed that the increase in pituitary and thyroid weights in females was probably due to a few outlying animals. The significant elevation or depression in the relative weight of certain organs at the highest dose was attributed to the concomitant change in body weight.

Table 47: Effect of paraquat on absolute and relative organ weight in mice (data shown for control and high-dose groups only)

Parameter	Dose (ppm)			
	0 ppm (control)		300 ppm	
	Male	Female	Male	Female
Terminal body weight (g)	45.7 ± 5.3	37.3 ± 4.1	42.7 ± 3.8*	35.5 ± 3.5*
Pituitary (mg)	2.2 ± 0.4	2.7 ± 0.4	2.2 ± 0.4	3.3 ± 1.0*
Heart (mg)	195 ± 22	144 ± 12	178 ± 15**	146 ± 13
Lung (mg)	193 ± 22	175 ± 12	197 ± 22	187 ± 17*
Liver (g)	2.23 ± 0.28	1.61 ± 0.19	1.96 ± 0.24**	1.63 ± 0.24
Kidneys (mg)	663 ± 103	385 ± 37	632 ± 91	420 ± 38**
Spleen (mg)	108 ± 31	104 ± 20	97 ± 20	126 ± 38*
Ovaries (mg)	-	20.7 ± 4.9	-	16.8 ± 3.7*
Muscle (mg)	220 ± 12	173 ± 17	203 ± 30*	172 ± 16
Relative pituitary wt	0.0049 ± 0.0008	0.0073 ± 0.0012	0.0052 ± 0.009 (19)	0.0092 ± 0.0027*
Relative thyroid wt	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.003	0.013 ± 0.004*
Relative lung wt †	0.43 ± 0.05	0.47 ± 0.07	0.46 ± 0.05*	0.53 ± 0.05**
Relative liver wt †	4.89 ± 0.39	4.32 ± 0.50	4.59 ± 0.45*	4.58 + 0.48
Relative kidney wt	1.45 ± 0.16	1.04 ± 0.12	1.49 ± 0.20	1.19 ± 0.14**
Relative spleen wt	0.24 ± 0.06	0.28 ± 0.06	0.23 ± 0.04	0.36 ± 0.11*
Relative ovary wt	-	0.056 ± 0.012	-	0.048 ± 0.010*

Results expressed as mean ± 1 SD (n=20 unless indicated); *p<0.05; **p<0.01; wt = weight; † = relative organ weight = (organ weight/body weight) x 100.

Histopathology: No histopathological abnormalities were observed at or below 100 ppm. Pulmonary oedema was confirmed in the 2 dead females treated with 300 ppm paraquat which was indicative of heart failure. In the surviving mice, 17/20 males and 12/18 females showed eosinophilic swelling of alveolar epitheliocytes of the lung.

Conclusions: The NOEL in CRJ-CRJ mice following 13 weeks of dietary exposure to paraquat was 100 ppm (equivalent to 11.50 and 13.8 mg/kg bw/d paraquat cation in males and females, respectively) based on mortality, clinical signs and macroscopic lung abnormalities in females, and decreased body weight gain and histopathological lung abnormalities at 300 ppm (equivalent to 35.8 and 41.9 mg/kg bw/d paraquat cation in males and females, respectively).

Comments: The following limitations were noted: stability analysis was performed at 5°C while the experiment was performed at approximately 24°C; no standard deviations/errors were provided for the mean food and water consumption, and organ weight data; storage and/or treatment conditions for blood and urine samples were unspecified.

4.5.1.2 Rats

Maita K, Saito T, Tsuda S & Shirasu Y (1980) Report on subacute toxicity of AT-5 in rats. Report no. RIC2565. Lab and Sponsor: Toxicology Division, Institute of Environmental Toxicology, Kodaira, Tokyo, Japan. Study duration: unspecified. Report date: 19 December 1980.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Groups of 20 male and 20 female, Fisher CDF (F344) CRJ strain SPF rats (Charles River Japan Inc., Japan) received paraquat (93.3% purity; batch no. and source unspecified) for 13 weeks by admixture in the diet (fundamental powder chow MF; Oriental Yeast Co., location unspecified) at 0, 10, 30, 100 or 300 ppm (equivalent to 0, 0.10, 0.30, 1.0 and 3.0 mg/kg bw/d). The amount of paraquat cation in the diet was indicated by the study authors to be equal to 0, 7, 22, 72 and 217 ppm, respectively (equivalent to 0, 0.7, 0.22, 0.72 and 2.17 mg/kg bw/d, respectively). The dose selection was based on unspecified preliminary tests.

On arrival, rats were 4 weeks old and were acclimatised for one week prior to the study. At the start of the study, the mean body weight of males and females was 83 and 77 g, respectively. Rats were housed under standard laboratory conditions (5/cage) and provided with diets and water *ad libitum.*

All rats were examined daily for clinical signs. Body weights were measured weekly. Food and water consumption were recorded twice weekly. At day 91, blood samples (unspecified volume) were collected under light anaesthesia from the posterior vena cava of 10 rats/group. The following haematology parameters were measured: Hb, RBC, WBC, platelets, Hct, MCV, MCH, MCHC and WBC-DC. The following clinical chemistry parameters were measured: ALP, LDH, BUN, glucose, cholesterol, ALT, AST, total bilirubin, direct type bilirubin, GGT, Ca, Na, K, total protein, albumin, globulin and A/G ratio. Urine samples were collected from the remaining rats in each group by the groin compression method. The following urinary parameters were measured: specific gravity, pH, protein, glucose, ketones and occult blood.

The following organs were weighed: brain, pituitary, thyroid, heart, lung, thymus, liver, kidneys, spleen, adrenals, gonads and skeletal muscles. The above organs/tissues in addition to the following were histopathologically examined: sciatic nerve, stomach, duodenum, jejunum, ileum, colon, caecum, urinary bladder, urethra, prostate, epididymis, seminal vesicle, coagulating gland, lymph nodes (cervical and mesentery), salivary gland, trachea, eyeballs, Harderian gland, inner lacrymal gland, bone marrow (femur, sternum) and any abnormal lesions.

Statistical analysis: A Student's t test was used to determine any statistical differences between control and treatment groups.

Results

Paraquat intake: In males, the mean paraquat cation intake of the 10, 30, 100 and 300 ppm groups was 0.678, 1.99, 6.55 and 19.6 mg/kg bw/d, respectively, while for females it was 0.719, 2.11, 7.10 and 21.1 mg/kg bw/d, respectively.

Mortality & clinical signs: There were no mortalities or clinical signs observed during the experiment.

Body weight & food consumption: In males, the mean body weight of all treatment groups was significantly lower than the control (p=0.001-0.05) at week 1. Thereafter, only the 300 ppm group had a significantly lower body weight (p=0.001) and this effect was consistent over the entire experimental period. In females, only the 300 ppm group had a significantly lower body

weight compared to the control (p=0.01-0.001) and this effect was also consistent over the entire study period. There was no treatment-related effect on mean food consumption at or below 100 ppm, while at 300 ppm there was a slight (though statistically insignificant) reduction in food consumption in males. Over the 13 week study period, the difference in total food consumption between the control and 300 ppm groups was 139 and 49 g for males and females respectively. This equates to a decrease of 10.4% in males and 5.2% in females relative to the controls.

There was no treatment-related effect on food conversion efficiency [(body weight gained/food intake) x 100] in females while in males, the 300 ppm group exhibited a slight though statistically insignificant depression (2.1% overall) in food efficiency. At 300 ppm in both males and females, group mean water consumption was consistently lower than the control over the entire study period. The difference in total water consumption between the control and 300 ppm groups was 204 and 187 mL/rat for males and females respectively. This equates to a decrease of 10% relative to the controls.

Haematology & clinical chemistry: Significant variations were observed in some haematological and clinical chemistry parameters but in the absence of any dose-response relationships, consistent effects in males or females, or the provision of historical control data, none of these were considered to be treatment-related. Additional comparisons with age and sex-matched historical control data for Fisher F-344 rats (Derelanko 2000) by the reviewing toxicologist also indicated that all clinical chemistry parameters fell within the normal range.

Urinalysis: There was no treatment-related effect on any of the urinary parameters tested.

Pathology: No gross abnormalities were observed following macroscopic examination. Variations in absolute and relative organ weights were observed predominantly at the highest dose, (300 ppm). Males exhibited significantly reduced brain (2%), pituitary (8%), thyroid (16%), heart (13%), liver (19%), kidney (10.4%), spleen (13%), and muscle (9%) weights compared to the control (see Table 48 below). Females had lower heart (4%) and liver (9%) weights, and increased lung weight (7%) relative to the control. Significant variation in the relative weights of some organs was also determined. As the magnitude of these elevations/depressions in absolute and relative organ weights was small (ie below 20%), and in the absence of any dose-response relationships, these observations were not attributed to paraquat.

Table 48: Effect of paraquat on absolute and relative organ weight in F344 rats (data shown for control and high-dose groups only)

Parameter	Dose (ppm)			
	0 ppm (control)		300 ppm	
	Male	Female	Male	Female
Body weight (g)	323 ± 17	188 ± 6	273 ± 9***	178 ± 12 *
Brain (mg)	1907 ± 49	1749 ± 44	1865 ± 39**	1764 ± 54
Pituitary (mg)	9.0 ± 0.7	13.0 ± 1.5	8.3 ± 0.6**	12.5 ± 2.7
Thyroids (mg)	17.6 ± 3.6 (19)	12.3 ± 2.6	14.7 ± 2.0**	12.7 ± 1.9
Heart (mg)	889 ± 97	571 ± 38	770 ± 68***	546 ± 41*
Lung (mg)	1004 ± 169	713 ± 68	946 ± 59	764 ± 58*
Liver (g)	10.50 ± 1.06	5.87 ± 0.59	8.48 ± 0.48***	5.32 ± 0.44**
Kidneys (mg)	2143 ± 158	1286 ± 72	1920 ± 93***	1273 ± 107
Spleen (mg)	583 ± 38	392 ± 31	508 ± 22***	404 ± 27
Muscle (mg)	1975 ± 162	1201 ± 79	1805 ± 117***	1234 ± 100
Relative brain wt†	0.59 ± 0.03	0.93 ± 0.03	0.68 ± 0.03***	0.99 + 0.06***
Relative pituitary wt†	0.0028 ± 0.0002	0.0069 ± 0.008	0.0030 ± 0.0002***	0.007 ± 0.0014
Relative lung wt†	0.31 ± 0.04	0.38 ± 0.04	0.35 ± 0.03**	0.43 ± 0.02**
Relative kidney wt†	0.66 ± 0.04	0.68 ± 0.03	0.70 ± 0.04**	0.72 ± 0.05
Relative spleen wt†	0.18 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	0.23 ± 0.01***
Relative adrenals wt†	0.012 ± 0.001	0.025 ± 0.001	0.014 ± 0.002*** (19)	0.025 ± 0.003
Relative ovary wt†	-	0.030 ± 0.004	-	0.034 ± 0.007*
Relative testes wt†	0.90 ± 0.04	-	1.01 ± 0.11***	-
Relative muscle wt†	0.61 ± 0.03	0.64 ± 0.04	0.66 ± 0.04	0.70 ± 0.05***

Results expressed as mean ± 1 SD (n=20 unless indicated); * p<0.05; ** p<0.01; wt = weight; † = relative organ weight = (organ weight/body weight) x 100.

Histopathology: Treatment-related histopathological effects were confined to the lungs of males administered 300 ppm, with 6/20 animals exhibiting alveolar epithelial hypertrophy compared to 0/20 in the control or other treatment groups. There was an increased incidence of brown pigmentation in the spleen of females administered 300 ppm (9/20) compared to the control group (3/20). No other treatment-related histopathological abnormalities were detected.

Conclusions: The NOEL in rats following 13 weeks of dietary administration of AT-5 (paraquat) was 100 ppm (equivalent to 6.55 and 7.10 mg/kg bw/d paraquat in males and females, respectively), based on a significant decrease in body weight gain, histopathological lung (alveolar epithelial hypertrophy) and splenic abnormalities (brown pigmentation) in males and females, respectively, at 300 ppm (equivalent to 19.6 and 21.1 mg/kg bw/d paraquat in males and females respectively). A slight decrease in food consumption and efficiency in males, and water consumption in both males and females was also observed at 300 ppm.

Comments: The following limitations were noted: the concentration, homogeneity and stability of paraquat in the diet were not determined; storage and/or treatment conditions for blood and urine samples were unspecified; standard deviations were absent from mean food and water consumption, and food efficiency data.

4.5.1.3 Dogs

Sheppard DB (1981b) Paraquat thirteen week (dietary administration) toxicity in beagles. Report no. CTL/C/1027. Lab: Hazelton Laboratories Europe Ltd., Harrogate, England. Sponsor: ICI, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Study duration: 25 March – 24 June 1980. Report date: 17 February 1981.

Guidelines & GLP: Non GLP and QA study. No test guidelines were provided.

Materials & Methods

Paraquat was supplied as a technical grade liquor (32.2% paraquat cation; batch no. Y00061/009/004; ICI Ltd., Mond Division, Runcorn, Cheshire, UK) and administered to four randomly-assigned groups of Beagle dogs (3/sex/group) (ICI Ltd., Alderley Park, Macclesfield, Cheshire, England) for 13 consecutive weeks at dietary levels of 0 (control), 7, 20, 60 or 120 ppm paraquat cation (equivalent to 0, 0.175, 0.5, 1.5 and 3 mg/kg bw/d paraquat cation, respectively). The diet was prepared by adding a suitable volume of paraquat to a small amount of expanded and reground Laboratory diet A (B.P. Nutrition UK Ltd., Stepfield Witham, Essex, UK) in a pestle and mortar to form a pre-mix. This pre-mix was diluted with untreated diet to produce a 20 kg batch.

The homogeneity, stability and concentration of paraquat was analysed by passing aqueous dietary extracts through a cation exchange column and quantifying the amount of eluted paraquat cation via a colorimetric assay. For the homogeneity tests, triplicate samples (approximately 200 g) from the 7 and 120 ppm diets were collected from the bottom, middle and top of each batch. For the stability tests, diets containing 7, 20, 60 and 120 ppm paraquat were stored for 14 days at ambient temperature and sampled at day 1, 7 and 14. For determination of paraquat content, samples were taken from each batch of diet during weeks 1, 3, 5, 7, 9, 11 and 13.

Prior to delivery, dogs were treated for parasites, and vaccinated against distemper, infectious canine hepatitis, *Leptospira canicola* and *L. icterohaemorrhagiae*. On arrival dogs were 5-6 month old and 7.3-18.2 kg bw, while at the start of treatment they were 7–9 months of age. Dogs were offered 400 g of treatment or control diet once daily. Any remaining diet was removed and weighed on the following day. Water was available *ad libitum* throughout the study. Dogs were housed individually under standard laboratory conditions.

All dogs were observed periodically for clinical signs during the test period. A detailed clinical examination was performed prior to treatment and at 6 and 12 weeks. Body weight and food consumption were recorded weekly and daily, respectively. Fasted blood samples (unspecified volume) were collected by jugular venipuncture at approximately 18 h prior to treatment and then after 3, 6 and 12 weeks. The following haematology parameters were measured: Hb, MCV, RBC, MCH, PCV, MCHC, WBC, WBC-DC, platelet count, PT and APTT. The following clinical chemistry parameters were measured: glucose, cholesterol, BUN, bilirubin, total protein and protein electrophoresis, ALP, CPK, ALT, AST, Na, K, Ca and Cl.

Urine samples were collected from water-deprived dogs at 3 and 6 weeks using a catheter. The following urinary parameters were measured: specific gravity, pH, protein, ketones, glucose, blood, bilirubin, reducing agents, urobilinogen and microscopy of spun deposits. At 6 and 12 weeks of treatment, urine samples were collected from all dogs over a period of 24 h in the absence of food and water, using diuresis cages. These urine samples were stored at -20°C until analysis. Ophthalmoscopy was performed on all dogs using a hand held Keeler direct ophthalmoscope once prior to treatment, at week 6 and again pre-terminally. Eyes were treated with 1% tropicamide approximately 15 minutes before examination. Auscultation was performed on all dogs prior to treatment, at week 6 and pre-terminally.

Dogs were sacrificed by an iv overdose of thiopentone sodium and exsanguinated immediately by severance of the major neck vessels. A full internal and external post-mortem examination was performed on all animals. The following organs were weighed: adrenals, brain, pituitary, spleen, thymus, lungs, liver, kidneys, gonads, heart and thyroids. The following organs/tissue were histopathologically examined: adrenals, aorta, bone marrow (femoral), brain, caecum, colon, duodenum, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, rib and bone marrow, sciatic nerve, skin and mammary gland, spinal cord, rib and bone marrow, spleen, stomach, salivary gland (submaxillary), testes, trachea, thymus, thyroids, tongue, skeletal muscle (quadriceps), urinary bladder, uterus (corpus and cervix) and all unusual lesions.

Statistical analysis: Group body weight and organ weight data were compared to the control group using an ANOVA followed by a student's t-test.

Results

Dietary analysis & paraquat intake: Analysis of duplicate samples of the 7 ppm diet revealed that the paraquat cation concentration in the top, middle and bottom samples were 9.3 and 6.5, 6.5 and 7.5, and 7.2 and 16.0 μg/g, respectively (mean ± 1 SD: 8.8 ± 3.66 μg/g). Analysis of duplicate samples of the 120 ppm diet revealed that the paraquat cation concentration in the top, middle and bottom samples were 177 and 113, 97 and 117, and 120 and 97 μg/g, respectively (mean ± 1 SD: 120 ± 29.55). Collectively, these data indicated that the diet was homogenous despite the inter and intra-sample variability. There was no loss in paraquat over 14 days at ambient temperature which indicated that paraquat was stable in the diet. Mean (± 1 SD) analytical paraquat concentrations of < 4, 8.3 ± 1.48, 24.1 ± 6.87, 71.8 ± 12.15 and 150.93 ± 17.86 μg/g (calculated by the reviewing toxicologist) were within 19-26% of the nominal concentrations of 0, 7, 20, 60 and 120 ppm throughout the 13 week study period.

There was a positive trend with regard to the dietary intake of paraquat and the concentration of paraquat measured in the urine at week 6 and 13 of the study (see Table 49 below). Additionally, the urinary concentration of paraquat was greater at week 13 than at week 6. Collectively these observations suggested that the absorption (and excretion) of paraquat was proportional to dietary intake.

Table 49: Concentration (μg/mL) of paraquat cation in urine samples from Beagle dogs following 6 and 12 weeks of dietary exposure

Group	0 ppm	7 ppm	20 ppm	60 ppm	120 ppm
Wk 6	(n=3)	(n=3)	(n=3)	(n=3)	(n=1)
Male	0	0	0.61 ± 0.07	5.41 ± 3.07	8.1
Female	0	0.07 ± 0.12	0.53 ± 0.04	3.71 ± 0.63	11.4
Wk 13					
Male	0	0.63 ± 0.09	2.33 ± 0.58	9.18 ± 2.23	14.4
Female	0	0.56 ± 0.06	1.84 ± 0.66	5.13 ± 2.41	27.6

Results are expressed as the mean ± 1 SD and were calculated from the raw data by the reviewing toxicologist.

Mortalities & clinical signs: Two males from the 120 ppm group were sacrificed in a moribund condition on day 16 and 23, while 2 females from the same group were sacrificed on day 18 and 23. All four dogs showed dyspnoea 0–2 days prior to sacrifice. Additionally, the male that was sacrificed on day 16 had a reduced heart rate and inappetance, with auscultation revealing

harsh dry rales and a slow mucous membrane refill time on day 15. The female that was sacrificed on day 18 had marked alopecia around the haunches and hyperventilation from day 17, and increased respiratory sounds and a slow irregular heart beat upon auscultation on the day of sacrifice. There were no further deaths or clinical signs observed during the study with the exception of one male from the 20 ppm group who exhibited a non-specific pyrexia during week 3 and 7, but this subsided following 2 courses of different antibiotics.

Body weight & food consumption: There was no treatment-related effect on body weight gain at or below 60 ppm. In males, the control group maintained a relatively stable body weight over the entire study period. At 120 ppm, the 2 males that were sacrificed showed a 450 and 650 g reduction in body weight (relative to their immediate pre-dose body weights) prior to death, however both dogs showed a progressive reduction in body weight from week 3 pre-dose. These reductions equated to a less than 5% loss of body weight. The surviving male from the 120 ppm group lost 800 g (~5%) from week 1-12 of treatment. An ANOVA revealed that there was no statistical difference in body weight between any group of males.

In females, the control group gained 870 g over the 12 week study period while the two high-dose dogs that were sacrificed showed a 350 and 200 g body weight loss prior to sacrifice (ie < 5%). The surviving female from the 120 ppm group lost 1200 g from week 1–12 of the study (~10%). Although statistical analysis showed that the body weight gain of females from week 1 pre-dose to week 13 of treatment was significantly lower than the control at 7–60 ppm ($p<0.01–0.05$), the lack of a dose-response effect suggested that the result was not treatment-related at and below 60 ppm.

There was no clear treatment-related effect on food consumption. One of the males from the 120 ppm group that was sacrificed in a moribund condition, consumed 250 g (9%) less food one week prior to death, a result which corresponded with clinical signs of inappetance in this dog. In contrast, both females from the 120 ppm group that were sacrificed in a moribund condition showed no reduction in food consumption prior to death. The surviving female from the 120 ppm group showed a 26-67% reduction in food consumption from week 8 of treatment (ie 740–1880 g/wk). One female from the 60 ppm group showed an erratic level of food consumption throughout the pre-treatment and 13-week treatment period.

Haematology, clinical chemistry & urinalysis: There was no treatment-related effect on any haematology, clinical chemistry or urinary parameters tested.

Ophthalmoscopy & Auscultation: There was no treatment-related effect on the incidence of ophthalmoscopic abnormalities. At 120 ppm, the male that was sacrificed at week 3 showed engorged retinal vessels in both eyes and small areas of haemorrhage on the retina while the male that was sacrificed at week 4 showed no ophthalmoscopic abnormalities. The surviving high-dose male exhibited no ophthalmoscopic abnormalities. Examination of the surviving female from the 120 ppm group at week 13 revealed that both eyes had retinal vessels that were very injected, however, injected retinal vessels were also observed in single females from the 7 and 20 ppm groups. Similarly, there was no treatment-related effect on the incidence of auscultation findings. Slight to marked increases in respiratory sounds were recorded across all groups.

Pathology: The mean relative lung weight of males at 60 and 120 ppm was higher (0.996 and 1.735% respectively) than the control group (0.904%). In females, relative lung weights were

higher at 7 (1.00%), 20 (0.936%), 60 (1.068%) and 120 ppm (1.685%) compared to the control (0.826%). Statistical analysis, however, revealed no significant difference in relative lung weight between paraquat-treated and control groups. There was a trend of increased relative spleen weight in females (0.226, 0.236, 0.278 and 0.282% for 0, 7, 20 and 60 ppm groups respectively), but in the absence of a similar trend in males and the absence of statistical analysis, this result was not considered to be treatment-related.

At necropsy, macroscopic lung lesions were detected in 1/3 and 3/3 male and females respectively in the 60 ppm group. These were described as large, irregular, dark red, depressed areas affecting most lobes. At 120 ppm, all dogs showed gross lung lesions which ranged from slight patchy darkening of all lobes to multi-focal areas of haemorrhage. No treatment-related abnormalities were detected in any other organs/tissues.

Histopathology: There was a clear treatment-related effect on the incidence of alveolitis which was detected in all dogs at 120 ppm, and all but one male at 60 ppm. No other treatment-related abnormalities were observed.

Conclusions: The NOEL in Beagle dogs following 13 weeks of dietary exposure to paraquat was 20 ppm (equivalent to 0.5 mg/kg bw/d paraquat cation) based on macroscopic lung lesions and alveolitis at 60 ppm and above (equivalent to 1.5 mg/kg bw/d paraquat cation). At 120 ppm (equivalent to 3 mg/kg bw/d paraquat cation), mortalities, a decrease in body weight gain in females ($p<0.01$) and a statistically insignificant elevation in relative lung weight were observed.

Comments: Statistical analysis was performed only on body weight and organ weight data. No standard deviations were provided for the mean haematology or clinical chemistry parameters. No group means were given for any urinary parameter. The storage conditions for urine and plasma samples were unspecified.

4.6 Chronic Studies

4.6.1 Mice

4.6.1.1 80 -week Carcinogenicity Study in Mice

Fletcher K, Flegg R & Kinch DA (1972) Paraquat: Carcinogenic study in the mouse. Study no: not stated. Lab: Imperial Chemical Industries Ltd, Industrial Hygiene Research Laboratories, Alderley Park, Macclesfield, Cheshire, UK. Sponsor & study duration: not stated. Report no: RO/IH/P/21. Report date: April, 1972.

Pre-dates GLP and test guidelines.

Study: Four groups of 70 male and 50 female mice (Alderley Park, initial bw: 31-32 g and 29-30 g for males and females, respectively) were fed with paraquat (paraquat cation content 25.86%, source and batch unspecified) in the diet at 0, 25, 50 or 75 ppm (equivalent to approximately 0, 3.75, 7.5 and 11.25 mg/kg bw/d, respectively) for 80 weeks. It was stated that the diets were analysed periodically for paraquat concentration, but the frequency of analysis was unspecified. The animals were housed by sex (10/cage) under standard laboratory conditions and provided with the test diets and water *ad libitum.* No further details on experimental methods were provided.

Observations: Animals were observed daily for any abnormalities and mortalities. Commencing from week 20, cumulative mortality was calculated at 10-week intervals. Food consumption per cage was recorded weekly for the first 12 weeks, and then for one seven-day period every fourth week. Body weights were determined initially at weekly intervals for the first 12 weeks, and fortnightly thereafter. A detailed necropsy examination was conducted on all mice found dead, moribund and sacrificed prematurely, and those that survived the full study period and sacrificed at termination (method unspecified). In addition to tumours and suspected neoplasms, the following tissues were processed for histological examination: pituitary, salivary gland, thyroid, thymus, heart, lungs, liver, adrenals, kidney, pancreas, spleen, stomach, duodenum, jejunum, ileum, caecum, colon, gonads, uterus or epididymis, seminal vesicles, prostate, urinary bladder and voluntary muscle.

Findings

Mortality: Survival rates among the test groups at 60 weeks were greater than 50% and was consistent with the current US EPA test guidelines for assessing the adequacy of carcinogenicity studies. Survival data at 72 weeks were not provided. At termination (80 weeks) survival was 20%, 34%, 17% and 21% in males, and 24%, 46%, 30% and 24% in females for the control, 3.75, 7.5 and 11.25 mg/kg bw/d groups, respectively. There was no treatment-related increase in mortality during the study. It was stated that the deaths during the first half of the study occurred mainly among males, and were largely associated with physical aggression. Isolated cases of respiratory diseases were also observed in all groups including controls, but no necropsy or pathology details on these were provided.

Food consumption & body weights: A slight reduction in the average individual food consumption was seen in all treatment groups at the majority of observation times, with no apparent dose-response relationship (see Table 50 below). At termination, the deficit in food consumption in high-dose males was approximately 11% compared to parallel controls, but an increase of approximately 18% was seen in high-dose females.

Table 50: Food consumption at selected observation times (g/mouse, M/F)*

Dose (mg/kg bw/d)	Weeks on test				
	8	24	48	72	80
Control	38/37	32/31	38/33	33/30	35/32
3.75	34/36	29/28	31 (18%)/37	28 (15%)/29	32/30
7.5	35/31 (16%)	30/28 (10%)	35/30	38/28	28 (20%)/33
11.25	32 (16%)/32	37/28	29 (24%)/35	34/33	31 (11%)/38

*Values in parentheses represent percent reductions compared to parallel controls. The values are typical of those seen at other observation times.

Similarly, decreases in group-mean body weights were seen across all treatment groups at the majority of observation times (see Table 51 below). In particular, the body weight reductions in all treated females at 48 and 72 weeks were dose-related and could be attributed to treatment.

Table 51: Group mean body weights at selected observation times (g, M/F)*

Dose (mg/kg bw/d)	Weeks on test				
	8	24	48	72	80
Control	49/41	59/53	64/64	59/60	55/45
3.75	49/39	57/48	62/57 (11%)	57/53 (12%)	51/48
7.5	48/39	56/46 (17%)	56 (18%)/54 (16%)	58/48 (20%)	51/45
11.25	48/37 (10%)	53 (10%)/43 (19%)	55 (14%)/48 (25%)	54 (8%)/44 (26%)	54/41 (9%)

*Values in parentheses represent percent reductions compared to parallel controls and are typical of those seen at other observation times.

Pathology: Congested and consolidated lungs were seen in all groups including controls. The incidence of haemorrhagic lungs was higher in treated males compared to the controls, together with an increased incidence of enlarged kidneys at the mid- and high-dose (see Table 52 below) compared to the corresponding controls. The gross pathology data for the animals sacrificed during the study were not provided.

Table 52: Non-neoplastic necropsy findings in mice (M/F) treated with paraquat

Observation	Dose (mg/kg bw/d)			
	Control	3.75	7.5	11.25
Number of animals/group	14/12	24/23	12/15	15/12
Haemorrhagic lungs	1/5 (7%/40%)	11/2 (45%/9%)	3/1 (25%/7%)	9/3 (60%/25%)
Enlarged kidneys	4/0 (28%/0)	0/0	9/0 (75%/0)	9/0 (65%/0)

Values in parenthesis represent percent incidences.

Histopathology & tumour incidence: No treatment-related tissue abnormalities were seen upon histopathological examination. Similarly, there were no treatment-related increases in tumour incidence. While there were no lung tumours in control animals, 2 tumours (1 adenoma, 1 carcinoma) in low-dose males (n=37), 4 tumours (3 adenomas, 1 carcinoma) in mid-dose females (n=36), and 4 tumours (3/38 males and 1/38 females, adenomas) at the high-dose were observed (no historical control data were provided). The lesions seen in the lungs were not considered to be toxicologically significant because there was no apparent dose-response relationship in the tumour incidence or signs of tumour progression (from adenoma to carcinoma) in either sex.

Conclusions: There was no evidence that paraquat was carcinogenic to mice. A NOEL for this study was not established due to the decreased group mean body weight in treated females and the increased incidence of gross pathological lesions in all treated animals compared to controls. The LOEL was 3.75 mg/kg bw/d. Interpretation of the study findings was difficult due to the unsatisfactory dose selection and poor survival rate among the test animals. Consequently, the regulatory value of this study is limited.

4.6.1.2 Lifetime Feeding Study in Mice

Sotheran M, Banham PB, Godley MJ, Lindsay S, Pratt I, Taylor K & Woollen BH (1981) Paraquat: Lifetime feeding study in the mouse. Study no: PM0006. Lab: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: not specified. Study duration: Commenced on October 25, 1977. Report no: CTL/P/556. Report date: June 17, 1981.

***Also includes*:**

Smith LL (1986) Paraquat: Lifetime feeding study in the mouse. Supplementary data for Japanese regulatory authorities. Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report no: CTL/P/556A. Report date: April 9, 1986, and

Smith LL (1990) Fourth supplement to paraquat: Lifetime feeding study in the mouse. Tumour summary and tumour incidence tables to support Shoroku submission. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report no: CTL/P/556/4. Report date: September 27, 1990.

Quality assured non-GLP study. No test guidelines were cited.

Study: Paraquat technical (paraquat cation content: 32.7%, batch: S358/1, ICI Ltd, Mond Division, UK) mixed with pulverised feed (BP Nutrition, UK Ltd) was fed to Swiss-derived mice (from a laboratory-maintained colony, 35 days old, initial mean bw 29.8-30.2 and 25.1-26.0 g for males and females, respectively, 60 animals/sex/dose) at 0, 12.5, 37.5 or 100/125 ppm (equivalent to approximately 0, 1.9, 5.6 and 15/18.8 mg paraquat ion/kg bw/d) for 97-99 weeks. There were 2 control groups in the study. The dose levels were based on the results of two preliminary studies. The initial high-dose of 15 mg/kg bw/d was increased to 18.8 mg/kg bw/d at 36 week onwards, as there were no adverse effects, other than some decreases in food consumption at 35 weeks. Further groups of 10 mice/sex were fed the same dose levels as above for 52 weeks to determine the paraquat levels in the kidney, lung and plasma at termination. Prior to commencement of the study, animals were quarantined for approximately 1-2 weeks.

During the pre-experimental period and then for approximately 10 months during the study, Expanded Porton Rat Diet with vitamin E supplement was used, which then changed to Porton Combined Diet for the remaining part of the study. Although some details on the procedure of diet preparation were provided, the frequency of preparation was unspecified. Animals were housed by sex (5/cage) under conventional laboratory conditions and provided with the prepared diets and water *ad libitum*. Animals that were maintained for the analysis of tissue paraquat levels were sacrificed by an overdose of halothane vapour at 52 weeks. The study was terminated between weeks 97 and 99 because the overall mortality rate among the test groups was at or approaching 80%. Blood samples were collected by cardiac puncture. Moribund animals and those surviving were sacrificed similarly and autopsied at termination.

Observations: Animals were observed daily for clinical signs and behavioural abnormalities. Body weights were recorded prior to commencement of feeding, weekly during the first 12 weeks, fortnightly for the next 24 weeks, weekly for the next 4 weeks, and fortnightly thereafter. Food consumption was measured weekly during the first 12 weeks, monthly from week 12 to 36, weekly from week 36 to 40 and from week 40 until termination. Daily food wastage was measured during the period when food consumption was recorded. Paraquat levels in the urine were determined using samples collected (from the cage) at 3 monthly intervals (main study, 2 cages/sex). The following organs were histologically examined: voluntary muscle, salivary glands, cervical lymph nodes, pancreas, spleen, liver with gall bladder, adrenals, kidneys, urinary bladder, heart, lungs, thyroid, trachea, oesophagus, thymus,

mesenteric lymph node, jejunum, ileum, duodenum, colon, caecum, stomach, pituitary, brain, spinal cord, skin, aorta, eyes, gonads, Harderian glands and sciatic nerves. Statistical differences between the control and the treatment groups were tested using either a Student's t-test (two-sided) or a one-sided Fisher's exact test. Analysis of mortality data involved pair-wise comparisons of all groups and an overall test for trend with dose.

Findings:
Paraquat was shown to be stable in diets at least for approximately 6 weeks with the paraquat content in the majority of the samples falling within 10% of the nominal concentrations. Mortality data are presented in Table 53 below. It was reported that the mortality rate in mid-dose males and high-dose females differed significantly from that of the combined controls, but no further details or the level of significance was provided for an independent evaluation. Only mortality in males at 99 weeks exhibited a weak dose-related trend. In females, a dose-relationship was seen at 80 weeks and at termination at the mid-dose and above.

Table 53: Percentage mortality at selected observation times

Observation week	Dietary concentration (mg/kg bw/d)			
	Control*	1.9	5.6	15/18.8
Males				
20	1.7	5.0	1.7	3.3
44	3.3	11.7	13.3	8.3
60	15.8	21.7	26.7	11.7
80	36.7	46.7	55.0	40.0
99	66.5	75.0	78.3	80.0
Females				
20	1.7	1.7	3.3	1.7
44	9.2	13.3	11.7	8.3
60	24.1	18.3	21.7	28.3
80	41.6	31.7	48.3	53.3
98	72.5	68.3	78.3	86.7

*Mean of combined controls.

Clinical signs: Swelling and sores in the genital area, incontinence and alopecia were frequently seen in both sexes across all groups. The number of observations of swelling in the genital area appeared to be approximately 1.5- to 2-fold greater at the high-dose compared to the controls. However, the group incidence of this clinical sign did not show any dose-effect relationship. A dose-related increase in the incidence of incontinence was seen in mid- and high-dose males, whilst this was slightly elevated in all treated females (1.3- to 1.7-fold) compared to controls (see Table 54 below). However, the number of animals that exhibited this clinical sign did not vary greatly between the control and treated groups, and therefore it's clinical significance was unclear.

Table 54: Clinical observations in mice fed paraquat

Observation	Dietary concentration (mg/kg bw/d)			
	Control*	1.9	5.6	15/18.8
	Number of observations (number of animals affected)			
Males				
Swelling in genital area	192 (33) *5.8*	254 (37) *6.8*	160 (34) *4.7*	290 (33) *8.7*
Sores in genital area	54 (13) *4.1*	52 (13) *4.0*	110 (15) *7.3*	66 (19) *3.5*
Incontinence	341 (44) *7.7*	373 (46) *8.1*	667 (41) *16.2*	816 (52) *15.7*
Females				
Swelling in genital area	8 (4) *2*	2 (2) *1*	3 (3) *1*	27 (8) *3.3*
Sores in genital area	22 (5) *4.4*	17 (7) *2.4*	4 (4) *1*	66 (16) *4.1*
Incontinence	184 (29) *6.3*	314 (38) *8.2*	243 (36) *6.7*	280 (36) *7.7*

*Mean of combined controls. Values in italics indicate the average number of observations/animal.

Food consumption: During the first 24 weeks, food consumption in all treated animals was low compared to the control, reaching statistical significance ($p \leq 0.01$ or 0.05) at the majority of observation times. It was consistently low at the high-dose during the remaining period of the study, showing statistical significance ($p \leq 0.01$ or 0.05) at 36, 52, 56 and 60 weeks in males and at the majority of observation times in females. After 24 weeks, females in the remaining dose groups also consumed less food compared to the parallel controls. Low-dose animals exhibited significant decreases at 60, 64 and 76 weeks and mid-dose animals showed similar depressions at 40, 56, 60, 72 and 84 weeks ($p \leq 0.01$ or 0.05) with a dose-related trend at 56, 72 and 84 weeks. The decreases in food consumption in females were attributed to the test substance. No treatment-related effects were observed in food utilisation values.

Body weights: Depressions in body weights were seen at the high-dose (~3-7% and 2-19% in males and females, respectively) compared to controls (see Table 55 below). This was consistent in both sexes and could be attributed to the test substance.

Table 55: Group mean body weights (g) at selected observation times

Observation week	Dietary concentration (mg/kg bw/d)				
	Control		1.9	5.6	15/18.8
Males					
20	54.5	54.7	54.6	55.5	52.9 (3%)
44	61.7	61.2	61.9	61.0	60.0 (3%)
60	61.1	61.4	60.4	59.0	58.7 (4%)
80	56.4	55.2	55.0	54.6	53.3 (3%)
96	52.5	51.2	49.2	52.2	48.8 (7%)
Females					
20	43.2	43.6	42.5	43.1	42.5 (2%)
44	52.6	52.6	51.5	52.4	49.8 (5%)
60	51.5	52.2	51.7	50.8	48.7 (6%)
80	51.3	52.2	49.6	49.3 (5%)	47.4 (8%)
96	47.9	47.0	46.7	46.5 (2%)	38.3 (19%)

Values in parenthesis represent percent depression compared to the mean of combined concurrent controls.

Consistent with the above data, high-dose animals showed decreased weight gain throughout the study (see Table 56 below). High-dose males showed approximately 3-7% depressions in weight gain at 20, 44, 60 and 80 weeks compared to the controls. The females at this dose level exhibited statistically significant depressions in weight gain ($p \leq 0.01$ or 0.05) at the majority of observation times, commencing from week 44. At termination, the weight gain in high-dose females was depressed by approximately 45% compared to the controls.

Table 56: Body weight gain (g) in mice treated with paraquat

Observation week	Dietary concentration (mg/kg bw/d)			
	Control¶	1.9	5.6	15/18.8
Males				
20	24.5	24.9	25.3	23.2 (5%)
44	31.2	32.6	30.8	30.2 (3%)
60	30.8	31.4	28.7	29.1 (5%)
80	25.8	26.5	24.4	24.1 (6.6%)
96	21.2	23.2	23.8	21.0
Females				
20	17.0	17.4	17.0	16.5
44	26.5	26.3	26.2	23.4**(12%)
60	25.8	27.1	25.0 (3%)	21.9**(15%)
80	25.8	24.4 (5%)	24.4 (5.4%)	21.2** (17%)
96	23.5	20.5 (13%)	18.9 (20%)	12.9** (45%)

¶Mean of combined controls. **Significantly different from corresponding controls ($p \leq 0.01$). Values in parenthesis represent percent depression compared to the concurrent controls.

Although no statistical significance was achieved, mid-dose females showed approximately a 20% depression in weight gain compared to controls at termination. The only time that a dose-response relationship for depressed weight gain was noticed was during the last 2-3 months of the study, when mortality was very high. The decreases in weight gain at the mid- and high-dose groups at termination were considered to be treatment-related.

Pathology: Non-neoplastic abnormalities observed during histopathological examination of lung, liver and kidney tissues are summarised in the Table 57 below. The incidence of renal tubular degeneration was elevated at the high dose upon termination, and in mid-dose males and in high-dose animals that died during the study compared to the controls. Mild to marked fatty-type vacuolation in the liver was seen predominantly in mid- and high-dose males that died or were sacrificed during the study. It was reported that the incidence of this lesion reached statistical significance in mid- and high-dose males (p value unspecified). Renal and hepatic lesions noted in mid- and high-dose males at termination and intercurrent deaths could be attributed to the test compound.

Table 57: Non-neoplastic findings in mice at terminal sacrifice and intercurrent deaths (M/F)

Observation	Number affected				
	Dietary concentration (mg/kg bw/d)				
	Control¶		1.9	5.6	15/18.8
Terminal sacrifice					
Renal tubular degeneration	0/0 (15/12)	0/2 (25/21)	0/0 (15/19)	1/0 (13/14)	6/5 (12/8)
Intercurrent deaths					
Number examined	(45/48)	(35/39)	(45/41)	(47/46)	(48/52)
Liver fatty-type vacuolation (mild, marked)	0/2	2/1	3/1	7/1	6/1
Renal tubular degeneration (without dilatation)	8/1	3/5	9/2	13/3	16/15
Renal tubular pigmentation	0/0	1/0	1/0	1/1	3/5
Lung focal hypercellularity	2/0	2/0	3/0	½	6/5

¶Number of observations for the 2 control groups. Values in parenthesis represent the number of animals examined for each sex.

Tumour incidence: The incidence of pulmonary adenomas was approximately 2.5-fold greater in high-dose animals that died during weeks 79-98, compared to controls (see Table 58 below). However, this was not seen in rats that were sacrificed at the end of the study and was therefore not attributed to treatment. Other tumours reported in the study were spontaneous in nature, and hence were not regarded as treatment-related. Overall, no treatment-related increase in tumour incidence was evident.

Table 58: Neoplastic findings in mice at terminal kill and intercurrent deaths (M/F)

Observation	Number affected				
	Dietary concentration (mg/kg bw/d)				
	Control¶		1.9	5.6	15/18.8
Lung adenomas					
Terminal kill	4/4 (15/12)	5/1 (25/21)	2/4 (15/19)	2/1 (13/14)	3/0 (12/8)
Intercurrent deaths (weeks 79-98)	3/4 (21/24)	2/2 (16/15)	2/1 (20/22)	3/1 (15/19)	7/8 (24/20)
Total	8/8 (60)	11/5 (60)	7/5 (60)	9/4 (60)	11/10 (60)

¶Number of observations for the 2 control groups. Values in parenthesis represent the number of mice examined for each sex.

Conclusions: Based on decreased body weight gain and non-neoplastic abnormalities seen (renal tubular degeneration) at 5.6 mg/kg bw/d, the NOEL was 1.9 mg/kg bw/d. The experimental design including the dose selection was unsatisfactory. The high mortality among both the control and test animals made the interpretation of the study findings difficult.

4.6.1.3 104-week Feeding Study in Mice

Toyoshima S, Sato R, Kashima M & Motoyama M (1982a) AT-5: Chronic toxicity study result – 104-week dosing study in mouse. Study no: not stated. Lab: Nippon Experimental Medical Research Institute, Haruna Laboratory, 3303-58, Hanatate, Ohdo, Agatsuma-machi, Agatsuma-gun, Gumma-ken 377-09, Japan. Sponsor: not stated. Study duration: February 27, 1979 to February 20, 1981. Report no: CTL/C/1871A. Report date: March 10, 1982.

Non-GLP, quality assured study. No test guidelines were cited.

Study: Paraquat technical (AT-5, as paraquat dichloride, purity 98%, Lot no: 540108, Asahi Chemical Industry Co, Ltd, Kanagava-ken, Japan) mixed with pulverised feed (CE-2, Japan Clea Laboratories Co Ltd) was fed to JCL:ICR mice (3 weeks old, initial bw 25-29 and 23-26 g for males and females, respectively, 80 mice/sex/dose) at 0, 2, 10, 30 or 100 ppm (equivalent to approximately 0, 0.3, 1.5, 4.5 and 15 mg/kg bw/d) for 104 weeks. The dose levels were based on the results of pilot studies. Animals were quarantined for 1 week prior to commencement of the study. Test diets were prepared once every five months. Animals were housed by sex (5/cage/dose) under standard laboratory conditions and provided with the prepared diets and water *ad libitum*. Groups of 10 mice/sex/dose were sacrificed at 26 and 52 weeks, and all survivors were sacrificed at 104 weeks. Animals showing debility and those *in extremis* were isolated in separate cages and observed, but not sacrificed.

Observations: Animals were observed twice daily for clinical signs and mortality. Growths or nodules on the skin were checked weekly by palpation. Body weights and food and water consumption were recorded weekly during the first 26 weeks and once every 2 weeks thereafter. Food efficiency was calculated using the group mean body weights at 26, 52 and 104 weeks, and the respective food consumption data. The achieved dose was calculated by dividing the group mean food consumption by the group mean body weight and multiplying the resultant value by the nominal concentration of paraquat. Blood samples collected from animals of intercurrent sacrifice and all survivors, and used to determine the following haematological and clinical chemistry parameters: RBC, WBC, Hct, Hb, WBC-DC, platelet count, and serum AST, ALT and ALP activities, A/G ratio, blood glucose, total protein, BUN, cholesterol, Na^+, K^+, Cl^-, creatinine, and brain, RBC and serum ChE activities. Urine samples were collected from the above groups by suppressing the upper surface of the urinary bladder and tested for the following: occult blood, ketones, glucose, protein, pH, urobilinogen and bilirubin. The animals dying during the study, those sacrificed by design and at termination were necropsied. The absolute weights of the following organs were recorded and the relative weights were calculated: brain, pituitary, thyroid, thymus, heart, lung, liver, spleen, pancreas, kidneys, adrenals, testes, seminal vesicles, prostrate, ovaries, uterus and urinary bladder. In addition to the organs above, tissue samples from the following were processed for histological examination: stomach, duodenum, small and large intestines, skeletal muscle, bone marrow, salivary glands, mesenteric lymph nodes, trachea, oesophagus, sciatic nerve, eye ball, skin and any macroscopically abnormal tissue.

Statistical differences between the control and treatment groups were tested using a Student's t-test. Mortality and tumour incidence data were analysed using a χ^2-test.

Findings:

The average amounts of paraquat ingested by the animals were 0, 0.26, 1.31, 3.92 and 13.09 mg/kg bw/d in males and 0, 0.26, 1.32, 3.82, 13.03 in females for the nominal concentrations of 0, 0.3, 1.5, 4.5 and 15 mg/kg bw/d, respectively.

Mortality, clinical signs: There was only one death during the first 52 weeks. Mortality during the latter part of the study was higher in all groups including controls. The proportions of animals surviving at termination were approximately 28%, 38%, 30%, 28% and 30% for males

and 43%, 36%, 47%, 35% and 30% for females in the control, 0.3, 1.5, 4.5 and 15 mg/kg bw/d groups, respectively. Although no treatment-related group differences were seen in males, the mortality in females at 15 mg/kg bw/d was approximately 30% higher compared with controls. Clinical signs such as lowered mobility, loss of coat lustre and piloerection were consistently observed in moribund animals, including controls.

Food consumption & body weights: Food consumption in males at 1.5, 4.5 and 15 mg/kg bw/d was slightly reduced during the first 34 weeks, but there were no further group differences in either sex thereafter. Terminal body weight in males at 15 mg/kg bw/d was depressed by approximately 6% compared to controls, but no statistical significance was achieved. Water intake and food efficiency were unaffected by treatment.

Clinical chemistry and necropsy: Males receiving the highest paraquat dose showed significantly reduced ($p \leq 0.05$) Hct, Hb and RBC, WBC and lymphocyte counts at 26 weeks, and reduced Hct, RBC and WBC at 52 and 104 weeks. In high-dose females, significantly reduced ($p \leq 0.05$) Hb levels were seen at all 3 observation times, together with similar decreases in WBC and RBC counts and Hct at 26, 52 and 104 weeks, respectively. Terminal necropsy showed approximately a 2-fold increase in the incidence of nodular changes in the lung in males at 15 mg/kg bw/d, which could possibly be attributed to the test substance (see Table 59 below). There were no treatment-related increases in the tumour incidence in the treated groups compared to controls.

Table 59: Necropsy findings in lungs at termination (M/F)

Necropsy findings	Incidence[a]				
	Dose (mg/kg bw/d)				
	0	**0.3**	**1.5**	**4.5**	**15**
Number of animals	**17/26**	**23/22**	**18/28**	**17/21**	**18/18**
Hepatoid changes	1/3	0/0	0/3	1/2	2/3
Nodules	4/6	4/5	7/4	4/8	9/4

[a]Values represent the number of animals affected and those in parentheses indicate percent incidence.

Pathology: Statistically significant ($p \leq 0.05$) changes in relative and absolute organ weights were noted at 26, 52 and 104 weeks at 15 mg/kg bw/d. These included decreases in relative and absolute thyroid and adrenal weights and an increase in lung weights in males at 26 weeks, and elevated absolute heart weight in males at 52 weeks. At termination, absolute, liver and thyroid weights in males and absolute brain weight in females were significantly reduced. No treatment-related inter-group differences were seen in histopathology or tumour incidence.

Conclusions: There was no evidence that paraquat was carcinogenic in mice. Based on statistically significant perturbations in haematological parameters (reduced Hct, Hb, and RBC, WBC and lymphocyte counts at 26 weeks; reduced Hct, RBC and WBC at 52 and 104 weeks), relative and absolute organ weights at 26, 52 and 104 weeks, and reduced body weights at 104 weeks at 15 mg/kg bw/d, the NOEL was set at the nominal dose of 4.5 mg/kg bw/d, equivalent to an actual dose of 2.84 mg/kg bw/d and 2.77 mg paraquat ion/kg bw/d in males and females respectively (after taking into account the amount of diet consumed).

4.6.2 Rats

4.6.2.1 104-week Feeding Study in Rats

Toyoshima S, Sato R, Kashima M, Motoyama M & Ishikawa A (1982b) AT-5: Chronic toxicity study result - 104 Week dosing study in rat. Study no: not stated. Lab: Nippon Experimental Medical Research Institute, Haruna Laboratory, 3303-58 Hanatate, Ohdo, Agatsuma-gun, Gumma-ken 377-09, Japan. Sponsor: not stated. Study duration: February 27, 1979 to February 20, 1981. Report no: CTL/C/1870A. Report date: March 10, 1982.

Quality assured non-GLP study. No test guidelines were cited.

Study: Paraquat technical (AT-5, as paraquat dichloride, purity 98%, Lot no: 540108, Asahi Chemical Industry Co, Ltd, Kanagava-ken, Japan) mixed with pulverised feed (CE-2, Japan Clea Laboratories Co Ltd) was fed to JCL: Wistar rats (4 weeks old, initial mean bw 100-130 and 90-120 g for males and females, respectively, 62 rats/sex/dose) at 0, 6, 30, 100 or 300 ppm (equivalent to approximately 0, 0.6, 3.0, 10 and 30 mg/kg bw/d) for 104 weeks. Rats were quarantined for one week prior to the commencement of the study. Diets were prepared once every four months. Rats were housed by sex (2/cage/dose) under standard laboratory conditions and provided with the prepared diets and water *ad libitum*. Groups of 6 rats/sex/dose were sacrificed at 26 and 52 weeks. All survivors were sacrificed at 104 weeks. Rats showing debility and those *in extremis* were isolated in separate cages and observed, but not sacrificed.

Observations: Animals were observed for clinical signs twice daily. Mortalities were recorded immediately after discovery. After 26 weeks on study, they were examined weekly by palpation for growths or nodules on the skin. Body weights, food and water consumption were recorded weekly during the first 26 weeks, and then fortnightly. Food efficiency was calculated by using the group mean body weights at 26, 52 and 104 weeks and respective food consumption data. The achieved dose was calculated by dividing the group mean food consumption by the group mean body weight and multiplying the resultant value by the nominal concentration of paraquat. Blood samples collected from the animals of intercurrent sacrifices and at termination were used to determine the following haematological and clinical chemistry parameters: RBC, WBC, Hct, Hb, WBC-DC, platelet and reticulocyte counts and PT, and AST, ALT, ALP activities, A/G ratio, blood glucose, total protein, BUN, cholesterol, Na^+, K^+, Cl^-, creatinine, brain, RBC and serum ChE activities. Urine samples collected (12-h) from intercurrent sacrifices and survivors were tested for the following parameters: occult blood, ketones, glucose, protein, pH, urobilinogen, bilirubin and Na^+ and K^+ levels. The animals dying during the study and those sacrificed by design and at termination were necropsied. The weights of the following organs were recorded and the relative weights determined: brain, pituitary, thyroid, thymus, heart, lung, liver, spleen, pancreas, kidneys, adrenals, testes, seminal vesicles, prostate, ovaries, uterus and urinary bladder. In addition to the above organs, samples of the following were processed for histopathology: stomach, duodenum, small intestine, skeletal muscle, bone marrow, salivary glands, mesenteric lymph nodes, trachea, oesophagus, sciatic nerve, eye ball, skin and any macroscopically abnormal tissue. Ophthalmoscopy was performed at 26, 52 and 104 weeks. Statistical differences between control and treatment groups were examined using a Student's t-test.

Findings:
Mortality: Except for one death (male) at 3 mg/kg bw/d at 52 weeks, there were no mortalities during the first year. Survival rates at 72 weeks and termination are given in the Table 60 below. Although there was no apparent dose-related trend, generally, the survival rates in all treated males at both observation times (except for males at 30 mg/kg bw/d at 72 weeks) appeared to be lower than controls. Percent survival in females at the highest-dose was depressed by approximately 17% at termination compared to controls. Increased mortality at this dose level could be attributed to treatment. It was stated that the deaths were consistently preceded by lowered spontaneous motility of the animals for approximately 3-7 days, and loss of coat lustre and piloerection.

Table 60: Percent survival* (M/F)

Observation time	Dose (mg/kg bw/d)				
	Control	0.6	3.0	10.0	30.0
At 72 weeks	94/98	88/94	72/100	78/92	94/92
At termination	60/58	56/54	44/62	54/56	34/48

According to the study authors, no clinical signs or behavioural abnormalities attributable to paraquat were noted in any group during any part of the study. Food and water consumption, and food utilisation efficiency were unaffected by treatment. The achieved dose, calculated using the food consumption data and the concentration of paraquat in the diet, ranged from 49 to 59% of the nominal dose (see Table 61 below).

Table 61: Actual dose levels of paraquat ingested following dietary administration

Nominal dose (mg/kg bw/d)	Mean test substance (mg/kg bw/d)*	
	Males	Females
0.6	0.25 (58%)	0.30 (50%)
3.0	1.26 (58%)	1.5 (50%)
10.0	4.15 (58%)	5.12 (49%)
30.0	12.25 (59%)	15.29 (49%)

*Values in parentheses represent percentages of target concentrations.

Body weights: Body weights in males at 30 mg/kg bw/d were depressed at the majority of observation times showing approximately a 4% deficit in body weight at termination compared to the controls. Sporadic depressions in group mean body weights were also seen at 3 and 10 mg/kg bw/d. At termination, the overall weight gain in males at 3, 10 and 30 mg/kg bw/d were depressed by approximately 6%, 3% and 5%, respectively, compared to the controls. The females at 30 mg/kg bw/d showed consistently low group mean body weights from the study week 20 onwards, reaching statistical significance at 34, 42-48 and 54 weeks ($p \leq 0.05$). At termination, the animals in this group exhibited a deficit of approximately 5% in overall weight gain compared to controls (see Table 62 below). However, there was no clear relationship between decreased body weight gain and treatment.

Table 62: Group mean body weights (g, M/F) at selected observation times

Dose (mg/kg bw/d)	Observation time (week)			
	26	52	72	104
Control	541/306	612/363	640/409	504/364
0.6	557/303	622/365	648/413	509/356
3.0	550/305	615/352	636/423	481/378
10.0	548/308	624/369	637/423	497/375
30.0	538/300	609/351	628/396	487/351

Haematology: Treatment-related and statistically significant ($p \leq 0.05$) perturbations in haematology were limited to the 30 mg/kg bw/d groups. At 26 weeks, males had significantly depressed RBC, Hct and Hb levels and increased reticulocyte counts and significantly decreased RBC and Hb levels were observed in females. At 52 weeks, significantly reduced RBC counts were seen in both sexes, in addition to significant decreases in Hb levels and WBC counts in females. At termination, significantly depressed RBC counts, Hct and Hb levels in both sexes, and elevated reticulocyte counts were seen in males.

Clinical chemistry: Statistically significant changes in clinical chemistry parameters that could be attributed to treatment were also confined to the 30 mg/kg bw/d dose groups ($p \leq 0.05$). Significantly reduced total protein levels were seen in both sexes at all sacrifice intervals. In addition, blood glucose levels in males were significantly depressed at 52 and 104 weeks. RBC ChE activity in males was significantly depressed ($p \leq 0.05$) at 52 weeks. There were no further inter-group differences in either sex at the other observation times.

Urinalysis at 26 and 52 weeks did not reveal any inter-group differences. At termination, positive occult blood reactions and fluctuating urinary protein levels were seen in all groups including controls, with no apparent dose-relationship.

Necropsy at 26 weeks revealed abnormalities in the lung and kidney. These included liver-like changes in the lung (1/6 male each at 3 and 10 mg/kg bw/d) and renal oedema (1/6 male each at 0.6 and 10 mg/kg bw/d, and 1 female in the control group). Necropsy at 52 weeks showed abnormalities in the kidney, the pituitary and uterus, and included renal oedema (1/6 male at 30 mg/kg bw/d), pituitary (1/6 female each at 3 and 10 mg/kg bw/d) and uterine oedema (1/6 female each at control and 10 mg/kg bw/d). Only a small number of animals appeared to have affected, and none of these abnormalities showed any dose-response relationship.

Pathology: Macroscopic tissue abnormalities seen at termination are given in the Table 63 below. An increased incidence of hepatoid changes in the lung of females (except for 10 mg/kg bw/d) and congestion in the lung in all treated males, and in high-dose females were noted. However, in the absence of any clear dose-response effect, the association between these abnormalities and treatment was equivocal. In addition, there was an increased incidence of testicular abnormalities at 30 mg/kg bw/d compared to controls, but again no dose-response relationship was evident.

Table 63: Terminal necropsy findings in rats (M/F) fed paraquat for 104 weeks*

Observation	Incidence				
	Dose (mg/kg bw/d)				
	Control	0.6	3.0	10.0	30.0
Number of rats	**30/29**	**28/27**	**22/31**	**27/28**	**17/24**
Lungs					
Hepatoid changes	15/8 (50/27)	10/11 (36/41)	5/10 (23/32)	13/6 (48/21)	5/10 (29/42)
Congestion	5/5 (16/17)	10/5 (36/18)	9/5 (41/16)	9/3 (33/11)	4/10 (23/42)
Testis					
Softening	3 (10)	6 (21)	3 (14)	5 (18)	4 (23)
Atrophy	3 (10)	5 (18)	3 (14)	4 (15)	4 (23)

*Values in parenthesis represent percent incidences.

Gross pathology: Necropsy findings in rats dying during the study are given in the Table 64 below. Increased incidence of hepatoid changes in the lung in both sexes of rats was seen at all doses compared to controls. In addition, there were 2- to 5-fold increases in the incidence of testicular atrophy in all dose-groups. However, due to lack of a clear dose-response relationship, these changes were not considered to be treatment-related.

Table 64: Necropsy findings in rats (M/F) that died during the study

Observation	Incidence				
	Dose (mg/kg bw/d)				
	Control	0.6	3.0	10.0	30.0
Number of rats examined	**20/21**	**22/23**	**28/19**	**23/22**	**33/26**
Lungs					
Hepatoid changes	0/0	3/2	2/3	2/3	6/3
Nodes	9/0	4/2	15/2	9/1	15/1
Congestion	0/6	6/4	9/6	13/5	5/6
Metachromatism	8/0	4/2	15/3	9/3	17/6
Testis					
Atrophy	1	2	5	6	4

Treatment-related and statistically or toxicologically significant organ weight changes observed at 26, 52 and 104 weeks were restricted to the 30 mg/kg bw/d groups. The changes seen at 26 and 52 weeks were significant increases in absolute kidney weights in both sexes (~10-17%) and absolute ovary weight (~28-38%, $p \leq 0.05$), and approximately 7-13% decreases in relative liver, heart and brain weights in females ($p \leq 0.05$ or 0.01) compared to controls. At termination, the absolute and relative heart weights in males were depressed by approximately 13-14%, whilst the absolute and relative liver weights in females were reduced by approximately 11-14% compared to the concurrent controls ($p \leq 0.05$). In females, absolute heart weight was reduced by 10% compared to controls ($p \leq 0.05$).

Histopathology: Thickening of lung alveolar epithelium was observed in 1-3 animals/sex/dose including controls at 26 and 52 weeks. In females, one rat each at 3 and 10 mg/kg bw/d and 2 rats at 30 mg/kg bw/d showed slight liver bile duct proliferation. Terminal histopathology showed approximately a 8% increase in incidence of slight peribronchiolitis at 30 mg/kg bw/d, an approximately 4- to 6-fold increased incidence in slight suppurative pneumonia, and a 7-14% increased incidence in slight inflammation of the trachea in males at all doses compared to controls. The only histological change seen in females was a 1.4- to 1.7-fold increase in the

incidence in slight suppurative pneumonia in the lung at 10 and 30 mg/kg bw/d. Due to lack of consistent histological evidence in both sexes, the relationship between the abnormalities noted above and treatment appeared somewhat equivocal.

Histological findings in animals dying during the study are given in the Table 65 below. Abnormalities included an increased incidence of thickening of lung alveolar epithelium and haemostasis in the adrenals in females of all doses, pneumonia in males and adenofibroma in the mammary gland in females at 3, 10 and 30 mg/kg bw/d. Incidence of adenofibroma in the mammary gland increased dose-relatedly (~1.7- to 3-fold) from 3 mg/kg bw/d and above compared to the controls, but the incidence fell within the historical control range (18-45%) for this rat strain (Poteracki & Walsh, 1998). The incidence of other tumours was unaffected by treatment.

Table 65: Histological findings in animals (M/F) that died during the study

Observation	Incidence*				
	Dose (mg/kg bw/d)				
	Control	0.6	3.0	10.0	30.0
Number of rats examined	**20/21**	**22/23**	**28/19**	**23/22**	**33/26**
Lungs					
Thickening of alveolar walls (slight)	1/2 (5/9)	1/4 (4/17)	1/2 (4/10)	3/3 (13/14)	1/6 (3/23)
Pneumonia (slight to moderate)	12/0 (60/0)	9/6 (41/26)	18/7 (64/37)	17/6 (74/27)	19/9 (57/35)
Females: Adrenals					
Haemostasis (slight)	1 (5)	3 (13)	5 (26)	5 (23)	12 (46)
Mammary gland					
Adenofibroma	2 (9)	1(5)	3 (16)	4 (18)	7 (27)

*Values in parentheses represent percent incidences.

Ophthalmoscopy revealed a slight elevation in the incidence of eye cataracts in both sexes at 52 weeks; incidences were approximately 3-6% at 3 mg/kg bw/d and above compared to approximately 2-3% in the control and 0.6 mg/kg bw/d groups. However, no inter-group differences were noted at 26 weeks or at termination.

Conclusions: There were number of equivocal findings (effects on body weight, pathology) in all groups including controls. However, due to lack of clear dose-response relationships, the regulatory value of the findings of this rat study were limited. Based on increased mortality in males at the next highest dose, the NOEL was established at an actual dose of 3 mg paraquat ion/kg bw/d, after taking into account the amount of diet consumed.

4.6.2.2 Combined Toxicity and Carcinogenicity Study in Rats

Woolsgrove BW (1983) Paraquat: Combined toxicity and carcinogenicity study in rats. Study no: not stated. Lab: Life Science Research, Stock, Essex CM4 9PE, UK. Sponsor: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study duration: April 6, 1978 to August 21, 1980. LSR Report no: 82/ILY217/328 (CTL/C/1225). Report date: October 27, 1983. Also include,

Ashby R & Finn JP (1983) Paraquat: Toxicity and carcinogenicity study in dietary administration to rats. Interim report 1: 0-52 weeks. Lab: Life Science Research, Stock, Essex. CM4 9PE. Report no: 80/ILY217/271 (CTL/C/1001).
Report date: June 23, 1983,

Ishmael J & Godley M (1983) Paraquat: Lifetime feeding study in rats. Histopathological examination of lungs. Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL/P/738). Report date: July 28, 1983,

Brown MP & Whitney JC (1984) Paraquat: Combined toxicity and carcinogenicity study in rats. Photomicrography of selected tissues. Lab: Life Science Research, Elm Farm Laboratories, Eye, Suffolk IL23 7PX, England. LSR Report no: 84/ILY 217/171 (CTL/C/1225). Report date: March 22, 1984,

Life Science Research Institute (1984) Paraquat: Combined toxicity and carcinogenicity study in rats. Volume IX, Final report. Stock, Essex CM4 9PE, UK. Report no: 84/ILY217/042. CTL Report no: CTL/C/1225. Report date: March 30, 1984,

Woolsgrove BW & Ashby R (1985) Paraquat: Combined toxicity and carcinogenicity study in rats. Supplementary information on numbers of protocol tissues examined. Amended supplement to LSR Report no: 82/ILY217/328. Lab: Life Science Research, Elm Farm Laboratories, Eye, Suffolk IL23 7PX, England. LSR report no: 85/ILY 217/460 (CTL/C/1225), Report date: August 23, 1985, and

Ishmael J (1987) Paraquat: Lifetime feeding study in rats. A histopathological review of slides from the head region. CTL Study no: PR0010, Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire UK. Sponsor: ICI PLC. Report no: CTL/P/1984. Report date: May 8, 1987.

Quality assured non-GLP study. No test guidelines were cited.

Study: Paraquat technical (batch: S 358, paraquat cation content: 32.69%, ICI Ltd) in the diet was administered to F344 rats (70 rats/sex/dose, 5 weeks old, initial bw: 60-70 g, Charles River Labs, USA) at 0, 25, 75 or 150 ppm (equivalent to approximately 0, 2.5, 7.5 and 15.0 mg/kg bw/d) for period of at least 113 (males) or 122 (females) weeks. Animals arrived in two batches, one week apart. During the study, these two batches were maintained separately. There were two control groups in the study (70 rats/sex). At 52 weeks, 5 rats/sex from one control and each treatment group were used for the determination of tissue paraquat levels. Animals were acclimatised for 7 days prior to commencement of the study. Test diets were prepared weekly, using powdered diets (Spratt's Laboratory Animal Diet No: 2, Spratt's Patent Ltd, UK). Paraquat concentrations in the diets were determined fortnightly using a method supplied by the sponsor. Homogeneity was checked using 6 samples obtained from the diets prepared for the first and 44th study weeks. The stability of paraquat in prepared diets was determined over 14 days using samples from the diets prepared for the first study week. Although the study was initially planned for 104 weeks, this period was extended to a life-span study because of low mortality seen upon completion of that period. Animals were housed (5/sex/cage) under standard laboratory conditions and provided with the prepared diets and water *ad libitum*. After 1 year of feeding, 10 rats/sex/group were sacrificed for histopathology. Terminal sacrifice was commenced after 113 and 122 weeks of treatment for males and females, respectively.

Observations:
Serial: Mortality and clinical signs were checked twice daily. From week 118 onwards, clinical observations were conducted once daily except during weekends and on public holidays. Rats were examined once weekly by palpation for superficial tumours. Food consumption was determined weekly. Water intake was recorded by daily measurements during 3-day periods in weeks 1 to 4, 13, 26, 41, 52, 65, 78, 92 and 101. Body weight and achieved dosages were determined on the day that treatment commenced, weekly for the next 12 weeks, then fortnightly until week 68, and again weekly thereafter. The efficiency of food utilisation was assessed weekly during study weeks 1-12, 13-26, 27-40 and 41-52. Ophthalmoscopic examinations were conducted on 20 rats/sex before commencement of the study, at 4, 14, 26, 52 and 79 weeks and on all surviving rats at 103 weeks. Retro-orbital blood samples were collected from 5 rats/sex prior to commencement of treatment and 10 rats/sex after 14, 26, 40, 53, 66, 79, 92 and 102 weeks of treatment, and then after 111 or 112 weeks for males and after 118 or 119 weeks for females. The following haematological parameters were determined: Hb, RBC, WBC, WBC-DC, PCV, platelet count, reticulocyte count, PT, APTT, MCHC, MCV. Additional tests conducted were: platelet counts and PT and APTT (5 rats/sex from the control and mid-dose groups) after 17 weeks; reticulocyte counts in control, mid- (5 females) and high-dose animals (5 rats/sex) PT and APTT (5 rats/sex/dose) after 29 weeks; PT and APTT in control and low-dose animals (5/sex) and in 5 females of all groups after 54 weeks; and PT and APTT in 5 females/group, after 96 weeks. In addition, plasma separated from the blood samples taken at the above sampling times was used the following clinical chemistry parameters: urea and glucose concentration, ALT and AST activities.

During the closing stages of the study, the following were examined in some decedents that had clinical signs (see Findings): ALP, total bilirubin and direct bilirubin concentrations. Urinalysis was performed on 5 rats/sex prior to commencement of feeding and after completion of 13, 15, 26, 39, 52, 65, 77 or 78, 92 and 101 weeks. It was stated that when possible urine samples were collected from those animals that were used for haematology. During sample collection (approximately 28 h), animals were kept in individual metabolism cages. Urine samples were analysed for the following: volume, pH, specific gravity, reducing substances, glucose, protein, ketones, bile pigments, urobilinogen, blood pigments, cells, casts and crystals. Urinary paraquat levels were determined after 15, 27, 41, 52, 65, 79, 92 and 102 weeks of treatment using pooled urine samples collected from 10 rats/sex/dose. During sample collection food and water were withheld from the animals.

Terminal: Those animals found moribund during the study and 10 rats/sex/group after 52 weeks, and all surviving at termination, were sacrificed by CO_2 asphyxiation and necropsied. These groups and those found dead during the study were subjected to following procedures: macro- and microscopic pathology, organ weights (adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, spleen, testes, thymus and thyroid glands) and histopathology of a range of tissues. Samples from liver, lungs kidneys, skin, plasma and urine were collected (5 rats/sex/group) for tissue paraquat level determination using a radio-immunoassay method. Tumour classification was largely based on the criteria specified in the publications of the International Agency for Research on Cancer (IARC). Where appropriate the following statistical procedures were used to analyse the data: χ^2-test, Cox's test, Fisher's exact test, Student's t-test, Covariance analysis and Armitage's test.

Findings:
Analysis of the diet did not show any appreciable changes of homogeneity or stability of the test substance with time. Achieved concentrations of paraquat in the test diets were in reasonable agreement with the targeted concentrations (~ ± 25%, 10-29% and 11-14% for the 2.5, 7.5 and 15 mg/kg bw/d dose groups, respectively). As the study progressed, achieved dosages of paraquat were decreased due to reduced energy requirement and the consequent decline in food consumption (see Table 66 below).

Table 66: Achieved paraquat dosage ranges (mg/kg bw/d) in the study

Nominal dose mg/kg bw/d	Achieved dose range (mg/kg bw/d)	
	Males	Females
2.5	2.9-0.8	2.8-0.9
7.5	8.5-2.4	8.4-3.0
15.0	16.8-4.8	16.2-5.6

Mortality: No treatment-related inter-group differences were seen in either sex. Percent survival at termination was 30%, 38%, 42% and 55% in males for the combined control, 2.5, 7.5 and 15 mg/kg bw/d groups, respectively. In females, approximately 48% of the animals in each group survived the full study period.

Clinical signs: In treated females, there was a dose-related increase in the incidence of opacity in one or both eyes (as evaluated by cage-side observation without the use of an ophthalmoscope) compared to combined controls. In males, this abnormality was seen at the mid-dose and above. An approximately 2-fold increase in ptosis/swollen eyelids was seen in high-dose females (see Table 67 below) compared to the controls. All these abnormalities could be attributed to treatment.

Table 67: Incidence of ocular lesions in rats (M/F) treated with paraquat*

Clinical sign	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15
	1	2			
Opacity	4/3	1/1	3/6	5/14	22/35
Ptosis/swollen eyelids	0/4	3/4	2/3	2/5	1/7

*Total incidence for the 2 batches of animals. Total number of animals examined = 60/sex.

No further treatment-related clinical signs were observed, and according to the study author, the behaviour of the animals during the study was similar to that of the controls.

Food consumption: At termination (at 113 weeks), weekly food consumption at the high-dose was depressed by approximately 11% and 6% in males and females, respectively, compared to controls. During the next 8 weeks of the study, food consumption in high-dose females was reduced by approximately 3-12% compared to controls, with no further inter-group differences seen when the study terminated at 122 weeks. Group-mean water consumption was depressed at the high-dose by approximately 7-37% in males and by approximately 3-35% in females compared to controls. In addition, sporadic reductions in water consumption were seen in both sexes at the mid-dose. There were no marked inter-group differences in food utilisation efficiency in treated rats, except for high-dose females, whose food conversion ratio was depressed by approximately 50% at 11 and 12 weeks compared to controls.

Body weights: Generally, treated males gained less weight compared to controls, exhibiting a dose-related trend throughout the study. Statistically significant deficits in body weight were

seen in males at the mid-dose (~2%) at 26 and 52 weeks and at the high-dose (~6-8%) at 78, 104 and 113 weeks ($p \leq 0.001$ or 0.05) compared to controls. High-dose females showed approximately 2-9% depressions in weight gain at the majority of observation times reaching statistical significance at 52, 78, 104, 117 and 122 weeks compared to controls (see Table 68 below). The body weight reductions seen at the high-dose were considered to be toxicologically significant.

Table 68: Group mean body weights (g) in rats treated with paraquat

Study week	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Males					
26	404	401	399	395* (2%)	374** (7%)
52	480	478	475	471* (2%)	440** (8%)
78	493	500	497	491	457** (7%)
104	448	448	445	437	418** (7%)
113	428	422	412	406	400** (6%)
Females					
52	237	235	243	236	231* (2%)
78	295	286	299	287	269* (7%)
104	315	309	318	308	287* (8%)
117	318	313	317	300* (5%)	286** (9%)
122	312	301	309	295	277** (9%)

Significantly different from controls *($p \leq 0.05$), ** ($p \leq 0.001$). Values in parentheses represent percent reductions compared to combined controls.

Eye lesions: After 52 weeks of treatment, the onset and progression of eye cataracts and/or opacity (see Table 69 below) were accelerated dose-relatedly at and above 7.5 mg/kg bw/d. These lesions, together with secondary lesions such as glaucoma and/or iritis in some animals of all dose groups were seen in both sexes at termination. Ophthalmoscopic examinations on all surviving animals were not carried out earlier than 103 weeks.

Table 69: Ocular lesions in rats treated with paraquat

Study week	Lesion	Group incidence				
		Control		2.5	7.5	15.0
		1	2			
103 (M/F)	Posterior polar cataract	3/0	0/0	1/0	8/5	19/30
	Radial cataract	0/0	0/0	-/0	2/2	8/5
	Posterior polar opacity	3/0	0/0	1/0	8/5	19/30
	Posterior capsular opacity/cataract	0/2	0/5	0/4	3/6	24/12
	Posterior polar opacity/cataract	3/0	0/0	1/0	8/5	19/30
	Cataract	1/1	1/1	2/1	3/1	5/4
Total number of animals examined		44/49	46/51	42/45	43/44	46/47
112/113 (M)*	Posterior polar cataract	4	2	1	14	4
	Radial cataract	0	0	0	2	29
	Posterior capsular cataract	0	1	3	0	9
	Posterior polar opacity	2	2	5	12	1
	Glaucoma	0	0	0	2	2
	Iritis	1	0	0	2	6
	Total cataract	0	0	0	1	3
Total number of animals examined		33	34	33	28	37
118/119 (F)*	Posterior polar cataract	4	4	6	16	0
	Radial cataract	2	2	1	12	24
	Posterior capsular cataract	1	2	1	1	0
	Posterior polar opacity	2	4	1	0	0
	Glaucoma	0	0	1	3	1
	Iritis	0	2	3	2	5
	Total cataract	1	1	2	3	3
Total number of animals examined		35	36	31	32	33

*The data for only male/female rats were provided.

Haematology and clinical chemistry: There were several statistically significant, but isolated and minor perturbations in various parameters especially during the first 18 months of the study. These include statistically significant ($p \leq 0.001$, 0.01 or 0.05) decreases in RBC counts at the mid and/or high-dose at 14, 26 and 79 weeks, decreased total WBC counts at 14 (females at all dose levels), 26 (males at all dose levels) and 53 (males at the mid- and high-dose groups) weeks, accompanied by an increased APTT in high-dose males at 17, 66 and 102 weeks ($p \leq 0.05$). Although the decreases in WBC counts reached statistical significance at all dose levels females (at 14 weeks) and males (at 26 weeks), toxicological significance was restricted to mid- and high-dose groups. No toxicological significance was attributed to any of the other isolated statistically significant differences. There were no significant inter-group differences in haematology in either sex at termination. Bone marrow and blood smears evaluated at termination did not reveal any treatment-related effects.

Clinical chemistry showed several isolated, but statistically significant perturbations in all study parameters. Most of these occurred during the first year of the study. The majority of them, however, did not show any consistent dose-related trend in either sex and therefore were considered to be unrelated to treatment. Urinalysis did not show any treatment-related changes. Excretion of paraquat in the urine was largely related to its dietary concentration.

Analysis of tissue paraquat levels showed dose-related concentrations of the test compound in the kidney and blood plasma at all doses. At the mid- and high-dose, paraquat levels in the lung tissue were relatively lower than those found in the kidneys. Paraquat was also detected in some

skin samples of mid-dose males and high-dose rats. In the liver tissue, paraquat was detected only in high-dose females at the lower limit of detection.

Pathology: Treatment-related and statistically significant changes in organ weights at intercurrent and terminal sacrifice were confined to the high-dose. At termination, relative brain and lung weights in both sexes were significantly increased (~7% and 16% in males, and ~12% and 14% in females, respectively, $p \leq 0.05$) compared to controls. Statistically significant depressions ($p \leq 0.01$ or 0.001) were seen in absolute liver weights in both sexes (~17% in males and 12% in females) and in relative liver weight (~10%) in males compared to controls. At termination, absolute heart weight at the high-dose was reduced by approximately 8% and 18% in males and females, respectively, compared to controls ($p \leq 0.01$ or 0.05). Absolute and relative testes weights were reduced by approximately 22% and 17%, respectively, compared to controls ($p \leq 0.01$ or 0.05). The organ weights changes seen at the high-dose group were attributed to treatment.

In addition to previously described eye lesions, the other macroscopic abnormalities that could be attributed to treatment was an increased total incidence in chronic pneumonitis in males at the mid- and high-dose ($p \leq 0.01$, total incidences were 0/69, 0/70, 0/70, 2/70 and 7/69 for the 2 control, low-, mid- and high-dose groups, respectively). Histopathology in rats sacrificed at termination showed focal subpleural abnormalities at all dose levels and proliferative lesions in alveolar epithelium at the high-dose.

In line with the study author's description, the histopathological abnormalities that could be attributed to treatment included hydrocephalus, degeneration of the nerve fibres of the sciatic nerve and an increase in the numbers of cysts or cystic spaces in the spinal cord. However, no dose-response relationship was apparent and there were inconsistencies in the incidences of these lesions between the animals that died during the study and those that were killed at termination (see Table 70 below).

Since the mortality was unaffected by treatment, the study authors considered that statistical analysis of the combined incidence of hydrocephalus for all affected animals was necessary. This evaluation showed that, there was a statistically significant increase in the incidence of hydrocephaly in females at 7.5 and 15.0 mg/kg bw/d ($p<0.05$), and the NOEL for hydrocephalus was, therefore, 2.5 mg/kg bw/d. The toxicological significance of hydrocephalus remains equivocal because there was no clear dose-response relationship in decedent animals, and also males were unaffected by treatment. The study authors stated that the degree of hydrocephaly, seen as a dilatation of the fourth ventricle, was not ‘marked’ in any case. However, no supporting historical control data were provided to substantiate this claim.

Further, there was a statistically significant increase in the incidence of degeneration of the sciatic nerve in decedent males at the mid- and high-dose ($p<0.001$). At all dose levels, females were unaffected. As the incidences in decedents and survivors were disproportionate, and the incidence in male controls was lower compared to female controls, the toxicological significance of this lesion is again unclear. Nonetheless, the study authors selected the low-dose, ie 2.5 mg/kg bw/d dose level as the NOEL for this lesion.

The group incidences of cysts or cystic spaces in the spinal cord were significantly higher ($p<0.05$, 0.01 or 0.001) in decedent males compared to the corresponding controls. However,

there was no clear dose-response relationship and no significant effect was found in decedent females. In addition, the incidences of this lesion were very low in all surviving rats (only in 1 high-dose male rat). It was therefore considered that the occurrences of cysts or cystic spaces in the spinal cord in decedent males were fortuitous and unrelated to treatment.

Table 70: Incidences of hydrocephalus, degeneration of the sciatic nerve and cysts or cystic spaces in the spinal cord (M/F)

Group	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Decedents					
Hydrocephalus	4/3 (30/31)	1/2 (24/29)	2/8* (36/30)	6/9* (35/27)	0/9* (26/30)
Degeneration of sciatic nerve	6/15 (30/30)	4/11 (24/30)	8/10 (36/30)	17***/11 (35/27)	19***/11 (26/30)
Spinal cord cysts or cystic spaces	0/5 (30/31)	0/1 (23/30)	6**/6 (36/30)	4*/3 (35/27)	7***/7 (26/30)
Survivors					
Hydrocephalus	0/4 (29/28)	1/0 (31/30)	2/1 (23/29)	2/3 (25/29)	2/11** (28/29)
Degeneration of sciatic nerve	13/5 (29/28)	7/7 (31/30)	11/2 (23/29)	15/6 (25/29)	13/10 (33/29)
Spinal cord cysts or cystic spaces	0/0 (29/28)	0/0 (31/30)	0/0 (23/29)	0/0 (25/29)	1/0 (33/29)

Values in parentheses represent the total number of animals examined. *Significantly different from controls ($p \leq 0.05$); **Significantly different from controls ($p \leq 0.01$)

Pathological changes in the eyes of animals sacrificed at 52 weeks, and for all rats regardless of the time of death, are presented in the Table 71 below. No treatment-related effects were seen in animals sacrificed at 52 weeks. The eye lesions, in particular the lenticular abnormalities observed in the latter group were attributed to treatment. Although the dose-time relationship of these lesions was not evaluated by the study authors, from the data, it is apparent that the onset and progression of this abnormality may have commenced after 52 weeks of treatment.

Table 71: Incidence of lenticular changes in the eyes[a] (M/F)

Observation	Dose (mg/kg bw/d)			
	Control[b]	2.5	7.5	15.0
At 52 weeks				
Number of eyes examined	39/40	20/20	20/20	20/19
Peripheral morgagnian corpuscles (slight)	1/1	0/1	0/1	2/0
Peripheral lenticular degeneration (slight)	0/0	0/0	1/0	1/0
'Pear shaped' posterior peripheral lenticular change	0/0	0/0	0/0	1/0
For all rats (regardless of time of death)				
Number of eyes examined	219/226	112/112	114/107	115/114
Peripheral morgagnian corpuscles (marked)	12/34	19**/31***	35***/52***	69***/84***
Peripheral lenticular degeneration (moderate)	8/33	13*/30**	39***/31**	34***/43***
Peripheral lenticular degeneration (marked)	1/7	4/4	6*/10***	22***/32***
Mid zonal lenticular degeneration (moderate)	0/12	4*/13	19***/27***	39***/37***
Mid zonal lenticular degeneration (marked)	0/0	0/3*	3**/23***	29***/27***
'Pear shaped' posterior peripheral lenticular change	6/42	11*/32	51***/48***	73***/74***

[a]Not all parameters provided at all sampling times; [b]Combined controls; *p≤0.05, **p≤ 0.01 or ***p≤0.001.

Tumour incidence: Preliminary lung histopathology revealed that there was a possible relationship between the incidence of alveolar epithelialisation and treatment. The findings of the incidence of this lesion as well as the lung adenoma/carcinoma incidence in treated rats was then re-evaluated by a consultant pathologist for the performing laboratory (Life Science Research, LSR). Results of this re-evaluation are provided in (i) Table 72 to (iii) Table 74.

Findings of the pathologist from the performing laboratory:

(i) Table 72: Animals[a] (M/F) bearing primary pulmonary neoplasms

Observation	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0[c]
	1	2			
Adenoma	1/0	2/0	3/1	5/2	4/8***
Carcinoma[b]	1/0	0/0	1/1	1/1	3/2
Total neoplasms	2/0	2/0	4/2	6/3	7/10***

[a]Total number of animals examined = 69 or 70; [b]Carcinomas include: broncho-alveolar and squamous carcinomas or both these types. [c]One animal in this group had a carcinoma and a separate adenoma and each was recorded. ***Significantly different from controls ($p \leq 0.001$).

(ii) Table 73: Animals[a] (M/F) bearing pulmonary alveolar epithelialisation (adenomatosis)

Observation	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Alveolar epithelialisation (with or without changes)	2/3	2/7	2/5	7/8	8/3

[a]Total number of animals examined = 69 or 70. Excluding those in which pulmonary neoplasia was seen in the same lobe.

(iii) Table 74: Animals[a] (M/F) bearing proliferative lesions of probable alveolar type II cell origin

Observation	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Alveolar epithelialisation + pulmonary adenoma + pulmonary carcinoma	4/3	4/7	6/7	13/11	14/13

[a]Total number of animals examined = 69 or 70. Excluding those in which pulmonary neoplasia was seen in the same lobe.

The conclusions of the LSR pathologist were as follows: 'Paraquat has induced proliferation of alveolar type II cells. Statistical support for this conclusion is present at 7.5 and 15 mg/kg bw/d for all proliferative changes both neoplastic and non-neoplastic. Furthermore, statistical support is present for the association between treatment at 15 mg/kg bw/d paraquat and the formation of pulmonary adenoma in female rats. There is a more general conclusion for the study, that treatment with paraquat at 2.5. 7.5 and 15 mg/kg bw/d resulted in a significant increase in the incidence of pulmonary tumours (both benign and malignant) over the control incidence'.

The above findings were then reviewed by the pathologist of the sponsoring agency (ICI), and are presented in (i) Table 75 to (ii) Table 76.

Findings of the pathologist from the sponsor:

(i) Table 75: Incidence of pulmonary adenoma and carcinoma[a] (M/F)

Observation	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Adenoma	0/0	0/0	2/0	1/1	1/0
Carcinoma	1/0	1/0	2/1	1/1	3/0

[a]Total number of animals examined = 69 or 70.

(ii) Table 76: Group distribution of adenomatosis[a] (M/F)

Observation	Dose (mg/kg bw/d)				
	Control		2.5	7.5	15.0
	1	2			
Total	2/4	4/4	5/5	8/4	11**/13**

[a]Number of animals examined = 10. **Significantly different from controls ($p \leq 0.01$).

The conclusions of the ICI pathologist were as follows: '(1) there was a range of lung lesions characterised by proliferation of epithelial cells in a proportion of animals, (2) a small number of these lesions were considered to be neoplasms, both benign and malignant, but these did not show a clear relationship to treatment with paraquat, and (3) a third proliferative lesion, designated adenomatosis and which was considered to be hyperplastic and reactive in nature rather than neoplastic, occurred at 15 mg/kg bw/d in males only'.

As there were apparent differences in the interpretation of the data, two further series of slides were prepared jointly by the LSR and ICI, using representative (for the type and severity of lesions) lung samples. These slides were then re-evaluated by two other independent pathologists. The conclusion of one of the pathologists was that 'they were difficult slides, which challenged some of the criteria traditionally used to differentiate adenomatosis from neoplasia. There has been a question whether the two conditions are associated, and in this instance, it appears that neoplasms are rising in areas of adenomatosis in some cases. I would, however, classify adenomatosis, in itself, as a non-neoplastic, proliferative lesion usually associated with a pulmonary interstitial reaction'. Following evaluation, the other pathologist described the lung pathology as 'bronchiolo-alveolar hyperplasia associated with centriacinar or interstitial inflammation (depending on extensiveness of the lesion)', and suggested that the use of the term 'adenomatosis' should be avoided for this lesion, even though it exists in the literature.

When the foregoing ICI and LSR assessments are viewed in the light of the differing diagnoses of the two consultant pathologists, it appears that the proliferative lung lesions seen in this study were not compartmentalised into non-neoplastic or neoplastic, or into adenoma or carcinoma, although the criteria used by all the pathologists were apparently similar. It was reported that all criteria set for any class of lesion were rarely met, and therefore the diagnosis was clearly dependent upon the weighting that each pathologist placed on each criteria.

It has been reported that spontaneous pulmonary neoplasms (ie alveolar/bronchiolar adenomas or carcinomas) are rare in rats, with background control rates of less than 3% in either sex (Dixon & Maronpot 1991). Viewed against such a background, it is apparent that the present study failed to provide unequivocal evidence of neoplastic transformation in the lung tissue in response to long-term paraquat exposure. However, the study suggested that paraquat induced

proliferative lesions of the alveolar epithelium at the high-dose, a result which was statistically significant. The data at the mid-dose were suggestive of an effect of treatment, but at the low-dose no toxicologically-significant increase in this lesion was seen compared to controls. Incidences of other tumours (benign fibroepithelial tumours of the mammary gland, pancreatic islet cell adenomas, thyroid parafolicular cell adenomas and carcinomas, testicular interstitial cell tumours, skin and subcutis fibromas and lipomas, and monocytic leukaemia) were age-related neoplastic lesions commonly seen in this strain of rats.

Conclusions: This study did not provide unequivocal evidence of paraquat-induced carcinogenicity following long-term dietary exposure in rats. However, evidence suggested that paraquat caused proliferative lesions of the alveolar epithelium at the highest dose of 15 mg/kg bw/d, a result which was statistically significant. No NOEL could be established because of treatment-related and statistically significant increases in the incidence of macroscopic and microscopic ocular lesions in both sexes at the lowest nominal dose of 2.5 mg/kg bw/d, equivalent to an actual dose of 1.25 mg paraquat ion/kg bw/day, after taking into account the amount of diet consumed.

4.6.3 Dogs

4.6.3.1 One-year Feeding Study in Dogs

Kalinowski AE, Doe JE, Chart IS, Gore CW, Godley MJ, Hollis K, Robinson M & Woolen B (1983a) Paraquat: 1 Year feeding study in dogs. Study no: PD0413, Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: Not stated. Study duration: January 6, 1981 – January 21, 1982. Report no: CTL/P/734. Report date: April 20, 1983 and

Kalinowski AE, Doe JE, Chart IS, Gore CW, Godley MJ, Hollis K, Robinson M & Woolen B (1983b) Paraquat: 1 Year feeding study in dogs. Individual animal data. Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report no: CTL/P/734S. Report date: June 16, 1983.

Quality assured non-GLP study. No test guidelines were cited.

Study: Paraquat (reference: S358/2, paraquat cation content: 32.2%, source: ICI PLC, UK) mixed with pulverised diets (BP Nutrition UK Ltd) was fed to Beagle dogs (20-24 weeks old, initial bw 12-12.5 kg, 6/sex/dose) at 0 (control), 15, 30 or 50 ppm (as paraquat cation) for one year. Based on the paraquat intake data provided by the study authors, the actual mean amounts of paraquat cation ingested by the animals (males/females) at 15, 30 and 50 ppm were 0.45/0.48, 0.93/1.0 and 1.5/1.58 mg/kg bw/d, respectively. All dogs were dewormed and vaccinated against distemper, infectious hepatitis, parvovirus and leptospirosis prior to allocating them into treatment groups. Animal grouping was also based on pre-treatment haematology, clinical biochemistry and health status. The acclimatisation period was 5-6 weeks. The test diets were prepared in 10-kg batches at approximately one-week intervals and analysed for paraquat content at intervals of approximately 4-weeks. Each dog was given 400 g of the prepared diet every morning. Dogs were individually housed under standard laboratory conditions and were provided with drinking water *ad libitum*.

Observations: Clinical examinations, which included chest auscultation, ophthalmoscopy and checks for clinical and behavioural abnormalities were performed twice daily, prior to commencement of the study, after weeks 13, 26, 39 on study and between the study weeks 48-51. Body weight was checked weekly during the acclimatisation period, on the first day of dosing and then weekly. Food consumption was measured daily. Haematological parameters were checked pre-experimentally and at 4, 8, 12, 16, 20, 26, 39 and 52 weeks (Hct, Hb, RBC, WBC and WBC-DC counts, MCV, MCHC and platelet count). Bone marrow smears were obtained from all animals at 26 and 52 weeks. Using blood samples collected from the jugular vein prior to feeding, the following clinical chemistry parameters were determined pre-experimentally, and at 4, 8, 12, 16, 20, 26, 39 and 52 weeks: AST, ALT, creatinine kinase, ALP, glucose, urea, albumin, total protein, triglycerides, calcium, potassium and cholesterol.

All dogs were placed in metabolism cages for urine collection before the study commenced, and then at 8, 16, 24, 39 and 50 weeks. Water only was available during urine collection. Urine was tested for the following parameters: glucose, ketones, urobilinogen, pH, specific gravity, protein and cytology. At termination, dogs were anaesthetised with sodium pentobarbitone, sacrificed by exsanguination and autopsied. The weights of the following organs were recorded: heart, lung, liver, kidney, gonads, thyroid, adrenals, brain, pituitary and thymus. The following tissues were sampled and processed for histopathology: adrenals, aorta, bone marrow, brain, caecum, cervix, colon, duodenum, epididymis, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, mammary gland, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, skin, spinal cord, spleen, stomach, testes, thymus, thyroids/parathyroids, trachea, urinary bladder, uterus, voluntary muscle, and any abnormal tissue. Urine samples collected from all dogs at 29 weeks (using urinary catheters), and samples of the kidney, liver and lung, were analysed for paraquat content. Where appropriate, the statistical difference between the control and treatment groups were tested using either an ANOVA, an analysis of covariance or a two-sided Student's t-test.

Findings:
According to results of the dietary analysis, concentrations of paraquat in the test diets were within 10% of the nominal concentrations. Paraquat was found to be stable in the diet for more than 8 weeks. Paraquat intake, in terms of paraquat cation ingested/kg bw, decreased slightly with time in all groups. There were no mortalities during the study.

Clinical signs: Hyperpnoea was seen in some dogs across all groups, but the incidence of this clinical sign and vesicular sounds was greater in the high-dose group, and could be attributed to treatment (see Table 77 below). Both these clinical signs were first observed after 13 weeks of treatment.

Table 77: Incidence of hyperpnoea and vesicular sound (M/F)

Clinical sign	Number of dogs affected*			
	Dose (mg/kg bw/d)			
	Control	0.45/0.48	0.9/1.0	1.5/1.6
Hyperpnoea	1/1	1/1	-/2	4/4
Vesicular sound	-	-	1/1	3/4

*In some animals, the signs were observed more than once.

Reddening of the dorsal surface of the tongue was frequently observed in all animals, including controls, the incidence of which was elevated and appeared to be dose-related in the mid- and high-dose groups. Ophthalmoscopy did not show any treatment-related effects.

Food consumption & body weights: A summary of food consumption data showed approximately 15-30% reductions in food intake, mainly in females in all groups (namely in 3, 3, 4 and 7 bitches at the control, low-, mid- and high-dose groups, respectively), and in 1 male dog in the high-dose group. This suggested that there was a paraquat-related depression in food intake at the high dose. It was reported that one female each in the low- and high-dose group left approximately 15-60% and 30-60%, respectively, of their ration uneaten during several weeks on study, but no further supporting data were provided. Group mean body weights and weight gain of the animals were unaffected by treatment.

Haematology & clinical chemistry: Reduced eosinophil counts were seen in all males (approximately 26-58%), and in mid- and high-dose females (~40%) compared to pre-treatment values. There were statistically significant perturbations in a few other haematological parameters, but they were sporadic in nature, and showed no dose-response relationships. Plasma urea levels in females, particularly at the low-dose were significantly low ($p \leq 0.01$ or 0.05) at the majority of sampling times, and may have been due to high control values. Plasma ALP activity was elevated at the high-dose, especially in females, with a dose-related increase at termination (see Table 78 below). However, the clinical significance of this finding is unclear due to lack of other pathological evidence.

Table 78: Plasma ALP activity (mU/mL) **at selected observation times**

Study week	Dose (mg/kg bw/d)			
	Control	**0.45/0.48**	**0.9/1.0**	**1.5/1.6**
Males				
Pre-treatment	136	150	145	138
4	131	124	125	143
20	98	85	97	114
52	112	101	113	113
Females				
Pre-treatment	164	165	144	153
4	124	125	130	130
20	83	90	89	116*
52	91	98	124*	136**

*$p \leq 0.05$, **$p \leq 0.01$

Evidence of increased plasma cholesterol was observed in high-dose females. In comparison to controls, the increases were statistically significant ($p \leq 0.05$) at the high-dose at 4, 20 and 39 weeks, and could be attributed to the test substance. Except for one occasion, the plasma triglyceride levels in high-dose males were elevated by approximately 13-28% at all sampling times compared to controls. Similar increases (except at 8 weeks) were noted in high-dose females, with approximately 8-46% increases, and reaching statistical significance at 26 weeks. There were several statistically significant, but isolated and toxicologically insignificant perturbations in various clinical chemistry parameters. The trends seen were not dose-related and the magnitudes were inconsistent between groups. Urine protein levels in high-dose males were depressed by approximately 45% and 32% compared to the concurrent controls at 39 and 50 weeks, respectively. In females, urine protein levels appeared to be depressed in both the mid- and high-dose groups (approximately 21-66% and 42-66%, respectively), showing a consistent dose-related trend throughout the study. However, the clinical significance of these findings is unclear.

Pathology: Treatment-related changes in organ weights seen at termination were restricted to the high-dose groups. Relative and absolute lung weights in males and females were significantly ($p \leq 0.01$) increased by approximately 36% and 61%, respectively, compared to controls. Relative and absolute liver weights in males were reduced by approximately 6% and 7%, respectively, with no changes observed in females. Absolute spleen weights were higher in both sexes, with increases of approximately 49% and 38% in males and females ($p \leq 0.05$), respectively, compared to controls. Although, increased mean spleen weight in males was partly attributable to gross spleen enlargement in a single animal, the group mean still remained elevated compared to the controls. Significantly reduced kidney weight (approximately 10% in absolute and relative weight ($p \leq 0.01$ or 0.05, respectively) in low-dose males could be an isolated incidence and no toxicological significance was attributed to this finding.

Chronic pneumonitis associated with yellow discolouration and consolidated areas of the lung were consistently observed in all animals, including controls. However, the incidence of this lesion was greater in the mid- and high-dose groups. The extent of severity was dose-related at these two dose levels and varied from minimal (total score 1-10) to moderate (total score 21-30) at the mid-dose, and from minimal (total score 1-10) to marked (total score 30 and above) at the high-dose. Animals in the control and low-dose groups showed either no or minimal pathological changes. The yellow consolidated lesions observed in the lungs were also associated with a range of histopathological changes, namely, peribronchial mononuclear cell infiltration, peribronchiolar/interalveolar fibrosis and erythrophagocytosis in bronchial lymph nodes. A benign papilloma in the ear pinna and a parafollicular adenoma was seen in a male and a female, respectively, in the mid-dose group, but these were considered to be incidental and unrelated to treatment. No histological changes were seen in association with the reddened tongue.

Mean paraquat cation concentration in the urine sampled at 29 weeks was dose-related. Paraquat concentrations in the lung and kidney showed a similar trend at the mid-dose and above.

Conclusions: Because of increased incidence of pulmonary lesions associated with chronic pneumonitis observed at and above 0.9 mg/kg bw/d, the NOEL was set at 0.45 mg/kg bw/d.

4.7 Reproduction Studies

4.7.1 Rats (oral)

Igarashi A (1980) AT-5 Rats Three Gen IIAR 1. Study no: not stated. Lab: Imamachi Institute for Animal Research, 1103 Fukaya, Dejima-mura, Niihari-gun, Japan. Sponsor: not stated. Study duration: June, 1979-September, 1980. Report no: RIC2814. Report date: not stated.

Pre-GLP study. No QA statement provided or any test guideline cited.

Study & observations: Three-week old Wistar-Imamichi rats (30/sex/dose, initial bw 61-92 and 68-94 g for males and females, respectively) were used in the study. Prior to commencement of treatment, rats were acclimatised for one week. Paraquat technical (as paraquat dichloride, purity 98%, source: unspecified) was administered to rats at 0 (control), 20, 100 or 200 ppm (equivalent to approximately 0, 2, 10 and 20 mg/kg bw/d, respectively) in the diet (MM-1,

Funabashi Nojo KK, Japan) for 3 parental generations of animals and their offspring throughout all phases of the study. Details of diet preparation and analysis of the homogeneity of paraquat in the diet were not provided.

At 14 weeks of age, F0 females that were confirmed to have undergone 2 vaginal plug cycles were mated thrice by housing them together with males on 1:1 basis (mating period unspecified), to produce the F1a, F1b and F1c litters. The F1 generation was mated twice, whilst the F2 generation was not mated. F0 dams received a 10-day rest period after weaning prior to the second mating (for F1b litters). Pregnancy was confirmed by the observation of a vaginal plug or sperm in the vagina. Following confirmation (day 0), the animals were individually housed during gestation and lactation. After a 21-day nursing period, the pups in each generation were weaned. Pups from each 'b' litters (30 and 20 per sex from F0 and F1 generation, respectively) were selected to become the parents of the next generation. The pups in 'a' litters of the F0 and F1, and the F2 generation was not mated. The F0 and F1 litters were sacrificed and necropsied after 21 days and 13 weeks, respectively. F1c pups were removed by caesarean section, and were subsequently examined and necropsied. The experimental design of the study is outlined in Table 79 below. An unspecified number of dams in the F0 generation were sacrificed to obtain foetuses for teratological examination. It was reported that since the mortality among dams in the F0 generation was high, F1c litters were used as the F1 generation animals, and for the F1 dams, F2b litters were used.

Table 79 - Experimental design (Igarashi, 1980)

Generation/litter	Procedures
F0 generation (30 males and 30 females)	
First litter (F1a)	Nursing observation. On day 21 of nursing the weaned pups were necropsied
Second litter (F1b, 30 litters/group)	After completion of nursing observation (21 days), the animals to become the next generation parents were selected. Remainder necropsied.
Third litter (F1c)	Dams underwent caesarean section on day 21 of gestation and foetuses were examined.
F1 generation (30 males and 30 females)	
First litter (F2a)	Nursing observation. On day 21, the weaned pups were necropsied
Second litter (F2b, 20 litters/group)	After nursing observation, animals to be used for producing the next generation were selected and the remaining pups were necropsied
F2 generation (15 males and 15 females)	All were necropsied after 13 weeks; organ weights of 10 pups/sex were determined.

Mortality and clinical signs were recorded daily during rearing. Abortions, premature difficult delivery and abnormal nursing behaviour were examined daily during reproduction periods. Body weight, food consumption and water intake were determined weekly during rearing, on days 0, 7, 14 and 21 of gestation, and on days 0, 3, 7, 14 and 21 of nursing. Food efficiency and the actual doses ingested by the test animals were calculated using the mean food consumption and the body weight data. The following maternal and offspring parameters were determined: copulation and pregnancy rates, gestation length, number of pups, offspring body weight, sex, external abnormalities, body weight of nursing pups and the number of perinatal deaths, numbers of corpora lutea, implantations, viable foetuses, live/dead ratio. Based on these data, perinatal survival, nursing pup survival, implantation and foetal mortality rates were calculated. Nursing pups were examined for pinnal opening (day 3 post partum), incisor eruption (days 8, 9 and 10 post partum), eyelid opening (days 14, 15 and 16 post partum) and

coat development. From these, the differentiation rate [(number of pups with positive differentiation)/(number of nursing pups) x 100] was calculated for each observation day. Males were sacrificed and necropsied after 27 weeks of treatment for F0 and F1 generations and after 13 weeks for the F2 generation. Females were sacrificed and necropsied after production of the F0 and F1 generations, and after 13 weeks for the F2 generation. At necropsy, weight of the following organs (10 rats/sex/group) were determined: liver, kidney, spleen, heart, lung, brain, thymus, pituitary, thyroid, adrenal, testis or ovary, epididymis, prostate, seminal vesicles and uterus. One-half of the viable foetuses were processed for the examination of skeletal anomalies/variants and the extent of ossification using alizarin red S. The remaining foetuses were examined for visceral anomalies using the free-hand razor method and micro-dissection technique. Statistical differences among group mean values were tested using a Student's t-test, χ^2 or Rank sum tests.

Findings:
General condition & mortality: The high-dose resulted in mortality rates of 36.7% and 24.1% in the F0 and F1 generation females, respectively. F0 dams (11/30) died after the nursing periods of the first or second litter and in all cases the deaths were attributed to respiratory disturbances. F1 dams (7/29) died due to either difficulty in delivery (2/29) or respiratory difficulty (5/29). It was stated that the majority of these deaths were also associated with emaciation due to marked weight loss. Clinical signs observed in high-dose F0 and F1 dams were tachypnoea, staggering gait, piloerection and two-stage abdominal respiration. No clinical abnormalities were noted in males or F2 dams.

F0 generation:
Parental: Dose-related and statistically significant ($p \leq 0.001$, 0.01 or 0.05) depressions in group mean body weights were observed in males from weeks 8 to 16 (except for week 15) at the mid-dose (~3-4%), and from weeks 2 onwards and throughout the rearing period at the high-dose (~4-14%) compared to controls (see Table 80 below). In females, approximately 4-20% body weight depressions were noted at the high-dose during the rearing (from weeks 3 to 9), gestation and lactation periods. A toxicologically significance reduction in body weight was restricted to the high-dose, because of consistent effects seen in both sexes. Mean food consumption at the high-dose was depressed by approximately 10% and 8% in males and females, respectively. Food utilisation efficiency and the actual dose ingested by the test animals were unaffected by treatment. There were no inter-group differences in any of the reproductive parameters. It was reported that all dams, excluding those dying, nursed the offspring normally.

Table 80: Body weights (g, mean ± SD) of F0 parents at selected observation times

Observation time (weeks)	Dose (mg/kg bw/d)			
	Control	2	10	20
Males				
0	82 ± 6	82 ± 5	83 ± 6	82 ± 6
5	373 ± 17	371 ± 20	372 ± 19	349 ± 22***
10	501 ± 27	491 ± 34	482 ± 23**	443 ± 35***
15	571 ± 36	563 ± 42	555 ± 29	502 ± 48***
20	603 ± 49	602 ± 47	591 ± 33	528 ± 58***
25	643 ± 53	638 ± 54	624 ± 41	555 ± 61***
Females				
0	79 ± 6	79 ± 5	79 ± 5	79 ± 5
3	210 ± 10	221 ± 15	211 ± 11	204 ± 12*
6	272 ± 15	293 ± 20	274 ± 21	259 ± 14***
9	302 ± 19	332 ± 26	313 ± 26	290 ± 15**

*(p≤0.05), **(p≤0.01), ***(p≤0.001).

At necropsy, high-dose females (13/30) showed signs of chronic interstitial pneumonia and the lungs appeared to be hard. The lung abnormalities were frequently associated with emphysematic alveolar dilatation. The lung surface was brown in colour, lacked elasticity and resembled hepatic cirrhosis. There were several statistically significant, but minor perturbations in absolute and/or relative weight of some organs in both sexes. However, the effects that could be attributed to treatment were confined to the high-dose group. These included statistically significant increases in relative lung and brain weights in both sexes (see Table 81 below). Histopathology of the lung showed abnormalities such as interstitial pneumonia associated with fibrosis (in animals that died during the study), thinning of the alveolar wall, sporadic fissures, fusion of alveolar sacs, pulmonary emphysema, eosinophil infiltration, fibroblast proliferation in the alveolar septum and diffuse fibrosis.

Table 81: Relative organ weights (mean ± SD) of the F0 generation

Organ	Dose (mg/kg bw/d)			
	Control	2	10	20
Males (post mating for F1c)				
Body weight (g)	*685 ± 63*	*702 ± 64*	*669 ± 47*	*615 ± 46**
Brain (mg)	316 ± 29	309 ± 25	317 ± 19	340 ± 27*
Lungs (mg)	261 ± 31	274 ± 28	270 ± 49	296 ± 30*
Females (post F1b weaning)				
Body weight (g)	*390 ± 19*	*413 ± 47*	*386 ± 28*	*352 ± 40**
Brain (mg)	491 ± 26	480 ± 52	502 ± 23	552 ± 78*
Lungs (mg)	391 ± 43	362 ± 31	413 ± 34	746 ± 312*

*(p≤0.05)

Offspring:

F1a litters: At the high-dose, both male and female pup body weights were depressed by approximately 8-12% compared to controls, achieving statistical significance at the majority of observation times (p≤0.001, 0.01 or 0.05).

F1b litters: Pups in the high-dose group showed statistically significant reductions (~5% compared to the corresponding controls) in body weights only on day 21 of the rearing period.

F1c litters (caesarean section): Similar to the effects seen in F1b pups, the offspring at the high-dose of these litters had reduced group mean body weight (~6-7%) showing statistical

significance only in males ($p \leq 0.05$) compared to controls. The reductions in pup body weights seen at the high-dose of these litters were considered to be treatment-related

External abnormalities such as strephendopodia in one control foetus and dwarfism in one foetus at the high-dose was seen, but were considered to be spontaneous occurrences and unrelated to treatment (litters unspecified). Except for a significantly reduced ($p \leq 0.05$) number of foetuses with ossified hind limb-metatarsal centres seen in high-dose F1c pups (due to maternal toxicity) in comparison to controls, there were no further inter-group differences.

F1 generation:
Parental: Mean body weights (see Table 82 below) in males at the high-dose were significantly depressed (~3-9%, $p \leq 0.001$, 0.01 or 0.05) from week one onwards and throughout the rearing period. High-dose females showed approximately 2-5% deficits in body weights showing statistical significance during weeks 2 and 3, and weeks 7 through 9 ($p \leq 0.001$ or 0.05) during the rearing period and during the production of the F2a litters (~2-10%, $p \leq 0.001$, 0.01 or 0.05). In mid-dose females, body weights were significantly reduced on gestation day 21 (~8%, $p \leq 0.001$) and on days 0 and 3 during the lactation period (~5%) of the first litter. The body weight reduction at the high-dose was attributed to treatment. Mean food consumption at the high-dose was depressed by approximately 11% and 8% in males and females, respectively. Food efficiency and the actual dose levels achieved were unaffected by treatment. No inter-group differences were seen in any of the reproductive parameters.

Table 82: Body weights (g, mean ± SD) of F1 parents at selected observation times

Observation time (weeks)	Dose (mg/kg bw/d)			
	Control	2	10	20
Males[a]				
0	78 ± 4	84 ± 11	87 ± 7***	86 ± 96***
5	364 ± 22	370 ± 21	380 ± 21**	351 ± 21*
10	498 ± 43	504 ± 34	496 ± 33	464 ± 33**
15	562 ± 57	579 ± 43	574 ± 45	519 ± 40**
18	587 ± 61	604 ± 46	604 ± 45	545 ± 40**
Females				
0	72 ± 2	75 ± 7	74 ± 5	75 ± 8
3	201 ± 14	198 ± 17	202 ± 10	193 ± 13*
6	270 ± 25	262 ± 24	277 ± 18	261 ± 21
9	306 ± 29	295 ± 25	311 ± 17	290 ± 24**

[a]No data provided for sampling intervals after 18 weeks. *($p \leq 0.05$), **($p \leq 0.01$), ***($p \leq 0.001$).

The organ weight changes that could be related to treatment were seen only in high-dose males (see Table 83 below). These included significant increases in relative brain, heart, liver (and absolute weight), lung and testis weights, and significant reductions in absolute and relative adrenal weights. Although statistical significance was not achieved for relative brain and lung weights in high-dose females, these weights were increased at termination by approximately 2% and 24%, respectively, compared to the corresponding controls. Necropsy of the high-dose F1 dams that died or were sacrificed by design, revealed lung lesions that are similar to those described for F0 animals.

Table 83: Organ weights (per body weight ratio, mean ± SD) of F1 parents

Organ	Dose (mg/kg bw/d)			
	Control	2	10	20
Males (post mating, F2b)				
Body weight (g)	*650 ± 53*	*634 ± 44*	*637 ± 46*	*583 ± 36***
Brain (mg)	335 ± 28	346 ± 22	338 ± 30	363 ± 20*
Liver (g)	3.9 ± 0.4	4.3 ± 0.2	4.0 ± 0.2	3.4 ± 0.2**
Heart (mg)	235 ± 11	244 ± 9	243 ± 24	252 ±12*
Lung (mg)	300 ± 43	323 ± 25	317 ± 39	338 ± 62
Testes (mg)	484 ± 44	508 ± 43	487 ± 58	568 ± 47***
Adrenals (mg)	11 ± 1	11 ± 1	11 ± 1	10 ± 1*
Females (at F2b weaning)				
Body weights (g)	*379* ± 22	*365 ± 22*	*385 ± 26*	*372 ± 21*
Brain (mg)	525 ± 28	524 ± 27	515 ± 38	534 ± 33
Lungs (mg)	367 ± 21	367 ± 27	368 ± 22	455 ± 153

*(p≤0.05), **(p≤0.01), ***(p≤0.001).

Offspring:
F2a litters: At the high-dose, body weights were depressed by approximately 6-9% compared to controls, and was statistically significant (p≤0.01 or 0.05) during the 21-day rearing period (except for female pups at 3 days).

F2b litters: Body weights were consistently low (~9-10%), again at the high-dose compared to controls, showing statistical significance in male pups on days 3 and 21 of the rearing period (p≤0.05). In visceral examination, approximately a 3-fold increase in the incidence of hydrourethrosis was seen at the high-dose compared to controls (p≤0.01). The foetal incidences of this abnormality were 7% and 26% at the control and high-dose, respectively [No historical control data were provided by the study authors. It has been reported that in Ctr:CD® BR rats, the foetal background incidence of hydroureter, ie distension of the ureter with urine due to blockage from any cause is approximately 5% (MARTA, 1993]. No inter-group differences were seen in any of the other pup parameters. There were no treatment-related external abnormalities.

F2 generation:
Parental: Statistically significant depressions (p≤0.001, 0.01 or 0.05) in body weights were seen in the high-dose groups (~3-9%) from week one onwards during the 13-week rearing period compared to corresponding controls. Mean food consumption in high-dose animals was depressed by approximately 12.5% and 8% for males and females, respectively. Food utilisation efficiency and the test substance incorporation were unaffected by treatment. No further inter-group differences were noted in any of the remaining study parameters.

Conclusions: Although the study was described as a 3-generation study, based on the data provided it appeared that only two parental generations were mated (F0 and F1), and hence it appears to be a 2-generation study. Nevertheless, paraquat had no effects on reproductive performance or the development of the reproductive organs in rats, when administered at dietary levels up to 20 mg/kg bw/d for 2 generations. The NOEL in the offspring was 10 mg/kg bw/d (equivalent to approximately 7.2 mg paraquat ion/kg bw/day) based on a significant depression in F0 and F1 pup body weights, and the increased incidence of hydrourethrosis in F2b pups, at 20 mg/kg bw/d. The NOEL for parental animals was also 10 mg/kg bw/d (equivalent to 7.2 mg paraquat ion/kg bw/day) based on significantly reduced body weights in F0 and F1 parents at 20 mg/kg bw/d.

Lindsay S, Banham PB, Godley, MJ, Moreland S, Wickramaratne, GA & Woolen BH (1982a) Paraquat: Multigeneration reproduction study in rats – Three generations. Study no: RR0151, Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: not stated. Study duration: December 19, 1979 to November 28, 1981. Report no: CTL/P/719, Report date: December 22, 1982, and

Lindsay S, Banham PB, Godley, MJ, Moreland S, Wickramaratne, GA & Woolen BH (1982b) Paraquat: Multigeneration reproduction study in rats – Two generations. Study no: RR0151, Lab: ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: not stated. Study duration: December 19, 1979 to April 10, 1981. Report no: CTL/P/649, Report date: February 26, 1982.

Non-GLP, quality assured study. No test guidelines were cited.

Study: Three-week old, Alderley Park rats, weighing 40-47 g were used. After an acclimatisation period of 8 days, animals were allocated to experimental groups (15 males and 30 females/group). Paraquat (as paraquat dichloride liquor, paraquat cation content 32.7%, ICI PLC, UK) was administered to rats at 0 (control), 25, 75 or 150 ppm (equivalent to approximately 0, 2.5, 7.5 and 15 mg/kg bw/d, respectively) in the diet (BP Nutrition UK Ltd) for 3 parental generations of animals and their offspring throughout all phases of this study. Four females and 2 males were included in each of the control and high-dose groups to obtain information on the microbiological status of the animals. A justification for the dose selection was not provided. Fresh diets were prepared at approximately 2-day to 4-week intervals. It was reported that the paraquat concentrations in diets were analysed throughout the study, with more frequent samples being tested during the littering phases, particularly at the high-dose. The homogeneity of paraquat in the diet was determined prior to commencement of the study. The stability of paraquat in the diet was confirmed during the study.

After 12 weeks of treatment, F0 parents were mated twice, firstly to produce the F1a litter and secondly the F1b litter (7 days after weaning of the 'a' litter). A different male was used at the second mating. During mating, 2 females were housed together with 1 male from the same group (mating period was unspecified). Daily examinations of vaginal smears were conducted to confirm mating success. Following confirmation of mating (day 0), the animals were individually housed and remained so throughout the gestation and lactation periods. Thirty females and 15 males from each 'b' litter were selected to become the parents of the next generation. The F1a litters were weaned at 21 days post partum, while subsequent litters were weaned at 28 days of age. Pups were housed by litter until selection, which was done at 35 days of age. The duration of the pre-mating period was 12 weeks for the F0, and 11 weeks for the next 2 generations (see Table 84 below for experimental design). All surviving male (after completion of mating to produce 'b' litters) and female parents (after weaning the 'b' litter) of each generation, those moribund and dams with imperforate vagina or of suspected infertility, and all survivors at termination were sacrificed by halothane overdose and necropsied. A range of tissues, including the lungs, reproductive tracts, and those suspected of any abnormalities were processed for histopathology. The animals of each generation were provided with the test diets and water *ad libitum* and housed under standard laboratory conditions throughout all phases of the study.

Table 84 - Experimental design (Lindsay et al, 1982a & 1982b)

Generation/litter	Procedures
F0 generation (15 males and 30 females/group)	
Pre-mating period	12 weeks
First litter (F1a)	Nursing observation for 21 days. On day 21, the pups were weaned and necropsied.
Second litter (F1b)	Nursing observation for 28 days but remained housed by litter for 7 more days. After this, the animals to become the next generation parents were selected. Remainder necropsied.
Parents	All males were killed after mating. Females were killed after weaning the 'b' litter. All were necropsied.
F1 generation (15 males and 30 females/group)	
Pre-mating period	11 weeks
First litter (F2a)	Nursing observation for 21 days. On day 21, the pups were weaned and necropsied.
Second litter (F2b)	Nursing observation for 28 days but remained housed by litter for 7 more days. After this, the animals to become the next generation parents were selected. Remainder necropsied.
Parents	All males were killed after mating. Females were killed after weaning the 'b' litter. All were necropsied and sampled for histopathology (10 males and 25 females/group).
F2 generation (15 males and 30 females/group)	
Pre-mating period	11 weeks
First litter (F3a)	Nursing observation for 28 days and necropsied.
Second litter (F3b)	Nursing observation for 28 days and necropsied.
Parents	All males were killed after mating. Females were killed after weaning the 'b' litter. All were necropsied.

Observations:
Parental: Animals were observed daily for clinical signs and behaviour, with a more detailed examination performed once a week. Body weights were recorded weekly (immediately before the first feeding for F0 and at the selection for F1 and F2 parents). After the pre-mating period, the males were weighed at 4-week intervals. Females were weighed at 0, 7, 14 and 21 days of gestation. Food consumption and utilisation for each cage of rats were determined weekly during the pre-mating periods. Individual urine samples were collected over a period of 3-4 h from 3 rats/sex/group at 8 weeks from the F0 parents and at 7 to 10 weeks from the F1 and F2 parents. Samples were pooled by sex and analysed for paraquat by RIA.

Offspring: Litters were examined at least once daily, and dead or grossly abnormal pups were removed for soft tissue examination. Litter size, sex of the pups, the number of live and stillborn pups and clinical signs were recorded within 24 h after birth and at 4, 10 and days post partum. Individual pup body weights were recorded within 24 h after birth and at 4, 10, 21 and 28 days.

Pups with gross abnormalities, those found dead up to and including 18 days of age and 50% of the 'a' litters were examined for malformations. Any pups found dead after 18 days of age were necropsied and samples of a range of tissues were processed for histopathology. Following selection of the next generation parents, 5 rats/sex/group from the F1b and F2b litters and 10 rats/sex/group from F3b litters were subjected to necropsy and a range of tissues were processed for histopathology.

The following reproductive parameters were determined: group-mean gestation length, live birth index, group mean litter weight gain, maternal neglect and survival indices. Statistical differences between the control and treatment groups was tested using, either a Student's t-test, Fisher's exact test or an ANOVA.

Findings: Dietary analysis showed that the concentrations of paraquat in the majority of the samples were within 10% of nominal concentrations. Paraquat was found to be stable in the prepared diets up to at least 7 weeks.

Overall mortality & clinical observations: Percent mortalities in F0, F1 and F2 parents are given in the Table 85 below. The majority of mortalities in each generation occurred among high-dose females, and was attributed to the test substance. It was stated that the animals died or became moribund during or shortly after rearing of either 'a' or 'b' litters. The mortalities observed among males appeared to be unrelated to treatment.

Table 85: Percent mortality in F0, F1 and F2 parents (M/F)

Generation	Dose (mg/kg bw/d)			
	Control	2.5	7.5	15.0
F0	6.7/0	0/3.3	6.7/3.3	0/26.7
F1	6.7/3.6	0/3.3	6.7/3.3	0/43.3
F2	13.3/0	0/0	3.3/3.3	0/16.7

F0 generation:
General: In males, significantly reduced ($p \leq 0.05$ or 0.01) body weight gains were seen in the mid-dose group throughout the study, with no statistically or toxicologically significant differences occurring in other groups. High-dose females showed statistically significant depression (~8%, $p \leq 0.05$) in group-mean body weight at 2 weeks, and approximately 2-5% reductions at 3 and 4 weeks compared to controls, with no further group differences at the other observation times. The changes seen in males were considered to be unrelated to treatment. Statistically significant increases ($p \leq 0.05$ or 0.01) in food consumption were seen in low-dose males, whilst no effect was seen in any other group. There was no treatment-related effect on food utilisation.

Reproductive performance: Body weight gain in pregnant rats at all doses was generally low compared to the controls, but no statistical significance was achieved. No treatment-related effects were seen in any of the reproductive parameters during the production of F1a or F1b litters. Isolated, but statistically significant reductions in gestation length were seen at the low- and mid-dose during production of either F1a or F1b litters, but these effects were not considered to be treatment-related.

Mortality rates in the control, low- and mid-dose F1b litters were slightly higher (survival 75-79%) compared to that of the other litters (survival 82-96%). The study authors attributed this finding to a slight increase in maternal neglect due to a noise disturbance at the time of littering. Pup body weights and other parameters were unaffected by treatment.

Gross pathological findings of the animals that either died or were sacrificed *in extremis* or at termination during this phase of the study were restricted to the high-dose groups. Pathological abnormalities noted at necropsy were, uniform dark red or purple discolouration of the lungs

often with pale surface foci. Histopathology showed lesions characteristic of acute or repeat-dose paraquat lung injury. These included generalised congestion with areas of alveolar oedema and consolidation, alveolar fibrosis, hyaline membrane formation, perivascular oedema, mixed inflammatory cell infiltration and variable alveolar exudate. A dose-related increase in the incidence and severity of focal alveolar histiocytosis was seen at termination (see Table 86 below), with approximately a 2- to 3-fold higher incidence in all treated females compared to that of the controls. Due to lack of any historical control data, and the absence of this anomaly in any of the subsequent generations, the relationship with treatment of this finding was equivocal. No treatment-related gross or histopathological abnormalities were found in the reproductive tract of either sex. Soft tissue pathology of the offspring showed either unilateral or bilateral pelvic dilatation of the kidney in all groups including controls, showing no apparent relationship to treatment.

Table 86: Prevalence of focal alveolar histiocytosis in F0 parents (M/F)

Observation	Dose (mg/kg bw/d)			
	Control	**2.5**	**7.5**	**15.0**
Focal alveolar histiocytosis				
Number of animals	14/29	15/28	14/29	15/21
Minimal	1/3	1/6	3/11	4/11
Mild	0/0	0/1	1/4	3/9
Moderate *(males)*	0	0	1	1
Total	1/3	1/7	5/15	8/20
Percent incidence	11/30	12/54	71/79	80/100

The only histopathological abnormality observed in pups was mild perivascular inflammatory cell infiltration in F1b pups (4/5 males and 2/5 females) at the high-dose at termination.

F1 generation:
General: No treatment-related effects were seen in any of the reproduction parameters.

Reproductive performance: Similar to the observations recorded for the F0 generation, the body weight gain during pregnancy of F1 rats (to produce the F1b litter) was generally low compared to controls, but no statistical differences were noted. No treatment-related effects were seen in any of the reproductive parameters during the production of F2a and F2b litters. Statistically significant increase in gestation length was seen at the low-dose, but this was not considered to be treatment-related. Pup body weight gain was unaffected by treatment.

At necropsy, macroscopic lung abnormalities seen in parents were again confined to the high-dose, and were similar to those seen in F0 parents. The incidence and severity of focal alveolar histiocytosis was increased in both sexes dose-relatedly at 7.5 mg/kg bw/d and above. Percent incidences were 28%, 28%, 61% and 82% in males, and 3%, 1%, 5% and 12% in females for the control, 2.5, 7.5 and 15 mg/kg bw/d groups, respectively. No treatment-related gross or histopathological abnormalities were observed in the reproductive tract or any other tissues of either sex. Soft tissue pathology of the offspring did not reveal any treatment-related effects, except for either unilateral or bilateral pelvic dilatation of the kidney in all groups including controls, but there was no relationship with dose.

One male F2b pup at the high-dose had marked internal hydrocephalus together with testicular hypoplasia and hypo-secretion, probably related to abnormal hypothalamic function, but no other toxicologically significant abnormalities were observed.

F2 generation:
General: No inter-group differences were seen in any of the reproduction parameters during the production of F3a and F3b litters. The body weight gain of F2 females during the production of F3a and F3b litters was normal.

No inter-group differences were seen in litter weight gain of F3a females. Statistically significant ($p \leq 0.05$) reductions in litter weight gain were observed in low-dose F3a males. Given that there was no apparent dose-related trend, this finding was considered to be unrelated to treatment and of no toxicological significance.

Necropsy of parental animals that either died prematurely or were sacrificed *in extremis* or at termination showed lung abnormalities characteristic of acute or repeat-dose paraquat toxicity. These were similar to those seen in the animals of the F0 and F1 generations, and were confined to the high-dose group. Histopathology revealed a dose-related increase in focal alveolar histiocytosis in females at and above 7.5 mg/kg bw/d, with the percent incidences being 11%, 0%, 10% and 50% in males, and 40%, 46%, 80% and 80% in females for the control, 2.5, 7.5 and 15 mg/kg bw/d groups, respectively. No paraquat-related abnormalities of toxicological significance were observed in the reproductive tracts or any other tissues. Except for either unilateral or bilateral pelvic dilatation of the kidney, which was unrelated to treatment and seen in all groups including controls, no treatment-related malformations were observed in any of the offspring. There were no treatment-related gross or histopathological abnormalities in any of the offspring.

In general, excretion of paraquat in urine by the animals of all 3 generations was related to the dose administered.

Conclusions: Under the conditions of the study, paraquat had no effects on reproductive performance or the development of the reproductive organs of rats, when administered at dietary levels up to 15 mg/kg bw/d for 3 generations. The NOEL in pups was 3.75 mg/kg bw/d based on perivascular inflammatory cell infiltration in the lungs of F1b pups at 15 mg/kg bw/d. The NOEL for parental animals was 1.25 mg/kg bw/d based on the dose-related increase in the incidence and severity of focal alveolar histiocytosis at and above 7.5 mg/kg bw/d.

4.7.2 Mice (ip)

Hausburg MA, Dekrey GK, Salmen JJ, Palic MR & Gardiner CS (2005) Effects of paraquat on development of preimplantation embryos *in vivo* and *in vitro. Reprod Toxicol 20(2):239-46.* Evaluation Part 1.

This part of the study concerned the effect of paraquat on reproductive outcomes in mice and was done to verify earlier work which found that of doses 0, 45, 90 and 125 paraquat/kg of feed, starting before pregnancy, the highest dose resulted in a decrease in the number of litters produced without a reduction in litter size in a two generation study (Dial & Dial 1987). This part is presented in this section of the Review and the work concerning the effect of paraquat

on the development of the preimplantation embryo is presented in the section on developmental toxicity (Hausburg *et al* 2005, Section 8 of this Review).

Study: Outbred non-Swiss albino mice [Hsd: NSA™ (CF-1®)] were selected for treatment. Virgin pubertal female mice were synchronised and superovulated by ip injection of 10IU equine chorionic gonadotrophin (eCG) followed 44-48 hours later, and just prior to placing the females with proven breeder males, by ip injection with 5IU of human chorionic gonadotrophin (hCG). On the following day (gestational day 0), the presence of copulation plugs was used as a positive selection criteria for animals to be used for further study.

Mice were treated with saline ip (vehicle) or paraquat (30 mg/kg bw ip) on the day of ovulation.

After collection, embryos were evaluated by light microscopy for indication of fertilisation, stage of development, quality of embryos, and abnormal features. Normal bred female mice (neither synchronised nor superovulated) were euthanized on day 17 of gestation. Uteri were dissected and examined for resorptions and foetuses were evaluated for gross malformations as described previously (Laub *et al* 2000).

Results: When compared to control mice, there were no significant differences in paraquat treated mice for body, liver or uterine weight in dams, or the number of foetuses per dam, the number of resorptions per dam, the total foetal weight per dam, individual foetal weight, or the number of foetal malformations.

However, the percent of dams that were pregnant on day 17 was significantly reduced (24%) by paraquat exposure. The data are shown in Table 87 below. No other observations were reported.

Table 87: Effects of paraquat exposure on breeding outcomes

Reproductive parameter	Treatment	
	Saline	Paraquat
No. of females with copulation plugs	62	63
Dam weight on day 17 (g)	51.7 ± 0.8	50.0 ± 1.1
Dam liver weight (g)	2.8 ± 0.1	2.7 ± 0.1
Dam uterine weight	16.7 ± 0.7	15.8 ± 0.8
No. of foetuses/dam	12.0 ± 0.6	11.4 ± 0.7
No. of resorptions/dam	2.0 ± 0.4	2.1 ± 0.4
Foetal weight (g)	0.91 ± 0.01	0.91 ± 0.01
No. of foetal malformations	0	0
Total foetal weight per dam (g)	11.1 ± 0.5	10.3 ± 0.6
Percent of dams pregnant on day 17 (full term)	93.1 ± 4.4%	70.6 ± 10.2* ($p < 0.05$)

Conclusion: A decrease in the number of pregnant dams without an increase in foetal resorptions suggested that paraquat exposure adversely affected preimplantation development or very early post-implantation development, although this is not a relevant dose route for human exposure.

4.7.3 Rats (dermal)

D'Souza UJA, Narayana K, Zain A, Raju S, Nizam HM & Noriah O (2006) Dermal exposure to the herbicide paraquat results in genotoxic and cytotoxic damage to germ cells in the male rat. *Folia Morphol (Warsz) 65(1):6-10.*

Animals: Adult male Sprague Dawley rats (200-220 g bw) were sourced from the University Sains Malaysia and maintained on standard laboratory chow and water *ad libitum.*

Dosage: The rats were acclimatised for 1 week before dermal treatment with paraquat. The treatment groups (6/group) received 6, 15 or 30 mg/kg bw/d, for 4 h/d (vehicle not specified) via the dermal route for 5 days. Control rats (4/group) received distilled water.

Methods: Approximately 24 h prior to the first application of paraquat the fur on the back of each rat was shaved without causing skin abrasions. Paraquat was applied uniformly over the shaved area (dimensions not specified) of skin with porous gauze for 4 h. The treated area was then covered with an adhesive tape and restrainers for 4 h, after which the site was rinsed with distilled water. Control rats underwent similar preparation but were exposed to distilled water. The rats were sacrificed by cervical dislocation after treatment-free periods of 7, 14, 28 and 42 days after the last treatment application (i.e., study days 12, 19, 33 and 47, respectively). *Sperm count:* The epididymis was separated from the testis and the cauda epididymis was minced in 1 mL of phosphate buffered saline (pH 7.4) and filtered through 80 µm nylon mesh. Sperm were stained with Eosin Y and diluted with PBS prior to counting using a Neubauer chamber (according to referenced procedures (Narayana *et al,* 2002a; Vega *et al,* 1988). *Sperm morphology:* Sperm morphology smears were prepared from the filtrate on clean glass slides and dried. Sperm (1000/rat) were screened and classified into normal and different types of abnormal spermatozoa (headless, double headed, microcephalous, defective at the cephalocaudal section, hookless, banana-shaped, amorphous, coiled, double tailed and broken tailed, Narayana *et al,* 2002b; Wyrobek & Bruce, 1975). Total sperm abnormality was expressed as percentage incidence per group. *Sperm motility and mortality:* The ductus deferens was removed and placed in 1 mL of normal saline and examined using a Makler's counting chamber (Makler, 1980). Dead and immotile sperm were considered as equivalent when assessing sperm motility.

Results: Treatment of rats with paraquat *via* the dermal route caused a decrease in sperm count on post-treatment day 7 and day 14, as shown in Figure 2 ($p < 0.05$, mean±SD, and percentage change values not presented). The effect was treatment-related on day 7 with 6 and 15 mg/kg bw/d being equi-effective (est. 40% decrease from control) and a further decrease (est. additional 10%) at 30 mg/kg bw/d and on day 14 in rats which received 15 or 30 mg/kg bw/d. The statistical significance assigned to the slight increase in the 6 mg/kg bw/d group in day 14 appears to be an error. Sperm mortality was increased on post-treatment day 7 and day 14 in rats which received 15 or 30 mg/kg bw/d (Figure 3). Sperm mortality was minimally affected on day 28 at 30 mg/kg bw/d. However, the methodology for sperm mortality also included immotile sperm as equivalent to dead sperm, and therefore the changes observed may represent transiently immotile sperm due to the experimental conditions rather than causing sperm death. Sperm motility data was not presented although the authors indicated an effect was apparent at 30 mg/kg bw/d (not significant) but not at lower doses.

Figure 2: The effect of dermal exposure to paraquat on epididymal sperm count in the rat. Data are expressed as means ± SD, n = 6 (from D'Souza *et al*, 2006)

Figure 3: The effect of dermal exposure to paraquat on sperm mortality in the rat. Data are represented as means ± SD, n = 6 (from D'Souza *et al*, 2006)

Paraquat treatment induced an apparent increase in the percentage of total sperm abnormalities which were described as headless, double-headed, microcephalus, cephalo-caudally defective, hookless, banana-shaped, amorphous, coiled tailed, double-tailed, broken-tailed. This diverse array of deformities occurred in an apparent random or sporadic manner and without a dose-relationship or trend with duration of the post-treatment period.

Overall, the statistical analysis of the data was poorly described. Statistical testing appears to have been performed only on the percentage of total abnormalities and not for individual deformities. All 'total' sperm abnormality percentages were statistically significantly different from the control percentage value. However, the only occasion when a slight treatment-related change occurred was on day 14, but the validity of this increasing trend is doubtful given the apparent large standard deviations and the 6-fold difference in applied dose. Collectively, there was no consistent pattern or effect on individual sperm morphology categories which could be assigned as the cause of the apparent increase in total abnormality score. The authors' claims that the data were consistent with an earlier study were considered to be of limited significance.

Conclusions: Paraquat exerted an apparent cytotoxic effect on epididymal sperm (as indicated by the day 7 data) and late spermatids (as indicated by the day 14 data) at high doses. The lack of effect on day 28 and day 42 indicates that paraquat did not affect spermatocytes and spermatogonia. Cytotoxicity was evident by increased incidences of sperm mortality (although also included immotile sperm) although the mode of cytotoxicity was not investigated. Treatment of rats with paraquat led to a diverse array of changes in the morphogenesis of rat spermatozoa at all dose levels on all post-treatment sampling days but without a clear dose-related pattern. The study author attributed the abnormal sperm morphology to the generation of free radicals by paraquat possibly resulting in oxidative damage in the testis.

This study presented weak evidence that paraquat may be cytotoxic to male germ cells in the rat and the treatment regimen appeared to interfere in sperm morphogenesis in a random or sporadic manner. However, robust evidence was not presented to support the authors' claim that paraquat was genotoxic or could cause point mutations in rat sperm and the potential effect of the observed (weak) cytotoxicity on functional male fertility was not investigated. The OCS considered that this study is not of sufficient regulatory value to regard this as evidence for cytotoxicity to germ cells.

4.8 Developmental Studies

4.8.1 Mice

Hodge MCE, Palmer S, Weight TM & Wilson J (1978a) Paraquat dichloride: Teratogenicity study in the mouse. Study no: RM 0053, Lab: Imperial Chemical Industries Ltd, Central Toxicology Laboratory. Sponsor: unspecified. Study duration: Commenced May 26, 1977. Report no: CTL/P/364, Report date: June 12, 1978.

Pre-dates GLP and test guideline

Study: Groups of at least 20 pregnant mice (Alderley Park, Cheshire, bw and age unspecified) were treated by po gavage with paraquat (purity 100%, batch: ADYM 76/G, ICI, UK) in 0.5% aqueous solution of Tween 80 (Polysorbate 80 BCP) at 0 (vehicle control), 1, 5 or 10 mg paraquat ion/kg bw/d from days 6 to 15 of gestation. The dosage volume was 10 mL/kg bw. Control animals received the same volume of 0.5% Tween 80 alone similarly. Two batches of each dosing solution were prepared and stored at room temperature, with the second batch being used for dosing of replacement animals. Mating was confirmed by the presence of a vaginal plug, and designated as day 0 of gestation. Initially, there were 120 mated animals, and according to the study authors an unspecified number of these animals littered early. Because there were insufficient numbers of litters available for the teratological examination, a further 42 females were mated 4-5 weeks after the first mating. The numbers of replacement animals allocated to different groups were unequal, and hence they were housed randomly using a shuffle card method. The test animals were sacrificed on day 18 of gestation by cervical dislocation and necropsied. At termination, there were 20, 22, 28 and 23 pregnant animals in the control, 1, 5 and 10 mg/kg bw/d groups, respectively.

Observations: Lungs and kidneys from at least 8 mice/group, and the heart, lung, kidney, spleen, liver, ovary and uterus from sick animals, and animals that had foetal abnormalities were processed for histopathology. During autopsy, the number of live foetuses and resorptions (early and late) were determined. The following foetal parameters were examined: body weights, external abnormalities, sex and the number of runts (foetuses <1.0 g). Alternate foetuses from each litter were eviscerated and the viscera were grossly examined. These were then stained with Alizarin Red for skeletal examination. The remaining foetuses were preserved and decalcified in Bouin's fixative, sectioned through the head, thorax and kidneys and examined. The abdomen was examined by dissection. Statistical differences between the control and treatment groups were examined using either a Student's t-test, double arcsine transformation of Freeman and Turkey, 1-way ANOVA, χ^2-test or by 2 x 2 Contingency Tables.

Findings: Results of the dose solution analysis, carried out prior to dosing commenced revealed that all but batch 2 of the 5 mg/kg bw/d solution were within 10% of the nominal concentrations (batch 2 had 80% of the nominal concentration). A re-analysis of batch 1 solutions, after dosing had finished, showed that the paraquat cation content in the 1 and 10 mg/kg bw/d solutions were 25% higher than the nominal concentrations. The study authors attributed this to evaporation of dosing solutions due to the inadequate sealing of the container lids, and to a 6-week gap between cessation of dosing and reanalysis. Nominal concentrations were used to report the findings of the study.

Maternal: Two animals (1 each in the control and high-dose groups) died due to intubation accidents. One mid-dose animal was ill for an unspecified period of time during the latter part of the dosing period. The study authors attributed this to a perforated oesophagus. No treatment-related clinical signs were seen in any of the treated animals. Body weight gain was depressed by approximately 15% and 11% at the mid- ($p \leq 0.05$) and high-dose, respectively. There was no dose-response relationship, and in the absence of any numeric data on food consumption, the interpretation of this finding was difficult. At necropsy, no treatment-related gross pathological abnormalities were seen in any group.

Foetal: A slight dose-related increase in the incidence of late resorptions and a depression in mean foetal body weight were seen in treated groups in comparison to controls. The mean litter weight, however, was unaffected by treatment. Alterations in foetal body weights and late resorptions (see Table 88 below) might be consequent to the reduced maternal body weight gain, particularly at the mid- and high-dose. However, because no statistical significance was achieved and historical control data were lacking, these findings were difficult to interpret. No further inter-group differences were seen in the pregnancy or remaining litter data.

Table 88: Litter data

Dose (mg/kg bw/d)	Number of viable foetuses	Resorptions[a]		Foetal weight (g)[b]
		Early	Late	
Control	236	29 (11)	0	1.36
1.0	272	41 (13)	2 (0.6)	1.32 (3%)
5.0	311	47 (13)	5 (1.4)	1.30 (4%)
10.0	276	28 (9)	7 (2.3)	1.28 (6%)

[a]Values in parentheses represent percentages. [b]Values represent percent change from the controls.

A small number of external foetal abnormalities were seen in all treated groups. These included umbilical hernia (8/312 and 1/272 at the mid- and high dose, respectively), malrotated left hind limb (3/272 at the low-dose) and foetuses with no genital opening (2/312 at the mid-dose). Five of the foetuses with umbilical hernia were seen in one litter of the mid-dose, whilst 3 further foetuses were described as runts. In the absence of any apparent dose-response relationship, these abnormalities were considered to be incidental and unrelated to treatment.

Isolated, but statistically significant ($p \leq 0.01$ or 0.05) incidences of foetal skeletal anomalies such as incompletely, partially or non-ossified sternebrae, forelimb or hind limb digits were seen in all treated groups, showing no evidence of a dose-related effect. The incidences of soft tissue abnormalities were unaffected by treatment.

Conclusions: No developmental toxicity was observed, when paraquat was administered to mice by po gavage at levels up to 10 mg/kg bw/d from days 6-15 post coitum. Although there was no clear dose-response relationship, maternotoxicity, characterised by reduced body weight gain was evident at the mid-dose and above. The NOEL for maternotoxicity was 1.0 mg/kg bw/d. The NOEL for foetotoxicity was 10 mg/kg bw/d, the highest dose tested.

Palmer K (1992a) Paraquat (technical) Y00061/160/001 Oral (Gavage) Mouse developmental toxicity, Dose range finding study. CTL Study No: RM0590, Lab: Toxicol Laboratories Ltd, Bromyard Road, Ledbury, HR8 1LH, England. Sponsor: ICI Central Toxicology Laboratory, Alderley Park, North Macclesfield, Cheshire, SK10 4TJ England. Study duration: April 29 to May 13, 1992. Report No: CTL/C/289, Report date: April, 1993.

Quality assured GLP (US, OECD & Japan) study. No test guidelines were cited.

Study: This dose range-finding study in mice was conducted to establish suitable dose levels for a subsequent developmental toxicity study. Five groups of time-mated female mice of Crl:CD1 (ICR) BR strain (10 mice/dose, Charles River UK Ltd, 8 weeks old, bw at mating: 25.7-32.0 g) were dosed with aqueous solutions of paraquat (as paraquat dichloride, batch: YF6219, source: ICI, paraquat cation content: 38.2%) by po gavage at 0, 10, 20, 30 or 40 mg/kg bw/d once daily, during days 6 through 15 of gestation. Mating was accomplished by caging

females with sexually mature males (1:1) overnight from approximately midnight. Animals were acclimatised to the laboratory conditions for approximately 51-62 days prior to mating. Matings were confirmed the following morning by the presence of a copulation plug either *in situ* in the vagina or in the cage tray. The dosing volume was 10 mL/kg bw. The control group received the vehicle only. Dosing solutions were prepared once prior to the start of dosing. Separate solutions were prepared and corrected for paraquat cation content for each dose level. The accuracy of the dose solution preparation was checked using a validated method (data provided). The homogeneity and stability of the dosing solutions were not assessed. Four mice at 10, 20 and 30 mg/kg bw/d were also mistakenly dosed on day 5 of gestation. The animals at 40 mg/kg bw/d were dosed approximately 3 h later than the other groups, in case there were any adverse effects at 20 and 30 mg/kg bw/d, in which case, the dosing at 40 mg/kg bw/d was discontinued. Animals were housed (3/cage) under standard laboratory conditions and provided with pelleted rodent diet (SQC Rat and Mouse No. 3 Breeder, Special Diet Services, Essex, UK) and water *ad libitum*.

Observations: Clinical signs were examined daily from day 0 of gestation. Body weights were recorded on day 0, 6 through 15 and 18 of gestation. Food consumption was determined for the following periods: day 0 through 6, 6 through 9, 9 through 12, 12 through 15 and 15 through 18 of gestation. The following pregnancy and developmental parameters were determined: pregnancy status, weight of the gravid uterus, early and late resorptions, dead or live foetuses, foetal weight and sex, external abnormalities. At termination, animals were sacrificed by CO_2 asphyxiation and necropsied (including those that died or were sacrificed prematurely). The lungs plus trachea and kidneys were removed, weighed and fixed in buffered formol saline. Statistical differences between the control and treatment groups were tested using, either a Student's t-test, the double arcsine transformation or an ANOVA.

Findings: Treatment at 30 and 40 mg/kg bw/d resulted in maternal mortality and clinical signs, including piloerection, lethargy, hunched posture, pale extremities and/or laboured respiration. At 40 mg/kg bw/d, 4 dams were found dead, and 2 were sacrificed prematurely one due to poor clinical condition and the other following an intubation accident. The remaining animals in this group were sacrificed on day 15 of gestation. Two mice at 30 mg/kg bw/d were sacrificed prematurely and two further animals at this dose level were found dead between days 14 and 17 of gestation. One animal at 20 mg/kg bw/d found dead on day 17 of gestation, but showed no clinical signs prior to death. There were no mortalities or clinical signs at 10 mg/kg bw/d.

At 40 mg/kg bw/d, mean food consumption was reduced by approximately 12-40% during days 6-15 compared to controls, reaching statistical significance on days 12 to 15 ($p<0.05$). At 30 mg/kg bw/d, food consumption was depressed by approximately 25-57% on days 12 to 18 compared to controls, showing statistical significance ($p<0.01$) during days 15-18 of gestation. At 20 mg/kg bw/d, decreases in food consumption during days 12-15 and 15-18 were approximately 16% and 8%, respectively. The depressions in food consumption seen at the 30 and 40 mg/kg bw/d groups were considered to be treatment-related.

No significant body weight loss or retarded weight gain were seen in dams at 40 mg/kg bw/d up to day 15, when the remaining animals were killed (data not shown), although the study author reported such an effect. At 30 mg/kg bw/d, retarded body weight gain was seen from day 6 to termination, reaching statistical significance on days 12 to 18. The mean weight gain at 30 mg/kg bw/d, after adjusting for gravid uterine weight, was depressed by approximately 48% compared to the controls, but no statistical significance was achieved.

Table 89: Maternal body weight gain (g, mean ± SEM)

Dose (mg/kg bw/d)	Days of gestation				
	12–15	15–18	6–15	0–18	0-18†
0	7.5 ± 1.7	10.7 ± 2.3	15.1 ± 3.1	27.8 ± 5.0	6.8 ± 2.2
10	7.4 ± 1.5	11.0 ± 1.6	14.8 ± 2.2	28.1 ± 3.6	7.4 ± 2.2
20	7.4 ± 2.3	13.0 ± 2.9 (7)	15.7 ± 3.1	31.3 ± 6.5 (7)	7.7 ± 2.8 (7)
30	3.6 ± 3.0**	4.8 ± 7.9**(3)	9.7 ± 4.3**	19.8 ± 7.9*(3)	3.5 ± 4.1 (3)
40	8.1 ± 1.6 (6)	-	15.8 ± 3.1 (3)	-	-

†Weight gain after adjusting for gravid uterine weight. Values in the parentheses represent the number of animals. Significantly different from controls *($p<0.05$)**($p<0.01$).

Necropsy of animals either found dead or sacrificed prematurely showed dark red lungs in 1, 2 and 6 dams at 20, 30 and 40 mg/kg bw/d, respectively. Statistically significant ($p<0.01$) increases in absolute and relative lung and trachea weights, and relative kidney weights were seen at 30 and 40 mg/kg bw/d. Absolute and relative lung and trachea weight at 20 mg/kg bw/d were elevated by approximately 20% and 25%, respectively, compared to controls, and were attributed to treatment, (see Table 90 below) even though these changes did not reach statistical significance.

Table 90: Maternal organ weights (mean ± SEM)

Dose (mg/kg bw/d)	Carcass weight (g)	Absolute (g)		Relative	
		Lung & trachea	Kidneys	Lung & trachea	Kidneys
0	52.8 ± 10.2	0.31 ± 0.06	0.53 ± 0.08	6.52 ± 3.6	10.4 ± 2.6
10	53.2 ± 10.0	0.31 ± 0.05	0.56 ± 0.07	6.14 ± 2.1	10.9 ± 2.5
20	51.5 ± 14.7	0.37 ± 0.11	0.53 ± 0.08	8.14 ± 4.3	11.0 ± 3.0
30	36.0 ± 9.2**	0.45 ± 0.14**	0.48 ± 0.05	13.2 ± 4.3**	13.8 ± 3.1**
40	36.4 ± 7.2**	0.49 ± 0.22**	0.52 ± 0.06	14.2 ± 6.9**	14.5 ± 2.1**

**Significantly different from controls ($p<0.01$)

Pregnancy data at 10, 20 and 30 mg/kg bw/d were comparable to those of the controls. The study authors stated that the pregnancy data of the animals at 40 mg/kg bw/d were not formally assessed as these dams were sacrificed prematurely. Foetal sex was unaffected by treatment. Foetal body weights (see Table 91 below) in all treatment groups were depressed by approximately 4-20% compared to controls, showing statistical significance at 30 mg/kg bw/d for all foetuses ($p<0.01$), and at 10 mg/kg bw/d for male foetuses ($p<0.05$). There was no dose-related trend, and the study author stated that the values at 10 and 20 mg/kg bw/d were within the historical control ranges (no supporting data were provided). At 30 mg/kg bw/d, gravid uterine weight was decreased by approximately 22% compared to the controls, but no statistical significance was achieved. No treatment-related major or minor foetal abnormalities were observed.

Table 91: Foetal and gravid uterine weights (g)[a]

Dose (mg/kg bw/d)	Foetal weight			Gravid uterus weight
	Male	Female	All foetuses	
0	1.42 ± 0.09	1.31 ± 0.11	1.38 ± 0.07	21.0 ± 5.9
10	1.31 ± 0.07*	1.27 ± 0.05	1.28 ± 0.06	20.6 ± 3.1
20	1.36 ± 0.10	1.30 ± 0.06	1.32 ± 0.08	23.6 ± 4.4
30	1.13 ± 0.30**	1.10 ± 0.30**	1.11 ± 0.30**	16.3 ± 4.1

[a]Mean ± SD; Significantly different from controls ($p<0.01$)** or ($p<0.05$)*

Conclusions: No treatment-related major or minor foetal abnormalities were observed. Based on a statistically significant depression in foetal body weights at 30 mg/kg bw/d, dose levels of 7.5, 15 and 25 mg/kg bw/d were chosen for the main developmental toxicity study.

Palmer K (1992b) Y00061/160/001 Oral (gavage) mouse developmental toxicity study. Lab: Toxicol Laboratories Ltd, Bromyard Road, Ledbury, HR8 1LH, England. Sponsor: ICI Central Toxicology Laboratory, Alderley Park, North Macclesfield, Cheshire, SK10 4TJ England. Study duration: May 15 to June19, 1992. Report no: ICL/19/92. Report date: November, 1992.

Quality assured GLP study. No test guidelines were cited.

Study: Paraquat (as paraquat dichloride, paraquat cation content 38.2%, batch no: YF6219 Ex. no. 9, Product Stock, ICI, UK) in distilled water was administered once daily to timed-mated female mice [Crl: CD1 (ICR) BR strain, initial bw 25-29 g, 26 mice/dose, age unspecified, Charles River, UK Ltd] by gavage at 0 (vehicle control), 7.5, 15 or 25 mg/kg bw/d on days 6 through 15 post coitum. Mating was accomplished by caging females with sexually mature males (1:1) overnight from approximately midnight. Matings were confirmed on the following morning by the presence of a vaginal plug either *in situ* or in the cage tray, and the day on which a vaginal plug was observed was designated as day 0 of gestation. The dose volume was 10 mL. The dose administered was adjusted according to the animal's most recent body weight. The dose levels used in this study were selected by the sponsor, and were based on the results of a preliminary study. Dosing solutions were prepared once prior to the start of dosing, and divided into appropriate quantities, corrected for paraquat cation content and then stored at room temperature protected from sunlight until required. Animals were acclimatised for 11 days prior to mating, and were individually housed under standard laboratory conditions and provided with a pelleted diet (SQC Rat & Mouse NO 3 Breeder, expanded, Special Diet Services, Witham UK) and water *ad libitum* throughout the study. The paraquat content in the dosing solutions was determined by a HPLC method. Animals were sacrificed on day 18 of gestation by CO_2 asphyxiation and necropsied.

Observations: Clinical signs were recorded daily. Body weights were determined on days 0, 6 to 15 inclusive and 18 of gestation. Food consumption was measured over the following periods: days 0 through 6, 6 through 9, 9 through 12, 12 through 15 and 15 through 18 of gestation. At necropsy, the lungs with trachea and kidneys were removed, weighed and fixed in buffered formalin. The following parameters were determined: pregnancy status, gravid uterine weight, early and late resorptions, dead and live foetuses, foetal weights, sex and external abnormalities. One half of the live foetuses were fixed in Bouin's fluid and subsequently examined for visceral abnormalities by a combined sectioning/dissection technique. The remaining foetuses were fixed in 70% alcohol for evisceration and the viscera were examined. These foetuses were then cleared in potassium hydroxide, stained with alizarin red S and examined for skeletal variants and abnormalities. Structural congenital abnormalities that impair or potentially impair the survival or fitness of the foetuses were classified as major abnormalities, whilst the other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 18 foetus, together with common variations in the extent of renal pelvic cavitation and ureter dilatation, were recorded as variants. Statistical differences between the control and treatment groups were tested using either a Student's t-test, the double arcsine transformation, an ANOVA or the Fisher's exact probability test.

Findings:
General: The paraquat cation content in two of the dosing solutions (7.5 and 25 mg/kg bw/d) were within 10% of the nominal concentrations, but the 15 mg/kg bw/d solution had approximately 29% more paraquat than the nominal value. One dam at the high-dose was found dead on day 16 of gestation, and 4 further animals in this dose group were sacrificed *in extremis* on days 15, 16 or 17 of gestation. Clinical signs observed in these animals were piloerection, laboured respiration, hunched posture, hypothermia, hypoactivity and/or pale extremities and eyes.

Maternal: Group mean body weights at the high-dose were depressed by 7% and 9% at 15 and 18 days of gestation, respectively, achieving statistical significance at both these observation times ($p \leq 0.05$ and 0.01, respectively). The mean food consumption in high-dose dams was decreased by 8-20% during days 12 through 18 compared to controls, but no statistical significance was achieved. The body weight gain in dams at this dose level was reduced by 7-34% during days 6 through 18 compared to controls, reaching statistical significance on days 12 through 18 ($p \leq 0.01$). After adjusting for the gravid uterine weight, the body weight gain at the high-dose was depressed by approximately 18% at termination compared to controls. The depressions in maternal food consumption, body weights and body weight gain at the high-dose were attributed to paraquat.

Necropsy revealed dark red lung lobes in four high-dose dams, and in animals that either died or were sacrificed *in extremis*. The study authors stated that the dam that died prematurely, and those sacrificed *in extremis,* were pregnant, and had live foetuses *in utero* at the time of maternal death. No further treatment-related macroscopic tissue abnormalities were seen in other dams in this group or any other groups. Treatment-related organ weight changes were confined to the high-dose group. These included significant increases in absolute and relative lung weights (31% and 66%, respectively, $p \leq 0.01$), and approximately a 15% increase in relative kidney weight compared to controls. The weight of the gravid uterus was reduced by approximately 17% compared to controls ($p \leq 0.01$).

The mean numbers of implantations and live foetuses were depressed by approximately 10% at the high-dose compared to controls, but the values fell within the historical control range (data provided), and the changes were not considered to be due to paraquat.

Foetal: At the high-dose, mean foetal body weight was depressed by approximately 9% compared to controls ($p \leq 0.01$). Slight reductions (approximately 2-3%) in mean foetal body weight, and gravid uterine weights were observed at the mid-dose compared to controls, but the data were within the historical control ranges, and these changes were not considered to be toxicologically significant.

There were no treatment-related foetal malformations. Spina bifida in one foetus at the mid-dose and cleft palate and kidney agenesis in 2 different foetuses at the high-dose were observed. These were considered to be spontaneous occurrences and unrelated to treatment. The incidence of renal pelvic cavitation was increased significantly (~ 3- to 4-fold on a litter basis) at the low- and mid-dose groups (on both the foetal and litter basis, $p \leq 0.01$ and 0.05, respectively) compared to controls. At the high-dose, the increase was approximately 2-fold compared to controls, but no statistical significance was achieved. Although the incidence of this abnormality in the treatment groups was above the historical control data range (0-2.2%,

on a foetal basis), they were not considered to be treatment-related, because of lack of a dose-response relationship and statistical significance at the high-dose. No treatment-related effects were seen in either major or minor skeletal abnormalities.

There was a significant increase ($p \leq 0.01$ or 0.05) in the incidence of foetuses with six or less caudal centra (also at the low- and mid-dose), incompletely ossified occipital and non-ossified astragalus at the high-dose (on both a foetus and litter basis). The incidence of foetuses with 3 or less caudal neural arches was slightly increased on both a foetus and litter basis (~2-fold) at the high-dose compared to controls, reaching statistical significance only on a litter basis. There were no apparent dose-response relationships and the data were within or slightly above the historical control ranges (data provided), and hence they were not attributed to treatment. These developmental effects, though equivocal, could be related to frank maternal toxicity at the high-dose.

Table 92: Incidence of foetal skeletal variants

Variant	Dose (mg/kg bw/d)			
	Control	**7.5**	**15**	**25**
Foetal incidence [number of foetuses affected (% incidence)]				
Total number of foetuses examined	157	134	143	83
Six or less caudal centra	5 (20.8)	11* (52.4)	11* (50)	88* (57.1)
Incompletely or non-ossified astralgus	14 (7.5)	18 (12.8)	13 (9.9)	30* (31.5)
3 or less caudal neural arches	2 (1)	5 (3.1)	1 (0.8)	5 (5.2)
Incompletely ossified occipital bone	6 (3.1)	6 (4.3)	6 (4.4)	8 (8.6)
Litter incidence [number of litters affected (% incidence)]				
Total number of litters examined	24	21	22	14
Six or less caudal centra	5 (20.8)	11 52.4	11 50	8* 57.1
Incompletely or non-ossified astralgus	5 (20.8)	8 (38.1)	7 (31.8)	8* (57.1)
3 or less caudal neural arches	2 (8.3)	4 (19)	1 (4.5)	4 (28.6)
Incompletely ossified occipital bone	2 (8.3)	5 (23.8)	3 (13.6)	6* (42.9)

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

Conclusions: There was no evidence that paraquat was teratogenic when administered orally to mice from days 6-15 post coitum up to 25 mg/kg bw/d. Maternotoxicity, characterised by mortality, clinical signs, reduced food consumption, body weight gain, and increased lung and relative kidney weights were observed at the high-dose and therefore the NOEL for maternotoxicity was 15 mg/kg bw/d. The effects on embryonic/foetal development, characterised by reduced foetal weight, increased incidences of retarded ossification of a

number of skeletal variants were only apparent at the maternotoxic dose level (25 mg/kg bw/d) and therefore the NOEL for foetotoxicity was 15 mg/kg bw/d.

Hausburg MA, Dekrey GK, Salmen JJ, Palic MR & Gardiner CS (2005) Effects of paraquat on development of preimplantation embryos *in vivo* and *in vitro. Reprod Toxicol 20(2):239-46.* Evaluation Part 2.

The aspect of this study concerned with the effects of paraquat on reproductive parameters is presented elsewhere (Hausburg *et al* 2005, Section 7 of this Review). This part of the study presented here was concerned with the effect of paraquat exposure on the development of preimplantation embryos.

Study:

In the studies below, GSH content in embryos was determined using HPLC. Pools of embryos were used (20-375) for analysis as required to permit detection. No degenerate embryos were analysed.

In vitro:

1. Embryos were isolated from synchronised, superovulated and bred mice approximately 36 h after ovulation. Embryos were cultured under light paraffin oil in 10 µL drops of culture medium (10 embryos per drop) containing paraquat at 0, 8, 40, 200, or 1000 µM for 24 h. After culturing, embryos were evaluated by light microscopy for indication of fertilisation, stage of development, quality of embryos, and abnormal features (Laub *et al* 2000).

2. In a separate experiment with embryos cultured the same way, compacted morrulae were isolated and examined for cell number by fluorescence microscopy.

3. To examine the overall impact of paraquat exposure *in vitro* on preimplantation development, embryos were cultured the same way except that they were cultured in concentrations of paraquat of 0, 8, 40, 200, or 1000 µM for 4 days, or to approximately day 5 post ovulation. After 1, 2, 3, and 4 days of culture, the embryos were evaluated for developmental for developmental stage to determine the percentage of total embryos that had developed to each developmental stage.

In vivo:

1. To determine if preimplantation embryos are sensitive to paraquat induced toxicity *in vivo*, embryos were isolated on day 1 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on the day of ovulation (Treatment Protocol #1 below).

The treatment and embryo collection protocols used are shown in Table 93 below.

Table 93 - Treatment and embryo collection protocols (Hausburg et al, 2005)

Gestation day	-3	-2	-1	0**	1	2	3
Treatment protocol No 1							
Treatment	eCG*	-	hCG* & Bred	Paraquat injection	Embryo collection		Embryo collection
Treatment protocol No.2							
Treatment	eCG	-	hCG & Bred	-	-	Paraquat injection	Embryo collection

*eCG = equine chorionic gonadotrophin 10IU ip; hCG = human chorionic gonadothrophin 5IU ip; paraquat or saline injections were given ip

**0 = the day of ovulation

2. To determine if paraquat exposure could alter the development of embryos beyond the 2-cell stage, embryos were isolated on day 3 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on 1 of 2 different days: day 0 or day 2 (Treatment Protocols above).

Results:

Exposure of preimplantation embryos (collected on the day after ovulation) to paraquat *in vitro* for 24 h at concentrations as low as 8 µM caused a significant decrease in the percentage of 8-cell embryos and an increase in the percentage of compacted morulae. Altered embryo development was most likely due to premature compaction because a 42% decrease in cell number per compacted morulae was observed in embryos exposed to paraquat at 1 mM. Exposure of preimplantation embryos to paraquat *in vitro* for 4 days at 200 at µM or higher eliminated development beyond the blastocyst stage. Exposure of bred female mice to paraquat at 30 mg/kg bw on day 2 after ovulation led to a small but significant decrease in the percentage of 8-cell embryos on day 3 without a detectable increase in the percentage of compacted morulae. No detectable change in preimplantation embryo development was found following paraquat exposure on the day of ovulation (day 0).

The data indicated that paraquat could adversely impact the development of preimplantation embryos *in vitro* and *in vivo.*

Bus JS, Preache MM, Cagen SC, Posner HS, Elliason BC, Sharp BW & Gibson JE (1975) Foetal toxicity and distribution of paraquat and diquat in mice and rats. *Toxicol Appl Pharmacol 33: 450-460.*

[Only the developmental toxicity data from this study were considered in the following evaluation.]

Study & observations: Nulliparous Swiss-Webster mice (Spartan Research Animals Inc, MI, USA, bw: unspecified) were administered with paraquat (paraquat cation content: 29.1%, a commercial preparation) on days 8 through 16 of gestation either by po gavage at 20 mg/kg bw/d single or ip at 1.67 or 3.35 mg/kg bw/d. Both the 3.35 mg ip and 20 mg/kg bw/d po doses were considered as $1/10^{th}$ of the respective LD_{50} values (unpublished observations of the study author). The day that a vaginal plug was detected in mated mice, was designated as day one of gestation. On day 19 of gestation, animals were sacrificed by ether anaesthesia and the uterine horns were removed. The number and position of live, dead and resorbed foetuses were

recorded. Foetuses were removed by cautery of the umbilical cord, then dried, weighed and examined for gross abnormalities. Litters were equally divided for fixation in Bouin's solution or 95% ethanol. Foetuses fixed in Bouin's solution were hand sectioned and examined under a dissecting microscope for soft tissue abnormalities, whilst those fixed in ethanol were cleared, stained with Alizarin red S and examined for soft tissue abnormalities. Data were analysed statistically by ANOVA. The level of significance chosen was $p<0.05$. No further information on experimental methods, including treatment of controls, was provided.

Findings: There were no significant deviations in foetal resorption rate or foetal body weight in mice receiving the 1.67 mg/kg bw/d ip dose (see Table 94 below) compared to controls. However, a statistically significant (~ 5-fold) increase in foetal resorption rate was seen in mice receiving paraquat at 3.35 mg/kg bw/d ip compared to controls. The increased foetal resorption rate was also associated with increased maternal mortality rate (71%), with only 2/7 pregnant mice at this dose level surviving the 8-day treatment period. No significant group differences in the above study parameters were noted in mice given paraquat orally. Foetal body weights of all treated groups were comparable to those of corresponding controls.

Table 94: Effect of paraquat on pregnancy and foetal parameters

Dose (mg/kg bw/d)	Route	Number pregnant		Mean response/litter ± SEM		
		Treated	Surviving	Number of foetuses	% resorptions	Body weight (g)
0	ip	7	7	11 ± 0	4.8 ± 1.7	1.31 ± 0.04
1.67	ip	6	6	10 ± 2	17.8 ± 16.5	1.21 ± 0.04
3.35	ip	7	2	9 ± 2	22.5 ± 3.5*	1.37 ± 0.02
0	po	8	8	13 ± 1	6.4 ± 1.9	1.27 ± 0.02
20	po	7	7	12 ± 1	3.9 ± 1.9	1.25 ± 0.04

*($p<0.05$)

No significant inter-group differences were seen in the incidence of other gross or soft tissue anomalies (see Table 95 below). However, following the 1.67 and 3.35 mg/kg bw/d ip or 20 mg/kg bw/d po dose, retarded foetal skeletal development as manifested by a treatment-related increase in the rate of either absent or non-ossified sternebrae was observed on per litter basis (~2- to 4-fold) compared to the corresponding controls.

Table 95: Skeletal anomalies observed in the offspring

Anomaly	Incidence* – Dose (mg/kg bw/d)				
	0 (ip)	1.67 (ip)	3.35 (ip)	0 (po)	20 (po)
Number of litters examined	7	6	2	8	7
Sternebrae absent or not ossified	6.9 ± 3.2	21.2 ± 8.2	25.0 ± 25.0	13.2 ± 5.8	33.4 ± 12.3
Vertebrae absent	0	3.3 ± 3.3	12.5 ± 12.5	0	0
Phalanges absent or not ossified	0	0	12.5 ± 12.5	0	0

*Values are the mean percentage response/litter ± SEM

Conclusions: There was no evidence that paraquat was teratogenic in mice following ip or po administration up to 3.35 and 20 mg/kg bw/d respectively. No NOELs were set as the design and dose selection of this study limited its usefulness for regulatory purposes

Bus JS & Gibson JE (1975) Postnatal toxicity of chronically administered paraquat in mice and interactions with oxygen and bromobenzene. *Toxicol Appl Pharmacol 33: 461-470.*

[Only the developmental toxicity data from this study were considered in the following evaluation.]

Study & observations: Mated Swiss-Webster mice (number of animals/group, age & bw: unspecified, Spartan Research Animals, MI, USA) were treated with paraquat dichloride (paraquat cation content 240 µg/mL, Chevron Chemical Co, CA, USA) in drinking water at 0 (control), 50, 100 or 150 ppm from day 8 of gestation to 42 days postnatally. Assuming that the body weight of a mouse is approximately 20-30 g and the water consumption of a mouse/d is approximately 3-7 mL, the quantities of the test substance ingested by the test animals were equivalent to approximately 5-17, 10-32 or 15-52 mg/kg bw/d, respectively.

In a separate experiment, pregnant mice were exposed to 100 ppm paraquat (10-32 mg/kg bw/d) from day 8 of gestation to 28 days after birth (weaning), and then given water without paraquat until 42 days postnatally, or placed on 10-32 mg/kg bw/d dose regimen only during days 28 and 42 postnatally.

In both studies, mating was accomplished by natural means (1 male: 5 females). The day that vaginal plugs were found was designated as day one of gestation. Animals were housed individually under standard laboratory conditions and provided with food and paraquat-containing water *ad libitum.* Following delivery of pups on day 20 of gestation, litters were normalised to 10 mice, all were weaned on day 28 postnatally and segregated by sex. Total litter weights and mortality were recorded weekly from day one after delivery to the end of the study (42 days postnatal). At termination, litters were examined for developmental defects macroscopically. The stability of the paraquat solutions was confirmed by a colorimetric assay. Data were analysed statistically using a Student's t-test and an ANOVA. The level of significance chosen was $p<0.05$.

Findings: In the first study, all pregnant mice receiving the high-dose died before delivery, starting from the first day of treatment. Cumulative mortality at the mid-dose was 33% and 67% at postnatal day 7 and 42, respectively, and was found to be statistically significant. Further, it was also reported that the postnatal cumulative mortality rate among the low-dose and controls was less than 7% (no supporting numeric data provided). Based on the percentage mortality data, the increased mortality in the mid-dose animals appeared to be biphasic, with an initial rapid increase during the first week after birth, a plateau during the second week, and then another increase in the mortality occurring during the postnatal third week. Paraquat did not affect the average litter body weight compared to controls (data provided in graph form). Further, the inclusion of paraquat in the drinking water did not alter water consumption of the animals. Paraquat did not affect the number of live pups born compared to the controls. However, no supporting data for any of these claims were provided.

In the second study, pregnant mice exposed to the mid-dose from day 8 of gestation to 28 days postnatally, and then transferred to tap water for 2 more weeks, yielded a mortality rate of approximately 26% by day 28 after birth. It was reported that this mortality rate was not significantly different from the 28-day mortality rate observed in mice that had been exposed to the mid-dose continuously throughout the development period. Changing to tap water

resulted in a 42-day cumulative mortality that was not different from the 28-day cumulative mortality, and thus eliminated the second rapid phase of increased mortality seen in the first study. Exposure of mice postnatally to the mid-dose from days 28 to 42 after birth resulted in approximately a 46% mortality rate. No gross malformations were observed in any of the offspring from treated females.

Conclusions: Administration of paraquat to mice in drinking water at 5-17, 10-32 or 15-52 mg/kg bw/d resulted in 100% mortality in high-dose animals. Paraquat did not alter the growth of the surviving animals or show any evidence of developmental toxicity. NOELs for maternal animals and their offspring were not established as the regulatory value of this study was limited due to lack of experimental detail, and the absence of numeric data which would allow an independent evaluation of the study.

Hodge MCE (1992a) Paraquat: Comparison of toxicity in pregnant and non-pregnant mice. Study no: RM0566, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI, Study duration: October 31 to November 18, 1991, Report no: CTL/L/4318, Report date: April 01, 1992.

Non quality assured study. No GLP statement was provided and no test guidelines were cited.

Study & observations: The objective of this study was to provide supporting evidence for the selection of 10 mg of paraquat/kg bw/d as the top dose in the previous developmental toxicity study (Hodge *et al* 1978a). A dose level of 20 mg/kg bw/d was selected for this single dose study, on the basis of the results of the previous study and a range-finding study in non-pregnant mice (no supporting data for the latter study were provided). In the present study, a group of 20 Swiss-Webster mice aged 7-9 weeks were treated by po gavage with paraquat (as paraquat dichloride, batch: ASY144, purity: 100%, paraquat cation content 72.37%, ICI) in 0.5% aqueous Tween 80 at 20 mg/kg bw/d on days 7 through 16 of gestation. The day that the mating was confirmed was designated as day one of gestation. The dose volume was 1.0 mL. Control groups of the same numbers of mated and unmated mice were similarly dosed daily for 10 days during the same period with 0.5% aqueous Tween 80 alone. The dosing solution was analysed for the achieved concentration and stability, but not for its homogeneity.

Clinical signs were observed daily. Body weights were recorded on days 1, 4, 7 through 16 (inclusive) and 19 of gestation. Food consumption was recorded at 3-day intervals. At termination, the surviving animals were sacrificed (method unspecified), the uteri were removed, and if gravid, the number and position of each implantation including live foetuses and early and late uterine deaths, were recorded. Both mated and non-mated mice were examined for macroscopic abnormalities in the major abdominal and thoracic organs and the lungs were stored for possible future examination.

Findings: Analysis of the dosing solutions showed that the achieved concentration was 99% of the nominal level, and was found to be stable in the vehicle for over 20 days.

There were no paraquat-related clinical signs or mortalities. One mated animal was sacrificed following an intubation accident. Food consumption data did not show any inter-group differences. It was stated that the body weight gain in paraquat treated, pregnant mice showed ‘little difference’ compared to that of the controls. However, no supporting data on this finding were provided.

The pregnancy rate of the paraquat treated group was 55% (11/20) compared to that of the controls, which was approximately 85% (17/20). Two paraquat-treated mice littered early, on an unspecified day during gestation, and another began to litter while awaiting necropsy. It was stated that there were no inter-group differences in the remaining tested reproductive parameters. Again, no supporting data were provided for any of these parameters. The depression in pregnancy rate in the treated group compared to the controls was considered to be unrelated to treatment. No further information on maternal or foetal parameters was provided.

Conclusions: No treatment-related adverse effects on maternal animals, foetal survival or any of the tested reproductive parameters or any evidence of teratogenicity were observed following po administration of paraquat to pregnant mice at 20 mg/kg bw/d. However, the relevance of the findings was limited, as there were no supporting data on any of the study parameters.

4.8.2 Rats

Hodge MCE, Palmer S, Weight TM & Wilson J (1978b) Paraquat dichloride: Teratogenicity study in the rat. CTL Study no: RR0052. Lab: Imperial Chemical Industries Ltd, Central Toxicology Laboratory. Study duration: not stated. Report no: CTL/P/365. Report date: June 5, 1978.

Pre-dates GLP and test guidelines.

Study: Paraquat (batch: ADY M 76/G, purity: 100%, ICI, UK) in 0.5% aqueous solution of Tween 80 (polysorbate 80 BCP) was administered once daily to mated Alderley Park strain female rats (initial bw: 237-242 g, 29 or 30 rats/dose, age & source: unspecified) by po gavage at 0, 1, 5 or 10 mg paraquat ion/kg bw/d on days 6-15 post-coitum. The dose levels were based on the findings of a preliminary range finding study (data provided). Test solutions were prepared freshly each day prior to dosing and the dosage volume was 10 mL/kg bw. Control animals received similar volume of the vehicle. Mating was accomplished by natural means overnight. Vaginal smears were collected on the following morning to confirm successful mating. The day that spermatozoa were detected in vaginal smears was considered to be day 0 of gestation. Animals were individually housed under standard laboratory conditions and provided with feed (Alderley Park rat cubes) and water *ad libitum*. On day 21 of gestation, rats were sacrificed by cervical dislocation.

Observations:
Maternal: The animals were observed daily for any abnormalities. Body weights were recorded at 0, 3, 6, 8, 12, 16 and 21 days. Food and water consumption were observed but not measured. At necropsy, a range of maternal tissues was examined macroscopically. Samples of the lung and the kidney from at least 11 rats/group together with samples of the heart, lung, kidney, adrenal, spleen, liver, ovary, uterus and placenta (only if there were any foetal abnormalities) of any sick animals were processed for histopathology.

In addition, the following reproductive parameters were determined: number of corpora lutea and live foetuses, resorptions (early or late), foetal weight, sex, external abnormalities and the number of runts (those <3.5 g bw).

Foetal: Alternate foetuses from each litter were eviscerated and fixed in 70% methanol, the viscera being macroscopically examined for abnormalities. These foetuses were stained with Alizarin Red for skeletal examination. Ossified bones were examined both for abnormalities and the degree of ossification. The overall ossification of forelimb and hind limb digits was assessed on a seven point scale (details provided), although all individual bones were examined. The remaining foetuses were preserved and decalcified in Bouin's fixative for at least ten days prior to examination. Sections through the head, thorax and kidneys were made and examined. The abdomen was examined by dissection. Statistical differences between the control and treatment groups were determined using either a Student's t-test, the double arcsine function of Freeman and Turkey, χ^2-test, 2x2 Contingency Tables or an ANOVA.

Findings: Dose solution analysis showed that the concentrations of solutions of 1 and 10 mg/kg bw/d were within 10% of nominal concentrations. However, the dose solution of 5 mg/kg bw/d had a concentration of 4 mg/kg bw/d (20% less paraquat content). Re-analysis of the 5 mg/kg bw/d dose solution towards the end of the study (date unspecified) yielded a concentration that was in close agreement with the previous results showing approximately 14% less paraquat in the solution compared to the nominal level.

Maternal: There were 12 mortalities (either died or sacrificed *in extremis*) in the study. Four of these deaths (one each in the control and high-dose, two in the mid-dose) were due to inadvertent dosing, whilst two dams at the mid-dose and six at the high-dose either died or were sacrificed. One dam in the high-dose group littered on day 21, and therefore, was excluded from the study. Clinical signs observed in the majority of the mid- (16/29) and high-dose (25/30) animals were hypersensitivity, piloerection, weight loss, hunched back appearance, staining around the eyes, nose, head or genital area, and respiratory distress in some dams. Treatment-related and statistically significant reductions ($p \leq 0.001$) in average weight gains were seen at 5 and 10 mg/kg bw/d (~24% and 29%, respectively) compared to controls.

At necropsy, the tissues of six high-dose dams that either died or were sacrificed were examined. The lungs of these animals were described as red and patchy. Histopathology of the lung tissue showed oedema fluid in the alveoli and polymorph infiltration, whilst widespread degenerative changes were seen in the kidney proximal tubules. The study authors attributed these abnormalities to paraquat. It was reported that the lungs and kidneys of the surviving dams at 0, 5 and 10 mg/kg bw/d did not show any signs of paraquat toxicity. The tissues of the low-dose dams were not examined. One mid-dose dam had 12 resorptions out of 14 implants.

Foetal: The two surviving pups of the mid-dose dam that had 12 resorptions, were oedematous and runts. The study authors analysed the litter data by both including, and excluding these findings (see Table 96 below). The number of litters were reduced in all groups compared to the controls, showing 15%, 11% and 33% depressions at the low-, mid- and high-dose, respectively. The reduction (approximately 33%) in the number of litters noted at the high-dose was attributed to the test substance (no historical control data were provided), as there was no dose-response relationship for this effect at lower doses. Mean foetal weight was significantly reduced by 4% and 6% ($p \leq 0.05$) compared to controls at the mid- and high dose, respectively. At these dose levels, the mean litter weight was depressed by approximately 3.5% and 4.5%, respectively, compared to controls, but no statistical significance was reached. Reduced foetal and litter weights may have been the consequences of maternotoxicity due to paraquat noted at these 2 dose levels.

Table 96: Group mean litter data

Observation	Dose (mg/kg bw/d)			
	Control	**1.0**	**5.0[a]**	**10.0**
Number of litters	27	23	24	18
Mean implants	13.1	13.1	13.5 (13.5)	13.78
Viable foetuses	12.5	12.2	11.9 (12.4)	12.6
% resorptions[b]	0.59 *(0)*	0.87 *(0.3)*	1.54 (1.09) *(0.9)*	1.09 *(0.4)*
Transformed (viable/implants)	2.66	2.62	2.49 (2.57)	2.54
Foetal weight (g)	5.2	5.2	4.9 (5.0)	4.9*
Litter weight (g)	64.8	63.2	60.0 (62.5)	61.9

[a]Values in parentheses represent the data calculated excluding the data of the mid-dose rat, which had 12 resorptions out of 14 implants. [b]Values in italics represent late resorptions. *Significantly different from controls ($p \leq 0.05$).

The proportion of females with one or more resorptions was approximately 40% greater at the mid- (15/24) and high-dose (11/18) groups in comparison to controls, but no statistical significance was achieved. There was evidence of slight retardation in ossification of caudal vertebrae and forelimb and/or hind limb digits at the mid- and high-dose, which could be attributed to delayed foetal growth, consequent to the maternotoxicity seen at these two dose levels. No further skeletal abnormalities were seen. The incidence of soft tissue abnormalities was unaffected by treatment.

Conclusions: Under the conditions of the study, there was no evidence that paraquat was teratogenic. The NOEL for maternotoxicity was 1 mg/kg bw/d based on increased mortality, clinical signs and reduced body weight gain at 5 and 10 mg/kg bw/d. The NOEL for foetotoxicity was also 1 mg/kg bw/d based on reduced mean foetal and litter weights at 5 and 10 mg/kg bw/d.

Hodge MCE (1992b) Paraquat: Developmental toxicity study in the rat. Study no: RR0593, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Chemicals. Study duration: May - June, 1992. Report no: CTL/P/3864, Report date: November 30, 1992.

Quality assured study. Conducted in accordance with the US EPA, UK, OECD and Japan GLP standards.

Study: Groups of 24 female Wistar-derived rats (initial bw: 209-297 g, 11 weeks old, Alpk: APfSD, ICI Pharmaceuticals, UK) were dosed by po gavage with paraquat (batch: YF6219, paraquat cation content 38.2%, ICI, UK) in deionised water once daily at 0, 1, 3 or 8 mg paraquat/kg bw/d on days 7 through 16 post-coitum. The dose volume was 10 mL/kg bw. Control animals received deionised water similarly. Mating was accomplished by natural means overnight. Vaginal smears were examined for the presence of sperm on the following morning for the confirmation of successful mating. The day that spermatozoa were detected in vaginal smears was considered to be day one of gestation. Animals were individually housed under standard laboratory conditions and provided with feed (CT1 Diet, Special Diet Services Ltd, Essex, UK) and water *ad libitum*. A sample of each dosing solution was analysed prior to the start of dosing to establish the achieved paraquat levels. The stability of paraquat in the

vehicle was determined using an HPLC method. On day 22 of gestation, the animals were sacrificed by halothane overdose and necropsied.
Observations:
Maternal: Animals were observed daily for clinical signs and behavioural abnormalities. Body weights were recorded on days 1 and 5, 7 through 17 and on days 19 and 22 of gestation. Food consumption of each animal over 3-day periods was measured by weighing the food left uneaten on days 4, 7, 10, 13, 16, 19 and 22. The following parameters were studied: intact gravid uterine weight, number of corpora lutea, number of live foetuses, early and late intra-uterine deaths, percent pre- and post-implantation losses and foetal weights. Foetuses were sacrificed by intra-cardiac injection of pentobarbitone sodium.

Foetal: All foetuses were examined for external abnormalities, cleft palate and visceral abnormalities, and then sexed, eviscerated and fixed in methanol. The head of each foetus was cut along the fronto-parietal suture line and the brain examined for macroscopic abnormalities. Carcasses were processed and stained with Alizarin Red S for examination of skeletal abnormalities and the degree of ossification of *manus* and *pes* (on a scale of 1-6, complete to poor ossification). Abnormalities were classified as major (rare or possibly lethal or both) or minor (deviations from normal that are not uncommon at external, visceral or at skeletal examination) defects. Variations were also recorded and classified as minor defects or variants depending on the incidence in the rat strain used (details provided). Statistical differences between the control and treatment groups were examined tested using either a Student's t-test, Fisher's exact test, the double arcsine transformation of Freeman and Turkey or an ANOVA.

Findings: The concentrations of paraquat in dosing solutions were within 8% of the nominal levels. Paraquat was shown to be stable in deionised water over 32 days.

Maternal: There were no mortalities or treatment-related clinical signs. Food consumption in all paraquat treated groups was marginally reduced during the treatment period compared to controls, but the changes did not indicate any dose-related effect. The high-dose dams, which had the highest initial group mean body weight showed a slight body weight loss (~1-2%) compared to controls. The adjusted group mean body weights at the high-dose showed statistical significance ($p \leq 0.01$ or 0.05) at the majority of observation times. The depression in body weight at this dose level may have been related to the test substance. The dose selection of the study made interpretation of this finding difficult. Group mean body weights at the mid- and high-dose were comparable to that of the controls (see Table 97 below). At necropsy, no treatment-related macroscopic lesions were seen in any of the dams.

Table 97: Adjusted group mean body weights (g) at selected observation times

Observation day	Dose (mg/kg bw/d)			
	Control	1.0	3.0	8.0
Pre-dosing				
1	251	248	242	252
During dosing				
10	291	290	291	287**
12	301	300	302	302**
14	312	310	313	308*
16	323	321	324	318*
Number of animals	24	21	24	22

** Significantly different from the corresponding controls ($p \leq 0.01$); *Significantly different from the corresponding controls ($p \leq 0.05$).

Based on the proportions of implants affected, pre-implantation loss at the low- and high-doses was higher compared to controls, achieving statistically significance at the high-dose ($p \leq 0.05$). Given that pre-implantation loss could occur prior to the commencement of dosing, this was not considered to be an effect related to the test substance. However, this resulted in a lower number of implants, live foetuses, and reduced gravid uterine and litter weights at these dose levels.

Foetal: Mean foetal body weights at the mid- and high-dose were depressed by approximately 3% compared to controls, possibly as a result of the marginally reduced maternal food consumption in these groups during treatment. Two high-dose litters had only one surviving pup/litter, weighing 2.9 and 4.0 g, respectively, compared to the group mean of 4.6 g. Gross examination of foetuses revealed bilateral microphthalmia (1/256 at the control), internal hydrocephaly (3/206) and malrotated hind limb (1/206) at the low-dose, and abdominal *situs inversus* (1/273 at the mid-dose), showing no indication of an association with treatment. According to the study authors, the incidence of internal hydrocephaly has been seen at the similar frequency in 2 control litters, in a study conducted by the study author's laboratory using the same strain of rats (data provided).

With regard to minor foetal defects, the only observation that showed statistical significance ($p \leq 0.05$) was slightly dilated ureters in the high-dose pups, with the incidences being 0.4%, 1.9%, 0% and 2.9% for the control, low-, mid- and high-dose groups, respectively. Given that there was no dose-response relationship and the overall incidences were within the historical control range, these findings were considered to be unrelated to treatment.

Minor skeletal abnormalities such as partially or non-ossified and/or misaligned vertebrae, sternebrae, transverse processes, ribs and the 5^{th} sternebrae bipartite were seen across all groups including controls, with no evidence of an association to treatment. The incidences were significantly elevated at the low- and high-dose groups compared to controls ($p \leq 0.01$). In the absence of a dose-effect relationship or an effect on per litter basis, and also because the incidences were within the historical control ranges, this abnormality was not considered to be an effect related to treatment. An assessment of the degree of ossification of digits and toes showed slightly higher and statistically significant ($p \leq 0.01$ or 0.05) scores at the low- and high-dose (score of 4-6) compared to controls. Given that the overall mean litter scores did not show any statistically significant differences, and a dose-response relationship was lacking, this finding was also considered to be unrelated to treatment. The remaining study parameters were unaffected by treatment.

Conclusions: Paraquat was not teratogenic following administration to rats by po gavage at 1, 3 and 8 mg/kg bw/d on days 7-16 of gestation. The maternotoxicity NOEL was 3 mg/kg bw/d based on significant weight loss in dams at 8 mg/kg bw/d. The NOEL for foetotoxicity was 8 mg/kg bw/d, the highest dose tested.

4.8.3 Rabbits

Hodge MCE (1990) Paraquat: Embryotoxicity study in the rabbit. CTL study no: RB0503, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals. Study duration: not stated. Report no: CTL/L/3423, Report date: November 26, 1990.

Non-quality assured study. No GLP statement was provided and no test guidelines were cited.

Study & observations: In this study, groups of ten artificially inseminated NZW rabbits (age, bw & source: unspecified) were dosed by po gavage with paraquat in deionised water at 0 (control), 2.5, 5, 10 or 20 mg/kg bw/d on days 7 through 19 of gestation. The dose levels selected for the study were based on the results of previous toxicity studies in rabbits, rats and mice. The day of insemination was designated as day 1 of gestation. The control group received deionised water alone. It was reported that, due to a mistake, the initial batch (Y00061/159) of paraquat liquor contained the emetic PP796 and was used for the initial 3 days of dosing. A new batch, (Y00061/160) containing no emetic was used for dosing thereafter. Animals were observed daily for clinical signs and behavioural abnormalities. Body weights were recorded on days 1, 4, 7 through 19, 22, 26, and 30 of gestation. Food consumption was determined over 3-day periods throughout gestation. On day 30 of gestation, the animals were sacrificed, and the weight of the gravid uterus, the number of live foetuses, and intra-uterine deaths were determined. The foetuses were weighed, sacrificed, examined for external abnormalities and cleft palate, and then discarded. Animals that showed signs of abortion or premature delivery, or that were *in extremis,* were sacrificed by an iv overdose of Euthatal. These animals and any animal found dead during the course of the study, were necropsied. No further information on experimental methods was provided.

Findings: Three does at 20 mg/kg bw/d, and 1 at 10 mg/kg bw/d, were found dead on day 10 of the study. The surviving rabbits at 5, 10 and 20 mg/kg bw/d were sacrificed *in extremis* between days 9 and 15. Four animals receiving the 2.5 mg/kg bw/d dose were sacrificed *in extremis* on days 16-17. One rabbit in this group aborted and was sacrificed on day 22. The number of pregnant animals in each group, including controls, was rather low, being 1, 6, 4, 2 and 6 for the 0, 2.5, 5, 10 and 20 mg/kg bw/d groups, respectively.

The major treatment-related clinical signs observed in the study were subdued behaviour and few/no faeces. It was reported that the test animals also lost weight during dosing, but no supporting numeric data were provided. There was a slight reduction in food consumption at 2.5 mg/kg bw/d. There was evidence of stomach irritation in a number of treated animals, with signs of haemorrhagic areas and sloughing off of the stomach mucosa, which were attributed to treatment. There was no evidence of any adverse effect on the number of surviving young *in utero*, or on mean foetal weight. No external foetal malformations were observed. However, only limited litter data were available for embryotoxicity assessment, as there were only small numbers of pregnant rabbits in most of the treatment groups.

Conclusions: The low pregnancy rate in all groups and the high degree of mortality among treated animals made the evaluation of embryotoxicity difficult. Although the limited data demonstrated that paraquat was not teratogenic in rabbits, the regulatory value of this study was limited due to the lack of details on experimental animals and methods, very low pregnancy

and high mortality rates in treated animals. Doses of 1.0, 1.5, 2.0 and 2.5 mg paraquat (without emetic)/kg bw/d were selected for a future embryotoxicity study in the rabbit.

Tinston DJ (1991a) Paraquat: Second embryotoxicity study in the rabbit. CTL study no: RB0508, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Chemicals & Polymers NW Ltd, Cheshire, UK. Study duration: February 21 to March 21, 1991. Report no: CTL/T/2730, Report date: July 12, 1991.

Non-quality assured study. No GLP statement was provided and no test guidelines were cited.

Study: Time-mated, female NZW rabbits (8/group, Interfauna UK Ltd, Huntington, UK, bw on day 3 of gestation: 3.1-3.9 kg, age: unspecified) were treated by po gavage with paraquat at 0, 1.0, 1.5, 2.0, or 2.5 mg/kg bw/d (Y00061/160, paraquat cation content: 33.6%, ICI Chemicals & Polymers NW Ltd, UK) in deionised water on days 7 through 19 of gestation. Mating was accomplished by caging females with sexually mature males overnight (1:1). Control animals received the vehicle alone similarly. The dose volume was 1 mL/kg bw. Prior to commencement of dosing, a sample of each dosing solution was analysed to verify the achieved concentrations of paraquat cation. The stability of paraquat was determined by re-analysis the dosing solution at the end of the dosing period. The day of mating was designated as day one of gestation. Animals were individually housed under standard laboratory conditions and provided with pelleted diets (CRB, Labsure Animal Diets, UK) and water *ad libitum*. No further experimental details were provided.

Observations: Clinical signs and behavioural abnormalities were checked at least once daily. Body weights were recorded on days 3, 4, 7-19, 22, 26 and 30 of gestation. Food consumption was recorded on days 4, 7, 10, 13, 16, 19, 22, 26 and 30. Animals *in extremis* and all survivors at termination (day 30) were sacrificed by an iv injections of pentobarbitone sodium. These animals and those found dead during the study were necropsied. The following parameters were determined: gravid uterine weight, number of corpora lutea, live foetuses, early and late intrauterine deaths and foetal weight. Foetuses were sacrificed by intracardiac injection of pentobarbitone sodium. Mean values of the study parameters were tested for statistical significance using a 2-sided Student's t-test.

Findings: Initial analysis of the dosing solutions revealed that the achieved concentrations were higher than the nominal concentrations by up to 8%. It was reported that there was approximately a 14% reduction in paraquat concentration in deionised water over the 19-day study period, but since the initial concentrations were higher than the nominal levels, it was assumed that the final paraquat levels were within 10% of the nominal concentrations. It was also stated that this apparent fall in concentration was inconsistent with previous data, which demonstrated satisfactory stability of the test substance in deionised water.

Five animals showed signs of abortion and were sacrificed (one each at 1.0 and 2.0 mg/kg bw/d, and three at 2.5 mg/kg bw/d). Three animals were sacrificed *in extremis* following weight loss and/or poor clinical condition (one in the control, and one each at 1.0 and 2.0 mg/kg bw/d groups), and one doe at 2.5 mg/kg bw/d was found dead. There were no mortalities at 1.5 mg/kg bw/d. In addition to treatment-related clinical signs, which included signs of abortion and hunched appearance at 2.5 mg/kg bw/d, the incidence of few/no faeces and diarrhoea were elevated in the 2.0 and 2.5 mg/kg bw/d groups.

Food consumption at 2.0 and 2.5 mg/kg bw/d was depressed by approximately 26% and 72% ($p \leq 0.01$) during the dosing period, and by approximately 6% and 18% for the full 30-day study period, respectively, compared to controls. No inter-group differences were seen after cessation of dosing (during days 19-30). On day 19 of gestation, body weights at 2.0 and 2.5 mg/kg bw/d were depressed by approximately 4% and 7%, respectively, in comparison to controls, with some signs of recovery during days 19-30. Overall body weight gain in animals at 2.0 and 2.5 mg/kg bw/d during the 30-day gestation period was reduced by approximately 13% and 20%, respectively, compared to controls. These reductions in body weight gain were attributed to paraquat (see Table 98 below).

Table 98: Inter-group comparison of maternal body weight gain (g, mean ± SD)

Days during gestation	Dose (mg/kg bw/d)				
	Control	1.0	1.5	2.0	2.5
3-7	47.4 ± 86.8	-5.4 ± 125.8	57.8 ± 83.7	51.6 ± 96.2	1.3 ± 32.8
7-19	264.3 ± 106.1	226.2 ± 115.7	285.2 ± 119.3	10.2 ± 380.0	-2.5 ± 347.9
19-30	259.6 ± 130.0	173.4 ± 161.4	328.5 ± 87.9	437.8 ± 165.4	459.3 ± 79.5*
3-30	571.3 ± 162.3	394.2 ± 235.7	671.5 ± 121.7	499.6 ± 252.3	458.0 ± 281.6

Values in parentheses represent percent decreases compared to controls. *Significantly different from controls ($p<0.05$).

Treatment-related necropsy findings were restricted to the stomach of the does at 2.0 and 2.5 mg/kg bw/d. In addition to abnormal or discoloured stomach contents, lesions such as haemorrhagic areas and sloughing off of gastric mucosa were reported.

Pregnancy data: There was an increase in the incidence of abortion at the top dose (incidences were 0, 1, 0, 1 and 3 for the 0, 1.0, 1.5, 2.0 and 2.5 mg/kg bw/d group, respectively) compared to controls, resulting in a decrease in the total number of live foetuses/group at termination. No further inter-group differences were seen in the remaining pregnancy related study parameters.

One foetus at 1.0 mg/kg bw/d had major abnormalities such as flexure of both fore-paws, left anophthalmia, left ear attached back to front, exposed heart and exencephaly. One foetus at 2.5 mg/kg bw/d had meningocoele. These anomalies were considered to be isolated occurrences and unrelated to treatment.

Conclusions: There was no evidence of teratogenicity in rabbits given paraquat at dose levels up to 2.5 mg/kg bw/d. The maternotoxicity NOEL was 1.5 mg/kg bw/d based clinical signs, and reductions in food consumption and body weight gain at 2.0 and 2.5 mg/kg bw/d. The NOEL for foetotoxicity was 2.0 mg/kg bw/d based on an increased incidence of abortion at 2.5 mg/kg bw/d.

Tinston DJ (1991b) Paraquat: Teratogenicity study in the rabbit. CTL study no: RB0519, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals. Study duration: April 23 to June 01, 1990. Report no: CTL/T/2749. Report date: September 21, 1991.

Non-quality assured study. No GLP statement was provided and no test guidelines were cited.

Study: Groups of artificially inseminated female NZW rabbits (20 animals/group, Interfauna UK Ltd, Huntington, UK, bw: 2.9-4.2 kg, age: unspecified) were treated by po gavage with paraquat at 0, 1.0, 1.5 or 2.0 mg/kg bw/d (Y00061/160, paraquat cation content: 33.6%, ICI Chemicals & Polymers NW Ltd, UK) in deionised water from days 7-19 of gestation. Control animals received the vehicle alone similarly. The dose volume was 1 mL/kg bw. Prior to the start of dosing, a sample of each dosing solution was analysed to verify the achieved paraquat cation concentration. The stability of paraquat was determined by re-analysis of the 1 and 2 mg/kg bw dosing solutions after 28 days. Prior to insemination, the animals were acclimatised to the laboratory for approximately a week, and the day of insemination was designated as day one of gestation. Animals were individually housed under standard laboratory conditions and provided with pelleted diets (CRB, Labsure Animal Diets, UK) and water *ad libitum.*

Observations: Clinical signs and behavioural abnormalities were examined at least twice each day throughout the study. Body weights were recorded on days 1, 4, 7-19, 22, 26 and 30 of gestation. Food consumption was calculated on days 4, 7, 10, 13, 16, 19, 22, 26 and 30. The animals *in extremis* and all survivors at termination (on day 30) were sacrificed by an intravenous injection of pentobarbitone sodium. These animals and the animals found dead during the study were subjected to a detailed necropsy. The following parameters were determined: gravid uterine weight (minus ovaries and connective tissue), number of corpora lutea, live foetuses, early and late intrauterine deaths and foetal weight. The foetuses were sacrificed by intracardiac injection of pentobarbitone sodium.

Foetal assessments: Each foetus was examined for external abnormalities and cleft palate. They were then examined for visceral abnormalities, sexed, eviscerated and fixed in methanol. After 24 h, the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to methanol for subsequent processing and staining with Alizarin Red S. Stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. Individual bones of the *manus* and *pes* were assessed using a 6 point grading scale. The observations were classified as major (permanent structural or functional deviations that are considered likely to be incompatible with survival or rarely seen) or minor (small, generally transient deviations that are considered not to be incompatible with life). Mean values were tested for statistical differences using either a one- or two-sided Student's t-test, Fisher's exact test, double arcsine transformation of Freeman and Tukey or an ANOVA. Mean *manus* and *pes* scores were not analysed statistically as a value of 2 was recorded for every foetus in the study. No further experimental details were provided.

Findings: Analysis of the dosing solutions showed that the achieved concentrations of paraquat in deionised water were within 6% of the nominal concentrations. It was stated that the stability of paraquat in the 1.0 and 2.0 mg/kg bw dose solutions was 'satisfactory' over a 28-day period.

There were four premature mortalities. One rabbit at 1.0 mg/kg bw/d was found dead on day 10, with no previous clinical findings or weight loss. One dam at 1.5 mg/kg bw/d was sacrificed *in extremis* on day 22 due to excessive weight loss. Two does at 2.0 mg/kg bw/d aborted their litters on days 24 and 25, respectively, and were sacrificed. Treatment-related clinical signs were observed in the treated animals (increased incidence of diarrhoea and few/no faeces) at 1.5 and 2.0 mg/kg bw/d. Unspecified 'other findings' were either low in incidence and/or showed no dose-response relationship, and therefore were considered to be incidental. A summary of reproductive performance and intercurrent deaths is presented in the Table 99

below. The overall pregnancy rate in this study was low, being 55% compared to the rate of 87.5% observed in the previous study by this author (CTL study no. RB0508). Consequently, there were an inadequate numbers of pregnant animals in the study, as opposed to the minimum group size of 12 rabbits specified in the current OECD guidelines.

Table 99: Reproductive performance

Parameter	Dose (mg/kg bw/d)			
	0	**1.0**	**1.5**	**2.0**
Number inseminated	20	20	20	20
Number pregnant	12	11	8	13
Number of intercurrent deaths	0	1	1	2
Found dead	0	1	0	0
Sacrificed for humane reasons	0	0	1	0
Aborted	0	0	0	2
Number of does with live foetuses at termination	12	10	8	11

Food consumption in dams at 1.5 and 2.0 mg/kg bw/d was significantly reduced by approximately 37% ($p \leq 0.01$) during days 7-13 of the dosing period, with some evidence of a slight reduction during days 16-19, which could be related to treatment. A compensatory recovery in food consumption was seen during the latter part of the study. No treatment-related effects were seen at termination.

A marked loss of body weight was seen at 1.5 and 2.0 mg/kg bw/d ($p \leq 0.01$) during days 7 through 10 of the dosing period. These animals showed some recovery in weight gain during the subsequent part of the study. The depression in total body weight gain in these 2 dose groups during the dosing period was approximately 56% compared to controls, and was statistically significant ($p \leq 0.05$, see Table 100 below). The overall weight gain in animals at 1.5 and 2.0 mg/kg bw/d during the 30-day study period was depressed dose relatedly by approximately 16% and 20%, respectively, compared to controls. The reduction in weight gain (approximately 0.2 g) seen at 1.0 mg/kg bw/d during days 7-10 was attributed to a transient weight loss in a single animal, which subsequently recovered and gained weight comparable to controls and was therefore not considered to be treatment-related.

At necropsy, an increased incidence of haemorrhagic areas in the stomach was seen at 2.0 mg/kg bw/d, with some evidence for this lesion at 1.0 and 1.5 mg/kg bw/d. An increased percentage of pre- and post-implantation loss, with reductions in mean numbers of implantations and live foetuses were seen at 1.0 and 2.0 mg/kg bw/d. These losses resulted in reduced mean gravid uterine weights and litter weights at termination. It was stated that the increased percentages of post-implantation losses seen at 1.0 and 2.0 mg/kg bw/d were attributable to an exaggerated loss in single dams in each of these dose groups, each of which had 3 early intra-uterine deaths (no individual data were provided). No inter-group differences were seen in mean foetal weights.

Table 100: Intergroup comparison of maternal body weight gain (g)[a]

Study period (days)	Dose (mg/kg bw/d)			
	Control	1.0	1.5	2.0
Pre-dosing (total during days 1-7)	188.0	176.5	204.5	163.1
During dosing (total during days 7-19)	233.2	223.4	101.6* (56%)	102.6* (56%)
7-10	35.2	-0.2	-85.1**	-52.9**
10-13	62.8	76.6	18.4	16.0
13-16	101.9	84.1	85.1	108.5
16-19	33.3	62.9	83.3	31.1
Post dosing (total during days 19-30)	273.8	263.4	277.9	290.9
Overall	695.0	663.3	584.0 (16%)	556.6 (20%)

[a]Group mean values. Values in parentheses represent percent reductions compared to controls.
Significantly different from controls *($p<0.05$),**($p<0.01$).

A summary of the major foetal defects observed is presented in the Table 101 below. The incidence of microphthalmia was higher at 1.5 (unilateral) and 2.0 mg (bilateral)/kg bw/d compared to controls. Percent incidences of this anomaly (per foetus and per litter) were 1.7% and 12.5% at 1.5 mg/kg bw/d and 3% and 18% at 2.0 mg/kg bw/d, respectively. These values were above the published historical control values for this strain of rabbits. [Average foetal and litter incidences of unilateral microphthalmia are 0.012% and 0.106%, respectively; maximum historical control incidences for bilateral microphthalmia are 1.3% and 11.1% for per foetus and per litter basis, respectively. (MARTA & MTA, 1996)]

Table 101: Major foetal defects observed and their incidence

Abnormality	Dose (mg/kg bw/d)			
	0	1.0	1.5	2.0
Number of litters	12	10	8	11
Number of foetuses examined	92	57	59	64
Spina bifida	1	-	-	-
Microphthalmia (unilateral)	-	-	1 (1.7%) 12.5%	-
Microphthalmia (bilateral)	-	-	-	2 (3%) 18%
Cardiac anomaly, aorta reduced	-	-	1	-
Aorta extremely enlarged	1	-	-	-
Number of foetuses affected	2	0	2	2
Number of litters affected	2	0	1	2

Values in parentheses are foetal incidences and those in italics are litter incidences.

Minor skeletal anomalies characterised by reduced ossification and an increased incidence of asymmetric development of the pelvic girdle and clubbed ribs were observed at 2.0 mg/kg bw/d ($p\leq0.01$ or 0.05). The foetal incidence of clubbed ribs was elevated in all treated rabbits compared to controls [incidences were 2%, 4%, 3% and 4% (litter incidences were 2.2%, 7.0%, 5.1% and 6.3%) for the control, 1.0, 1,5 and 2.0 mg/kg bw/d groups, respectively]. These values also fell above the published historical control data ranges of this strain of rabbits (averages are 0.007% and 0.055%, with maximum values of 0.66% and 5.3% for per foetus and per litter basis, respectively; MARTA & MTA, 1996). The lack of statistical significance and a dose-response relationship, and narrow dose selection made it difficult to interpret these results. The incidence of partially ossified cervical vertebrae was higher at 1.5 and 2.0 mg/kg bw/d, compared to controls achieving statistical significance ($p\leq0.05$) at the top-dose. A dose-related

increase in the foetal incidence of asymmetrically aligned pelvic girdle was seen in all paraquat treated animals showing statistical significance at 2.0 mg/kg bw/d (per foetus basis). The foetal incidence of extra ribs (13th, normal length) was increased dose-relatedly, reaching statistical significance ($p \leq 0.01$) at 1.5 and 2.0 mg/kg bw/d. This may be a suggestive of paraquat-related foetotoxicity at these 2 dose levels. In addition, the incidences of several other skeletal variants (partially ossified lumbar transverse processes, non-ossified or partially ossified sternebrae, misshapen hyoid) in all paraquat treated groups were increased in comparison to controls, showing statistical significance on a per foetus basis occasionally, but showed no consistent dose-effect relationships.

Conclusions: There was some evidence of foetotoxicity, characterised by non- or retarded ossification of the skeleton, with an increased incidence of developmental anomalies (extra ribs, microphthalmia) at maternotoxic dose levels (1.5 mg/kg bw/d and above). The study author concluded that 'the number of pregnant dams and litters available for assessment was too low in the paraquat treated groups to allow an adequate evaluation of the teratogenic potential of the chemical'. Nevertheless, there was no conclusive evidence for teratogenic potential of paraquat at the dose levels used. The interpretation the findings of the study was difficult due to the narrow dose selection.

Tinston DJ (1991c) Paraquat: Second teratogenicity study in the rabbit. CTL study no: RB0547, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals. Study duration: March 04 to April 12, 1990. Report no: CTL/T/2763, Report date: September 27, 1991.

Non-quality assured study. No GLP statement was provided and no test guidelines were cited.

Study: Given that inadequate pregnancy rates were achieved in the previous developmental toxicity study, the study was repeated and the findings are presented in this report. The identity and purity of the test substance, the dose levels, animal model used, and experimental methods adopted in this study were essentially similar to those of the previous study (CTL study no: RBO519). The test animals (NZW rabbits, bw: 3.3-4.6 kg) were acclimatised to the laboratory conditions for at least 2 weeks prior to insemination.

Findings: Analysis of the dosing solutions showed that the achieved concentrations of paraquat cation in deionised water were within 8% of the nominal concentrations. The stability of paraquat cation in deionised water was shown to be 'satisfactory' for up to 28 days.

The mortality and pregnancy data are summarised in the Table 102 below. A dose-related increase in intercurrent deaths was seen among the paraquat treated groups (3, 8, and 11 at 1.0, 1.5 and 2.0 mg/kg bw/d, respectively). Two animals in the 2.0 mg/kg bw/d group were found dead during the study (days unspecified), and 2, 4 and 4 animals at 1.0, 1.5 and 2.0 mg/kg bw/d, respectively, were sacrificed due to excessive weight loss and poor clinical condition. In addition, 1, 4 and 5 dams aborted their foetuses, and were therefore sacrificed.

Table 102: Mortality and reproductive performance

Parameter	Dose (mg/kg bw/d)			
	0	1.0	1.5	2.0
Number inseminated	20	20	20	20
Number pregnant	17	15	16	19
Number of intercurrent deaths	0	3	8	11
Found dead	0	0	0	2
Killed for humane reasons	0	2	4	4
Aborted	0	1	4	5
Number of does with live foetuses at termination	17	11	9	8
Number with total resorptions	0	1	1	0

The incidence of animals with few/no faeces, thin appearance and signs of bleeding was increased in all paraquat treated groups, with an increased incidence of diarrhoea at 1.5 and 2.0 mg/kg bw/d compared to controls. Unspecified 'other findings', that were unrelated to dose, were considered as incidental by the study author.

Food consumption in all paraquat-treated groups (excluding those dams that had total resorptions, abortions, or died prematurely) was depressed dose-relatedly (5-26%) during days 7-13, achieving statistical significance at 1.5 and 2.0 mg/kg bw/d ($p \leq 0.01$ or 0.05). Food consumption in all animals showed statistically significant reductions ($p \leq 0.01$ or 0.05) during the dosing period at all dose levels, at the majority of observation times.

Consistent with the observations in the previous study, a reduction in food consumption and a marked body weight loss was seen at 1.5 and 2.0 mg/kg bw/d during days 7-10 of the gestation period. The overall reduction in body weight gain during the dosing period was dose-related and statistically significant ($p \leq 0.01$ or 0.05), and 50% and 60% at 1.0 and, 1.5 and 2.0 mg/kg bw/d, respectively, compared to controls. An indication of recovery in weight gain was seen after cessation of dosing, but the overall weight gain in treated dams during the 30-day study period was depressed by 26%, 46% and 40% at 1.0, 1.5 and 2.0 mg/kg bw/d, respectively, compared to controls. The reductions in food consumption and body weight gain at all 3 doses were attributed to treatment.

At necropsy, all treated dams including, those dying or sacrificed intercurrently showed a dose-related increase in lesions of the stomach, with a similar trend in liver and lung lesions at 1.5 mg/kg bw/d and above in comparison to controls.

The incidence of pre-implantation loss was higher in all paraquat treated groups than the controls, and this resulted in reductions in the mean number of implantations, live foetuses, gravid uterine weight and litter weight (see Table 103 below). However, this was not attributed to treatment. These reductions achieved statistical significance ($p \leq 0.01$ or 0.05) at all dose levels, except for the mean number of live foetuses and mean litter weight at 1.0 mg/kg bw/d. No inter-group differences were seen in mean foetal weights. The high post-implantation loss seen at 1.5 mg/kg bw/d was attributable to one early and 11 late intra-uterine deaths (out of 13 implantations) in one dam of that dose group. However, there were statistically significant ($p \leq 0.01$ or 0.05) reductions in the mean number of live foetuses at 1.5 (37%) and 2.0 (23%) mg/kg bw/d in comparison to controls, which could be attributed to treatment. However, this interpretation is confounded by the higher pre-implantation loss in paraquat treated groups, an

anomaly that occurred prior to commencement of dosing. As a proportion of the mean number of implantations compared to the mean number of live foetuses, the difference is not as evident.

Table 103: Litter data

Parameter	Dose (mg/kg bw/d)			
	Control	1.0	1.5	2.0
Number pregnant	17	11	9	8
Mean number of corpora lutea	11.5	10.2	10.4	9.6
Pre-implantation loss				
Mean percentage	8.3	15.3	20.9	18.3
Mean number of implantations	10.5	8.7*	8.6*	7.8*
Post-implantation loss				
Mean percentage	8.1	9.2	22.9	6.6
Percent incidence	47	45	66	50
Mean number of live foetuses	9.6	8.2	6.0**	7.4*
Mean gravid uterine weight (g)	621.5	530.2*	451.8**	489.5*
Mean litter weight (g)	399.8	344.9	265.0**	311.7*
Mean foetal weight (g)	42.4	43.6	42.4	42.6

*****Significantly different from controls ($p \leq 0.05$); **Significantly different from controls ($p \leq 0.01$)

Foetal assessment: A summary of the type and incidence of major foetal defects seen are given in the Table 104 below. Approximately a 3 to 4-fold increase in the mean percentage of foetuses with major defects was seen in all paraquat treated groups compared to controls. The only surviving foetus of one dam at 1.5 mg/kg bw/d showed three major types of defects. Two littermates at the top-dose and one foetus at 1.5 mg/kg bw/d had major head defects. Three foetuses in the same dose group also showed major limb defects. However, the foetal incidence of each of these anomalies was low, and none of them showed any consistent indication of an association with treatment.

Table 104: Foetal abnormalities and their incidence

Abnormality	Dose (mg/kg bw/d)			
	0	1.0	1.5	2.0
Number of litters examined	17	11	9	8
Number of foetuses examined	163	90	54	59
Number showing major defects	2	3	3	2
Mean percentage	0.6	1.8	2.2	2.5
Number showing minor defects	74	49	21	26
Mean percentage	44.8	58.2	42.0	45.2
Mean percentage of variants	91.8	99.1	95.0	98.8
Defects				
Acephaly (agenesis of the skull)	0	0	1	0
Oral atresia, nares absent, cyclopia, external hydrocephaly, gross malformation of the skull, major cervical defects	0	0	0	1
Persistent ductus arteriosus, single ventricle heart, aorta enlarged, pulmonary artery reduced	1	0	0	0
Aorta enlarged, pulmonary artery reduced	0	2	0	0
Aorta enlarged, pulmonary artery enlarged	1	0	0	0
Anomaly of the great vessels of the heart	0	0	1	0
Right hind limb malrotated, left hind limb extremely flexed	0	0	1	0
Forepaw extremely flexed	0	0	1	0
Hind limb extremely flexed	0	1	0	0

For other (minor) foetal anomalies, approximately a 3-fold increase in the incidence of asymmetric alignment of the pelvic girdle (incidences were 3.1%, 3.3%, 1.9% and 8.5% for control, 1.0, 1.5 and 2.0 mg/kg bw/d, respectively) was seen at 2.0 mg/kg bw/d compared to controls. No further evidence for an increase in minor external/visceral defects was observed in the treated groups.

Although none of the foetuses had any external/visceral variants, the majority of them showed at least one skeletal variant (see Table 105 below). The percentages of foetuses with 27 pre-sacral vertebrae and extra 13th ribs of normal length displayed dose-related increases at all 3 doses, reaching statistical significance ($p \leq 0.01$ or 0.05) at 1.5 and 2.0 mg/kg bw/d. The skeletal variants noted at 1.5 and 2.0 mg/kg bw/d were attributed to treatment, as these anomalies may have occurred in consequence to frank maternotoxicity seen at these two dose levels. No inter-group differences were seen in *manus* and *pes* scores.

Table 105: Incidence of external and visceral variants

Variant	Dose (mg/kg bw/d)			
	Control	1.0	1.5	2.0
Number of litters examined	17	11	9	8
Number of foetuses examined	163	90	54	59
27 pre-sacral vertebrae with any extra 13th rib	25 (15.3) *9 (52.9)*	20 (22.2) *9 (81.8)*	19** (35.2) *5 (55.6)*	31** (52.5) *8* (100)*
Any extra 13th rib	83 (50.9) *15 (88.2)*	62** (68.9) *10 (90.9)*	39** (72.2) *9 (100)*	47** (79.7) *8* (100)*

Values in parentheses represent percent incidence, whilst those given in italics indicate litter incidences.
Significantly different from controls *($p \leq 0.05$),**($p \leq 0.01$).

Conclusions: The overall pregnancy rate of this study (84%) was satisfactory in comparison to that observed in the author's previous investigation (55%). Yet, there were insufficient

numbers of pregnant dams at termination due to increased treatment-related mortalities and clinical signs in dams at all 3 dose levels, resulting in an inadequate number of litters to provide conclusive evidence on the teratogenic potential of paraquat. The maternotoxicity NOEL was not established based on increased mortality, clinical signs, reduced food consumption and body weight gain at all doses tested. Based on minor foetal skeletal variants from 1.5 mg/kg bw/d, the NOEL for foetal toxicity was 1.0 mg/kg bw/d.

4.9 Genotoxicity Studies

A summary of the submitted and published findings of genotoxicity studies with paraquat is shown in the Table 106 to Table 112.

Table 106: Summary of *in vitro* gene mutation assays

Assay	Strain or cell type	Concentration	Metabolic activation	Result	Reference
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537, TA 1538 (reverse mutation)	1-1000 µg/plate phosphate buffer vehicle (n=3)	+, -	-, - [a]	McGregor (1977)
		0.5-500 µg/plate distilled water vehicle (n=2)	+, -	-, - [b, c]	Shirasu *et al* (?)
		10–10000 µg/plate water vehicle (n=3)	+, -	-, -	Crichton *et al* (1978)
		1-50 µg/plate distilled water vehicle (n=3)	+, -	-, - [b]	Benigni *et al* (1979)
	TA 98, TA 100, TA 1535, TA 1538 (reverse mutation)	4-5000 µg/mL water vehicle (n=2 or 3)	+	- [b]	Longstaff *et al* (1976)
	TA 98, TA 100 (forward mutation)	0.01-1.0 mM distilled water vehicle, sample size unspecified	-	+ [d]	Moody & Hassan (1982)
	G46 (forward mutation)	0.5-500 µg/plate distilled water vehicle (n=2)	-	- [e]	Shirasu *et al* (?)
		0.1-1.0 µg/plate distilled water vehicle (n=3)	-	+	Benigni *et al* (1979)
	TA 102 (reverse mutation)	10 ng/plate distilled water vehicle (n=3)	-	-	Levin *et al* (1982)
	TA 1535, TA 92 (forward mutation)	0.1-1.0 µg/plate distilled water vehicle (n=3)	-	+	Benigni *et al* (1979)
Escherichia coli	WP2 hcr	0.5-500 µg/plate distilled water vehicle (n=2)	+, -	-, - [b, c]	Shirasu *et al* (?)

Assay	Strain or cell type	Concentration	Metabolic activation	Result	Reference
Mammalian cells	Hamster kidney fibroblasts (BHK21 cells)	10, 50, 250 μg/mL vehicle & sample size unspecified	+	- [b]	Longstaff *et al* (1976)
	Human diploid lung fibroblasts (WI-38 cells)			- [b]	
	Mouse lymphoma L5178Y	31.3-1000 μg/mL saline vehicle (n=2)	+, -	-, - [b, f]	Clay & Thomas (1985) [QA]
	Chinese hamster cell lines G12 & G10	200 & 300 μM Sample size unspecified	-	- [b]	Kitahara *et al* (1996)
	Chinese hamster cells (V79)	$2 \times 10^{-4} – 10^{-3}$ M Hank's balanced salt solution vehicle (n=2)	-	-	Speit *et al* (1998)

QA = study was Quality Assured. Positive and control substances were used in all assays and gave expected results. Metabolic activation was via the addition of S9 mix which was derived from a rat liver homogenate plus a co-factor solution. Rats were induced 5 days prior to sacrifice by an ip injection of Aroclor 1254 (500 mg/kg bw)

a = Cytotoxicity evident at and above 100 μg/plate, with a greater effect observed + S9 mix.
b = Concentration-related increase in cytotoxicity.
c = Greater cytotoxicity + S9 mix.
d = Due to cytotoxicity, a modified liquid incubation assay was employed. A concentration-related increase in the number of revertants per viable cells was observed with the effect in the presence of limited histidine up to 6-fold greater than that in the absence of histidine. Results also suggested that some growth is required for full expression of the mutagenic effect. The presence of oxygen in the medium was found to be important for the mutagenicity of paraquat. No statistical analysis was performed.
e = Cytotoxicity evident at and above 50 μg/plate.
f = A statistically significant (logit regression) concentration-related increase in mutation frequency was determined in 3/5 experiments + S9 mix but this was not considered to be treatment-related as there is no evidence that paraquat is metabolised and therefore results should have been the same ± S9 mix. Additionally there was large inter-experimental variability in the mutation and plating efficiencies for the positive and negative controls.

Table 107: Summary of *in vitro* DNA damage and repair assays

Assay	Strain or cell type	Concentration	Metabolic activation	Result	Reference
Unscheduled DNA synthesis (UDS)	EUE cells (human epithelial-like)	20-2000 µg/mL distilled water vehicle, sample size unspecified	-	± [a]	Benigni *et al* (1979)
	Primary rat hepatocytes	10^{-9}-10^{-2} M Williams incomplete medium 'E' vehicle (n=3)	-	- [b]	Trueman *et al* (1985) [QA]
Reversion, gene conversion and crossing over	*Saccharomyces cerevisiae JD1*	0.02-1.0 µg/mL Commercial grade (n=2)	-	- [c]	Parry (1977)
	Saccharomyces cerevisiae D7	100-900 ppm Gramoxone (100 g/L ai) (n=2)	-	+ [d, e]	Parry (1973)
		10-100 µL/mL Gramoxone (20% ai) sample size unspecified	-	+ [d, f]	El-Abidin Salam *et al* (1993)

QA = study was Quality Assured. Positive and negative control substances were used in all assays and gave expected results. Metabolic activation was via the addition of S9 mix which was derived from a rat liver homogenate plus a co-factor solution. Rats were induced 5 days prior to sacrifice by an ip injection of Aroclor 1254 (500 mg/kg bw)

a = Although a 3-5-fold increase in the mean number of grains per nucleus was reported at every paraquat concentration, this result was considered to be equivocal due to the absence of a dose-response relationship and performance of statistical tests.
b = Evidence of cytotoxicity at the highest concentration.
c = Although the study author concluded that paraquat was mutagenic based on a statistically significant increase ($p<0.01$-0.05) in the number of convertants (relative to the control) at every concentration tested, the evaluating toxicologist concluded that in the absence of any dose-response effect and a positive control, and the fact that the test strain and method were not validated, the result should be viewed as equivocal.
d = Concentration-related increase in cytotoxicity.
e = Induction of gene conversion at cytotoxic concentrations. No statistical analysis performed.
f = 2-10-fold increase over the control of convertants and revertants at and above 50 µL/mL, and in cross-overs at 100 µL/mL.

Table 108: Summary of *in vitro* chromosomal effect assays

Assay	Strain or cell type	Concentration	Metabolic activation	Result	Reference
Sister chromatid exchange (SCE)	Chinese hamster lung fibroblasts (Don cells)	1.2-124 μg/mL (-S9), 1.2-245 μg/mL (+S9) saline vehicle (n=2)	+, -	+, + [a]	Howard *et al* (1985) [QA]
	Human lymphocytes	250-4000 μg/mL distilled water vehicle, sample size unspecified	+, -	+, + [b]	Ribas *et al* (1997/98)
Clastogenicity (cytogenetic test)	Chinese hamster fibroblasts (CHL)	0.2-0.8 mg/mL saline vehicle, sample size unspecified	-	+ [c]	Sofuni & Ishidate Jr (1988)
	Chinese hamster cells (V79)	$2 \times 10^{-4} – 10^{-3}$ M Hank's balanced salt solution vehicle (n=2)	-	+ [d]	Speit *et al* (1998)
	Human lymphocytes	125-3500 μg/mL saline vehicle (n=2)	+, -	+, + [e]	Sheldon *et al* (1985a) [QA]
		15-60 μL/mL Gramoxone (20% ai) (n=4)	-	+ [f]	El-Abidin Salam *et al* (1993)
		1-50 mM distilled water vehicle, unspecified sample size	+, -	-, -	Ribas *et al* (1997/98)
Clastogenicity (comet assay)	Chinese hamster cells (V79)	$2 \times 10^{-4} – 10^{-3}$ M Hank's balanced salt solution vehicle (n=2)	-	-	Speit *et al* (1998
	Human lymphocytes	250-2000 μg/mL distilled water vehicle (n=2)	+, -	+, + [g]	Ribas *et al* (1995)
Micronucleus test	Human lymphocytes	500-4000 μg/mL distilled water vehicle, unspecified sample size	+, -	-, - [h]	Ribas *et al* (1997/98)

QA = study was Quality Assured. Positive and negative control substances were used in all assays and gave expected results. Metabolic activation was via the addition of S9 mix which was derived from rat liver homogenate plus a co-factor solution. Rats were induced 5 days prior to sacrifice by an ip injection of Aroclor 1254 (500 mg/kg bw)

a = Statistically significant ($p<0.01$; 1-sided Student's t-test) concentration-related increase in SCE/cell – S9 mix at all concentrations. Statistically significant ($p<0.01$) concentration-related increase in SCE + S9 mix at and above 24.5 μg/mL. The reduced effect + S9 mix could have been due to paraquat binding to S9 proteins.

b = SCE and cytotoxicity observed at the highest concentration (4000 μg/mL) ($p<0.05$-0.001; t-test for SCE, χ^2 test for cytotoxicity).

c = No evidence of cytotoxicity was reported. The frequency of cells with chromosomal aberrations reached 50% at the highest concentration. Almost all chromosomal aberrations were of the chromatid type and involved exclusively gaps and breaks. Induction of chromosomal aberrations was enhanced with diethyldithiocarbamate or diethyl maleate, and at a high oxygen concentration (80%)

d = Chromosomal aberrations only detected at cytotoxic concentrations. The majority of chromosomal aberrations were chromatid breaks.

e = Clastogenicity observed at cytotoxic concentrations $\pm$ S9 mix. A statistically significant ($p<0.01$; Fisher's Exact test) increase in chromosomal damage occurred at and above 2500 μg/mL – S9 mix, and at and above 1750 μg/mL + S9 mix.

f = Concentration-related increase in the number of chromosomal aberrations including gaps, breaks, fragments and deletions. Statistically significant but no p value given.

g = Concentration-related increase in DNA migration length which was statistically significant at most concentrations ($p<0.05 – 0.001$; t-test). Greater effect – S9 mix. No assessment of cytotoxicity.

h = Cytotoxicity observed at most concentrations (statistically significant reduction in the nuclear division index; $p<0.001$; χ^2 test).

Table 109: Summary of *in vivo* gene mutation and/or recombination assays

Assay	Species	Dose	Result	Reference
Dominant lethal	Mouse (CD-1)	0.04-4 mg/kg bw, PO, 0.5% Tween 80 in water vehicle (n=15 ♂ treated)	- [a]	Anderson *et al* (1976), McGregor (?)
	Mouse (Swiss-Webster)	66 mmol/kg, ip, physiological saline vehicle (n=5 ♂ treated)	- [b]	Pasi *et al* (1974)
Recessive lethal	*Drosophila melanogaster*	7.5 mL/L Gramoxone (20% ai), sample size unspecified	+ [c]	El-Abidin Salam *et al* (1993)
Host-mediated	ICR mice (♂) *S. Typhimurium G46 (his⁻)*	2x5 & 2x20 mg/kg bw over 24 h, ip (n=6)	- [d]	Shirasu *et al* (?)
mwh/flr SMART	*Drosophila melanogaster*	2-8 mM, 1% Tween & 5% ethanol vehicle, sample size unspecified	+ [e]	Torres *et al* (1992)
w/w$^+$ SMART	*Drosophila melanogaster* Oregon K (*Ok*) strain	4-10 mM, phosphate buffer vehicle (30 ♀ mated with 30 ♂)	+ [d, f]	Gaivao *et al* (1999)

Positive and negative control substances were used in all assays and gave expected results.

a = No anti-fertility effect on treated males.
b = Significantly reduced ($p<0.01$; χ^2 test) pregnancy rates were observed during the 3rd mating week only. This anti-fertility effect was thus restricted to post-meiotic spermatids.
c = Statistically significant increase ($p<0.05$; χ^2 test) in sex-linked recessive lethals in the 2nd and 4th broods with the overall difference between the control and treatment group statistically significant ($p<0.01$).
d = Dose-related increase in cytotoxicity
e = No dose-response effect in the frequency of small single spots, large single spots, twin spots or total spots. Statistically significant increase ($p<0.05$; 2 alternative hypotheses method) in the frequency of small single spots and total spots at 2, 6 and 8 mM.
f = The slope of the dose-response regression was statistically different from the vehicle control ($t=10.36$, $df=2$, $p<0.01$)

Table 110: Summary of *in vivo* DNA damage and repair assays

Assay	Species	Dose	Result	Reference
Unscheduled DNA synthesis (UDS)	Rat (Alpk:ALP)	45-120 mg/kg bw/d, PO, water vehicle, sample size unspecified	- [a]	Trueman & Barber (1987) [QA]

QA = study was Quality Assured. Positive and negative control substances were used in all assays and gave expected results.

a = Evidence of cytotoxicity at the highest dose

Table 111: Summary of *in vivo* chromosomal effect assays

Assay	Species	Dose	Result	Reference
Micronucleus test	Mouse (C57BL/6J), bone marrow	51.75 & 82.8 mL/kg bw, PO, deionised water vehicle (n=10; 5 ♂ & 5 ♀)	-	Sheldon *et al* (1985b) [QA]
	Swiss mice (♂) Bone marrow & peripheral blood	2 x 20 mg/kg bw, ip, saline vehicle, (n=10/group)	+ [a]	Ortiz *et al* (2000)
	Rat Spague-Dawley (♂), bone marrow	6, 15 & 30 mg/kg bw via dermal route (n=6/group)	+	D'Souza *et al* (2005)
Clastogenicity (cytogenetic test)	Rat (Wistar-derived) Bone marrow	15-150 mg/kg bw, PO, deionised water vehicle, (n=16; 8 ♂ & 8 ♀)	-	Howard *et al* (1987) QA
	BALB/c mice, bone marrow	1 x 7-23 mg/kg bw ip, 10 x 1.5-5 mg/kg bw/d ip saline vehicle (n=12; 6 ♂ & 6 ♀)	+ [b]	Rios *et al* (1995)
	BALB/c mice, germ cells	5 x 0.5-3 mg/kg bw/d ip, saline vehicle (n=6; ♂)	+ [c]	Rios *et al* (1995)

QA = study was Quality Assured. Positive and negative control substances were used in all assays and gave expected results.

a = Time-related increase in the number of micronuclei/polychromatic erythrocytes in both peripheral blood and bone marrow. Melatonin significantly inhibited ($p<0.001$; 1-way ANOVA & Student Newman-Keuls test) the formation of micronuclei in both peripheral blood and bone marrow. The results were reproducible.

b = Statistically significant ($p<0.01$; χ^2 test) increase in chromosomal aberrations in high-dose females given multiple doses, however decreased mitotic indices (ie cytotoxicity) were observed at all doses in females, and at and above 3 mg/kg bw/d in males.

c = No dose-response effect but a statistically significant increase in sperm head abnormalities at some concentrations ($p<0.05 – 0.01$; Poisson distribution) when cells were treated at 3 different stages (spermatozoa, spermatids, spermatogonial cells in preleptotene).

Table 112: Genotoxicity studies conducted on paraquat that had limited regulatory value due to poor experimental design and/or the lack of reporting detail

Assay	Strain, cell type or species	Concentration/Dose	Metabolic activation	Result	Reference
S. typhimurium	TA 1535, TA 92 (forward mutation)	NS	-	±	Longstaff & Callander (1992)
	TA 1535, TA 92 (forward mutation)	0-2.5 µg/plate distilled water vehicle (n=3)	-	+ [a]	Bignami & Crebelli (1979)
Mammalian cells	Mouse lymphoma L5178Y (forward mutation)	NS	+, -	-, -	Longstaff *et al* (1985b & c)
Sister chromatid exchange (SCE)	Chinese hamster lung fibroblasts (Don cells)	NS	+, -	+, +	Longstaff *et al* (1985e)
Clastogenicity (cytogenetic test)	Human lymphocytes	NS	+, -	+, +	Longstaff *et al* (1985a)
In vivo UDS	Rat	NS	-	-	Elliot *et al* (1986b)
Micronucleus test	Mouse	NS	-	-	Longstaff *et al* (1985d)
Clastogenicity (cytogenetic test)	Rat	150 mg/kg bw, sample size unspecified	-	-	Elliot *et al* (1986a)

a = Evidence of cytotoxicity at the highest concentration, NS= not stated

4.10 Neurotoxicity Studies

Consideration of the neurotoxicity of paraquat is considered in a separate technical report 'Supplement II: Neurotoxicity'.

Potential neurotoxicity was re-examined by the OCS following the finalisation of the toxicology report. This was undertaken due to a number of studies exploring an association between paraquat and neurotoxicity, or specifically Parkinson's disease being published in the open literature. In addition, further animal studies, undertaken by an approval holder to further investigate this association were made available to OCS. A detailed evaluation is presented in Supplement II: Neurotoxicity. The OCS concluded in this report that paraquat does not induce neurotoxicity via the oral route, dermal or intranasal routes; routes of relevance to human exposure to this herbicide.

4.11 Human Studies

4.11.1 Occupational Exposure

Chester G & Woollen BH (1981) Studies of the occupational exposure of Malaysian plantation workers to paraquat. *Brit J Indust Med 38: 23-33.*

[Only the data pertaining to urinary excretion of paraquat were considered in the following evaluation].

Study & observations: This study involving a group of plantation workers in Malaysia was conducted to obtain quantitative dermal and respiratory exposure estimates of paraquat during 'normal' working conditions. Paraquat used by the workers in this study was an agricultural concentrate containing 20% paraquat dichloride (equivalent to 14.5% paraquat cation). Paraquat was usually sprayed in combination with monosodium methane arsanate, diuron or amino triazole. The measured paraquat content in the spray-mix varied from 0.1% to 0.2%, and was sprayed using a standard knapsack sprayer (13.6 L). The application rate ranged from 135 to 169 L/ha. The duration of exposure was variable, ranging from 135-254 minutes.

The study consisted of 2 parts. Firstly, an evaluation of the potential dermal and respiratory exposure of 3 types of worker groups [spray operators (15 males and 4 females), carriers (3 males and 4 females) and rubber tappers (4 males); age & bw ranges unspecified], was undertaken according to the methods described in the WHO standard protocols (patch tests and air sampling). The second part of the study involved taking additional exposure measurements for spray operators and a more extensive evaluation of dermal exposure while the tappers were working in areas that were being sprayed.

In part 1 of the study, urine samples were collected from all workers immediately after completion of spraying. Care was taken not to contaminate samples via paraquat on the hands or clothing. Samples were stored at 4°C until analysis for paraquat by RIA. Appropriate corrections were made for the extraction efficiency. The percentage toxic dose/h was calculated for each worker who participated in the first study according to the method of Durham and Wolfe (1962). Respiratory exposure data were not included in the calculation as the respiratory minute volume data for South-east Asians was not accurately known. The dermal LD_{50} value

used in the calculation was 91 mg/kg bw (paraquat cation), and was based on the findings of the rat study of Chester (1979) using a concentrated paraquat formulation. The method of calculation attempted to relate an exposure/h in man to an exposure/24 h in rats, under an occlusive dressing.

Findings: The mean total dermal exposure for the spray operators was 1.1 mg/kg bw/h (range: 0-2.8 mg/kg bw/h), whilst for the carriers it was 0.3 mg/kg bw/h (range: 0-0.9 mg/kg bw/h). No paraquat was detected in any of the dermal exposure pads of the tappers, and hence their dermal exposure was considered to be zero. However, for tappers working in blocks where the spraying was taking place, the dermal exposure was higher, ranging from 0.01 to 0.08 mg/kg bw/h. The overall mean contribution of hand and leg exposure to total exposure was calculated to be equivalent to 0.13 mg/kg bw/h.

The mean paraquat concentration in the breathing zone of the spray operators was 0.97 µg/m^3 in the first study and 4.9 µg/m^3 in the second study. The study authors claimed that the larger value obtained in the second study was attributable to one very high reading due to accidental contamination of a sampler filter or a misdirected spray during application. It was stated that if this higher value was not included in the calculation, the exposure level would be 0.25 µg/m^3, which is of the same order as the previous value. The mean respiratory exposure value for the carriers was 0.24 µg/m^3. No paraquat was detected in the breathing zone of tappers.

Urine analysis detected paraquat residues only in 9/19 spray operators, and in 1/7 carriers, with the levels ranging from 0.05 to 0.76 mg/L (the limit of detection was 0.05 µg/L). No urinalysis data were provided for tappers.

The mean percentage toxic dose calculated for spray operators was 0.05%/h or 0.3%/working day (range: 0-0.25%/h or 0-1.5%/working day). One of the carriers received a percentage toxic dose of 0.06%/h or 0.36%/working day. The study authors stated that these calculations were based on the exposed body part data, and not total potential exposure. However, it was unclear whether the data were related to total dermal or dermal plus respiratory exposure.

Conclusions: Spray operators appeared to be the most exposed group of workers, with paraquat residues detected in the urine of 9/19 spray operators and in 1/7 carriers, with the levels ranging from 0.05 to 0.76 mg/L.

Howard JK, Sabapathy NN & Whitehead PA (1981) A study of the health of Malaysian plantation workers with particular reference to paraquat spraymen. *Brit J Indust Med 38: 110-116.*

Study: The health of 27 rubber and oil palm spraymen (age: 25-44 years = 14 persons, <25 years = 7 persons, >45 years = 6 persons), chosen from 6 plantations was investigated by performing pulmonary, liver and renal function tests, as well as a full haematological screen. The workers had an average of 5.3 years spraying experience, representing a mean of 8696 spraying hours/worker (minimum of 1000 h). The 6 estates participating in the study used approximately 950-2050 L of Gramoxone (paraquat 20% concentrate) per year, representing an estimated annual average of approximately 336 L of Gramoxone sprayed by each sprayman (equivalent to approximately 62.7 kg paraquat cation). Herbicides were sprayed on a regular and continuous basis, with the predominant chemical used being paraquat. Generally, paraquat

was sprayed at a concentration of approximately 0.1% paraquat cation mixed with diuron [3-(3,4-dicholrophenyl)-1, 1-dimethylurea] and monosodium methane arsenate.

There were 2 control groups in the study, which consisted of male workers chosen from other work areas. The first group (C1) consisted of 24 general workers (tappers, oil palm harvesters and general workers), while the second group (C2) was made up of 23 latex factory workers. Some members of the C1 group had received minimal exposure to paraquat as a result of working in areas of the plantation, in which spraying had recently been completed. None of the C2 workers had any known occupational exposure to paraquat. All workers were residing in the same estate villages, and worked 8 h/d for 6 days/week. Generally, the workers did not wear any protective clothing, but showered regularly after work and changed their clothes. They rarely washed before eating at work, and work clothing was laundered infrequently. The study population consisted of ethnic Chinese, Indians and Malays, with the data on the individual races presented separately.

Observations: Clinical examinations were conducted on all workers, with particular attention paid to the respiratory system and the skin. Blood samples were collected for haematology (Hb, RBC, WBC, PCV, MCHC, MCH and MCV, differential WBC), liver (ALT, AST and ALP) and renal (BUN), serum creatinine and urine albumin) function tests. Respiratory function was determined [forced expiratory vital capacity (FVC), forced expiratory volume during the first second (FEV_1) and percent forced expiratory volume during the first second (FEV_1%) using a standard spirometer. Transfer factor (D_{co}), an estimate of alveolar diffusion, was measured by the single breath method. To test the effect of occupational exposure to paraquat, sprayers, factory workers and general workers were compared for each of the 15 clinical measurements taken. The significance of occupation was tested with allowance made for differences in the distributions of race, age, height, and smoking history among the three groups. This was achieved by fitting multiple regression equations with race, age, height, and smoking history as the independent variables and then refitting the equations with occupation added as a further independent variable. The decrease in the residual sum of squares thus achieved was tested was tested for statistical significance using the F-test. However, the validity of this test is equivocal, as the number of subjects in each study group was relatively small.

Findings: Although personal monitoring was carried out during spraying to obtain estimates of the degree of actual exposure to paraquat, these data were not included in this publication. Skin irritation or rashes on the hand, leg or groin, were the commonest form of adverse reactions reported by workers. Reported incidences of groin or buttock rashes were attributed to leaking knapsacks that allowed spraymix to run down the back of the sprayman. According to medical records, all skin responses (11/27, approximately 41%, with 1 or more incidents) were resolved rapidly after local treatment, which usually was a steroid cream. One case of an eye injury due to a splash was recorded, but this had resolved quickly and completely.

Haematology or lung function test findings of the spraymen were not significantly different from those of the controls. However, significant differences have been seen in both liver and renal function tests of factory workers compared to spraymen and general workers. Concentrations of both serum creatinine and blood urea nitrogen were significantly higher (p=2.8-3.4%) in factory workers, as was the serum ALP activity (p=0.3%). Serum ALT activity was similar between spraymen and general workers, but was twice that of factory workers (see

Table 113 below). All group means fell within the levels quoted as the normal range for the laboratory conducting the assays.

ALP activity in spraymen was approximately 20% lower than that in the factory workers, but approximately 10% higher than that in the general workers. Similarly, creatinine levels in spraymen were approximately 8.5% lower than that in factory workers, but approximately 13% greater than that in the general workers. BUN levels in spraymen were similar to that of the general workers, but 17-18% lower than that of factory workers. Although the study authors claimed that all clinical chemistry parameters determined in the study were within normal ranges, the elevated BUN, creatinine and ALP levels in factory workers may be attributable to some unidentified factors in the work environment.

Table 113: Clinical chemistry data of spraymen and two control groups

Parameter	Group	Malay	Indian	Chinese
ALT (IU/L)	Spraymen	39.5	59.0	31.0
	General workers	36.4	54.2	28.6
	Factory workers	14.7	21.9	11.5
ALP (IU/L)	Spraymen	32.4	30.7	28.4
	General workers	29.0	27.5	25.5
	Factory workers	39.7	37.7	34.9
Creatinine (mg/dL)	Spraymen	0.97	1.14	0.98
	General workers	0.84	0.99	0.85
	Factory workers	1.06	1.24	1.07
BUN (mg/dL)	Spraymen	10.7	10.1	10.4
	General workers	10.5	9.9	10.3
	Factory workers	12.8	12.1	12.4

Conclusions: No evidence of any adverse effects on lung function, blood parameters, liver or renal function from regular long term use of low concentration paraquat spray was observed in this study, despite the lack of use of adequate PPE. However, the study is limited as paraquat exposure measurements were not reported.

Hoffer E & Taitleman U (1989) Exposure to paraquat through skin absorption: Clinical and laboratory observations of accidental splashing on healthy skin of agricultural workers. *Human Toxicol 8: 483-485.*

Study & observations: This study evaluated some clinical parameters of a group of agricultural workers, who were accidentally exposed to paraquat. Plasma and urine samples of 15 workers, submitted to the Israeli Poison Information Centre after incidents involving skin or eye splashing, were analysed for paraquat content by RIA. In all cases sampling was initiated after the worker complained of skin burns, usually 1-3 days after the actual exposure. No further information on experimental procedures was provided.

Findings: The relevant data on the 15 cases are presented in the Table 114 below. The limit of detection of paraquat was 50 ng/mL. Cutaneous effects after splashing of concentrated paraquat solutions (paraquat content unspecified) on previously healthy skin were presented in photographic form for 2 cases, and showed second or third degree burns. Paraquat was detected in both the plasma (25 to 50 ng/mL) and urine (0.025-0.15 µg/mL) of 3 persons, and only in the urine of 2 workers (0.05 and 0.07 µg/mL). For these individuals, the delay between

exposure and sampling was 1-2 days. The only treatment that had been recommended to them was washing the contaminated area with plenty of water after the exposure took place.

It was reported that no systemic effect of paraquat was found in any of these workers at the time of clinical examination or in follow up assessments. The levels of paraquat in the urine and plasma were low (unspecified), when sampled more than 12 h after the exposure (data not provided). The study authors suggested that paraquat is more likely to be detected in biological fluids, when skin blisters are present (ie absorption via damaged skin).

Table 114: Data on 15 cases and the plasma and urinary paraquat levels

Age (yrs)	Sex	Exposed area	% Body surface	Paraquat content in the solution spilled (%)	Paraquat concentration (µg/mL)		Time lapse between exposure and sampling (days)	Skin response
					Plasma	Urine		
24	M	Face	4	-	ND	ND	3	Burns II
30	M	Bottom	5	0.75	ND	ND	3	Vesicles, burns
28	M	Legs	1.5	2	0.05	0.1	1.5	Contact dermatitis
17	M	Hand, arm	9	-	-	ND	1	Vesicles
30	M	Neck	1	2	0.05	0.15	1	Vesicles
25	M	Inner thighs	5	1	ND	0.07	1	Burns I
-	M	Face	0.7	-	ND	ND	1	None
20	M	Pelvic area	0.2	2.6	Not done	0.05	2	Vesicles
30	M	Hands	2.3	-	ND	ND	1	Burns II
16	F	Face, hands	6.3	-	ND	ND	1	None
23	F	Eye, face	4	2.5	0.25	0.025	1.5	Irritation, conjunctivitis
17	M	Face	4	-	ND	ND	2	Chemosis
22	M	Eye	-	-	ND	Not done	1	
35	M	Axilla, deltoid region	<1	-	ND	ND	1	Burns II
4	M	Knee	<1	-	ND	ND	2	Burns II-II

ND = Not detected (< 0.05 µg/mL, which is the lowest detection limit of the assay method used). Burns I = First degree burns, Burn II = Second degree burns.

Conclusions: From the data, it appeared that a single exposure of healthy skin or eyes to paraquat solutions of low concentrations caused no systemic effects, but caused local lesions.

Woolen BH (1989) Analysis of serum and urine samples from an exposure study involving tea plantation workers using paraquat. CTL Study no: XH1063, Lab: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: ICI Agrochemicals. Study duration: unspecified. Report no: CTL/L/2117 (Revised). Report date: October 9, 1989.

Quality assured GLP study.

Study & observations: This study reported the clinical investigations (serum and urine analyses) obtained in an occupational exposure study in paraquat spraymen conducted in Sri Lanka in 1987. Urine samples were collected from 12 tea plantation workers over a period of 12 days (from day 0 through 12, total of 156 samples) in addition to some serum samples (n=48). Serum and urinary paraquat, creatinine and 24-h urine volumes and were determined. Paraquat was measured by RIA. No further details on the workers who participated in the study, their exposure conditions, sample collection procedures or experimental methods and the data analysis were provided.

Findings & conclusions: The report provided only data on urinary volumes and creatinine levels. It was reported that paraquat was not detected in any of the urine or serum samples tested. According to the two data tables provided, urinary volumes and creatinine concentrations were generally consistent during the 12-day sampling period.

Gurutharan, Basnayake V, Hart TB & Tomenson JA (1990) The health of Sri Lankan tea plantation workers associated with long term exposure to paraquat: An epidemiological study. Study no: not stated. Lab: General Hospital (Teaching) of Peradeniya & The Faculty of Medicine, University of Peradeniya, Kandy, Sri Lanka. Sponsor & study duration: not stated. Report no: ICI Agrochemicals Report TMF 3589B. Report date: April 12, 1990.

Study: This cross-sectional epidemiological study was conducted to determine the health of a group of Sri Lankan tea plantation workers exposed to paraquat for 5 years or longer. Three groups of male plantation workers (chosen from 6 tea estates) matched for age and length of service were included in the study. Two of these groups were controls. The first control group (C1) consisted of factory workers, who processed freshly picked tea leaves (n=76, age range: 17-53 years, bw: 34-63 kg). The second control group (C2) was made up of general plantation workers, whose main task was the maintenance of roads and walls (n=79, age range: 21-52 years, bw range: 38-62 kg). All workers had a minimum of 5 years work experience. The study group consisted of spraymen and spray mixer-loaders (collectively termed 'spraymen'), all of whom had been exposed to paraquat on a regular basis for a minimum of 5, and an average of 12 years (n=85, age range: 22-55 years, bw range: 35-59 kg, work experience 5-20 years), with minimal or no direct contact with other pesticides used on the tea plantation. All workers were without any pre-existing, non-work related medical conditions. They were employed by the same employer in the same region. Apart from an absence of asthmatic sufferers in the study group in comparison to controls, all had similar medical history profiles.

Workers in the study group sprayed paraquat (Gramoxone, purity: 200 g paraquat cation/L) using knapsack sprayers fitted with a single lance and single or multiple nozzles. Prior to application, the concentrated formulation was diluted at least 40 times and then between 100 and 200 times to form the spray solution. Each worker sprayed approximately eight, 15 L tanks of spraymix/d. Estimated average daily exposure rate was approximately 48 g of paraquat/person. The sprayers applied the diluted formulation using their own backpacks, which they maintained and cleaned. The mixer-loaders prepared the spray from the concentrate in open drums. Personal protective equipment was not used by any of the workers, whose standard work dress was a short sleeve shirt and shorts or a sarong with bare feet. Most workers washed themselves in running water regularly throughout the day.

Observations: In addition to lung function tests and chest X-rays, all workers were subjected to the following clinical and laboratory tests: pulse rate and blood pressure, examination of the respiratory and nervous systems and the skin condition, Hb, Hct, renal function (urea and creatinine), liver function (ALP, AST and ALT, bilirubin, total protein and albumin. Health examiners, who conducted the general tests, were blind to the occupational history of the workers under examination. Exposure to paraquat by the study group was estimated from the average number of days they spent spraying paraquat (19-95 days) per year. In the data analysis, the group means of the study parameters were compared using the analysis of covariance method.

Findings: Clinical examination of spraymen revealed a relatively higher incidence of skin damage reported as cracks on the heels or sole of the feet (23.6% versus 11.8% and 15.2% for the study, C1 and C2 groups, respectively), nose bleeds (3.6% versus 1.3% and 0% for the study, C1 and C2, groups, respectively) and headache (21.2% versus 15.8% and 17.7% for the study, C1 and C2, groups, respectively), but a lower incidence of coughing (7.1% versus 13.2% and 8.9% for the study, C1 and C2, groups, respectively) than in the controls. Nail damage was not reported in C1 workers, but its incidence in C2 workers and in the study group was approximately 11%. Lung function tests, which were standardised for age, weight and height, found no clinically or statistically significant differences between the groups. The mean CO_2 transfer factor, which can be affected by paraquat toxicity in humans, was not different in spraymen compared to controls. Comparison of pulmonary function with the period of exposure to paraquat showed only one correlation, with FEV_1 (forced expiratory volume during the first second)/FVC (forced expiratory vital capacity), being positively associated with exposure. As this would imply an improved lung function through greater exposure, the study authors considered this finding to be an experimental artefact or suggested that it may have occurred due to some confounding factors. Chest X-rays did not reveal any paraquat-related lung abnormalities.

Hb and Hct were significantly lower in C1 workers (14.3% g/dL and 44.8%, respectively) than in C2 workers (14.8 g/dL and 46.4%, respectively) and lower than, but not statistically significant from exposed workers (14.5 g/dL and 45.4%, respectively). These differences were not regarded as clinically significant and no correlation between the periods of exposure with either value was seen. Clinical chemistry, liver and renal function tests did not show any clinically significant group differences. Although the values for the study group were consistently within the normal range, serum AST level was positively and significantly correlated with the total number of spraying days and with the number of spraying days over the previous 5 years. No inter-group differences were found in the other study parameters.

Conclusions: Long term exposure of unprotected workers to low concentrations of aqueous solutions of paraquat did not produce any detectable adverse clinical outcomes.

Jee SH, Kuo HW, Daniel WP, Chang CH, Sun CC & Wang JD (1995) Photodamage and skin cancer among paraquat workers. *Int J Dermatol 34 (7): 466-469.*

Study & observations: This study investigated skin conditions in a group of paraquat-manufacturing workers. The skin condition was described as ‘skin-malignancy’ or ‘premalignancy’, characterised by pigmentation and keratosis in the sun-exposed skin.

Histopathology was performed on 11 workers (age: 31-54 years, sex: unspecified, exposure period 6-14 months) selected from 4 factories, considered to be representative of affected individuals. Twenty-three biopsy specimens collected from these individuals were evaluated to identify the pathological features of the skin condition. In an epidemiological study, a total of 242 exposed workers from 28 paraquat-manufacturing factories were examined and interviewed during the period from 1983 to 1991. Approximately 2/3 of these factories produced paraquat from bipyridines, following centrifugation and crystallisation of chemicals in an open area. Following a clinical examination, 156 workers (age & sex unspecified, none had a history of previous irradiation, exposure to arsenic or of skin cancer), who were found to have skin lesions were selected for further study. The skin lesions were photographed and graded (grade 1 = multiple tiny freckles or scattered lentigines within a rectangular area, grade 2 = confluent solar lentigines and a few actinic keratosis lesions, grade 3 = heavy hyperpigmentation and multiple actinic keratoses or possibly malignancy). The severity grades of the skin lesions were used as an end point for epidemiological analysis.

Findings: According to histopathological findings, all workers except one had actinic keratosis. Of the lesions seen, 3 were solar lentigo, 11 were actinic keratosis, 3 were squamous cell carcinoma *in situ,* coexisting with actinic keratosis, and 3 were squamous cell carcinoma. Eighty-five percent of the workers (133/156) had skin lesions of varying severity (grade 1 to 3). Thirteen workers had severe (grade 3) skin lesions, and the odds ratio for developing grade 0, 1, 2, and 3 lesions were 1, 3.9, 3.2 and 85.7, respectively. There was a trend of association between heavy exposure and the severity of skin lesion.

Conclusions: The findings of the histopathological study showed that all the skin lesions occurred due to either photodamage or skin cancer. The strong trend in the correlation between the severity of photodamage and exposure to bipyridines led to speculation of a synergistic role of bipyridine exposure and sun exposure in causing these malignant and/or premalignant skin lesions. The validity of the study findings, however, was reduced, due to the lack of details on the chemical(s), exposure data, PPE used, the work environment and paraquat manufacturing conditions.

Castro-Gutierrez, McConnell R, Anderson K, Pacheco-Anton F & Hogstedt C (1997) Respiratory symptoms, spirometry and chronic occupational paraquat exposure. *Scand J Environ Health 23: 421-427.*

Study & observations: This cross-sectional study involving groups of workers in 15 banana plantations was conducted to evaluate the relationship between respiratory health and paraquat exposure. Paraquat was used heavily to control weeds in these plantations. During preparation of the spraymix, a 20% concentrate was transferred from approximately a 250-L drum into unlabelled containers (size unspecified). These containers were then carried by teams of 2-6 workers into the fields, where the solution was diluted with water to obtain a 0.1-0.2% spray solution, which was then applied from knapsack sprayers. Many of the workers carried, mixed, and loaded their own concentrated solutions in the fields, resulting in greater exposures than if they worked only as applicators. The workers wore rubber boots, long pants, short sleeved shirts, but no gloves or respiratory protection. They were assigned to full time spray teams for periods ranging from a few days to 6 months or more at a time during the heaviest spraying season of the year (June through December). The selection of workers for the study was based on the information they provided in response to a questionnaire. On the basis of their responses,

the workers were divided into 3 groups: (a) unexposed workers, who reported no history of work with paraquat (n=152), (b) exposed workers, who reported cumulative exposure as knapsack sprayers for 24 or more months, but had no history of rash or skin burn attributed to paraquat exposure (n=63), and (c) more intensely exposed workers, with at least 24 months of cumulative work as knapsack sprayers, who also reported a prior history of eye splashes and health problems (rash or skin burn, nail damage or loss of nails, bloody nose) attributed to paraquat (n=71).

Each worker was also interviewed using a standardised questionnaire (modified from the respiratory questionnaire of the British Medical Research Council), including questions approximately those illnesses that were unlikely to be related to paraquat exposure. All workers were examined for respiratory function [(Forced Vital Capacity (FVC), FVC during the first second (FCV_1)] using the Collins water sealed spirometer. Workers with a FVC of <80% of the predicted value were classified as having a restrictive defect; workers with a FCV_1: FCV ratio of <0.70 were classified as having an obstructive effect.

Given that concentrated paraquat or solutions of paraquat in contact with occluded skin may be more likely to cause skin damage and hence enhance percutaneous absorption, rash or skin burn was used as a surrogate for the intensity of exposure and to estimate the dose-effect relationship. Univariate and multivariate statistics and regression analyses were used to analyse the data. The estimation of relative risks was based on the Mantel-Haenszel procedure.

Findings: Of the 134 exposed workers, 53% reported problems such as skin rashes or burns, related to paraquat exposure. Epistaxis (nosebleed) was reported by 25%, nail damage by 58%, and eye splashes by 42%. Several workers provided anecdotal evidence for continued blurred vision, which they attributed to eye splash. There was a higher proportion with a history of skin rash or burn also reporting history of nail damage, compared with the exposed workers with no history of skin rash or burn (relative risk = 1.4). The incidence of nosebleeds and eye splashes were higher among workers, who were more intensely exposed to paraquat (relative risk = 2.2). Among the exposed workers, there was a larger proportion of workers with exertional dyspnoea of all grades (especially grade 3 dyspnoea: dyspnoea while walking at one's own pace on level ground) and episodic wheezing accompanied by shortness of breath. A consistent dose-response relationship was seen between the intensity of exposure (as indicated by the history of skin rash or burn) and exertional dyspnoea after adjustment for smoking, age and sex. No relationship was found between exposure and FEV_1 or FVC.

Conclusions: The high incidence of respiratory symptoms associated with paraquat exposure seen in these workers, in the absence of any spirometric abnormalities, could be attributed to unmeasured, pulmonary gas exchange abnormalities following long-term paraquat exposure. However, the validity of the findings was reduced due to lack of numeric data on exposure conditions. Further, the skin lesions such as rash or burns were not correlated with any estimates of paraquat exposure levels in the exposed individuals.

Schenker MB, Stoecklin M, Lee K, Lupercio R, Zeballos RJ, Enright P, Hennessy T & Beckett LA (2004) Pulmonary function and exercise-associated changes with chronic low-level paraquat exposure. ***Am J Respir Crit Care Med 170(7):773-779.***

Aim: This study was undertaken to test the hypothesis that chronic, low-level paraquat exposure causes restrictive lung function with gas transfer impairment.

Background: Respiratory failure from adult respiratory distress syndrome (ARDS) is a prominent outcome in fatal paraquat ingestions (Smith & Heath 1975), demonstrating the ability of paraquat in high doses to cause oxidative damage to the lung, pulmonary fibrosis, and respiratory failure. The radiologic appearance in paraquat poisoning begins with air-space consolidation, which then leads to end stage lung disease with fibrosis (Im *et al* 1991).

Study and observations: The study was conducted on banana, coffee, or palm oil farms throughout Costa Rica that were using similar paraquat application practices. Three hundred and thirty eight workers participated (203, 97 and 38 worked with banana, coffee and palm oil plantations respectively). Worker inclusion was dependent on their farm being a current user of paraquat and the farm contained sufficient numbers of workers to make data collection worthwhile.

Data collection included an interviewer-administered questionnaire, pulmonary function testing, and cardiopulmonary exercise testing. All data were collected at the work site with the cooperation of owners and managers. Demographic and lifestyle factors, work history, occupational exposures, and the presence of respiratory symptoms were assessed. Questions were taken from existing standardized questionnaires where possible (Ferris 1978).

Mean age of workers was 37 years (SD = 10), and 71% were normal weight (body mass index = 18.5-24.9. A quarter of the subjects reported current smoking. Workers classified as handlers reported 6 months or more work experience mixing, loading, or applying paraquat; non-handlers reported no experience with handling paraquat. Sixty six per cent of workers were classified as handlers and 33% as non-handlers. Handlers reported a mean of 8.5 years (25th-75th percentile = 2-13 years) experience with paraquat. The proportion of current smokers was similar among handlers and non-handlers.

Pulmonary function outcome measures were selected *a priori* to be evaluated for association with interstitial or restrictive lung disease. Outcome measures assessed included D_{LCO} (single breath carbon monoxide diffusing capacity), TLC_{SB} (alveolar volume measured with single breath), V_{O2} (peak oxygen uptake) assessed during a maximal exercise test. FVC, the ventilatory equivalent for CO_2 (V_E/V_{CO2}), O_2 pulse peak (V_{O2}/HR), and arterial oxygen desaturation from resting to peak exercise were also examined. Other pulmonary function measurements, FEV_1, FEV_1/FVC, mean mid-expiratory flow (FEF_{25-75}) were evaluated for their distribution and physiologic consistency.

The cumulative exposure index was calculated based upon work history reported by each worker, including the handling of paraquat in each job, the length of employment, the type of crop, and the use of protective equipment. In analyses, the cumulative paraquat exposure index was log transformed to improve the fit of the regression models. Values below the limit of detection for paraquat on this index were coded to one before log transformation, resulting in zero values on the log scale. Mean paraquat exposure on this log-transformed index was 1.25, SD = 1.12, and 25th-75th percentile was 0.0-2.25. The log scale exposure index was treated as a continuous variable in multivariate analysis.

The cumulative paraquat exposure index was added to logistic regression models after the inclusion of age and smoking status

Findings: Each unit increase in the total cumulative paraquat index was associated with a 1.8 increase in the odds of chronic cough (95% CI = 1.0-3.1) and a 2.3 increased odds of shortness of breath with wheeze (95% CI = 1.2-5.1). Increases in the cumulative paraquat index were not

significantly associated with chronic bronchitis, persistent wheeze, or ever having a diagnosis of asthma.

Models for chronic bronchitis, chronic cough, persistent wheeze, and shortness of breath with wheeze were also examined with asthma added as a covariate, with no change in the association between the paraquat exposure measure and respiratory symptoms.

Mean percent predicted was in the normal range for all of the spirometry and diffusion capacity outcome measures. A comparison of spirometry and diffusion capacity outcomes between handlers and non-handlers revealed no statistically significant differences in mean percent predicted for any of the pulmonary function measures.

Similarly, there was no significant difference in mean values for the cardiopulmonary exercise testing outcome measures in workers less than 40 years of age. (This study excluded participants of 40 years of age or more from exercise testing on the basis of American College of Sports Medicine Guidelines. Because those over 40 were more likely to include workers most exposed to paraquat, their exclusion was a study deficiency - acknowledged by the study authors).

TLC_{SB}, D_{LCO}, and V_{O2} all showed very small negative coefficients for cumulative paraquat exposure that were not statistically significant. In addition, cumulative paraquat exposure was not an independent predictor of other pulmonary function parameters, including FVC, FEV_1/FVC, and FEF_{25-75}. Among the other exercise outcome measures, the parameter estimate for V_{O2}/HR was small and non-significant. The total cumulative paraquat exposure index showed a statistically significant increase in V_E/V_{CO2} for each unit increase in exposure, although paraquat exposure accounted for a small portion of the overall variance.

These associations were also examined in models with paraquat exposure measured as a dichotomous variable (handler versus non-handler) and quartiles of the cumulative exposure index. Regression models with these exposure variables produced similar results as with the cumulative exposure index.

Logistic regression models, adjusted for age, weight, and smoking status, were used to assess the association of paraquat exposure with 5% or greater decrease in oxygen desaturation during the cardiopulmonary exercise test (ΔSp_{O2}). Each unit increase in the total cumulative paraquat exposure index was associated with a 1.7 increase in the odds of a 5% or greater decrease in oxygen desaturation (95% CI = 0.9-3.0).
Conclusions: The findings of small changes in V_E/V_{CO2} at maximal exercise and ΔSp_{O2} from rest to peak exercise suggested that subclinical function changes may be present, but long term, low level paraquat exposure in the population studied was not associated with clinically significant interstitial lung disease or impairment of gas exchange. Changes of chronic airflow obstruction with paraquat exposure were not observed, but as the study authors indicated the self-reported respiratory symptoms associated with cumulative paraquat exposure (chronic cough, shortness of breath with wheeze) warranted further study.

Dallie MA, London L & Myers JE (2006) Respiratory health effects due to long-term low-level paraquat exposure. ***Am J Respir Crit Care Med. 172(5):646-647 Letter to the editor commenting on the Schenker et al (2004) study above.***

These authors supported the observations of Schenker *et al* 2004 (above) that an association between long term paraquat exposure and inefficient ventilation and oxygen desaturation during exercise suggested that paraquat may cause subclinical gas exchange abnormalities.

They commented that the relationship between paraquat exposure and exercise induced oxygen desaturation amongst Costa Rican banana, coffee, and palm oil farm workers was consistent with what they found amongst South African deciduous fruit farm workers. This was despite the fact that in the Costa Rican study the handlers had a substantially shorter work history (median 8.5 compared with 16 years in the South African study and also used protective equipment while in the South African study population they hardly do.

Both studies did not identify clinical effects and it was possible that the lack of demonstration of clinical effects due to long term exposure to paraquat could be because the time period required for sub-clinical effects to achieve clinical relevance exceeds the study window of studies conducted so far.

The authors of the South African report calculated that a clinically relevant oxygen desaturation of 5% will result from a lifetime of paraquat exposure.

These two independent studies (in South Africa and Costa Rica), conducted in different study populations and in different crop sectors, both found similar results and supported the idea that chronic long term paraquat exposure results in sub-clinical respiratory impairment.

4.11.2 Poisoning Incidents

4.11.2.1 Introduction

A large number of poisoning incidents due to accidental exposure or deliberate ingestion of acutely toxic doses of paraquat solutions have been reported from many parts of the world. The earlier cases were mostly accidental and generally resulted from the habit of decanting the liquid formulations into unmarked or incorrectly labelled beer or soft drink containers. However, an increasing number of suicidal poisonings via ingestion has been noted in recent years (FAO/WHO 1986). Approximately 75% of pesticide-related fatalities that occur in the UK during 1990-1991 were due to paraquat (Thompson *et al,* 1995). A review of 1000 poisoning cases in Japan indicated that the most frequent cause of acute poisoning in that country was paraquat/diquat products (Yamashita *et al,* 1996). A study conducted in Sri Lanka involving 669 cases of suicidal pesticide poisoning identified paraquat as the commonest poisoning agent (Hettiarachchi & Kodithuwakku, 1989). According to these reports, the fatality rate following deliberate ingestion was approximately 75%. Paraquat has been one of the common causes of fatal pesticide poisoning in many other countries (De Alwis & Salgado, 1988; Hutchinson & Simmons, 1991; Nemeth *et al,* 1991; Perriens *et al,* 1989; Taylor *et al,* 1985; Tinoco *et al,* 1993; Tsatsakis *et al,* 1996). A few unusual cases in which concentrated liquid formulations have been improperly used (to treat body lice) have also been reported (Wohlfahrt, 1982).

Although difficult to determine, the lethal dose of paraquat in adults has been estimated to be approximately 3-5 g (50-80 mg/kg bw) which translates into approximately 10-15 mL of a 20% formulation (Pond, 1990). The following approximate dose-effect scale has been proposed by Reigart and Roberts (1999) and may be of use to base prognosis in cases of paraquat ingestion.

- Amount ingested: <20 mg paraquat cation/kg bw (<7.5 mL of 20% w/v formulation): No symptoms or only GI symptoms occur. Recovery is likely.
- Amount ingested: 20-40 mg paraquat cation/kg bw (7.5-15 mL of 20% w/v formulation): Pulmonary fibroplasia ensues. Death occurs in most cases, but may be delayed 2-3 weeks.

- Amount ingested: >40 mg paraquat cation/kg bw (>15 mL of 20% w/v formulation): Multiple organ damage occurs, but is more rapidly progressive. Often characterised by marked ulceration of the oropharynx. Mortality is essentially 100% in 1-7 days.

A selection of published reports of clinical and toxicokinetic relevance were reviewed and presented in the section below.

4.11.2.2 By Ingestion

Hong SY, Yang DH & Hwang KY (2000) Associations between laboratory parameters and outcome of paraquat poisoning. *Toxicol Letters 118: 53-59.*

Study & observations: This study reports the clinical biochemistry parameters of 147 patients admitted to the Institute of Pesticide Poisoning of Chunan Hospital in Korea in 1999, with a history of intentional (n=110) or accidental (n=37) ingestion of paraquat (purity: unspecified). The value of these parameters in predicting the patient's outcome was investigated (see Table 115 below. On admission, the vital signs (blood pressure, pulse and respiration rates) were checked, demographic details, medical history and paraquat poisoning were collected using a standardised questionnaire, and blood and urine samples collected (except urine samples from 8 patients). The level of exposure was assessed from the details on the patient's clinical history and by a urine paraquat test. Haematology and clinical chemistry parameters (RBC and WBC counts, Hb and Hct, liver and renal function tests, arterial blood gas and urine analysis) were checked prior to intensive medical therapy, which generally included either gastric lavage, administration of Fuller's earth or haemoperfusion.

Urinary paraquat levels were measured using a semi-quantitative method. The primary outcome of poisoning was defined as survivor or fatality at the time of discharge from the hospital. Statistical comparisons of the study parameters between survivors and fatalities were performed using either a Student's t-test or χ^2 test. Each parameter was categorised as normal or abnormal, based on the reference values and investigated as explanatory variables of the outcome. The strength of associations between the laboratory data and the outcome of paraquat intoxication was expressed as the odds ratio (OR).

Findings: The mean volume of paraquat solution ingested by patients was 54.5 mL (SD=104.9). The overall fatality rate was 44.2%. There were no significant differences in age, sex, alcohol intake, blood pressure, partial pressure of oxygen in arterial blood (PaO_2) and transfer time (the time between ingestion and medical facility) between the survivor and fatality groups. In the fatality group, the time interval from ingestion to first medical treatment was significantly shorter (1.7 h, $p<0.001$) compared to the survivor group (15.6 h). The patients in the fatality group ingested significantly larger quantities (102 mL, $p<0.001$) compared to the survivors (7 mL), and had more positive urine paraquat test results. Significantly increased levels of Hb and Hct, AST, ALT, bilirubin, creatinine and BUN, and elevated WBC counts were seen in the fatality group compared to those in the survivors ($p<0.001$). The elevated levels of AST, ALT, bilirubin and creatinine levels seen in the fatality group patients were indicative of probable liver and renal damage following paraquat intoxication. Arterial blood gas analysis showed metabolic acidosis and excretion of elevated levels ($p<0.001$) of WBC and protein in the urine in the fatality group patients compared to that of the survivors.

Table 115: Physiological and clinical chemistry data of intoxicated patients (mean ± SD)

Parameter	Group		P-value
	Survivors	Fatality	
Vital signs			
Systolic blood pressure (mmHg)	130.0 ± 22.6	130.3 ± 28.3	0.942
Diastolic blood pressure (mmHg)	82.9 ± 12.6	82.8 ± 17.7	0.950
Pulse/min	80.1 ± 12.6	85.8 ± 16.8	0.021
Respiration rate (per minute)	21.3 ± 7.8	23.5 ± 5.9	0.057
Haematological data			
WBC (count/mm^3)	10315 ± 4870	16840 ± 8287	<0.001
Hb (g/dL)	13.7 ± 1.8	14.5 ± 1.7	<0.001
Hct (%)	41.4 ± 5.5	43.3 ± 5.4	<0.001
Liver function			
AST (IU)	35.8 ± 31.8	87.2 ±151.9	0.003
ALT (IU)	29.1 ± 25.5	72.0 ± 100.1	<0.001
Bilirubin (mg/dL)	1.09 ± 0.54	2.44 ± 3.4	<0.001
Renal function			
BUN (mg/dL)	15.5 ± 12.4	23.9 ± 20.6	0.003
Creatinine (mg/dL)	1.05 ± 1.2	2.22 ± 2.04	<0.001
Arterial blood gas analysis			
pH	7.45 ± 0.05	7.38 ± 0.08	<0.001
$PaCO_2$ (mmHg)	36.9 ± 6.07	29.0 ± 7.56	<0.001
PaO_2 (mmHg)	91.9 ± 12.5	96.6 ± 30.9	0.213
*Urine analysis**			
WBC (%)	16 (19.8)	30 (51.7)	<0.001
RBC (%)	7 (8.6)	14 (24.1)	0.012
Protein (>trace) (%)	11 (13.6)	23 (39.7)	<0.001

*****For RBC and WBC, the values represent the number positive (3> high power field).

Significant correlations were found between AST (r=0.35, p<0.001), bilirubin (r=0.17, p<0.05), BUN (r=0.38, p<0.001), creatinine (r=0.29, p<0.001) and PaO_2 (r=0.30, p<0.001) and the time intervals from ingestion to hospital admission. Leukocytosis and elevated AST, ALT and renal dysfunction showed significantly higher ORs for paraquat fatality. Statistically significant ORs were found with patients that had more than two abnormal findings. At the least, an abnormal finding in renal function was a significant predictor of the patient's outcome. Acidosis status significantly predicted the fatality, with the highest OR in cases of 2 acidic findings (OR=35.25, p<0.001). When further analysis was conducted by adding urine paraquat levels or amounts of paraquat ingested to the study model, no change to the above associations were noted (no numeric data provided).

Conclusions: According to the findings of this study, the initial routine clinical biochemistry data could be used to predict the outcome of paraquat intoxication. It was suggested that the evaluation of acid-base status, renal and liver function should be conducted prior to administration of intensive medical therapy. However, the validity of the study findings is limited as only cross sectional laboratory data were used, with no evaluations being conducted on time related changes of organ damage during the process of paraquat poisoning.

Tsatsakis AM, Perakis K & Koumantakis E (1996) Experience with acute paraquat poisoning in Crete. *Vet Human Toxicol. 38 (2): 113-117.*

Study & observations: Ten cases of acute paraquat poisoning with lethal and non-lethal outcomes were reviewed. There were 7 males and 3 females in the study (age range: 16-58 years, the 16-year old female was approximately 6 weeks pregnant). Nine of these persons had ingested approximately 5-100 mL of paraquat solution (strength unspecified) deliberately with suicidal intent. One patient had a history of dermal exposure to paraquat with subsequent painful skin irritation in the lumbar and intergluteal regions after spraying the chemical. They were admitted to the study-performing hospital approximately 2-41 h after ingestion or exposure. Besides the standard supportive therapy for paraquat poisoning adopted by the hospital (bentonite, Laxatol and iv fluids containing manitol), generally, the treatment procedure used also included haemoperfusion/haemodialysis and/or plasmapheresis. Blood, urinary and post mortem tissue (kidney, liver, thyroid, testis, vitreous humor, conceptus and amniotic fluid) paraquat levels were determined using either a spectrophotometric or liquid chromatographic method.

Findings: Two persons (male and female) were found dead following ingestion. The common clinical signs seen were vomiting, abdominal pain, nausea, diarrhoea and oropharyngeal erythema, and dysphagia was observed in one patient. The paraquat content in various tissues of 2 patients is given in the Table 116 below. It is apparent that paraquat was mainly distributed to the kidney, lung and liver, while noticeable amounts were also present in the thyroid, testis, vitreous humor and cerebrospinal fluid. In both cases, kidney paraquat levels were approximately twice the concentrations in the lung, while the blood/liver concentration ratios were somewhat identical, being 206/165 and 88/73 for cases 4 and 5, respectively.

Table 116: Paraquat content in autopsy specimens of 2 patients (µg/g of tissue of µg/mL)*

Tissue	Case 4 (M)	Case 5 (F)
Kidney	807	185
Lung	479	95
Liver	206	88
Thyroid	64	32
Midbrain	11	ND
Spleen	180	45
Epinephron	210	ND
Blood	165	73
Vitreous humor	45	18
Cerebrospinal fluid	7.4	ND
Urine	530	250
Spinal medulla	9	ND
Testis	21	-
Brain	ND	16

*In both cases (M = male, F = female) the amount of paraquat solution ingested was >150 mL. Both these patients died after a few hours of ingestion, and approximately 30 and 20 g of paraquat were extracted from the gastric contents of case 4 & 5, respectively.

The pregnant female survived after treatment. Her aborted foetus contained 0.25 µg of paraquat/g of tissue, indicating that paraquat had crossed the placenta. It was reported that paraquat was concentrated in the foetus to a level 4-6 times that of the mother, with subsequent foetal death. However, for this case, the supporting maternal blood paraquat data were not

provided. The paraquat concentration in the amniotic fluid was 0.05 µg/mL. Despite 3-4 days long therapy, 3 patients died.

Conclusions: Ten cases of acute paraquat poisoning with lethal and non-lethal outcomes were reviewed. Despite 3-4 day long therapy, 3 patients died. The cause of death was determined to be circulation/respiratory collapse and/or multi-organ failure due to paraquat intoxication.

Ragoucy-Sengler C & Pileire B (1996) A biological index to predict patient outcome in paraquat poisoning. *Human & Exp Toxicol 15: 265-268.*

Study & observations: Eighteen patients (8 males and 10 females, mean age: 34.5 years) were admitted to hospital following accidental paraquat intoxication (n=2) or attempted suicide (n=16). The utility in predicting patient outcome based on the rate of plasma creatinine increase over a 5 h period following paraquat intoxication (solutions containing 40 g paraquat/L) was investigated. The findings of the present study were compared with previously published severity indices for paraquat poisoning. On admission, a general questionnaire was completed to establish demographic characteristics, previous medical history and life style factors. This was followed by specific questions on the intoxication (eg nature of the paraquat formulation, volume of paraquat solution ingested). Blood samples were collected on admission and then at 5-h intervals at least during the first day. Serum creatinine, potassium and bicarbonate were determined using an autoanalyser. Blood paraquat was determined by the method of Maruyama and Ide (1988). Results were analysed statistically using the K-W non-parametric test.

Findings: Twelve patients in the study died. The mean survival time after ingestion of paraquat was 152 h (range: 18-480 h). The mean volume of paraquat solution that had been ingested by these patients was 38.9 mL (range: 5-100 mL). The rate of increase in blood creatinine levels in intoxicated patients was linear during the first 24 h ($r=0.76$, $p<10^{-5}$). For deceased patients, the linear regression was represented by the following equation: Creat = 8.3t + 86.9. The rate of increase in creatinine was equal to a constant (zero order kinetics) for each patient. The rate of increase in creatinine over 5 h was not linked to the time elapsed since ingestion.

The authors stated that the proposed creatinine increase index correlated well with the patient's progress (no numeric data provided). In survivors, the mean rate of increase was 0.45 µmol/L/h, while that of the deceased was significantly different from this figure, being 14.8 µmol/L/h ($p<10^{-3}$). When survival time was selected, both the survivors and the deceased were well separated and no overlap was observed. The proposed index was also correlated with the volume of paraquat ingested by the patients ($r=0.69$, $p<0.05$), as were the linear relationships between the rate of increase in creatinine and patients survival time (ST; expressed as 1/ST, $r=0.94$, $p<10^{-5}$), and the rate of creatinine increase versus plasma paraquat concentration ($r=0.64$, $p<0.05$).

Conclusions: The proposed index (rate of plasma creatinine increase) appeared to be an efficient means in evaluating the severity of paraquat poisoning. The study authors claimed that the proposed method was accurate and reliable..

Gil HW, Yang JO, Lee EY & Hong SY (2005) Paraquat-induced Fanconi syndrome. ***Nephrology (Carlton).10(5):430-2.***

Fanconi syndrome is characterized by a generalized transport defect in the proximal tubules, leading to renal losses of glucose, phosphate, calcium, uric acid, amino acids, bicarbonate and other organic compounds. Fanconi syndrome may be inherited, and there are also many acquired causes, such as heavy-metal-induced nephrotoxicity, renal metabolic disorder, autoimmune disease and monoclonal gammopathy. However, Fanconi syndrome due to paraquat intoxication has not been reported in the literature. This case report concerns a patient in whom Fanconi syndrome presented as severe hypophosphataemia associated with acute tubular necrosis after paraquat intoxication.

A 44-year-old Korean woman was admitted to hospital approximately 7 h after ingesting approximately 5 mL of paraquat (no further details of exposure were provided). She had no history of suicidal attempts. She was alert upon admission, and her serum data were: creatinine, 71 µmol/L; phosphate, 0.61 mmol/L; and calcium, 2.31 mmol./L. Blood-gas analysis showed a pH of 7.447, PCO_2 of 26.1 mmHg, and bicarbonate of 18 mmol/L. Urine analysis showed a pH of 5.0, no protein or glucose, and on microscopy there were 5-7 red blood cells per high power field, and 0-2 white blood cells per high power field. Standard medical emergency procedures were performed. Briefly, after gastric lavage with 100 g of Fuller's earth in 200 mL of 20% mannitol, antioxidant medication was administered (glutathione, 50 mg/kg iv bolus every 4 h for 7 days; N-acetyl cysteine, 70 mg/kg iv bolus every 4 h for 7 days; deferoxamine 3000 mg/day iv for 7 clays; thioctic acid; and vitamin E). On the fifth day after admission, hypophosphataemia and a greatly decreased serum bicarbonate (8 mmol/L) with a normal serum anion gap ($Na+-(Cl^- + HCO_3^+)$ = 17.7 mmol/L) and positive urine anion gap (105 mmol/L) were noted. On the seventh day, the patient became drowsy and hypophosphatemic. Urine analysis showed a pH of 6.0, protein+, glucose+++, 20-25 red blood cells per high-power field, and 2-4 white blood cells per high-power field. Brain magnetic resonance imaging (MRI) did not reveal any abnormality. Phosphaturia was very severe, and hyperaminoaciduria was present on the 12th day. Thyroid function tests were within normal limits. On the seventh day, intravenous phosphate repletion (15 mmol/day) was started but hypophosphataemia persisted; however, on the 13th day the patient became alert following serum phosphate elevation. Intravenous phosphate repletion was continued at 30 mmol/day until the 16th day, when it was decreased to 15 mmol/day. Abdominal sonography revealed normal kidney size with increased renal echogenicity. A renal biopsy was performed on day 23 to distinguish the acute lesion from any underlying renal pathology. The renal biopsy revealed acute tubular necrosis.

Renal tubular acidosis was suspected because of the normal serum anion gap, the positive urine anion gap and hyperchloremic metabolic acidosis, but a bicarbonate loading test was not performed because the mental state of the patient was not stable. Metabolic acidosis later resolved. Intravenous phosphate repletion was stopped on the 17th day, upon which the serum phosphate level did not decrease and the patient remained alert. There was neither hypotension nor hypoxia during the hospitalization. She was discharged on day 24, and after one week there was no glycosuria, acidosis, hypophosphataemia or aminoaciduria.

Conclusion: This case report concerned a 44-year-old woman who presented with severe hypophosphataemia and reversible acute tubular necrosis after paraquat intoxication. Paraquat

can cause Fanconi syndrome and very severe hypophosphataemia presenting as deep drowsiness.

Sittipunt C (2005). Paraquat poisoning. *Respir Care. 50(3):383-5.*

This report describes a case of fulminant paraquat poisoning. The patient had a high blood paraquat level 6 hours after ingesting a large amount of concentrated paraquat, and developed multiple organ failure within 24 hours.

Fulminant poisoning is described as occurring when the intake of paraquat ion is > 40 mg/kilogram of body weight. These patients suffer multiple-organ failure leading to death within hours to a few days after ingestion. They may be asymptomatic soon after ingestion but deteriorate quickly within a few hours.

The patient was a 34-year-old woman admitted to Chulalongkorn Hospital, Bangkok, Thailand, 4 hours after ingesting about 20 mL of 24% paraquat (Gramoxone), with suicidal intent. She experienced nausea and vomiting shortly after ingestion and was brought to the hospital by her relatives. In the emergency room she reported sore throat and epigastric pain, but did not experience shortness of breath or breathing difficulties. She reported no underlying medical problems and had been working at a farm, where she had access to herbicides. Physical examination in the emergency room revealed an alert, fully conscious woman in no acute distress, with blood pressure 100/50 inm Hg, heart rate 110 beats/min, respiratory rate 16 breaths/min, and temperature 65.5° C. Her blood oxygen saturation (measured via pulse oximetry while breathing room air) was 95%. Her oral mucosa was erythematous and edematous. Both lungs were clear to auscultation.

Initial complete blood count, electrolyte, and liver function tests were within normal ranges. A chest radiograph was clear, without definite infiltrates, and the cardiac contour was normal. Electrocardiogram revealed sinus tachycardia. Urine dithionite test was strongly positive, confirming the presence of paraquat. A blood sample was obtained for paraquat estimation and the patient was given 100 g of activated charcoal plus 100 ml, of 70% sorbitol via nasogastric tube. The administration of intravenous fluid was initiated, and the patient was admitted to the medical intensive care unit for close observation and further evaluation.

After admission the patient was given a repeated dose of activated charcoal and sorbital. Other supportive treatments included intravenous fluids and analgesics to control her epigastric pain. In the following 24 hours, the patient experienced increasing epigastric pain, severe dysphagia, and progressive shortness of breath. A subsequent chest radiograph revealed bilateral lower-lobe and perihilar infiltration. Blood chemistries on day 2 revealed elevated blood urea nitrogen, creatinine, and liver enzymes. Her arterial blood gas values on day 2 were pH 7.48, P_{aCO2} 32 mm Hg. and P_{aO2} 56 mm Hg. She required increasing supplemental oxygen to keep her oxygen saturation above 88%.

On day 3 the chest radiograph revealed bilateral infiltrates, pneumomediastinum, and pneumopericardium. She became increasingly hypoxemic and required intubation and mechanical ventilation. Her renal function deteriorated, with markedly increased blood urea nitrogen and creatinine levels, and her urine output decreased. Haemodialysis was started. Progressive multiple-organ failure ensued, and she died on day 4 after admission.

The patient's initial blood paraquat level at 6 hours after ingestion was 1.98 µg/mL.

In categorising the severity of poisoning observed in this patient the author suggested that systemic manifestations depend on the amount of paraquat ingested, and that patients can be classified into 3 categories:-

1. Mild poisoning: < 20 mg paraquat ion per kilogram of body weight. These patients may have gastrointestinal symptoms but usually fully recover.

2. Severe poisoning: 20-40 mg paraquat ion per kilogram of body weight. These patients usually develop severe caustic lesions in the gastrointestinal tract, acute renal failure, and progressive pulmonary fibrosis. Death occurs in 2-3 weeks, from severe respiratory failure.

3. Fulminant poisoning: > 40 mg paraquat ion per kilogram of body weight. These patients suffer multiple- organ failure leading to death within hours to a few days after ingestion.

The patient described above ingested about 20 mL of 24% paraquat, which was about 67 mg paraquat ion/kg in a 50 kg person. Lung injury in severe or fulminant paraquat poisoning is characterized by diffuse alveolar damage, with destruction of alveolar epithelium. pulmonary edema, and hemorrhage in the early phase. The author indicated that such destruction can be expected to lead to ongoing intense proliferation of fibroblasts and collagen deposition in the lung, progressive pulmonary fibrosis, and hypoxemic respiratory failure within a few weeks.

Agarwal R, Srinivas R, Aggarwal AN & Gupta D (2006) Experience with paraquat poisoning in a respiratory intensive care unit in North India. *Singapore Med J. 47(12):1033-7.*

The efficacy of treatment with immunosuppressants was examined in a retrospective analysis of 84 poisoning patients five (5.9%) of whom were identified as poisoned by paraquat. All five had hepatic failure with median peak bilirubin being 22.1 ± 15.1 mg/dL. Four of the five patients had renal failure (median peak creatinine 3.8 ± 1.5 mg/dL) requiring renal replacement therapy. Following immunosuppressive therapy, two of the five survived. Three died because of severe acute respiratory distress syndrome and multi-organ dysfunction syndrome.

Paraquat poisoning is an uncommon entity in India but is associated with a high mortality rate. There was some potential for immunosuppressive therapy.

Dinis-Oliveira RJ, Sarmento A, Reis P, Amaro A, Remião F, Bastos ML & Carvalho F (2006) Acute paraquat poisoning: report of a survival case following intake of a potential lethal dose. *Pediatr Emerg Care. 22(7):537-40.*

This study reported a successful clinical case regarding the intoxication of a young girl who ingested a potentially lethal dose of paraquat.

A 15 year old girl (47 kg weight) deliberately ingested approximately 50 ml of a paraquat formulation (20% wt/vol paraquat dischloride salt), corresponding to approximately 10 g of paraquat ingested. The girl vomited 20 minutes later and was taken to hospital about 2 hours 30 minutes after ingestion.

Upon admission the girl underwent a gastric lavage procedure with NaCl solution and subsequently administered 100 g of mineral adsorbant (Fuller earth) before being transferred to intensive care. The girl was conscious, coherent, with a slightly increased heart and respiration rate and no other symptoms. The ingestion was confirmed through a urine test.

After confirming the paraquat intoxication through urine and serum analysis, an aggressive treatment was commenced that included 3-days of haemoperfusion with cyclophosphamide, methylprednisolone, desferrioxamine, vitamin E, and N-acetylcysteine. This was followed by long-term steroid therapy, antibiotics and prophylactic treatment to prevent stomach ulcers.

The patient did not develop renal or hepatic failure, but some lung damage was observed using computerized axial tomography (CAT) at day 7. The patient was discharged after 22 days with normal lung function and CAT scan results. Her recovery was confirmed upon follow-up examination 6-months later.

4.11.2.3 By Dermal Absorption

Prolonged contact with paraquat solutions at concentrations as low as 5 g paraquat cation/L can cause systemic poisoning which may be fatal (Smith 1988). Previous skin lesions can be aggravated by paraquat itself. This was the case in this patient who remained in contact with paraquat through paraquat moistened trousers despite the initial paraquat burn. Most fatalities via the dermal route are reported in developing countries. All cases are also associated with pre-existing skin lesions or prolonged contact of healthy skin with concentrated solutions (Garnier 1995; Smith 1988).

Newhouse M, McEvoy D & Rosenthal D (1978) Percutaneous paraquat absorption. An association with cutaneous lesions and respiratory failure. *Arch. Dermatol. 114: 1516-1519.*

This published report described the effects of cutaneous paraquat exposure in a 39-year old woman on a fruit farm. She received scratches to her arms and legs during pruning and was subsequently exposed while diluting paraquat and spraying it on fruit trees in the absence of any protective clothing or without showering immediately after spraying. The progression of clinical signs over an 8-week period, culminating in death, are summarised in Table 117 below

Table 117: Progression of paraquat poisoning in a 39-year old women following dermal exposure.

Time after exposure	Clinical signs	Laboratory/medical examinations	Treatment/outcome
3-5 days	Raised lesions evident on legs & right arm at the scratch sites. These became tender & painful, increased in size, crusted & exuded a clear-bloody fluid.	-	4 weeks of various topical medications and oral antibiotics – no improvement
4 weeks	Repeated headaches, breathlessness, chest tightness, dry cough, 9kg weight loss. Ulceration of the leg & arms at scratch sites; haemorrhagic necrotic crust & erythema around the ulcers.	Ulcer biopsy: coagulative necrosis of the epidermis & dermis. Pulmonary assessment revealed complaints of lower retrosternal discomfort on deep breathing without specific chest findings. Chest x-ray: soft infiltrates & streaky opacities in the left upper lobe. Pulmonary function studies = abnormal gas transfer, restrictive ventilatory defect. Needle lung biopsy: early interstitial & intra-alveolar foamy macrophages & pneumocytes, inflammatory cells, foreign body giant cells. Urinalysis normal. No bacterial or fungal pathogens detected. Hb 12 g/L, WBC 4000/mm^2, differential WBC 2% eosinophils, ESR 56 mm/h, pre-diabetic curve.	Topical therapy with Burrow's solution compresses and petrolatum gauze – improvement of ulcers within 14 days. Patient felt 'reasonably well' & was sent home despite progression of pulmonary lesions.
6-8 weeks	Initial improvement – went back to work in the orchard. Re-admitted to hospital due to increasing dyspnoea, wheezing, brown sputum, chest ache, fever, epistaxis, nausea, vomiting, and weight loss.	Physical exam: jaundiced, pale with ulceration of the nasal septum; both lungs dull to percussion. Previously healed skin lesions re-occurred. Additional extensive ulceration on the legs, hands and arms. Progressive deterioration of vital capacity to 0.8 L. Chest x-ray: increased upper lobe opacity. Hb = 11.9 g/L, ESR = 105 mm/h, WBC = 12000/mm^2, 95% neutrophils. Blood gas studies: marked hypoxemia & large physiological shunt. Hepatic dysfunction: increased LDH, SGOT, SGPT, ALP, bilirubin 3 mg/L. Diabetic curve. Renal dysfunction: serum creatinine = 2 mg/dL, BUN = 26 mg/dL.	Vigorous treatment with hydrocortisone, azathioprine & oxygen therapy. No improvement. Death at 8 weeks after initial exposure.

The study authors reported that pathological examination revealed lung abnormalities consistent with paraquat toxicity including a diffuse, fibrosing interstitial pneumonitis with marked intra-alveolar haemorrhage, thickening of the alveolar wall and inflammatory cell infiltration. Renal abnormalities included tubular epithelial cell degeneration with intraluminal proteinatous casts. Additionally, extensive superficial skin ulceration and intracanalicular and intracellular hepatic cholestasis were observed.

Although the study authors could not completely discount other exposure routes (eg inhalation) they concluded that the extensive ulceration and skin damage at the scratch sites provided evidence that they were the sites of paraquat absorption. In the absence of urinary and plasma paraquat analysis, the dose of paraquat received was unknown, however, the study authors hypothesised that only relatively small amounts of paraquat were absorbed at any one time

through the scratched skin. Additionally the suggestion was made that the progression of pulmonary changes was potentiated by oxygen therapy. They also concluded that death was the result of repeated exposure of damaged skin to paraquat. This case suggested that lethal quantities of paraquat can be absorbed by skin wounds.

Athanaselis S, Qammaz S, Alevisopoulos G & Koutselinis A (1983) Percutaneous paraquat intoxication (Letter to the editor). ***J Cutaneous & Ocular Toxicol. 2 (1): 3-5.***

Case study: A 64-year-old spray operator applied a 0.5% solution of paraquat to his fields using a knapsack sprayer, which leaked fluid freely down his back for approximately 3.5 h. He did not wash himself until late the same evening. On the following day, he had a burning feeling on his back, where the skin appeared to be irritated. Two days later he consulted a local physician and the examination revealed the affected skin area to be necrotic. The person also had anorexia and physical weakness. Although the doctor recommended immediate hospitalisation, the patient returned home. He became progressively ill over the next 3 days and then was admitted to hospital. In spite of therapy (procedures unspecified), the patient died 12 h after admission due to renal and respiratory failure.

Results & Conclusions: Necropsy revealed diffuse fibrosing interstitial pneumonitis with marked haemorrhage, renal tubular epithelial cell degeneration, with intraluminal proteinaceous casts, intracanalicular and intracellular hepatic cholestasis and dry blood necrosis of the skin at several sites on the back. Paraquat concentrations in the blood, urine, liver and kidneys were 0.08, 85.8 µg/mL, and 3.6 and 4.5 µg/g of tissue, respectively. These data together with histopathological findings confirmed that death was due to paraquat intoxication.

Tungsanga K, Israsena S, Chusilp S & Sitprija V (1983) Paraquat poisoning: evidence of systemic toxicity after dermal exposure. ***Postgraduate Medical Journal. 59: 338-339.***

This published report described a non-fatal case of renal and pulmonary failure following paraquat exposure to the intact perineum. A 44-year old male Thai farmer was exposed to Gramoxone (20% paraquat dichloride, unspecified source) after mistakenly using it in a toilet container to clean his perineum. The clinical progression of paraquat poisoning and subsequent treatment are summarised in Table 118 below.

Table 118: Progression of paraquat poisoning in a 44-year old Thai farmer following exposure of the perineum to Gramoxone (20% paraquat dichloride).

Time after exposure	Clinical signs	Laboratory/medical examinations	Outcome/treatment
0 days	Burning sensation	-	Immediately washed the affected area with soap & water
2-3 days	Ulceration	-	Admitted to local health centre
21 days	Ulceration	Rise in BUN & creatinine	Patient transferred to hospital 26 days after exposure
26 days	Localised erythematous, oozing, macerated denuded area of the scrotal sac with purulent discharge. No other clinical signs.	Haematology & urinalysis normal. Clinical chemistry: urea = 75 mmol/L, creatinine = 1025 μmol/L, total bilirubin = 10.3 μmol/L, SGOT = 1.28 μmol/s/L, ALP = 1.92 μmol/s/L, Na = 133 mmol/L, K = 3.8 mmol/L, Cl = 95 mmol/L, HCO_3 = 18.1 mmol/L, pH 7.43. PaO_2 = 72 mmHg. No paraquat detected in serum & urine. X-ray: interstitial and alveolar infiltration of the lungs. Daily urine output = 0.5-2 L.	Peritoneal dialysis started on 3rd day of hospitalisation. Cloxacillin & gentamicin given on the 9th day for staphylococcal peritonitis with a 'satisfactory response'.
33 days	Unspecified	Liver biopsy: mild swelling of hepatocytes with mild focal necrosis	Unspecified
36 days	Acute respiratory distress	PaO_2 = 30 mmHg	Mechanical ventilation for 5 days
48 days	Unspecified	Kidney biopsy: mild tubular necrosis, diffuse interstitial fibrosis & inflammatory cell infiltration	Unspecified
53 days	Unspecified	Renal function gradually recovered	Discharged from hospital

This case indicated that a single, short exposure of intact scrotal skin to a concentrated paraquat solution can lead to life-threatening systemic toxicity (renal and respiratory failure, hepatic damage).

Papiris SA, Maniati MA, Kyriakidis V & Constantopoulos SH (1995) Pulmonary damage due to paraquat poisoning through skin absorption. *Respiration 62: 101-103.*

Case: A 57-year-old farmer was accidentally exposed to a paraquat spraymix (250 g Gramoxone/14 L of water), which leaked freely down his back from a defective sprayer. After 5-6 h, he felt a burning sensation in the scrotum, where vesicles and bullae appeared; these later ruptured leading to erythema. Initially, a physician advised him to wash the affected area with normal saline and 2% eosin twice daily. Five days later, he was admitted to a provincial hospital because of mild breathlessness on exertion, cough and low-grade fever.

Findings: Clinical examination of the thorax was negative, the lesions on the scrotum were healed, and a chest X-ray showed interstitial infiltrates through the upper lung fields. Liver and renal function, and urine chemistry were normal (blood or urine paraquat levels were not measured). Over the following 2 days, the patient's condition worsened with the development of increasing breathlessness and high fever. Chest auscultation revealed inspiratory rales in the middle respiratory fields, while mild central cyanosis became evident. A subsequent roentgenogram showed progression of infiltrates in both lungs. Lung damage due to paraquat poisoning was diagnosed, and then the patient was treated with 40 mg of methylprednisolone

daily (iv). However, the patient's condition deteriorated further, showing central cyanosis, a severe restrictive pattern in a respiratory function test, and progression of confluent fibrosis, which was more evident in the middle and upper lung fields. An additional urinary paraquat screening test was negative. Subsequently, the dose of methylprednisolone increased to 60 mg/d, together with administration of vitamins C and E. After one month, the patient's condition was improved and remained stable on follow-up examinations, conducted 4 and 12 months later.

Conclusions: Evidence to support the hypothesis that lung damage caused by sublethal concentrations of paraquat does not always lead to fatal outcomes was presented. Administration of anti-inflammatory drugs and anti-oxidants led to an improvement of the patient's condition and survival, despite residual pulmonary fibrosis.

Peiro AM, Zapater P, Alenda C, Ramírez A, Gutiérrez A, Pérez-Mateo M & Such J (2007) Hepatotoxicity related to paraquat and diquat absorption through intact skin. ***Dig Dis Sci. Nov;52(11):3282-4. Epub2007 Feb 15.***

This case report concerned a 69-year-old man, a farmer, who was admitted because of continuous right-sided and central abdominal pain. He complained of coluria for the previous 2 weeks and the physical examination disclosed subconjuntival jaundice, conjunctivitis in the left eye, and hepatomegaly.

The patient was interrogated about a hypothetical exposure to toxic compounds and mentioned the professional use of herbicides without adequate skin protection. The herbicide contained a mixture of paraquat and diquat, and a case of hepatotoxicity to these compounds was suspected. The patient was a smoker and stated occasional alcohol intake. There was no history of any prescribed or over-the-counter drug(s).

Analytical results showed the following: AST. 115 IU/L (normal value, <37); ALT, 255 IU/L (normal value, < 41): alkaline phosphatase (ALP), 731 IU/L (normal value, 35-129); γ-glutamyl transpeptidase (GGT), 510 IU/L (normal value. <61); total bilirubin (TB i), 9.7 mg/dL (normal value, <1.3): and prothrombin index, 60%.

No antibodies against hepatitis virus A. B. and C, or autoantibodies (antinuclear, anti-mitochondrial. anti-smooth muscle) were found. Both abdominal ultrasonography and endoscopic retrograde cholangiography (ERCP) were normal.

A liver biopsy evidenced the existence of intrahepatic cholestasis and necrotic epithelium of bile ductules, associated with infiltration of neutrophils and histiocytes in the intraductal and periductal tissues, and no signs of alcohol injury or fibrosis were observed. An empirical treatment with intravenous steroids, intramuscular vitamin B12, oral folate supplementation, ursodeoxicolic acid, and N-acetyl-cysteine was started.

Four weeks later, total bilirubin had increased to 17.5 mg/dL without relevant changes in the rest of the analytical parameters except in the existence of a nonhaemolytic, normochromic, and macrocytic anaemia (haemoglobin, 11.8 g/dL [normal value. 12-16 g/dL]; hematocrit, 31.8% [normal value, 36-46%I, mean corpuscular volume, 103.6 fL, [normal value, 80100

fL]). Lipid peroxide levels were determined and showed a higher activity (LDL-p = 38.5 ft HDL-p = 45.2 pmol/L; normal, < 10 µmol/L.

Despite the empirical therapy, the blood tests continued to deteriorate. A second liver biopsy was done, and evidenced bile duct degeneration and ballooning degeneration of liver cells with marked cholestasis. Despite the advanced biochemical and histological damage, the patient did not show signs of hepatic encephalopathy, renal, or lung disease at any time. Due to the absence of clear clinical and analytical improvement and to the severity of the histological findings, the patient was evaluated for inclusion in the liver transplantation program. A hepatic vascular and cholangio-MRI and an abdominal Doppler ultrasound were without pathologic findings. The serum levels of total bilirubin gradually and spontaneously decreased, reaching normal values, and the patient was finally not considered for transplantation and was followed up at the outpatient clinic. Analytical controls were normal after 2 years. The patient was asymptomatic throughout this period.

Soloukides A, Moutzouris DA, Kassimatis T, Metaxatos G & Hadjiconstantinou V (2007) A fatal case of paraquat poisoning following minimal dermal exposure. ***Ren Fail. 29(3):375-7.***

This study reports a case of fatal paraquat poisoning after minimal dermal exposure.

An 81-year-old male presented to his family doctor because of a skin lesion of the right thigh after accidental contact with paraquat the previous evening. His relatives reported that the pesticide was spread on the trousers, and the man slept overnight without removing the clothes. The lesion was limited, producing only skin erosion, which was treated empirically with steroid ointments. Four days later, the patient complained of severe breathlessness and was admitted for the attention.
The patient complained of difficulty in breathing. He also reported decreased urine output over the last 48 hours. He did not report fever or other systemic symptoms. His past medical history was unremarkable except for mild hypertension during the last four years, which was treated with perindopril.

On initial physical examination, the patient was alert but highly distressed. Vital signs were (on admission): rectal temperature, 37.2°C; heart rate, 85/min; respiratory rate, 16/min; and blood pressure, 120/70 mmHg. Superficial chemical burn on the right thigh was calculated as covering 4% of the total body surface area. There were no mouth ulcers. The rest of the examination was negative.

Laboratory tests revealed the following: hematocrit, 42.5%; white blood cells count, $14.6x10^3/4$ (89.9% neutrophils); PLT, $160x10^3$/bL; urea, 246 mg/dL; creatinine, 10.1 mg/dL; Na, 140 mEq/L; K, 4.2 mEq/L; alkaline phosphatase, 102 IU/L; SGOT, 87 IU/L; SGPT, 100 IU/L; LDH, 431 TU/L; Ca, 9.4 mg/dL; P, 6.0 mg/dL; ESR, 107 mm; PT, 26%; and INR, 2.17. Blood gas analysis on two liters of oxygen showed pH, 7.46; $Pa0_2$, 49.1 mmHg; $PaCO_2$, 27.7 mmHg; and 0_2, saturation 83.5%. Chest radiograph showed findings compatible with acute respiratory distress syndrome (ARDS). Abdomen ultrasound was negative. Urine sodium dithionite test was negative for paraquat, and no pesticide was detected in blood sample. These findings indicated acute lung injury, acute renal injury, leucocytosis, and impaired hepatic function.

The patient was treated with hydration, oxygen supplementation, and intravenous antibiotics. Renal failure was managed by hemodialysis and haemoperfusion. The lung function continued to deteriorate. He was transferred to the Intensive Care Unit, intubated, and ventilated, but died two days later.

4.11.2.4 During Pregnancy

Talbot AR, Fu CC & Hsieh MF(1988) Paraquat intoxication during pregnancy: A report of 9 cases. *Vet Hum Toxicol 30 (1): 12-17.*

Study & observations: The details of 9 pregnant women (age range: 18-24 years) presenting to the Changhua Christian Hospital (CCH) in central Taiwan following deliberate ingestion of paraquat (24% paraquat) were reported. Some patients received treatment from the local hospital (unspecified) prior to admission to the CCH. On admission, full clinical examination with a chest X-ray, and haematology, respiratory, hepatic and renal function, and clinical chemistry tests were conducted on all patients. Paraquat levels in maternal, foetal and cord blood, urine and amniotic fluid were monitored. Postmortem examinations were conducted on all of the deceased.

Findings: Seven patients died due to acute respiratory distress in spite of therapy, which generally included mechanical ventilation, haemoperfusion, gastro-intestinal lavage, blood transfusion and forced diuresis. Although emergency Caesarean operations were performed on all cases, 7/9 foetuses died. One foetus was stillborn. Paraquat ingestion-related data are presented in Table 119 below.

Table 119: Obstetric and paraquat ingestion details of the patients

Case	Amount ingested	Time since ingested	Paraquat (µg/mL)		Gestation length	Time to death
			Blood	Urine*		
1	1 mouthful	7 days	-	-	12	17 days
2	1 mouthful	6 days	-	-	2	-
3	3-5 mouthfuls	12 h	0.85	+++	32	2 days
4	3 mouthfuls	7 h	1.67	+++	40	1 day
5	2 mouthfuls	4 h	-	+++	40	-
6	half a cup	6 h	5.6	+++	32	1 day
7	Unknown	4 h	6.3	+++	22	3 days
8	2 mouthfuls	1.2 h	3.6	++++	24	-
9	20 mL	24 h	0.08	+	6	-

*Scale not clearly defined.

Clinical signs observed in patients were ulceration of oral and/or pharyngeal mucosa (5/9), irritability (4/9), dyspnoea (3/9), icteric sclera (2/9), tachycardia (2/9), and oliguria (1/9), confusion (1/9) and tenderness over the epigastrium (1/9). Clinical chemistry details and serum paraquat levels of two patients, with those of their foetuses were provided (see Table 120 below). The level of paraquat in the cord blood of one patient (case 6) was 28.85 µg/mL, indicating that paraquat crosses the placenta, and concentrated to the levels approximately 4-6 times greater than maternal blood. The foetal data of another patient (case 8) revealed that the paraquat levels in amniotic fluid 7 days after ingestion was nearly twice that of the maternal serum paraquat level.

Table 120: Clinical chemistry and serum paraquat levels in two cases of paraquat poisoning in pregnant women

Parameter*	Case 6		Case 8	
	Mother	foetus	Mother	Foetus
AST (u/L)	288	6170	142	62
ALT (u/L)	89	540	65	20
SALP (u/L)	78	-	154	-
BUN (mg/dL)	15.5	31	17	8
Creatinine (mg/dL)	2.3	1.0	1.2	1.7
Serum paraquat (µg/dL)	5.6	20.6	ND	ND
Biochemistry of amniotic fluid of the foetus of case 8			**Amniotic fluid**	**Maternal serum**
Oestradiol (ng/mL)			14.2	5.5
α-fetoprotein (µg/mL)			6200	278
Creatinine (mg/dL)			5.5	4.3
Paraquat (µg/mL)			0.05	0.03

*SALP = Serum alkaline phosphatase, BUN = Blood urea nitrogen, ND = Undetectable

Although emergency Caesarean operations were performed in all cases, 7/9 foetuses died. At delivery, 2 foetuses showed signs of respiratory distress. A postmortem needle biopsy of the liver of one person (case 1) showed dilation of sinusoids with fine fat vacuoles in the cytoplasm of hepatocytes, central cholestasis, and clusters of coagulation necrosis. The portal area and the nucleus of hepatocytes were unaffected. Autopsy of case 8 showed no pathological effects in the placenta, but fatty metamorphosis of the foetal liver and heart and hyaline membrane abnormalities of the foetal lung were seen.

The protocol adopted for therapy emphasised aggressive fluid administration, forced diuresis, gastric lavage, with no supplemental oxygen. The adsorbent of choice was changed from bentonite to Fuller's earth, and then to kayexalate with continuous haemoperfusion (6-8 h/course) until no paraquat was detected in the serum and/or urine. Colchicine was included during treatment to prevent pulmonary fibrosis. Follow up studies revealed that one of the 2 survivors had a normal pregnancy since, with no evidence of teratogenicity following the earlier exposure to paraquat.

Conclusions: The data from one patient showed that paraquat crosses the placenta, and was concentrated to levels 4-6 times greater than maternal blood. The concentration of paraquat in the amniotic fluid of one foetus was nearly twice that in maternal serum. The treatment procedure adopted emphasised aggressive fluid administration, forced diuresis, gastric lavage, with no supplemental oxygen and was generally successful.

Jenq CC, Wu CD & Lin JL (2005) Mother and fetus both survive from severe paraquat intoxication. *Clin Toxicol (Phila). 43(4):291-5.*

A 21-year old female who was 27 weeks pregnant deliberately ingested approximately 40 ml of 24% paraquat solution. She was admitted to hospital 2-hours later with various symptoms including nausea, vomiting, mild oral mucosal pain, and haematemesis.The physical examination reported sensorium, anicteric sclera and ulcers on the oral mucosa and tongue, but all other observations were normal.

After conducting various blood and foetal tests, activated charcoal and magnesium citrate were administered orally followed by prophylactic treatment. Haematemesis was observed on day 3 and a blood transfusion was subsequently performed.
The patient received pulse therapy with methylprednisolone followed by 6 hourlydexamethasone (5 mg), tapered to twice daily (day 18), then daily (day 23) until discontinued on day 24 following good arterial blood oxygen concentration measurements.

Before discharge on day 29, the patient was well without respiratory symptoms. Foetal ultrasounds at week 30 and 35 showed an estimated gestational age similar to the actual age (29.4 *vs* 30 and 33 *vs* 35 respectively). At 41 weeks gestation (day 97 following paraquat intoxication), a baby girl was delivered via spontaneous vaginal delivery, with good Apgar scores.The child was of normal weight and size.

Five years later, the mother and the child were both alive and well without apparent complication of paraquat intoxication.

The authors discussed the possible reasons for the survival of mother and child. They relate to the application of various treatment steps at critical times after intoxication and will not be presented here.

Chomchai C & Tiawilai A (2007) Fetal poisoning after maternal paraquat ingestion during third trimester of pregnancy: case report and literature review. *J Med Toxicol. Dec;3(4):182-6.*

A 17-year old pregnant female (36 weeks gestational age) presented to hospital after ingesting 2 mouthfuls (approximately 30 ml) of 27.6% w/v paraquat formulation 5 hours prior. The patient had visible burns on her lips, but the heart, lungs, uterine height, and neurological examinations were normal.Gastric lavage was undertaken, followed by oral administration of activated charcoal and Fuller's earth solution. An emergency caesarean section was then performed approximately 7 hours following ingestion. The male infant had a slightly increased respiration rate which resolved within 24-hours. Chest x-ray results were normal. After consultation with a toxicologist, prophylactic treatment was not commenced. At 6 days of age, respiration rate again increased above normal levels along with subcostal retractions and xray revealed an infiltrate in one lung lobe. The infant was treated with oxygen and various courses of antibiotics for presumed pneumonia and his condition gradually improved until he was discharged after 22-days while still requiring oxygen treatment.

Subsequent medical check-ups revealed signs of chronic lung disease such as recurrent wheezing, high respiration rate and hypoxia. At 10-months of age, oxygen therapy was discontinued and the infant subsequently exhibited several episodes of respiratory illness which improved by 16-months of age.

Other case reports: Details from other similar case reports presented in the paper are summarised in Table 121 below.

Table 121: Selected summary of reported cases of mother-foetus paraquat exposure in the third trimester (gestational age ≥ 26 weeks)

Case specifics:	Maternal and Foetal Outcome
Talbot *et al*, 1988 Amount ingested: "Several mouthfuls" Gestational age: 32 weeks Maternity Urine DTT[a]: Positive 3+	Maternal: Serum paraquat level 0.85 µg/dL. Received haemoperfusion. Died of respiratory failure (3 days).
	Foetal: Inaudible heart sound (3 days).
Talbot *et al*, 1988 Amount ingested: ½ cup Gestational age: 32 weeks Maternity Urine DTT[a]: Positive 3+	Maternal: Developed oliguria and cyanosis. Blood paraquat level 5.6 µg/mL. Died.
	Foetal: Emergency caesarian section at 4 hours after ingestion. Developed oliguria, hypotension and bradycardia at 19 hours of life. Cord blood paraquat level 20.6 µg/mL. Died.
Prasarnphanich *et al*, 1988 Amount ingested: 3 mouthfuls Gestational age: 36 weeks Maternity Urine DTT[a]: Positive	Maternal: Died at home due to multi-organ failure
	Foetal: Normal spontaneous vaginal delivery Apgars 7 and 10. Total exchange transfusion at 10 hours of life. Developed renal failure and cyanosis at 24 hours of life. Died at 46 hours of life
Jenq *et al*, 2005 Amount ingested: Not known Gestational age: 26 weeks Maternity Urine DTT[a]: strongly positive	Maternal: Received haemoperfusion with charcoal cartridge at 4 and17 hour post ingestion. Pulse cyclophosphamide and dexamethasone started 17 hours.
	Developed haematemesis, anemia, and renal insufficiency.
	Had resolution of symptoms and was discharged home.
	Foetal: NSVD 14 weeks after exposure. Apgars 1 5. Continued to do well at 5 years.

[a] Urine DTT = urine dithionate test

Exposure to paraquat prior to the third trimester are typically fatal in the cases reported in Table 121 above.

In humans, there is evidence that paraquat may focus in the foetus at levels 4-6-times higher than that of the mother (Talbot *et al*, 1988).

Effects of maternal paraquat exposure on newborn infants can result from direct exposure of the foetus to amniotic fluid containing paraquat or the active re-uptake of paraquat into tissues. Symptoms include mucosal ulcerations and desquamation of the buccal mucosa. Exposure of the foetus to paraquat later in pregnancy can cause pulmonary effects and significant hepatic necrosis at high doses (Chomchai & Tiawilai, 2007).

Animal studies indicate that paraquat readily crosses the placenta and can concentrate in the lungs, kidneys, skin and salivary glands (Ingebrigtsen *et al* 1984; Anderson *et al* 1976). Re-uptake in the lungs of the foetus by type II pneumocytes has also been demonstrated in rat studies (Randell & Young, 1998).

Concluion: Paraquat exposure in pregnant woman poses a significant maternal and foetal risk. The case report by Chomchai & Tiawilai (2007) suggests that prompt treatment can reduce negative outcomes for both mother and child.

4.11.2.5 Mixed Exposure

Wesseling C, Hogstedt C, Picado A & Johansson L (1997) Unintentional fatal paraquat poisonings among agricultural workers in Costa Rica: Report of 15 cases. *American J Indust Med 32: 433-441.*

Study: This study analysed the exposure circumstances of 15 fatal occupational paraquat intoxications in Costa Rica. To evaluate the potential danger of dermal absorption and the amount needed to produce a fatal outcome in the event of oral ingestion, medical records and autopsy records were reviewed and the relatives of the deceased patients were interviewed.

Case identification & observations: The cases included in the study were selected by reviewing the paraquat-related fatality data that were collected in a national survey of hospitalisations and autopsies during the period of 1980-1986, and the records kept at all health centres where the deceased patients had received medical attention. The search for additional cases of fatal poisonings during 1987-1992 was limited to the discharge registers of 2 major hospitals and screening a list of diagnoses from autopsy reports during the same time period. From a pool of 400 fatalities, 36 work-related deaths were selected for further investigation. When possible, information on the exposure was obtained by interviewing the relatives of the deceased persons. The criteria used in case selection included the history of occupational exposure close to the onset of illness, clinical or patho-morphological evidence of lung injury, kidney or liver dysfunction, and a final diagnosis of paraquat poisoning. Following scrutiny, 15/36 cases were chosen for the main study.

Findings: All deceased workers were male farmers or farm labourers aged from 10 to 78 years. Ten workers ingested differing quantities of paraquat concentrate (purity unspecified) either because of confusion of bottles or under varying unspecified circumstances. The exposure route in 3 workers was dermal, whilst that of the remaining 2 workers was not identified. It was reported that the usual concentration of paraquat in spray solutions used on banana plantations in Costa Rica was 1-2 g/L, and chemical burns in the genital area were common injuries seen in paraquat spraymen.

All patients had died due to respiratory distress, pulmonary oedema, preceded by either renal (n= 14) and/or liver impairment (n= 10), and in a few cases myocardial or central nervous system dysfunction. According to clinical and autopsy reports, there were ulcers in the GIT in 9/10 ingestion cases, and also in another case without a history of oral exposure. No clinical or any pathological findings were provided for the dermally exposed persons. Residues of paraquat had been detected in the urine of 9 cases during hospitalisation and/or in tissues at autopsy. The mean survival time for the groups with intakes of a mouthful, <1 mouthful and without apparent oral ingestion was 5.1, 9.4 and 17.6 days, respectively. Histopathology revealed diffused alveolar damage characterised by various combinations and degrees of intra-alveolar and interstitial oedema, lymphocyte, macrophage and leukocyte infiltration, thickening, rupture or necrosis of the alveolar walls, hyperplasia and desquamation of pneumocytes, hyaline membranes and fibrotic changes.

In those cases with overt ingestion due to mix-up of bottles, the hospital diagnosis was ‘paraquat poisoning’. In the remaining cases, the physicians were initially uncertain approximately the cause and the hospital admission had often been denied. The illness had also

been misdiagnosed as pharyngitis, laryngitis, urinary sepsis, gastritis, septicaemia, diphtheria, infected contact dermatitis, methaemoglobinaemia, myocarditis, cyanotic congenital heart disease, hepatitis or infectious pneumopathies of various etiologies.

Conclusions: Circumstances involved in 15 fatal paraquat intoxications were described. The clinical, pathological and histological findings suggest that the deaths were due to respiratory failure associated with unintentional paraquat exposure. According to the study findings, ingestion of small quantities of paraquat or exposure to dilute solutions of the chemical via dermal absorption, or possibly by inhalation may lead to fatal outcomes.

4.12 Mechanistic studies and studies on target organ toxicity

Gage JC (1967) The effect of diquat and paraquat on cell respiration. Report no. IHR/223. Lab & Sponsor: ICI Ltd., Industrial Hygiene Research Laboratories, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: November 1967.

Mitochondrial, microsomal and soluble fractions were prepared from the livers of male Alderley Park SPF albino rats (unspecified body weight, age & source). Mitochondrial fragments were prepared from pig heart according to the method of Pumphrey & Redfearn (1960). The respiration (ie O_2 consumption) of each fraction was measured using an O_2 electrode at 27°C in the presence of aqueous solutions of paraquat or diquat (unspecified purity & batch no; ICI Plant Protection Ltd., Jealott's Hill, Berks, UK) at 0, 10^{-3}, 10^{-4}, or 10^{-5} M (paraquat cation unknown). The substrate for the mitochondrial fraction was p-hydroxybutyrate (unspecified concentration) and the medium used was that described by Clark *et al* (1965) which contained 125 nmoles adenosine diphosphate (ADP). For the mitochondrial fragments the substrate was NADH (0.8 μmoles), β-hydroxybutyrate (8 μmoles) plus NAD^+ (0.2 μmoles), or succinate (8 μmoles) in phosphate buffer (pH 7.4). For the microsomal or soluble fractions the substrate was nicotinamide (280 μmoles) and NADH or NADPH (1.33 μmoles) in phosphate buffer (pH 7.4).

Paraquat or diquat (10^{-3} M) was reported to increase the respiration of the rat liver mitochondrial fraction by approximately 3 nmoles/min. This slight increase was unaffected by amytal (2 μmoles) or antimycin A (0.1 μg). In the absence of ADP and inorganic phosphate, paraquat or diquat did not increase O_2 consumption, although no supporting data were provided to substantiate this finding. The study author suggested that the relatively weak effect of paraquat or diquat on mitochondrial respiration was due to their failure to penetrate the mitochondrial membrane.

Paraquat or diquat (10^{-3}, 10^{-4}, 10^{-5} M) had no effect on the respiration of pig heart mitochondrial fragments when succinate was used as the substrate. In contrast, a concentration-related increase in respiration occurred in the presence of diquat or paraquat when β-hydroxybutyrate plus NAD^+ were used as the substrate, and using antimycin A (0.05 mg/mL) to inhibit basal respiration (see Table 122 below). The effect of diquat was marginally greater than that of paraquat. In the presence of NADH and using amytal (0.1 M) to inhibit basal respiration, diquat caused a concentration-related increase in respiration which was approximately 2-fold greater than that seen with paraquat. EDTA (10^{-3} M) did not effect the stimulatory effect of either

diquat or paraquat. The study author concluded that diquat and paraquat interfered with the respiratory chain by interacting with a NADH-dehydrogenase.

Table 122: Effect of diquat or paraquat on the respiration of pig heart mitochondrial fragments

Treatment	O_2 uptake (nmoles O_2/min)	
	β-hydroxybutyrate plus NAD^+	NADH
10^{-3} M Diquat	46.5, 41.5	129, 118, 110
10^{-3} M Diquat + 10^{-3} M EDTA	44	103, 128
10^{-4} M Diquat	7.5	12.5, 7.5
10^{-5} M Diquat	0	2.5
10^{-3} M Paraquat	30, 27.5	50, 20, 47.5
10^{-3} M Paraquat + 10^{-3} M EDTA	27.5	-
10^{-4} M Paraquat	7.5	0.5
10^{-5} M Paraquat	0	-

Respiration before addition of diquat or paraquat was 6-12 nmoles O_2/min.

A dose-related increase in microsomal respiration occurred in the presence of diquat or paraquat (down to 10^{-5} M) when NADPH was used as the substrate (see Table 123 below). Neither EDTA (diquat or paraquat) nor carbon monoxide (diquat) inhibited this increase in respiration. When NADH was used as the substrate, the stimulatory activity of diquat was markedly reduced while paraquat appeared inactive. The study author suggested that diquat or paraquat react with microsomal NADPH-dehydrogenases.

Table 123: Effect of diquat or paraquat on the respiration of a rat liver microsomal fraction

Treatment	O_2 uptake (nmoles O_2/min)	
	NADPH	NADH
10^{-3} M Diquat	20, 35, 20, 35	25, 27.5, 15
10^{-3} M Diquat + CO/O_2 (3:1)	25	
10^{-3} M Diquat + 10^{-3} M EDTA	27.5	12.5, 20
10^{-4} M Diquat	12.5, 17.5, 17.5	0, 2.5
10^{-5} M diquat	15, 5	0, 0
10^{-3} M Paraquat	25, 20	0, 0
10^{-3} M Paraquat + 10^{-3} M EDTA	25	
10^{-4} M Paraquat	15	
10^{-5} M Paraquat	10, 10	

Respiration before addition of diquat or paraquat was 0-10 nmoles O_2/min with NADPH and 0 with NADH.

Respiration of the soluble rat liver fraction was stimulated with diquat when either NADH or NADPH were used as substrates (see Table 124 below). EDTA slightly inhibited this effect. However the magnitude of this stimulation was lower than that observed for the mitochondrial fragments or microsomal fraction. Paraquat caused a very slight increase in respiration only at the highest concentration (10^{-3} M).

Table 124: Effect of diquat or paraquat on the respiration of a rat liver soluble fraction

Treatment	O_2 uptake (nmoles O_2/min)	
	NADPH	NADH
10^{-3} M Diquat	10.5	20
10^{-3} M Diquat + 10^{-3} M EDTA	7.5	15.5
10^{-4} M Diquat	4.0, 5.5	4.0
10^{-3} M Paraquat	1.5, 1.0, 2.5	2.5
10^{-4} M Paraquat	0	0

Resting O_2 consumption was negligible.

Limitations to this study were that no supporting data were provided for the mitochondrial fractions. The use of pig heart mitochondrial fragments rather than rat was not justified. Uneven sample sizes were used and not all samples were tested in duplicate. No statistical analysis was performed.

Gage JC (1968) The action of paraquat and diquat on the respiration of liver cell fractions. *Biochem J 109: 757-761.*

This published study utilised data from Gage (1967) (see previous evaluation) with the following additions and/or changes:

(1) Rat (rather than pig) liver mitochondrial fragments were prepared by resuspending the mitochondrial pellet in 50 mM phosphate buffer (pH 7.4) and sonicating it for two 15 second bursts.
(2) Observations made using rat liver mitochondrial fragments were similar to those made using pig heart mitochondrial fragments – ie paraquat or diquat stimulated respiration when NADH or β-hydroxybutyrate, but not succinate, were used as substrates. The study author reported that the addition of catalase after stimulation of respiration with paraquat or diquat for a few minutes failed to liberate any O_2.
(3) The increase in microsomal respiration by paraquat or diquat was not inhibited by *p*-chloromercuribenzoate (10 μM) or catalase (400 units).

Crabtree HC, Fletcher K, Rose MS & Wyatt I (1973) Biochemical effects of diquat and paraquat: disturbance of the control of corticosteroid synthesis in rat adrenal and subsequent effects on control of liver glycogen utilisation. Report no. HO/IH/R/358. Lab & Sponsor: ICI Ltd., Industrial Hygiene Research Laboratories, Alderley Park, Macclesfield, Cheshire, UK. Study duration: unspecified. Report date: September 1973,

and

Rose MS, Crabtree HC, Fletcher K & Wyatt I (1974) Biochemical effects of diquat and paraquat - disturbances of the control of corticosteroid synthesis in rat adrenal and subsequent effects on the control of liver glycogen utilisation. *Biochem J 138: 437-443.*

Guidelines & GLP: No test guidelines or GLP statement were provided. This study was not quality assured.

Materials & Methods: Paraquat or diquat dichloride (unspecified purity & batch no.; Plant Protection Ltd., Jealott's Hill Research Station, Berks., UK) were dissolved in 0.9% NaCl and injected intraperitoneally into male Alderley Park (Wistar-derived) rats (180-220 g body weight, age and source unspecified) at 0 or 20 mg/kg bw (equivalent to 14.4 mg/kg bw paraquat cation; n=4-8). The dose volume was unspecified. Rats were maintained with or without food (unspecified source) for 24 or 48 h, sacrificed by an unspecified means, and liver glycogen measured. Similarly, 6-8 male Alderley Park SPF mice (30-40 g body weight, age unspecified) per group were fasted for 24 h, injected with diquat (0 or 20 mg/kg bw, ip), sacrificed 4 h later by an unspecified means and liver glycogen measured. Details of housing and feeding conditions were unspecified. In a separate experiment, adrenalectomized Alderley Park rats (age and body weights unspecified; ICI Ltd. Industrial Hygiene Laboratories, Alderley Park, Macclesfield, Cheshire, UK) were given an ip injection of diquat at 0 or 20 mg/kg bw and then fasted. Adrenalectomized rats had been maintained for at least 3 days on 0.1 mg aldosterone/rat/d prior to experimentation. Three rats/group were sacrificed at 0, 1, 3 and 6 h after diquat administration and liver glycogen measured according to the method of Hassid & Abraham (1957).

In a separate experiment, 3 male rats/group were sacrificed by an unspecified means at 0, 1, 2, 3, 4 and 7 h after administration of paraquat (0 or 20 mg/kg bw, ip; equivalent to 14.4 mg/kg bw paraquat cation). Rats were given 2 μCi of [U-^{14}C]glucose (Amersham, UK) iv one hour before sacrifice in order to determine the level of incorporation of glucose into liver glycogen. Liver glycogen was isolated and the amount of radioactivity determined by scintillation counting.

To determine the effect of paraquat or diquat on blood glucose, paraquat was administered intraperitoneally to Alderley Park rats, hypophysectomized SD CFY rats (age and body weight unspecified; Carworth Europe, Huntington, UK) or adrenalectomized Alderley Park rats at 0 or 20 mg/kg bw (equivalent to 14.4 mg/kg bw paraquat cation; n=4). The hypophysectomized rats were used 24 h after the operation and were not administered any supportive therapy. Blood (20 μL) was collected at 30 minutes, 1, 2, 4 and 7 h from the tail, and analysed for blood glucose.

To determine the effect of diquat on adrenal catecholamines, normal or hypophysectomized rats were given an ip injection of either reserpine (5 mg/kg bw) or diquat (0 or 20 mg/kg bw) and maintained without food. At 1, 2 and 24 h, rats were sacrificed by cervical dislocation, their adrenals removed and homogenised, and the concentrations of adrenaline and noradrenaline measured by the method of Welch & Welch (1969). To determine the effect of diquat on plasma corticosteroid concentrations, rats were given an ip injection of diquat at 0 or 20 mg/kg bw and maintained without food for up to 24 h. At 0, 30 minutes, 1, 2, 4, 6 and 24 h rats were sacrificed by decapitation (n=5-19) and an unspecified volume of blood collected for measurement of corticosteroids according to the method of Givner & Rockefort (1965). The effect of diquat on the removal of corticosteroids from plasma was determined by administering adrenocorticotrophic hormone (ACTH) to hypophysectomized rats followed 5 minutes later by an ip injection of diquat (0 or 20 mg/kg bw). An unspecified number of rats were sacrificed by decapitation at 0, 1, 2 and 4 h. An unspecified volume of blood was collected from the trunk and the concentration of corticosteroids measured.

In a separate experiment, rats were given an ip injection of diquat at 0 or 20 mg/kg bw and then maintained without food for up to 24 h. Three to four rats were sacrificed by decapitation at 30 minutes, 2, 4 or 24 h and an unspecified volume of blood collected for determination of ACTH activity by RIA.

The effect on adrenal cAMP levels was determined by giving rats or hypophysectomised rats an ip injection of 0 or 20 mg/kg bw diquat and maintaining them without food for up to 24 h. Rats were sacrificed by decapitation at 0, 10, 30 minutes, 2, 4, 6 and 24 h (n=4-12), the adrenals removed and homogenised, and the concentration of cAMP measured using the method of Brown *et al* (1971).

Results

Effects on liver glycogen: An approximately 100-fold reduction in liver glycogen was observed in control rats following fasting for 24 or 48 h while paraquat- and diquat-treated rats exhibited only a 2-5-fold reduction (see Table 125 below). Diquat was marginally more effective than paraquat at reducing the magnitude of glycogen depletion during fasting. There was no indication by the study authors as to whether any of these findings were statistically significant. These observations suggested that paraquat or diquat increased the synthesis, or inhibited the release/use of liver glycogen in rats. Graphically presented data illustrated that diquat-treated adrenalectomized rats showed a more rapid decrease in liver glycogen than controls which suggested that the effect of diquat on liver glycogen (ie synthesis or release/use) was mediated by the adrenals.

Table 125: Effect of paraquat (20 mg/kg bw, ip) on liver glycogen concentrations

Treatment	n	Glycogen (mg/g wet liver weight)
Control, fed (24 h)	4	67.2 ± 3.3
Control, starved (24 h)	8	0.8 ± 0.2
Control, starved (48 h)	4	0.7 ± 0.1
Diquat, starved (24 h)	4	27.7 ± 6.7
Paraquat, starved (24 h)	8	17.5 ± 3.2
Paraquat, starved (48 h)	8	12.8 ± 3.9

Results expressed as means ± 1 SEM

Four hours after the administration of diquat to mice that had already been fasted for 24 h liver glycogen was statistically higher ($p<0.05$, t-test; 11.5 ± 2.4 mg/g wet wt) than that of the controls (4.6 ± 1.4 mg/g wet wt, fasted for 24 h; 4.8 ± 1.1 mg/g wet wt, saline-injected, 28-hour fasted mice). These results suggested that administration of diquat stimulated liver glycogen synthesis in fasted mice for up to 4 h. Measurement of liver glycogen in diquat-treated adrenalectomized mice was not performed.

Incorporation of [U-^{14}C]glucose into liver glycogen: Graphically presented data showed that there was a time-related increased in the incorporation of [U-^{14}C]glucose into liver glycogen (ie glycogen synthesis) from one to 4 h after paraquat administration. Glycogen synthesis then appeared to plateau from 4-7 h. In the control group, a transient increase in the incorporation of [U-^{14}C]glucose into glycogen at one hour was followed by a decline over the remaining study period.

Effects on blood glucose: Graphically presented data indicated that blood glucose was rapidly increased following administration of paraquat or diquat (~25 and 110 mg glucose/100 mL

blood above controls, respectively), reaching a maximum at one hour post-dose and then decreasing to control levels over the remainder of the experiment. The increase observed in the presence of diquat was approximately 5-fold greater than that observed in the presence of paraquat, however the study authors reported that the effect of paraquat could be increased by using twice the LD_{50}. Graphically presented data also illustrated that diquat caused a similar pattern of increase when administered to hypophysectomized or adrenalectomized rats, however the magnitude of the effect was marginally lower in hypophysectomized rats and markedly lower in adrenalectomized rats. Blood glucose levels actually fell below control levels from 2 h in diquat-treated adrenalectomized rats, with the study authors reporting an unspecified number of deaths within 6 h. These results suggested that the effect of diquat on blood glucose was mediated by the adrenals.

Effect on adrenal catecholamines and plasma corticosteroids: Diquat had no significant effect on adrenal concentrations of adrenaline or noradrenaline while the positive control (reserpine) caused an approximately 50% reduction in both. Graphically presented data illustrated that the plasma concentration of corticosteroids was increased in both control and diquat-treated rats 30 minutes after injection, thereafter control rats showed a decline in plasma corticosteroids which reached an apparently stable level by 4 h. In contrast, diquat-treated rats showed a persistent though declining elevation in plasma corticosteroids over 24 h. The study authors reported that paraquat showed a similar persistent effect on plasma corticosteroids, however no supporting data were provided to substantiate this finding. Graphically presented data indicated that diquat had no effect on the removal of corticosteroids from hypophysectomized rats after the administration of ACTH. The study authors reported that diquat had no effect on plasma corticosteroids in hypophysectomized rats although no supporting data were provided.

Effect on ACTH: Graphically presented data revealed that plasma ACTH levels decreased over time in both control and diquat-treated rats, however levels were higher in diquat-treated rats than in control rats over the first 4 h.

Effect on adrenal cyclic adenosine monophosphate (cAMP): Graphically presented data indicated that control rats had an initial elevation in adrenal cAMP (~750 pmol/adrenal) but this fell to almost zero by 30 minutes. In diquat-treated rats, adrenal cAMP was highly elevated at 30 minutes (~1500 pmol/adrenal) and then decreased over the following 3 h. Overall, adrenal cAMP levels were significantly higher (no p value provided) in diquat-treated rats than in controls over the entire 24-hour sampling period. The study authors reported no significant increase in wet adrenal weight and no elevation in adrenal cAMP in diquat-treated hypophysectomized rats, however no data were provided to support these findings.

Conclusions: Paraquat or diquat (20 mg/kg bw, ip) increased the synthesis and/or reduced the utilisation of liver glycogen, and increased blood glucose in rats or mice, although the ip dose route is not relevant to humans. These effects appeared to be mediated by the adrenals as they were markedly or completely reduced in adrenalectomized rats. Diquat had no effect on the level of adrenal catecholamines (adrenaline and noradrenaline) and thus the effect on liver glycogen and blood glucose was probably due to the observed increase in plasma corticosteroids. The diquat-induced increase in plasma corticosteroids was shown to be related to increased synthesis as diquat had no effect on the metabolism or excretion of corticosteroids from the plasma of hypophysectomized rats. Adrenal cAMP and plasma ACTH levels were

also elevated with diquat. The study authors suggested that paraquat and diquat increase the response of the adrenal cortex to ACTH.

Comments: Limitations with this study include: no raw data were provided; uneven sample sizes; only a single dose was tested; some haematology/clinical chemistry parameters were not measured in in mice and results of statistical analyses were not always provided. A limitation of this study was that measurements of glucose, glycogen, catecholamines, corticosteroids, cAMP and ACTH were performed in separate groups of mice.

Phillips CE (1977) Haematological effects of paraquat in the rat. Report no. CTL/P/3263. Lab & Sponsor: Unspecified. Study duration: unspecified. Report date: 31 January 1977, and

Phillips CE & Sanderson JH (1977) Paraquat: Effects on peripheral blood and bone marrow cells in rats. Report no. CTL/P/363. Lab & Sponsor: Unspecified. Study duration: unspecified. Report date: 16 September 1977.

Guidelines & GLP: Pre-dates GLP and test guidelines.

Materials & Methods:

The aim of this study was to investigate the occurrence of red cell aplasia following paraquat poisoning in rats, based on a previous report in humans (Lautenschlager *et al* 1974).

Part I: Male rats (unspecified strain, body weight, age & source) were starved overnight and dosed with paraquat (unspecified purity, batch no. & vehicle; ICI Ltd, Plant Protection Division, unspecified location) at 0 (n=30), 20 (sc, n=50), 31 (po, n=30) or 125 mg cation/kg bw (po, n=50) at an unspecified dose volume. One control rat and 3 rats from the remaining groups were sacrificed with halothane on days 1, 2, 3, 7, 9, 14, 21 and 28. Bone marrow was collected from the femur of each rat. Smears were prepared and stained with Wright's stain, then microscopically examined.

Part II: Female rats (unspecified strain, body weight, age & source) were starved overnight and dosed with paraquat at 0 (n=30), 20 (sc, n=100), 31 (po, n=65) or 125 mg cation/kg bw (po, n=100). Eight rats/group were sacrificed with halothane on days 1, 2, 3, 4, 5, 6 and 7, and an unspecified volume of blood collected by cardiac puncture. The following haematology parameters were measured: Hb, Hct, MCV, RBC, WBC, MCHC and differential cell count.

Four or 5 rats were housed per cage (Wilmslow-type mobile rat units) with standard diet plus vitamin E (Oakes limited, Congleton, Cheshire, UK) and water (unspecified source) available *ad libitum*. No further experimental details were provided.

Results:

Mortality: At the low po dose (31 mg/kg bw), no deaths were observed. At the high po dose (125 mg/kg bw), 22% of males had died by day 14 and 60% of females had died by day 5, with the remaining females dying by the end of the study. Subcutaneous administration of 20 mg/kg

bw paraquat resulted in 54% mortality in males (to day 14) and 60% mortality in females (to day 5) with all females reported to have died after day 5.

Bone marrow effects (♂): The myeloid:erythroid ratio was increased in all paraquat-treated rats from day 3 or 7 but had returned to normal in all surviving rats by day 28. Rats dosed orally at 125 mg/kg bw or sc at 20 mg/kg bw showed a moderate to marked increase in the myeloid:erythroid ratio from day 3 to 21, while rats dosed orally at 31 mg/kg bw showed a moderate increase at day 7 and 9 only. The study authors considered these observation equivocal as there was no evidence of destruction of erythroid precursors and all stages of erythropoiesis appeared to be present in the bone marrow.

Peripheral blood effects (♀): There was no treatment-related effect on MCHC or MCV. There was a statistically significant elevation ($p<0.01$-0.05; t-test) in Hb in rats that were dosed orally at 125 mg/kg bw or sc at 20 mg/kg bw (see Table 126 below).

Table 126: Effect of a single po or sc dose of paraquat on haematology in female rats

Parameter	Control	20 mg/kg bw sc	31 mg/kg bw PO	125 mg /kg bw PO
Hb (g/dL)				
Day 1	14.08 ± 0.37	16.98 ± 0.96 **	14.66 ± 1.03	17.35 ± 0.85 **
Day 3	14.56 ± 0.51	17.40 ± 1.52 **	14.78 ± 1.43	18.55 ± 0.53 **
Day 5	14.68 ± 0.47	15.46 ± 2.28	13.90 ± 0.82 *	18.26 ± 2.26 **
RBC (10^{12}/L)				
Day 1	7.10 ± 0.32	8.52 ± 0.50	7.26 ± 0.49	8.82 ± 0.50
Day 3	7.58 ± 0.34	8.92 ± 0.84	7.57 ± 0.72	9.45 ± 0.48
Day 5	7.70 ± 0.36	7.80 ± 1.18	7.19 ± 0.42	9.25 ± 1.09
Total Leukocytes (10^9/L)				
Day 1	6.15 ± 0.85	4.25 ± 1.76 *	6.55 ± 0.95	5.01 ± 1.74
Day 3	7.64 ± 1.33	3.04 ± 0.67 **	6.66 ± 0.68	5.08 ± 1.47 **
Day 5	7.96 ± 1.45	7.89 ± 2.17 *	6.85 ± 1.83	4.61 ± 2.17 **
Lymphocytes (x $10^{-3}mm^3$)				
Day 1	5.29 ± 0.83	3.26 ± 1.34 *	5.79 ± 0.91	3.93 ± 1.87
Day 3	6.26 ± 1.08	2.40 ± 0.66 **	5.74 ± 0.77	3.56 ± 1.56 **
Day 5	6.41 ± 0.96	5.05 ± 2.34	5.98 ± 1.78	2.91 ± 2.00 **

Results expressed as means ± 1 SD; * $p<0.05$ (t-test); ** $p<0.01$ (t-test)

RBC and Hct (graphically presented data only) were also elevated in these same groups but these results did not appear to be statistically significant. The study authors suggested that these observations were due to fluid loss from the circulation as a consequence of paraquat-induced dehydration. Rats administered the highest po dose (125 mg/kg bw) or the sc dose (20 mg/kg bw) exhibited significant reductions ($p<0.01$-0.05, t-test) in total leukocytes and lymphocytes (see Table 126 above). A weakness of these findings was the absence of any pre-treatment baseline data.

Conclusions: At doses of paraquat which caused mortalities (125 mg/kg bw, po & 20 mg/kg bw, sc), a transient increase in the myeloid:erythroid ratio in male rats was observed in the absence of any destruction of erythroid precursors or perturbations in any erythropoietic stage. At these same doses in females, mortalities, elevations in Hb, RBC and Hct, and decreased total leukocytes and lymphocytes occurred, due possibly to fluid loss.

Oliveira MV, Albuquerque JA, Paixão AD, Guedes LS & Cabral AM (2005) High blood pressure is one of the symptoms of paraquat-induced toxicity in rats. ***Arch Toxicol.;79(9):515-8.***

This study investigated the hypothesis that paraquat-induced lipid peroxidation provokes changes in blood pressure and heart rate.

Study & Observations:

Male Wistar rats (250-350 g) were kept in a controlled environment on a standard diet and water *ad libitum*. TBARS -thiobarbituric acid reactive substances (formed with lipid peroxidation) and cardiovascular parameters were measured for the groups as specified in Table 127 below.

Table 127: Study groups and treatment measures

Group	Group size	Treatment
Control	8	Saline 0.1 mL/kg bw ip
Pq2h	12	Paraquat 35 mg/kg bw ip
Pq12h	7	Paraquat 35 mg/kg bw ip
SOD	4	Superoxide dismutase (CuZnSOD) 50,000 IU/kg bw iv – antioxidant
Pq2hSOD	8	Paraquat 35 mg/kg bw ip & CuZnSOD 50, 000 IU/kg bw iv administered simultaneously

Two catheters were inserted under ether anaesthesia; one was placed in the carotid artery to measure blood pressure (BP) and the second was introduced into the inferior vena cava through the femoral vein for saline or drug administration. The catheters were filled with heparinized saline (125 IU/mL) and were externalized at the dorsal neck level. Penicillin (24,000 IU) was injected and the animals were transferred to individual cages. Twenty-four hours later, the venous catheter was connected to an infusion pump and the arterial catheter was connected to a BP transducer, which was coupled to the pre-amplifier of a Hewlett Packard polygraph and to a digital-analog converter.

After a period of rest of 30-45 min, the basal levels of the systolic and diastolic blood pressure (SBP and DBP) were determined. Saline or paraquat was then administered intraperitoneally. Saline (5 mL/kg) was then infused into the inferior vena cava of each rat for 15 min, except for the SOD and Pq2hSOD groups, where the rats received SOD (50,000 IU dissolved in saline, 5 mL/kg) for the same length of time. The BP was recorded after two and 12 h following saline or paraquat administration. During the recordings, the rats could move freely, and were unstressed as they were housed in an acoustically isolated room and had no visual access to the researcher. The signal collected was stored on a microcomputer for later analysis, using the playback program of the Cale Package Windaq-200. Mean arterial pressure (MAP) and heart rate (HR) were calculated from the BP traces.

When the cardiovascular recordings were completed, the animals were anaesthetized with ether in preparation for removal of the liver, kidneys and lungs. These tissues were used to determine the level of TBARS. The animals were submitted to thoracotomy for puncture of the left ventricle and tissue perfusion with 150 ml physiological saline. Each organ was macerated in KCI (1.15%) in ice bath, at a proportion of 1 mL:1 g, for 15 min, and then transferred to test

tubes. Then 2 mL of the reagent (0.375% thiobarbituric acid and 15% trichloroacetic acid) was added to each millilitre of the mixture. Duplicate tubes were sealed and heated in a water bath (100 °C) for 15 min. After cooling, the protein precipitate was centrifuged for 10 min, the supernatant was separated, and absorbance was measured at 535 nm

Results:

Table 128 below shows the level of TBARS in tissues from the C, Pq2h, Pq12h and Pq2hSOD groups. Rats were evaluated 2 h after treatment with saline or after 2 h (Pq2h) and 12 h (Pq12h) after paraquat administration. The levels were higher in the kidneys of Pq2h group (104%, $p < 0.05$), than in their respective control. In contrast to the Pq2h group, the Pq2hSOD rats had significantly lower levels *(*$p < 0.05$) of TBARS in the liver (56%), kidney (72%) and lung (41%).

Table 128: Effects of paraquat on the levels of thiobarbiturid acid reactive substances (TBARS) (mmol malondealdehyde (MDA)/g tissue) in the liver kidney and lung

Group	TBARS (mmol MDA/g tissue)		
	Liver	Kidney	Lung
Control	8.3 ± 0.5	14.7 ± 1.6	11.0 ± 1.9
Pq2h	13.7 ± 2.7	30.1 ± 2.2	10.6 ± 4.2
Pq12h	10.5 ± 0.9	20.6 ± 2.1	9.7 ± 0.4
Pq2hSOD	4.6 ± 0.3	5.6 ± 0.6	5.7 ± 0.5

Cardiovascular parameters did not change in the control group after saline administration. In contrast, after paraquat administration, the levels of SBP, DBP and MAP were higher ($p < 0.05$) than basal levels in the Pq2h (+ 36, +23 and +30 mmHg, respectively) and Pq12h (+28, + 34 and + 32 mmHg, respectively) groups. The parallel values of HR were lower in both groups (-17 and -21 beats/min, respectively; $p < 0.05$). The parameters for the SOD group were not different from those of the control group. In the Pq2hSOD group, although the values of SBP after administration of paraquat and SOD were higher (+ 10 mm Hg, $p < 0.05$) than before the administration of these drugs, the levels of DBP and MAP remained the same. When compared to the Pq2h group, the levels of SBP, DBP and MAP were lower (-21, -24 and -23 mmHg, respectively; $p < 0.05$). Furthermore, all cardiovascular parameters presented by the Pq2hSOD group were similar to those exhibited by the control group.

Conclusion:

High blood pressure is one of the effects of acute paraquat poisoning in rats.

De Lavaur E, Siou G, Grolleau G & Carpentier-Le Sech J (1979) Comparative study of the action of diquat and paraquat on the digestive mucosa of mice, rats and rabbits. *Ann Zool Ecol Anim 11(2): 159-169.*

Aqueous diquat dibromide (270 g/L diquat ion) or paraquat dichloride (370 g/L paraquat cation) (batch numbers unspecified; SOPRA-ICI, Reaumur, Clamart Cedex) were administered to adult male Swiss mice (n=5 or 15), Wistar rats (n=5 or 10) or rabbits (cross bred from NZW and Grande Russe rabbits; n=2) (body weights & sources unspecified) via their drinking water at 0, 100, 250, 500 or 1000 mg/mL for an unspecified number of consecutive days. Some groups of rats and rabbits received water-only for 18-24 h prior to administration of paraquat

or diquat. Two groups of rats (n=5) also received paraquat by po gavage at 10 and 20 mg/kg bw. A number of animals in each group were sacrificed at various times after treatment began (see Table 129 below) by an unspecified means. The following organs were collected for histopathological analysis: oesophagus, stomach, duodenum, colon, lungs and tongue. No further experimental details were provided.

There was a concentration-related increase in mortalities in paraquat-treated rats and mice. In rats given a preliminary water diet, treatment with 1000 mg/kg bw paraquat resulted in high mortality (8/10). Deaths (2/5) also occurred in rats gavaged with 20 but not 10 mg/kg bw paraquat. Mortalities (2/5) were observed in rats treated with the highest concentration of diquat (1000 mg/mL). No mortalities were observed in paraquat- or diquat-treated rabbits.

Animals were reported to dislike drinking the paraquat or diquat solutions particularly at the highest concentrations, but this finding was not quantified. Therefore it was unclear whether mortalities were due directly to paraquat or to dehydration due to the repugnance of the animals for the paraquat solutions. Rabbits exhibited no clinical signs following diquat treatment, while atonia and increased salivation (unspecified incidences) were observed following treatment with paraquat. No clinical signs were reported for either mice or rats.
There were no macro or microscopic pathological abnormalities observed in paraquat-treated mice or rabbits. Additionally, there were no histopathological abnormalities observed in diquat-treated rats or rabbits. At 1000 mg/mL paraquat, the lungs of 2 rats showed serious exudative alveolitis and congestion, while no abnormalities of the GIT were observed. In rats that were placed on a preliminary water diet, most (unspecified) showed congestive or inflammatory lesions of the pulmonary parenchyma at 500 and 1000 mg/mL. Small blood clots in the gastric mucosa were observed in one rat given 1000 mg/mL paraquat, and 2 rats given 500 mg/mL, however in the absence of a dose-response effect this result was not considered to be treatment-related. Four rats treated with 1000 mg/mL paraquat showed regions of alveolitis and the presence of macrophages. Rats that were gavaged with paraquat showed no histopathological abnormalities.

Table 129: Effect of paraquat or diquat on mortalities in mice, rats or rabbits

Treatment	Mortalities	No. animals sacrificed	No. of days of treatment prior to sacrifice
Mice			
PQ 0 mg/mL (B)	0/5	5	16
PQ 100 mg/mL (B)	0/15	15	1-9-15
PQ 500 mg/mL (B)	7/15	8	1-7
PQ 1000 mg/mL (B)	8/15	7	1-7
Rats			
PQ 1000 mg/mL (B)	8/10	2	14
PQ 100 mg/mL (A)	0/5	5	7-12
PQ 500 mg/mL (A)	1/5	4	6-7
PQ 1000 mg/mL (A)	3/10	7	5-6-8
PQ 10 mg/kg bw, PO	0/5	5	3-11
PQ 20 mg/kg bw, PO	2/5	3	3-11
DQ 500 mg/mL (A)	0/5	5	20
DQ 1000 mg/mL (A)	2/5	3	8
Rabbits			
PQ 100 mg/mL (B)	0/2	0	6
PQ 250 mg/mL (A)	0/2	2	10
DQ 100 mg/mL (B)	0/2	0	6
DQ 500 mg/mL (A)	0/2	2	10

PQ = paraquat; DQ = diquat; (A) = with preliminary water diet; (B) = without preliminary water diet

In summary, treatment of mice, rats or rabbits with either paraquat or diquat via the drinking water (up to 1000 mg/kg bw) or by po gavage (up to 20 mg/kg bw) had minimal affect on the digestive mucosa, despite evidence of toxicity [mortalities (rats and mice), clinical signs (rabbits) and histopathological lung abnormalities (rats) typical of paraquat poisoning]. This study was considered to have limited regulatory value due to the absence of methodological and observational detail.

Podprasart V, Satayavivad J, Riengrojpitak S, Wilairat P, Wananukul W, Chavalittumrong P, Chivapat S & Yoovathaworn K (2007) No direct hepatotoxic potential following a multiple-low dose paraquat exposure in rat as related to its bioaccumulation. *Toxicol Lett 170(3):193-202.*

Whereas a single high dose of paraquat in man results in many signs of paraquat induced hepatotoxicity (Hong *et al* 2000; Bataller *et al* 2000; Erickson *et al* 1997) this study was conducted to investigate the repeat low dose effect of paraquat on liver function and xenobiotic-metabolizing enzyme activities, and to correlate the effects with tissue accumulation.

Dose-response study. Forty male Wistar rats (120-140 g bw) randomly allocated into four groups and treated with daily subcutaneous doses of 0 (water control), 4.0, 5.0, and 6.0 mg of paraquat/kg bw/day for seven consecutive days. The body weight and clinical signs of toxicity were observed.

Time-course study. Twenty-four rats were divided into two groups: control and paraquat treated groups (4.0 mg/kg bw/d, sc). They were treated for 3, 7, or 10 days.

Sample collection and biochemical analyses: At the end of each experimental period, blood samples were obtained for blood chemistry study. Aspartate aminotransferase (AST), alanine

aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, BUN, and creatinine were determined using an automatic hematology analyzer. Total protein and albumin concentrations were determined. Liver and lung specimens were collected for morphological evaluation using standard method for H&E staining. The remainders of the plasma and tissue samples were kept frozen at -80 °C.

Effect of PQ on xenobiotic-metabolizing enzyme activities: Hepatic post-mitochondrial supernatant and microsomal fractions were prepared for the determination of CYP activities. The O-deethylation of ethoxyresorufin, as CYP1A1 probe, was assayed by a fluorometric method. The *p*-nitrophenol hydroxylation, as CYP2E1 probe, was determined spectrophotometrically. Formaldehyde formation was measured for the determination of erythromycin N-demethylase activity, CYP3A4 probe. Liver microsomal protein concentration was also determined.

Bioaccumulation of paraquat in the biological samples: The frozen plasma and homogenized tissue samples were thawed and prepared for the determination of paraquat content. Ethyl viologen, as an internal standard, was added into the sample prior to the process of extraction. The obtained alkaline eluents were introduced onto a C18 p.Bondapak^{TM} column (10 p.m, 125 A, 3.9 mm x 300 mm) connecting with an Aligent 1100 Series HPLC system. The calibration curves of paraquat in plasma and tissues were constructed by plotting paraquat and internal standard peak area ratio against the respective paraquat concentrations. The standard curve data was subjected to least square linear regression analysis and the resulting equation was utilized for the calculation of paraquat concentration in plasma and tissue samples.

Study on the plasma and tissue paraquat concentrations after single and multi-dose exposures: For a single low dose paraquat exposure (6.0 mg/kg, sc), the animals were sacrificed at pre-assigned time points: 0.5, 1, 3, and 24 h post dose. For repeat-dose paraquat exposure, the same dose of paraquat was administered for 7 days and sacrificed at the same time points as in a single dose study after the last dose. Additional data at 24 h post-dose were obtained from the animals in the study of time-course of toxicity.

Results:

General signs of toxicity of paraquat: Piloerection and impairment of respiratory function shown as sunken thorax during breathing were observed in treated animals at all doses after four injection days. Animal had reduced locomotion and response to the stimuli with slight ataxia after 6 days of treatment. Over the 7-day treatment period, a rapid and significant weight loss was found in 30% treated animals.

Dose-response to paraquat and time-course of paraquat effect on the liver function: Plasma ALT and ALP tended to decrease with an increase in the dose of paraquat as shown in the Table 130 below. Paraquat produced a dose dependent decrease in the level of both albumin and total bilirubin, especially at the highest dose. A statistically significant decrease in BUN and serum creatinine levels, but not the total plasma protein level, was observed only at the paraquat dose of 6.0 mg/kg day.

Table 130: The effects of paraquat given subcutaneously at various doses for 7 days on the body weight and blood chemistry of male Wistar rats

Parameters	Paraquat (mg/kg bw/d)			
	0 (10)	4.0 (10)	5.0 (9)	6.0 (9)
BW gain (g)	38.55 ± 1.51	36.38 ± 1.71	25.78 ± 7.01*	12.15 ± 3.68***
AST (U/L)	77.10 ± 4.47	71.00 ± 3.96	71.22 ± 4.64	55.33 ± 3.28***
ALT (U/L)	26.30 ± 1.33	24.20 ± 0.89	25.56 ± 1.22	23.78 ± 2.13
ALP (U/L)	156.0 ± 5.47	161.0 ± 6.63	157.6 ± 5.57	144.3 ± 8.16
Total bilirubin (mg/dl)	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01*
Albumin (mg/dl)	4.10 ± 0.11	3.85 ± 0.11	3.72 ± 0.14*	3.33 ± 0.12***
Total protein (g/dl)	6.34 ± 0.19	6.41 ± 0.19	6.54 ± 0.18	6.72 ± 0.13
BUN (mg/dl)	19.00 ± 1.05	17.96 ± 0.51	17.87 ± 0.22	16.51 ± 0.45*
Creatinine (mg/dl)	0.52 + 0.01	0.52 ± 0.01	0.50 ± 0.01	0.43 ± 0.01***

Paraquat was subcutaneously injected at the doses of 4.0, 5.0, and 6.0 mg/kg/day, once daily, for seven consecutive days. Values are mean ± SEM. The number in the parenthesis indicates number of animals.
* $p < 0.05$, *** $p < 0.001$ when compared with control.

Low dose PQ given up to 10 days did not affect BUN, creatinine, total protein, total bilirubin, and plasma albumin. The other effects are shown in the Table 131 below.

Table 131: The time-course of effect of PQ on the body weight gain and plasma chemistry of male Wistar rats injected subcutaneously with 4.0 mg/kg bw/d

Parameters	Group	PQ treatment period (days)		
		3	7	10
BW gain (g)	Control	15.00 ± 1.26	42.39 ± 1.13	67.70 ± 7.80
	Treated	14.85 ± 0.29	37.29 ± 1.57	24.40 ± 13.02*
AST (U/L)	Control	80.50 ± .50	71.25 ± 4.11	76.25 ± 1.80
	Treated	82.75 + 2.75	76.75 + 5.17	59.33 + 3.28**
ALT (U/L)	Control	27.00 + 2.35	26.00 + 0.71	25.50 ± 1.04
	Treated	28.75 + 2.87	24.25 ± 1.44	19.00 + 2.31*
ALP (U/L)	Control	171.3 ± 8.86	157.8 + 2.87	176.0 + 6.52
	Treated	165.5 + 4.41	147.0 + 6.34	153.7 + 12.6

Paraquat was subcutaneously injected at the dose of 4.0 mg/kg/day, once daily, for up to 10 days and sacrificed during treatment and on day 10. Values are mean ± SEM from 4 animals.
* $p < 0.05$ and ** $p < 0.001$ when compared with its control.

Effect of paraquat on gross and cell morphology of the lung and the liver: Treatment for 7 days resulted in extensive pulmonary hemorrhage, thickening of alveolar septum as well as infiltration of inflammatory cells but no pulmonary fibrosis was observed. The hepatocytes of the control and treated groups were still intact, though slight atrophy of the hepatocytes around the central vein was observed. No swelling, dilation, infiltration, or proliferation and degenerative changes of bile ductule epithelia in the portal area were noted. Necrosis was not seen in the liver of paraquat treated rats.

Effect of paraquat on xenobiotic-metabolizing enzyme activities: Significant decrease in the ethoxyresorufin-*O*-deethylase activity, the CYP1A1 probe, was found in both paraquat 5.0 and

6.0 mg/kg bw/d treated groups as shown in Table 132 below. There was no change in the activities of p-nitrophenol hydroxylase and erythromycin-N- demethylase, the CYP2E1 and 3A4 probes, respectively, as compared to those of the control group. Paraquat seemed to dose-dependently reduce the hepatic microsomal protein content.

Table 132: Effect of multi-low dose subcutaneous administration of PQ on the hepatic CYP I AI, CYP2E1, and CYP3A4 activities

Parameter	PQ (mg/kg bw/d)			
	0	4.0	5.0	6.0
EROD	0.11+ 0.01	0.10± 0.01	0.05+ 0.01***	0.03+ 0.00***
PNPH	5.07+ 0.21	4.72+ 0.22	5.09+ 0.46	5.87± 0.40
ERY	1.09+ 0.06	1.29+ 0.07	1.03+ 0.04	1.11+ 0.07
Microsomal protein	19.13+ 0.65	16.29+ 0.83*	17.42± 0.60	14.73± 0.73***

PQ was subcutaneously injected, once daily, for seven consecutive days. Values are mean ± SEM from eight animals. The asterisks, * and ***, represent p-value of less than 0.05 and 0.005, respectively, when compared with control. Unit of ethoxyresorufin-O-deethylation (EROD)= nmole of resorufin formed/mg protein/min. Unit of p-nitrophenol hydroxylation (PNPH)= nmole of 4-nitrocatechol formed/mg protein/min. Unit of erythromycin-N-demethylase (ERY)= nmole of formaldehyde formed/mg protein/min Unit of microsomal protein content = mg/g wet liver weight.

Bioaccumulation of paraquat in tissues: The means % recovery of internal standard spiked in the biological specimens after solid-phase extraction were higher than 90%. Except for the plasma, paraquat accumulation in the lung and liver of rats treated for 7 days was dose related with the correlation coefficient (R^2) in a range of 0.90-0.99. Very high paraquat concentrations (2.00-2.25 µg of paraquat ion/g tissue) were detected in the lung compared with that of the liver. At the highest dose, paraquat concentration in the liver was only 8% of that detected in the lung.

Comparison of the concentration-time profiles of paraquat in the plasma, lung, and liver after a single and repeat-dose: There was no difference in the plasma concentration-time profiles of paraquat after single dose and repeat-dose exposures at any time point indicating a nearly complete paraquat elimination from the plasma within 24 h after each dose. In contrast lung and liver paraquat concentrations after repeat-dose paraquat exposure were much higher than those obtained from a single dose at all times. Paraquat concentrations in the lung and liver at 24 h post-dose were approximately two to three-fold higher than a single exposure.

Liver tissue in rats given a single dose of 6.0 mg/kg bw showed a paraquat concentration of 0.1 µg paraquat ion/g wet weight at 24 h post dosing compared to 0.25 µg paraquat ion/g wet weight at the same interval post dosing for rats that were given 6.0 mg/kg bw/d sc for 7 days. The respective concentrations for lung tissue were 1.2 µg paraquat ion/g wet weight and 2.6 µg paraquat ion/g wet weight.

Conclusion: It was apparent that repeat low dose sc injections of paraquat daily for up to seven days may affect liver function.

With these dosing regimes, plasma paraquat concentration did not indicate the degree of exposure and severity of paraquat toxicity.

Fletcher K & Wyatt I (1970) The composition of lung lipids after poisoning with paraquat. *Br J Exp Path 51: 604-610.*

Adult female SPF Wistar rats (Alderley Park strain, 175-230 g body weight, source unspecified) received an aqueous po dose of paraquat (unspecified purity, batch no. & source) at 0 or 125 mg/kg bw (equivalent to 90 mg/kg bw paraquat cation; n=4). Rats were maintained on a standard commercial diet (unspecified source) and sacrificed 'with a blow on the head' at 2 or 6 days after dosing. Lipids were extracted from isolated peritoneal and pulmonary macrophages, in addition to homogenised lungs, and analysed for phospholipids, neutral lipids, total fatty acids, gangliosides, cholesterol, glycerol and phosphates.

There was no treatment-related effect on the proportion of myristic, palmitic, stearic, oleic and linoleic acid in rat lung tissue. Arachidonic acid was elevated in both the neutral and total lipid fractions (16.6 $\pm$ 7.4 and 26.1 $\pm$ 6.1% respectively) 6 days after paraquat treatment, relative to the controls (2.0 $\pm$ 1.4 and 17.1 $\pm$ 0.7% respectively). In the absence of statistical analysis the significance of this finding was unclear. There was no treatment-related effect on total lung lipids, total lung phosphorus or lung phospholipids (phosphatidyl choline, phosphatidyl ethanolamine, phosphotidyl inositol, phosphatidal ethanolamine, phosphatidal choline, phosphatidal inositol, sphingomyelin). There was no treatment-related effect on neutral lipids (total cholesterol, gangliosides and glyceride) with the exception of ester cholesterol which was significantly elevated ($p<0.01$, statistical test unspecified) 6 days after dosing with paraquat (12.0 $\pm$ 2.6 %) relative to the control (4.1 $\pm$ 2.6 %).

Data were presented illustrating that the fatty acid and lipid composition of alveolar and peritoneal macrophages, isolated from 'normal' rats, differed from that of paraquat-treated rats, however the relevance of these experiments and their findings to the mechanism of paraquat toxicity was unclear. The main limitation to this study was the absence of statistical analyses.

Popenoe D (1979) Effects of paraquat aerosols on mouse lung. *Arch Pathol Lab Med 103: 331-334.*

An unspecified number of male BALB/c mice (2-3 months old, unspecified body weight & source) were exposed to aerosols of a 12 mg/mL paraquat dichloride solution (equivalent to 8.64 mg/mL paraquat cation; unspecified solvent, purity, batch no. & source) for 15 minutes at an unspecified temperature and humidity. An unspecified number of control rats were also tested. Aerosols were generated using an ultrasonic nebuliser with the exposure chamber consisting of a cylindrical tube (unspecified dimensions) with a small hole at one end so that only the nose of the mice were exposed to the aerosols. The air flow, particle size, shape and distribution were unspecified. Ten mice were housed per metal cage (unspecified source), with mouse chow and water (unspecified sources) available *ad libitum*. At 6 h, 1, 3, 7, 14 and 28 days after exposure, four paraquat-treated mice and one control mouse were sacrificed with an overdose of sodium pentobarbital and their lungs removed. For light microscopy, lungs were fixed with glutaraldehyde, embedded in paraffin, sectioned then stained with haematoxylin-eosin-azure II and Mallory's trichome. For electron microscopy, lungs were also fixed with glutaraldehyde, cut into small pieces, dehydrated, embedded in resin, sectioned, stained with uranyl acetate and alkaline bismuth subnitrate then examined with a Phillips EM 300 electron microscope.

All mice were reported to show signs of paraquat intoxication 24 h after exposure (lethargy, ruffled hair, ataxia, laboured breathing). The study author reported a 10% loss of body weight during the first week, however no data were provided to substantiate this finding. The study author also reported that 30% of mice not sacrificed by day 3 had died by day 7. All remaining mice appeared asymptomatic by week 2. Over the first 3 days following exposure, there was a time-related increase in microscopic lung damage (specifically necrosis) which was proceeded by a period of cellular proliferation and regeneration. Despite the decrease in lung lesions and regeneration of the epithelium, collagen deposition increased over the remaining 2 weeks so that by 28 days extensive pulmonary fibrosis was evident. The study author indicated that the initial destruction of the bronchial epithelium followed by its regeneration had not previously been observed following systemic administration, and was possibly due to paraquat reaching the lungs via the expired air rather than by the blood. The regulatory value of this study was limited due to the absence of methodological detail and quantitative observational data.

Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML & Carvalho F (2008) Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. ***Crit Rev Toxicol. 38(1):13-71. Review.***

Independently of the route of administration, the lung and the kidney are the organs showing the highest concentrations of paraquat (Murray & Gibson, 1972; Sharp *et al*, 1972; Ilett *et al*, 1974). Paraquat pulmonary concentrations can be 6-10 times higher than those in the plasma and the compound is retained in the lung even when blood levels start to decrease. Rose *et al* (1976) showed that lung was able to accumulate paraquat against a concentration gradient and that the mechanism of uptake is an adenosine triphosphate (ATP)-driven process which exhibited saturation kinetics. Paraquat is neither metabolised by the lung nor becomes covalently bound to any degree and it is apparent that its accumulation is mediated through binding to and subsequently translocating into cells by a carrier system. This involves the participation of the polyamine transport system abundantly expressed in the membrane of alveolar cells of type I, II, and Clara cells. The main molecular mechanism of paraquat toxicity involves redox cycling and intracellular oxidative stress.

Lock EA & Ishmael J (1979) The acute toxic effects of paraquat and diquat on the rat kidney. ***Toxicol Appl Pharmacol 50: 67-76.***

Materials & Methods: Groups of 5-33 fasted male Alderley Park (Wistar-derived) albino rats (150-180 g, age & source unspecified) received an LD_{50} dose of paraquat (680 μmol/kg bw, po; 108 μmol/kg bw, sc; equivalent to 127 and 20 mg/kg bw paraquat, or 91 and 14.4 mg/kg bw paraquat cation, respectively), or a po equimolar dose or LD_{50} of diquat (680 and 900 μmol/kg bw respectively). Paraquat and diquat (purity unspecified) were dissolved in saline and administered in a dose volume of 5 mL/kg bw. Control rats were dosed with saline and positive controls received $HgCl_2$ (7.4 μmol/kg, sc) at the same dose volume. Water was available *ad libitum*.

Rats were housed in individual metabolism cages and urine collected at 6 and 24 h for analysis of the following parameters: urinary flow, total protein, albumin, glucose, spun cell counts, ALP and β–D-glucosaminidase. Rats were sacrificed by an overdose of halothane and blood

collected by cardiac puncture for measurement of plasma urea. Kidneys were subject to histopathological examination.

Excretion studies were performed by administering [methyl-^{14}C]paraquat (30 mCi/mmol, unspecified purity) or [ethylene-^{14}C]diquat (29 mCi/mmol, unspecified purity) to an rats at 0 or 50 μCi/kg bw (n=5) via an unspecified route. The urinary concentration of radiolabelled paraquat and diquat was measured at 6 and 24 h.

Renal p-aminohippurate (PAH) and N'-methylnicotinamide (NMN) accumulation was measured both in an *in vitro* study where rat renal cortical slices were incubated in a medium with p-amine[^{3}H]hippuric acid or N'methyl[^{14}C]nicotinamide) and paraquat or diquat, in addition to an *in vitro* component on samples from rats treated with paraquat (680 μmol/kg bw, po; equivalent to 91 mg/kg bw paraquat cation) or diquat (900 μmol/kg bw, po). Results were expressed as the ratio of the radioactivity in the slice:medium.

$^{14}CO_2$ production (ie glucose oxidation) and O_2 consumption were measured in renal cortical slices in a respirometer where any $^{14}CO_2$ generated was trapped on filter paper and quantified by scintillation counting. This was conducted on samples incubated in a medium *in vitro* with [1^{14}C] glucose or [6^{14}C]glucose and 0, 0.1 or 1 mM paraquat or diquat. Positive controls were incubated with phenazine methosulphate. An additional experiment was performed using renal cortical slices from rats that had been treated with paraquat (680 μmol/kg bw, PO; equivalent to 91 mg/kg bw paraquat cation) for 0, 17 and 24 h.

[U-^{14}C]acetate incorporation into fatty acids (ie fatty acid synthesis) was measured in rat renal cortical slices following incubation in medium with [U-^{14}C]acetate and 0, 0.1 or 1 mM paraquat or 0, 0.01, 0.1 or 1 mM diquat. Positive controls were incubated with phenazine methosulphate. Fatty acids were extracted using the method of Gould *et al* (1953) and the amount of radioactivity measured by scintillation counting. An additional experiment was performed using renal cortical slices from rats that had been treated with paraquat (680 μmol/kg bw, PO; equivalent to 91 mg/kg bw paraquat cation) or diquat (900 μmol/kg bw) for 24 h.

Statistical differences were evaluated using a Student's t test. Results were considered to be statistically significant when $p<0.05$.

Results & Conclusions: The presence/absence of mortalities or clinical signs were unreported. Over the first 6 h, rats administered paraquat sc at 108 μmol/kg bw (14.4 mg/kg bw paraquat cation) showed a statistically significantly increased urine flow compared to the control group (see Table 133 below). All other groups were comparable to controls for this period, however for the 6-24 h period, all treatment groups had statistically significant increased urine flow. As the magnitude of this difference was not large, and the control group had a decreased urinary flow, this is not considered treatment related.

Urinary protein was significantly elevated in diquat-treated rats over the first 6 h while both paraquat- and diquat-treated rats had significantly elevated urinary protein over 6-24 h. The positive control group ($HgCl_2$) also showed a marked elevation in urinary protein over 6-24 h which was more than 2-fold greater than that observed in paraquat- and diquat-treated rats.

Urinary albumin was significantly elevated in rats given a sc injection of paraquat (108 μmol/kg bw) or an po dose of diquat (680 μmol/kg bw) over the first 6 h with the magnitude of this effect increasing considerably over the remaining 6-24 hour period. Urinary albumin was also significantly elevated in rats given the po dose of paraquat (680 μmol/kg bw) or $HgCl_2$ over 6-24 h, with the effect of $HgCl_2$ approximately 2-5-fold greater than that of either paraquat or diquat.

Glucose excretion was significantly elevated over 0-6 h in rats given a sc injection of paraquat (108 μmol/kg bw), and in paraquat-, diquat and $HgCl_2$-treated rats over 6-24 h. The magnitude of the effect seen with $HgCl_2$ was up to 3-fold greater than that with either paraquat or diquat.

There was an obvious increase in the number of cells shed in the urine 6-24 h after administration of paraquat, diquat or $HgCl_2$, however these results did not appear to be statistically significant due possibly to the large variability of the data.

There were no effects on urinary ALP or β–D-glucosaminidase activities following treatment with paraquat, diquat or $HgCl_2$ over the first 6 hour period. Over the 6-24 h period, rats given the po dose of paraquat had significantly higher levels of β–D-glucosaminidase activity (246 nmol 4-methylumbelliferone formed/h, n =10) than the control rats. For this same period, rats treated with the positive control ($HgCl_2$) showed a significant elevation in urinary ALP and β–D-glucosaminidase activities (767 and 464 nmol 4-methylumbelliferone formed/h respectively, n=6) relative to their corresponding controls (81 and 110 nmol 4-methylumbelliferone formed/h respectively, n=22 or 23). For this same period, there was no effect of diquat or the sc dose of paraquat on either parameter.

Table 133: Effect of paraquat or diquat on rat kidney function

Parameter	Treatment				
	Control	**PQ 680 µmol/kg bw, po (91 mg PQ cation/kg bw)**	**PQ 108 µmol/kg bw, sc (14.4 mg PQ cation/kg bw)**	**DQ 680 µmol/kg bw, po (91 mg PQ cation/kg bw)**	**$HgCl_2$ 7.4 µmol/kg bw, sc**
Urine flow (mL/h)					
0-6 h	0.31 ± 0.03	0.27 ± 0.03	0.99 ± 0.07*	0.30 ± 0.07	0.32 ± 0.03
6-24 h	0.24 ± 0.02	0.39 ± 0.03*	0.35 ± 0.03*	0.31 ± 0.06	0.33 ± 0.02*
Urinary protein (µg/h)					
0-6 h	170 ± 15	145 ± 42	270 ± 58	404 ± 79*	145 ± 18
6-24 h	154 ± 18	559 ± 65*	310 ± 45*	435 ± 64*	1158 ± 214*
Urinary albumin (µg/h)					
0-6 h	38 ± 4	46 ± 6	86 ± 12*	62 ± 13*	36 ± 6
6-24 h	48 ± 3	484 ± 39*	260 ± 50*	168 ± 35*	1060 ± 135**
Urinary glucose (µg/h)					
0-6 h	0.09 ± 0.01	0.11 ± 0.02	0.22 ± 0.03*	0.10 ± 0.01	0.06 ± 0.01
6-24 h	0.13 ± 0.01	6.95 ± 0.83*	4.80 ± 1.20*	3.05 ± 1.7*	10.88 ± 3.4*
Excreted cells‡ (cells/h x 10-3)					
0-6 h	0.39 (0-2.8)	-	5.0 (0-10.3)	1.0 (0-2.0)	0.3 (0-0.9)
6-24 h	1.2 (0-5.5)	26.5 (19-38.9)	12.0 (9.7-14.0)	13.4 (4.6-47.5)	103.0 (42-143)
Plasma urea (mM)					
6-24 h	9.9 ± 0.1	17.2 ± 1.1*	21.3 ± 1.5*	10.5 ± 1.4*	26.4 ± 2.3*
% of administered dose excreted in urine					
0-6 h	-	3.0 ± 0.5	91.0 + 3.1	1.3 ± 0.2	-
6-24 h	-	7.7 ± 0.9	9.6 + 0.7	6.2 ± 1.2	-

Results are expressed as means ± 1 SEM; PQ = paraquat; DQ = diquat; * $p<0.05$; ‡ = range given in parentheses.

Plasma urea levels were significantly elevated relative to the control following treatment with paraquat, diquat or $HgCl_2$ for 24 h (see Table 133 above).

After po administration, approximately 10% of paraquat and 7% of diquat was excreted by the kidneys over 24 h. In contrast, virtually all of a sc dose of paraquat was excreted via the urine over 24 h, with the majority (~90%) excreted over the first 6 h.

Hydropic degeneration in proximal tubules was reported in the majority of rats treated orally with paraquat or diquat, and inconsistently in rats dosed by sc injection ofparaquat, although no data were provided to substantiate these findings. The positive control ($HgCl_2$) was reported to cause extensive proximal tubular necrosis.

Paraquat and diquat had no significant effect on the accumulation of PAH either when sections were incubated directly with the test substances or rats were treated *in vivo.* Although *in vivo* treatment of rats with paraquat or diquat had no effect on the subsequent accumulation of NMN by renal cortical slices, direct incubation of cortical slices with either test substance caused a significant reduction in its accumulation (3.65 and 2.04 slice:medium ratio, respectively; n=4 or 10) relative to the control (4.44 slice:medium ratio; n=20).

Paraquat or diquat had no significant effect on O_2 consumption by renal cortical slices when either compound was incubated directly with the slices, or following *in vivo* treatment of rats

with paraquat. In contrast, phenazine methosulfonate caused a significant reduction in O_2 consumption relative to the control [133 mol/h/g wet wt (n=27) versus 149 mol/h/g wet wt (n=37)].

Oxidation of [1-^{14}C]glucose (ie generation of $^{14}CO_2$) by renal cortical slices was significantly elevated ($p<0.05$) when slices were incubated directly with 1 mM paraquat (651 dpm $^{14}CO_2$/mg wet wt, n=10), 0.1 or 1 mM diquat (623 and 743 dpm $^{14}CO_2$/mg wet wt, n=7 and 3 respectively), or 0.1 mM phenazine methosulfate (1306 dpm $^{14}CO_2$/mg wet wt, n=11) compared to the control (480 dpm $^{14}CO_2$/mg wet wt, n=31). Paraquat or diquat had no significant effect on the oxidation of [6-^{14}C]glucose while 0.1 mM phenazine methosulfate significantly elevated ($p<0.05$) oxidation (759 dpm $^{14}CO_2$/mg wet wt, n=8) relative to the control (454 dpm $^{14}CO_2$/mg wet wt, n=28). In contrast, no effect on the oxidation of 1- or 6-^{14}C-glucose by renal cortical slices occurred following *in vivo* treatment of rats with paraquat.

There was a concentration-related inhibition (up to 66%) of fatty acid synthesis (ie ^{14}C-acetate incorporation) when renal cortical slices were incubated with paraquat or diquat (see Table 134 below). Phenazine methosulphate (0.1 mM) also caused a significant inhibition of fatty acid synthesis, the magnitude of which was greater than that seen with paraquat or diquat. No significant effect on fatty acid synthesis by renal cortical slices was observed following *in vivo* treatment of rats with paraquat or diquat.

Table 134: Effect of paraquat and diquat on fatty acid synthesis by rat renal cortical slices

Treatment	Fatty Acid Synthesis [U-^{14}C]acetate incorporation into fatty acid (dpm/mg fatty acid)	% Inhibition
Control	1052 ± 59 (n=22)	0
Paraquat 0.1 mM	874 ± 61 (n =12)	17
Paraquat 1 mM	362 ± 26* (n =12)	66
Diquat 0.01 mM	1256 ± 153 (n =12)	0
Diquat 0.1 mM	661 ± 120* (n =12)	37
Diquat 1 mM	643 ± 26* (n =12)	39
Phenazine methosulphate 0.1 mM	299 ± 47* (n =12)	72

Results are expressed as means ± 1 SEM; * $p<0.01$

In summary, po or sc administration of paraquat (680 or 108 μmol/kg bw respectively; equivalent to 91 and 14.4 mg/kg bw paraquat cation respectively) or po administration of diquat (680 μmol/kg bw) caused renal damage in rats as shown by the significant increase in urinary glucose, protein and albumin, increased urinary shedding of renal cells, and plasma urea. Mild focal hydropic degeneration in proximal tubules was also reported following administration of paraquat or diquat. Paraquat (680 μmol/kg bw, po) also caused an increase in urinary β–D-glucosaminidase activity. Paraquat and diquat were shown to inhibit the accumulation of N'-methylnicotinamide, to inhibit glucose oxidation and fatty acid synthesis in renal cortical slices. However none of these later effects were observed in cortical slices that were isolated from rats that had been treated *in vivo* with paraquat or diquat. As the magnitude of all of these effects was markedly lower than that seen with $HgCl_2$, the study authors concluded that paraquat and diquat had only mild renal damaging effects.

Although faeces were apparently collected, no experimental measurements were performed on them. Uneven sample sizes were used in the majority of experiments. No histopathological data were provided.

Lock EA (1979) The effect of paraquat and diquat on renal function in the rat. *Toxicol Appl Pharmacol 48: 327-336.*

Materials & Methods: Fasted (16 or 40 h) male Alderley Park (Wistar-derived) albino rats (150-180 g, age & source unspecified) received an LD_{50} of paraquat (680 μmol/kg bw, po; 108 μmol/kg bw, sc; equivalent to 127 and 20 mg/kg bw paraquat, or 91 and 14.4 mg/kg bw paraquat cation, respectively), or a po equimolar dose or LD_{50} of diquat (680 and 900 μmol/kg bw respectively). In one experiment rats were dose orally with 540 μmol/kg bw diquat. Paraquat and diquat (purity unspecified) were dissolved in saline and administered in a dose volume of 5 mL/kg bw. Controls received an equivalent dose of saline. Water was available *ad libitum*. Food was available prior to some experiments.

Renal clearance was measured according to the method of Dicker and Heller (1945). At various times up to 24 h after dosing, rats were injected with inulin (5% w/v, sc) at 0.5 mL/100 g bw and 1 μCi/mL [hydroxymethyl-^{14}C]inulin (10.9 mCi/mmol) or [^{3}H]inulin (900 mCi/mmol) in aqueous gelatin (32% w/v). In some experiments, 5% w/v p-amino[^{3}H]hippuric acid (PHA) , 2 μCi/mL N'-methyl[^{14}C]nicotinamide (NMN) at 1.5% w/v and 0.5 μCi/mL, [methyl-^{14}C]paraquat at 0.04% w/v and 5 μCi/mL, or [ethylene-^{14}C]diquat at 0.04% (w/v) and 2 μCi/mL were also administered sc. All rats were pre-dosed with water to ensure ensure adequate urine flow prior to commencement of the study.. Following dosing, rats were housed individually in metabolism cages for one hour and all urine collected. Urinary volume, urea and radioactivity were measured. Rats were sacrificed with an overdose of halothane, blood collected and plasma urea and radioactivity measured.

Twenty-four hours after the administration of paraquat or diquat, rats (n=5-29) were injected iv with [^{125}I]-plasma and [^{51}Cr]dichromate and sacrificed after 5 minutes with halothane. The renal veins and arteries were rapidly clamped and blood collected by cardiac puncture. Radioactivity was measured in samples of blood, injected material and kidneys by γ-counting. Hct was also measured. Whole animal and kidney plasma and red cell volumes were calculated based on the dilution of injected material after correction for the volume of injected material. F_{cells} was calculated by dividing the whole body Hct by the large vessel Hct. The total weight of both kidneys was also recorded.

Rats (n=6) were dosed with paraquat, placed in individual metabolism cages without food or water for 24 h, and urine and faeces collected. Rats were sacrificed with an overdose of halothane, blood collected by cardiac puncture and the following organs collected: GIT, liver, muscle, skin, kidney and lung. Tissue water content (weight loss on drying to a constant weight at 105°C) was measured in each of these organs as well as in the blood.

Statistical differences were determined using a Student's t test. Results were considered to be statistically significant when $p<0.05$.

Results & Conclusions: There was a clear dose-related increase in plasma paraquat concentrations and excretion rates following treatment of rats with increased doses of [methyl-

^{14}C]paraquat (see Table 135 below). Renal clearance of [methyl-^{14}C]paraquat and [^{3}H]inulin were constant over all doses with paraquat being cleared at a slightly higher rate than inulin (means of 1.18-1.38 mL/min/100 g bw for paraquat compared to 0.92-1.07 mL/min/100 g bw for inulin). Based on these later observations the study author concluded that paraquat was slightly actively secreted. There was no treatment-related effect on Hct while a significant increase in plasma urea occurred at the highest dose (6.7 µmol/mL) relative to the lowest dose (5.2 µmol/mL). A significant depression in urine flow also occurred only at the highest dose (0.005 mL/min/100 g bw) relative to the lowest dose (0.020 mL/min/100 g bw).

Table 135: Effect of increased doses of paraquat on plasma paraquat concentrations and paraquat excretion

Dose (µmol/kg bw)	**11**	**27**	**54**	**108**	**269**
Plasma PQ (nmol/mL)	2.6 ± 0.4	6.6 ± 1.5	14.0 ± 1.7	27.6 ± 7.6	70.5 ± 6.4
PQ Excretion rate (nmol/min)	3.4 ± 0.2	7.3 ± 1.4	16.5 ± 1.4	34.6 ± 6.7	102.2 ± 14.1

Results are expressed as means ± 1 SEM; PQ = paraquat

In rats that had been feed *ad libitum*, there was no significant difference in plasma urea, renal clearance of urea, [methyl-^{14}C]paraquat and [^{3}H]inulin, or in Hct, between paraquat-treated and control rats (see Table 136 below). Urine flow in paraquat-treated rats was significantly higher than that of the controls.

Fasting caused control rats to excrete significantly less urea and [^{14}C]-paraquat ($p<0.05$), and significantly increased Hct and plasma urea compared to the fed control rats. Twenty-four hours after a single sc injection of paraquat (108 µmol/kg bw, sc) fasted rats exhibited a significant elevation in plasma urea and Hct relative to the fasted control group. Additionally, fasted paraquat-treated rats showed significant reductions in the renal clearance of urea, [^{14}C]-inulin and [^{14}C]-paraquat, while urine flow was unaffected. These results suggested that fasting increased the renal toxicity of paraquat.

Table 136. Effect of fasting on renal clearance 24 h after paraquat treatment (108 µmol/kg bw, sc)

Treatment	**Control (fed)**	**PQ (fed)**	**Control (fasted)**	**PQ (fasted)**
Plasma Urea (µmol/mL)	6.4 ± 0.1 (43)	6.7 ± 0.3 (19)	9.6 ± 0.3 (42) *	21.3 ± 1.5 (28) **
Urea Clearance (mL/min/100 g bw)	0.29 ± 0.01 (38)	0.31 ± 0.02 (14)	0.26 ± 0.01 (36) *	0.13 ± 0.02 (14) **
^{14}C-Inulin Clearance (mL/min/100 g bw)	0.99 ± 0.07 (39)	0.94 ± 0.06 (14)	0.96 ± 0.06 (21)	0.41 ± 0.05 (15) **
^{14}C- PQ Clearance (mL/min/100 g bw)	1.26 ± 0.04 (40)	1.06 ± 0.10 (5)	0.88 ± 0.05 (15) *	0.40 ± 0.07 (5) **
Urine Flow (mL/min/100 g bw)	0.015 ± 0.001 (37)	0.022 ± 0.002 (14) *	0.018 ± 0.001 (41)	0.015 ± 0.002 (16)
Hct (%)	47.5 ± 0.4 (38)	49.3 ± 0.5 (13)	52.5 ± 0.5 (35) *	57.1 ± 0.8 (15) **

PQ = paraquat; results are expressed as means ± 1 SEM; sample sizes are shown in parentheses; * statistically different to fed control ($p<0.05$); ** statistically different to the fasted control ($p<0.05$).

Rats given a single po dose of paraquat (680 µmol/kg bw) showed a statistically significant time-related increase in plasma urea from 17 h (see Table 137 below). A time-related decrease in urea and inulin clearance, and in urine flow was also observed in paraquat-treated rats, with

the results statistically significant from 12 or 17 h after dosing. Hct was significantly elevated from 12 h post-dose but this effect did not increase over time. These observations indicated that paraquat caused a time-related decrease in renal function (ie 24 h).

Table 137: Renal function at various times after dosing with paraquat (680 μmol/kg bw, po~ 91 mg paraquat ion/kg bw)

Time after dosing (h)	0	8	12	17	24
Plasma Urea (μmol/mL)	7.5 ± 0.3 (39)	5.5 ± 0.3 * (5)	7.3 ± 0.8 (5)	17.7 ± 2.1 * (11)	21.8 ± 1.7 * (28)
Urea Clearance (mL/min/100 g bw)	0.38 ± 0.02 (31)	0.33 ± 0.04 (4)	0.29 ± 0.04 (5)	0.10 ± 0.02 * (11)	0.04 ± 0.01 * (14)
Inulin Clearance (mL/min/100 g bw)	0.99 ± 0.05 (27)	0.94 ± 0.04 (4)	0.90 ± 0.15 (4)	0.41 ± 0.11 (7)	0.21 ± 0.05 (19)
Urine Flow (mL/min/100 g bw)	0.03 ± 0.02 (24)	0.024 ± 0.007 (4)	0.017 ± 0.002 * (5)	0.010 ± 0.002 * (7)	0.004 ± 0.001 * (14)
Hct (%)	50.9 ± 0.6 (24)	50.0 ± 0.7 (5)	56.6 ± 1.5 * (5)	55.0 ± 0.7 * (7)	56.4 ± 0.4 * (15)

Results are expressed as means ± 1 SEM (sample sizes shown in parentheses); * $p<0.05$

Treatment of rats with either paraquat (680 μmol/kg bw, po) or diquat (540 μmol/kg bw, po) caused a significant reduction in the renal clearance of urea and radiolabelled inulin, PAH, NMN, paraquat and diquat (see Table 138 below). There were only marginal differences in the clearance of these compounds between paraquat- and diquat-treated rats. Urine flow was also significantly decreased in paraquat- and diquat-treated rats, and filtration fraction (clearance of inulin/clearance of PAH) was increased in diquat-treated rats only.

Table 138: Effect of paraquat or diquat on renal clearance in rats

Parameter	Clearance (mL/min/100 g bw)		
	Control	PQ (680 μmol/kg bw, po)	DQ (540 μmol/kg bw, po)
Urea	0.26 ± 0.01 (49)	0.05 ± 0.01 (12) *	0.10 + 0.03 (10) *
Inulin	0.96 ± 0.04 (33)	0.36 ± 0.09 (6) *	0.54 + 0.12 (6) *
PAH	2.97 ± 0.19 (16)	1.14 ± 0.26 (6) *	0.85 + 0.39 (4) *
NMN	2.57 ± 0.26 (17)	0.87 ± 0.33 (6) *	0.44 + 0.16 (4) *
PQ	1.14 ± 0.07 (15)	0.40 ± 0.10 (6) *	-
DQ	1.14 ± 0.11 (15)	-	0.57 + 0.14 (6) *
Urine Flow (mL/min/100 g bw)	0.019 ± 0.001 (56)	0.003 ± 0.001 (12) *	0.004 ± 0.001 (12) *
Filtration fraction	0.32	0.35	0.64

Results expressed as means ± 1 SEM (sample sizes shown in parentheses); PQ = paraquat; DQ = diquat; PAH = p-aminohippurate; NMN = N'-methylnicotinamide; * $p<0.05$

Red cell volumes, F_{cells}, kidney weights and renal blood volume were unaffected by treatment with either paraquat or diquat. The plasma volume of rats treated orally with diquat or sc with paraquat were significantly lower than the control (see Table 139 below). Hct was significantly elevated following paraquat or diquat administration and this was likely due to the concomitant reduction in blood volume. Renal plasma volume was significantly reduced only in rats given a po dose of 900 μmol/kg bw diquat.

Table 139: Effect of paraquat and diquat on plasma and red cell volumes

Treatment	Plasma Volume (mL/100 g bw)	Hct (%)	mL plasma/g kidney
Control	3.91 ± 0.07 (27)	52.4 ± 0.3 (27)	0.119 + 0.004 (26)
PQ, 680 µmol/kg bw, PO	3.66 ± 0.12 (5)	54.2 ± 0.8 (5) *	0.121 + 0.003 (5)
PQ, 108 µmol/kg bw, sc	3.34 ± 0.15 (9) *	57.2 ± 1.1 (9) *	0.119 + 0.013 (9)
DQ, 680 µmol/kg bw, PO	3.38 ± 0.08 (5) *	54.0 ± 0.4 (5) *	0.107 + 0.007 (5)
DQ, 900 µmol/kg bw, PO	3.12 ± 0.22 (5) *	58.7 ± 1.5 (5) *	0.098 + 0.005 (5) *

Results expressed as means ± 1 SEM (sample sizes shown in parentheses); PQ = paraquat; DQ = diquat;

Paraquat had no significant effect on the water content of the muscle, skin and kidney. When administered either sc or orally, paraquat caused a significant dehydration ($p<0.05$) of the blood and lung, and significantly increased urinary volume (see Table 140 below). Significant liver dehydration also occurred in rats that had been dosed orally with paraquat. The effect of paraquat on the water content of the GIT appeared to depend on the administration route, with significant dehydration occurring in rats dosed sc, and significantly increased fluid observed in rats dosed orally.

Table 140: Effect of paraquat on the water content of organs

	Water content (g H_2O/g dry weight)		
	Control	PQ, 108 µmol/kg bw, sc (14.4 mg paraquat ion/kg bw)	PQ, 680 µmol/kg bw, PO (91 mg paraquat ion/kg bw)
GIT	3.58 ± 0.12	3.31 ± 0.04 *	5.89 ± 0.26 *
Blood	3.64 ± 0.04	2.90 ± 0.05 *	3.18 ± 0.08 *
Liver	2.40 ± 0.02	2.32 ± 0.06	2.27 ± 0.03 *
Lung	3.62 ± 0.03	3.22 ± 0.11 *	3.17 ± 0.07 *
Urine volume (mL/24 h)	4.0 ± 0.6	13.2 ± 0.9 *	7.6 ± 0.4 *

Results expressed as means ± 1 SEM; * $p<0.05$

In summary, this study showed that a single dose of paraquat (680 µmol/kg bw, po or 108 µmol/kg bw, sc) or diquat (540, 680 or 900 µmol/kg bw, po) adversely affected renal function in rats. This was evidenced by the following statistically significant ($p<0.5$) effects: reduced excretion of inulin, urea, p-aminohippurate and N'-methylnicotinamide; decreased urine flow; increased plasma urea; decreased plasma volume; increased Hct. The increase in Hct was likely due to the reduced plasma volume. Paraquat was also shown to dehydrate the blood, liver and lung when given sc, while the GIT actually retained water when rats were dosed orally. There renal toxicity of paraquat was also shown to increase over time and was exacerbated by fasting.

4.13 Antidote Studies

Table 141 below summarises the results of a wide range of antidote and treatment studies conducted for paraquat. A description of these studies follows.

Table 141: Results of antidote studies

Species	PQ Dose	Treatment	Result	Reference
Mice				
♂ ddY, unspecified age	200 mg/kg bw, (144.8 mg/kg bw cation), po Aqueous vehicle	Dextran sulphate Cellulose sulphate Chondroitin sulphate Sucrose sulphate Glucose sulphate Dextran Sucrose Glucose All given @ 2000 mg/kg bw (po) in distilled water vehicle, immediately after PQ administration (n=10-11).	+ [a] + [a] + [b] + [a] + [a] - - -	Tsuchiya *et al* (1989)
♂ Alderley Park, unspecified age	200 mg/kg bw cation, po Deionised water vehicle	BeDS: 2000 mg/kg bw, po NDS-2: 2000 mg/kg bw, po NTS: 2000 mg/kg bw po Deionised water vehicle; All given 1 h after PQ administration (n=5).	+ [c] + [d] -	Farnworth & Heylings (1994)
Rats				
♂ &♀, unspecified strain & age	0.05% cation, po (diet)	Butazolidine: 100mg/kg bw/d, po Chlorpromazine: 10 mg/kg bw/d, sc Prednisolone: 17-25 mg/kg bw/d, sc Cortisone: 25 mg/kg bw/d, sc Vehicle unspecified; Treatment started 5 days after ingestion began & continued 5 days/week (n=10)	- [e] - - [e] - [e]	Weston Hurst (1965)[†]
	0.025% cation, po (diet)	Chlorpromazine: 5 mg/kg bw/d Prednisolone: 25 mg/kg bw/d Cortisone: 50 mg/kg bw/d Vehicle & administration route unspecified; Treatment started 3 days after ingestion began & continued for 57 days (n=10)	± [f] - ± [g]	
	100, 150, 200, 300 mg/kg bw/d (cation unspecified), po Vehicle unspecified	Cortisone: 50 mg/kg bw/d, sc for 15 days; Vehicle unspecified; Treatment began 1 day after PQ administration (n=20)	-	

Species	PQ Dose	Treatment	Result	Reference
♂ Alderley Park (Wistar-derived), adult	0, 20 or 25 mg/kg bw cation, sc Vehicle unspecified	Cysteamine: 1.30 mg/m^3 Ascorbate: 1.36 mg/m^3 Glucose: 1.37 mg/m^3 Spermidine: 1.32 mg/m^3 Ethanol: 2000 ppm vapour Cystamine: 1.33 mg/m^3 Putrescine: 1.31 mg/m^3 Promethazine: 1.32 mg/m^3 Paraffin: 24.1 mg/m^3 Selenite: 1.86 mg/m^3 Niacinamide: 1.33 mg/m^3 Ethacrynate: 1.52 mg/m^3 Methyl GAG: 1.62 mg/m^3 Vitamin: E 7.25 mg/m^3 BHT: 9.31 mg/m^3 M128036: 1.85 mg/m^3 Amsonate: 0.91, 1.40, 1.45 mg/m^3 Brufen: 2.41 mg/m^3 Mannitol: 3.31 mg/m^3 Aqueous vehicle except for vitamin E & BHT (paraffin); Exposure to aerosols immediately after PQ dosing for 24 or 48 h (n=10)	- [h] - [h] - ± [h] - - - - - - - - - - - ± [h] - [h] - -	Collinge *et al* (1981)
♂ SD, unspecified age	30 mg/kg bw (cation unspecified) x 2, ip, 24 h apart Distilled water vehicle	Niacin (500 mg/kg bw/d, ip) ± thiamine (100 mg/kg bw/d, ip) for 5 days starting 24 h after PQ administration (n=25)	+ [i]	Brown *et al* (1981)
♀ unspecified strain & age	200 mg/kg bw cation, po Deionised water vehicle (n=3)	Triquat: 100 mg/kg bw, ip, 1 h after dosing with PQ	-	Henderson (1982)[†]
♂ SD, unspecified age	30 mg/kg bw (cation unspecified), ip Vehicle unspecified	N-acetylcysteine (50 mg/kg bw, ip) @ 8 & 24 h after PQ administration; unspecified vehicle (n=15)	- [j]	Hoffer *et al* (1993)
♀ unspecified strain & age	125, 150 mg/kg bw (cation unspecified), po Vehicle unspecified	$MgSO_4$ (300 mg/kg bw, sc) 0, 1, 2, 3 h after paraquat administration; water vehicle (n=5)	+ [k]	Heylings & Trebilcock (1994)
♀ Alderley Park, unspecified age	100, 125, 150, 200 mg/kg bw cation, po, 10, 20 mg/kg bw cation, sc Deionised water vehicle	BeDS: 1000 mg/kg bw, po NDS-2: 1000 mg/kg bw, po, sc NTS: 1000 mg/kg bw, po 1, 2, 3, or 4 h after dosing with PQ; deionised water vehicle (n=5)	+ [l] + [l, m] + [l]	Treblicock & Heylings (1995)
	125, 150 mg/kg bw cation, po Deionised water vehicle	Fuller's earth 1000 mg/kg bw, po @ 1, 2, 3 or 4 h after dosing with PQ (n=5)	+ [n]	

Species	PQ Dose	Treatment	Result	Reference
	150 mg/kg bw cation, po Deionised water vehicle	Activated charcoal 1500 mg/kg bw, po, further treatment details unspecified (n=5)	+ [o]	
♂ Alderley Park (Alpk:Apf CD), unspecified age	100 mg/kg cation, po Deionised water vehicle	N^G-nitro-arginine methyl ester (50 mg/kg bw, ip) immediately, 4 h, 24 h & 35 h after PQ dosing; deionised water vehicle (n=5)	- [p]	Farnworth & Simpson (1995)
	100, 125 mg/kg bw cation, po Deionised water vehicle	SNP: 1, 1.5, 3 mg/kg bw x 3, sc; given @ 0, 3 & 6 h post PQ Molsidomine: 100 mg/kg bw, po, given immediately after PQ SNAP: 0.5, 1 mg/kg bw x 3, sc, given @ 0, 3 & 6 h post PQ Isosorbitol dinitrite: 32 mg/kg bw x 2, po; given @ 0 & 8 h post PQ Water vehicle (n=5); Given immediately after PQ administration	- - - -	Farnworth & Simpson (1996)
♂ Wistar & SD, adult	50-480 mg/kg bw (cation unspecified), ip Saline vehicle (n=5)	Melatonin (50 mg/kg bw, ip, ethanol vehicle) @ 30 min before & 2, 6, 10 & 14 h after PQ administration (n=5)	+ [q]	Melchiorri *et al* (1996)
Alderley Park rats, age & sex unspecified	100, 125, 150 mg/kg bw cation, po Deionised water vehicle	Astaxanthin: deionised water vehicle, 1, 10, 25, 100 μmol/kg bw, ip @ 0, 3, 6 & 24 after PQ administration (n=5) Crocetin: DMSO vehicle, 2, 8.5 mmol/kg bw, ip, @ 0, 3, 6 & 24 after PQ administration (n=5) Glycine: 200, 400 mmol/kg bw, ip, @ 0, 3, 6 & 24 after PQ administration (n=5) Melatonin: ethanol/water vehicle, 10 mg/kg bw, ip @ 0, 6, 12 & 24 h after PQ administration (n=5)	± [r] - - ± [s]	Farnworth & Heylings (1997)
Dogs				
♂ Beagle, age unspecified	5.43 mg/kg bw cation, iv Vehicle unspecified	Untreated (n=3); Carbon haemoperfusion (n=3) 2 h after PQ administration for 2 h; Cation exchange haemoperfusion (n=3) 2 h after PQ administration for 2h	± [t]	Maini & Winchester (1975); Maini *et al* (unspecified date) [†]
Beagles, adult, sex unspecified	10 mg/kg bw, iv pure PQ or equivalent of Gramoxone, po, Vehicle unspecified	30% Fuller's earth, 5 % $MgSO_4$, 250 mL/4 h; charcoal haemoperfusion 3 h after dosing, continued for 6-10 h, blood flow rates 100-200 mL/min (n= 2-5)	+ [u]	Widdop *et al* (1975)
	Gramoxone, 10 mg/kg bw cation, po Aqueous vehicle	Haemoperfusion with activated charcoal for 10-12 h (n=12); 5 dogs treated @ 3 h post-dose, 2 @ 6 h, 3 @ 12 h & 2 @ 18 h; blood flow 100-200 mL/min; dogs sedated with pentobarbitone & valium during treatment	+ [v]	Widdop *et al* (1977)

Species	PQ Dose	Treatment	Result	Reference
	Gramoxone, 40, 50 mg/kg bw cation, po, Vehicle unspecified, 1♀ & 1 ♂ @ each dose	Haemoperfusion with cation exchange resin (Zerolit 225 SRC 221) or coated charcoal (haemocol) 6 h after PQ dosing for 10 h @ 100 mL/min; dogs were maintained under pentobarbitone sodium (60 mg/mL) anaesthesia during PQ administration & perfusion	- [w]	Harling *et al* (1978) [†]
♀ & ♂ Beagles, adult	20 mg/kg bw (cation unspecified), sc Saline vehicle	Chlorpromazine 3.0, 5.0 mg/kg bw/d, im for 5 d, 30 min after PQ administration; saline vehicle (n=7)	-	Yamada *et al* (1993)
Monkeys				
♂ cynomolgus monkeys	85 mg/kg bw (61.54 mg/kg bw cation), po Water vehicle	Forced diuresis under alphaxalone/alphadolone anaesthesia 4 h following PQ administration & lasting for 24 h; n=3 PQ, n=3 PQ & diuresis, n=1 diuresis only	- [x]	Purser (1976)
Humans				
Human	Case 1: 40 g Case 2: 20 g Case 3: 40-60 g Cation unspecified	Fuller's earth/$MgSO_4$ every 4 h; Haemoperfusion within 7-30 h of ingestion	-	Widdop *et al* (1975)
	96 poisoning cases, 0-200 mL concentrate , po 28 cases $\geq$ 50 mL, 68 cases < 50 mL Cation unspecified	No treatment, iv fluid, mannitol, antibiotics, hydrocortisone, cortisone, prednisone, fluorouracil, diuresis, lung transplant, furosamide and/or O_2, parenteral fluids, stomach wash, charcoal, dialysis, haemodialysis, thioctic acid, heparin, gastric lavage, enemas, peritoneal dialysis, exchange transfusion, steroids, Fuller's earth, bentonite, azathioprine or cyclophosphamide	$\pm$ [y]	Fletcher & Cavalli (1976). Review.
	60-80 mL Gramoxone-S® (200 g/L cation)	Gastric lavage & instillation of bentonite from 16-23 h after admission; haemodialysis & haemoperfusion 1 h after admission	- [z]	Okoneck *et al* (1976a & b)
	30-300 mL concentrate, po Cation unspecified 9 ♂, 1 ♀	Gastric lavage 10 min – 3 h after ingestion of PQ (n=9). Fuller's earth/Mg SO_4, 250 mL/4 h for 24-48 h. Charcoal haemoperfusion, 3-30 h after ingestion of PQ lasting between 3 and 21.5 h	- [aa]	Vale *et al* (1977)

PQ = paraquat; † = considered to have limited regulatory value due to the absence of methodological and/or observational detail; suitable vehicle controls were used in each experiment; BeDS = m-benzene-di-sulphonate; NDS-2 = sodium 2,6-naphthalene-di-sulphonate; NTS = sodium 3,6-naphthalene-tri-sulphonate; DMSO = dimethyl sulphoxide; SNP = sodium nitroprusside; Molsidomine = N-[ethyoxycarbonyl]-3-[4-morpholinyl]sydnone imine; SNAP = S-nitroso N-acetyl penicillamine

a = 100% survival after 14 days compared to 0% for the control. The optimal time for treatment was within 10 minutes of PQ administration. Using a 4-fold greater proportion of sugar sulphate to PQ caused an approximately 10-fold increase in the LD_{50} (from 140 mg/kg bw to over 1000 mg/kg bw)

b = 73% survival after 14 days compared to 0% for the control.

c = All antidote-treated mice survived and no effect on body weight gain was observed. This contrasts with the low survival in the control group (1/5) and the PQ-related depression in body weight gain.

d = 4/5 mice survived compared to only 1/5 in the control group, and only 1 mouse showed decreased body weight gain compared to all control mice.

e = Increased survival by 2 days, no reduction in the severity or extent of macroscopic lung lesions
f = Increased survival by 7 days, no reduction in the severity or extent of macroscopic lung lesions
g = Increased survival by 24 days, no reduction in the severity or extent of macroscopic lung lesions
h = Possible decrease in mortality with spermidine & M128036 (20-30% relative to untreated rats) however the significance of these results is questionable as no statistical analysis was performed. Additionally there was large variability in the incidence of death in control rats (range 1-10/10). Increased mortality observed with cysteamine, ascorbate & amsonate (20-40%). Mannitol & amsonite increased the uptake of PQ by the lungs, while cysteamine halved lung PQ uptake.
i = Significantly increased survival and smaller weight losses ($p<0.05$; statistical test unspecified) occurred in niacin or niacin/thiamine treated rats compared to the control. Niacin did not completely prevent PQ-induced respiratory symptoms, weight loss or deaths. Niacin therapy also maintained liver NAD and niacin levels which were both lowered in animals that had died from paraquat poisoning.
j = No effect on survival but a decrease in the paraquat-induced release of chemoattractants for neutrophils in the bronchoalveolar fluid and a reduction in the infiltration of inflammatory cells into the lungs.
k = 4/5, 5/5, 5/5 and 5/5 rats survived after 10 days following treatment at 0, 1, 2 & 3 h after PQ treatment (125 mg/kg bw, po), respectively, while only 1/5 control rats survived. Plasma, lung & kidney PQ levels were approximately 3-fold lower than the control following po, sc or po/sc administration of $MgSO_4$ (no statistical analysis performed, no raw data).
l = BeDS achieved 100% survival (after 10 days) when given 1 h after PQ administration (125 mg/kg bw PQ), 20 % after 2 and 0 % after 3 & 4 h. NDS-2 & NTS achieved 80-100% survival when given up to 4 h after po administration of 125 mg/kg bw PQ, 40% following 150 mg/kg bw and 0% after 200 mg/kg bw.
m = Statistically lower PQ levels ($p<0.01$-0.05; Student's t-test) were detected in the plasma (4 & 24 h), kidney (2, 24, 48 h), lung (24, 48 h) and stomach (24 h) following treatment with NDS-2 (1000 mg/kg bw, po) one hour after PQ administration (150 mg/kg bw, po) relative to the control. Plasma (4 h), kidney (24 h) and large intestine PQ levels (24 h) were also significantly reduced ($p<0.01$-0.05) with NDS-2 (1000 mg/kg bw, po) one hour after a sc injection of PQ (10/20 mg/kg bw). This same treatment regime also increased ($p<0.05$) paraquat levels in the large intestine (4 h) and stomach (24 h). Oral dosing with PQ (150 mg/kg bw) followed 1 h later with a sc dose of NDS-2 (100 mg/kg bw) increased ($p<0.01$-0.05) the concentration of paraquat in the plasma (24 h), kidney (24 h) and large intestine (24 h), while decreasing ($p<0.05$) the concentration in the stomach (24 h).
n = 60% survival (after 10 days) when given 1 h after administration of PQ; 40% survival after 2h and 100 % survival after 3 and 4 h. Significant decrease ($p<0.01$-0.05; student's t-test) in plasma, lung and kidney PQ concentrations 24 h after paraquat administration (150 mg/kg bw, po)
o = Significant reduction ($p<0.05$; student's t-test) in plasma, lung and kidney PQ concentrations.
p = Exacerbation of PQ toxicity.
q = LD_{50} increased from 79 to 251 mg/kg bw.
r = MLD increased from approximately 100 to 150 mg/kg bw. This result was considered to be equivocal due to the small group sizes and the fact that the difference in the number of survivors between treated and control groups was only 1-2.
s = Although the study authors indicated that melatonin had no antidotal activity, 5/5, 1/5 and 5/5 rats survived following administration of 100, 125 and 150 mg/kg bw PQ respectively and 10 mg/kg bw melatonin. Survival in the corresponding controls was 3/4, 3/5 and 3/5 respectively.
t = Plasma PQ levels were significantly reduced ($p<0.05$; unspecified statistical test) compared to controls, with cation exchange perfusion more effective than activated carbon perfusion. Reported elevation of PQ levels following cessation of perfusion. No survival or raw data were provided.
u = 7/8 control dogs died; 1/7 treated dogs died; 6.6-17.5% of the applied dose was removed
v = 6/6 controls died; 3/10 treated dogs died; 1-19% of the applied dose removed
w = No dogs survived beyond 4 d & there was no difference between cation exchange and activated charcoal treatment; greater elimination of PQ using activated charcoal than cation exchange resin; no untreated control group was examined; the small group sizes were a considerable deficiency of this study.
x = No effect on plasma PQ concentration or survival however diuresed animals showed less severe renal impairment (perturbations in plasma urea & creatinine) than undiuresed animals.
y = Doses > 50 mL, 50% survival following treatment, 0% survival untreated. Doses < 50 mL, 73% survival treated, 13% survival untreated.
z = Death 77 h after ingestion; haemodialysis had no effect on PQ clearance; haemoperfusion cleared approximately 0.2 ppm

aa = 9 patients died from 12 h to 19 days after PQ ingestion; 7 died from multiple organ failure and 2 from pulmonary fibrosis. The one patient that recovered was reported to have ingested the smallest amount of PQ.

The effectiveness of sodium sugar sulfates to treat acute paraquat toxicity was investigated by administering dextran, cellulose, sucrose or glucose sulphate orally to male mice at 2000 mg/kg bw immediately after a 200 mg/kg bw po dose of paraquat dichloride (equivalent to 144.8 mg/kg bw paraquat cation). Survival was 100% at 14 days compared to 0% for the control group. The use of a 4-fold greater proportion of sugar sulphate to paraquat increased the LD50 from 140 mg/kg bw to over 1000 mg/kg bw. Similar treatment with chondroitin sulphate resulted in 73% survival, while dextran, sucrose and glucose showed no antidotal activity (Tsuchiya *et al,* 1989).

The use of aromatic sulphonates as an antidote for paraquat poisoning was investigated by administering a single po dose of m-benzene-di-sulphonate (BeDS), sodium 2,6-naphthalene-di-sulphonate (NDS-2) or sodium 1,3,6-naphthalene-tri-sulphonate (NTS) at 2000 mg/kg bw to male mice one hour after a 200 mg/kg bw po dose of paraquat cation. Nine days after treatment, survival was 100, 80 and 40% with BeDS, NDS-2 and NTS, respectively, compared to 20% for the untreated paraquat control group. The majority (4/5) of control mice lost between 10-25% of their body weight over 9 days, while BeDS and NTS-treated animals were protected from this effect. Mice treated with NDS-2 were not protected from paraquat-induced body weight loss (Farnworth & Heylings, 1994).

Attempts were made to modify the pulmonary lesions caused by paraquat by treating rats for 5 days with butazolidone (100 mg/kg bw/d, po), chlorpromazine (10 mg/kg bw/d, sc), prednisolone (17-25 mg/kg bw/d, sc) or cortisone (25 mg kg bw/d, sc) starting 5 days after dietary administration of 0.05% paraquat cation. None of these compounds reduced mortality or the severity or extent of macroscopic lung lesions. At a lower dietary level of paraquat cation (0.025%), treatment with chlorpromazine (5 mg/kg bw/d) or cortisone (50 mg/kg bw/d) for 57 days extended survival by 7 and 24 days, respectively, but still failed to reduce the severity or extent of macroscopic lung lesions. In this same experiment, prednisolone (25 mg/kg bw, sc) was ineffective at prolonging survival and actually proved quite toxic to the animals as evidenced by their ill-looking appearance and 41 g loss of body weight over 8 days. In another experiment, treatment of paraquat-poisoned rats (100-300 mg/kg bw, po gavage) with cortisone (50 mg/kg bw/d, sc) for 15 days did not improve survival or the incidence/severity of lung lesions. These studies were considered to have limited regulatory value due to the lack of reporting detail (Weston Hurst, 1965).

The effectiveness of a number of putative antidotes given as aerosols were investigated via mortality studies and by determining the ability of lung slices to accumulate 14C-paraquat in vitro. Male rats were dosed subcutaneously with 20 or 25 mg/kg bw paraquat cation and then immediately exposed to aerosols of cysteamine (1.30 mg/m^3), ascorbate (1.36 mg/m^3), glucose (1.37 mg/m^3), spermidine (1.32 mg/m^3), ethanol (2000 ppm), cystamine (1.33 mg/m^3), putrescine (1.31 mg/m^3), promethazine (1.32 mg/m^3), paraffin (24.1 mg/m^3), selenite (1.86 mg/m^3), niacinamide (1.33 mg/m^3), ethacrynate (1.52 mg/m^3), amsonate (0.91, 1.40, 1.45 mg/m^3), methyl GAG (1.62 mg/m^3), vitamin E (7.25 mg/m^3), BHT (9.31 mg/m^3), M128036 (1.85 mg/m^3), brufen (2.41 mg/m^3) or mannitol (3.31 mg/m^3) for 24 or 48 h. There was a 20-30% increase in survival following treatment with spermidine or M128036 while some

compounds (cysteamine, ascorbate, amsonate) actually exacerbated paraquat toxicity or the uptake of paraquat by the lungs (mannitol, amsonite) (Collinge *et al* 1981).

The effect of daily niacin therapy on paraquat-poisoned male rats was investigated by administering niacin (500 mg/kg bw/d x 5, ip) ± thiamine (100 mg/kg bw/d x 5, ip) 24 h after 2 injections of paraquat (30 mg/kg bw, ip; amount of cation unspecified) which were spaced 24 h apart. There was a significant increase in survival, smaller body weight loss and delayed respiratory distress in niacin- and niacin/thiamine-treated rats, however neither were observed to completely prevent respiratory symptoms, weight loss or death. Niacin therapy also maintained liver NAD and niacin levels which were both lowered in animals that had died from paraquat poisoning (Brown *et al,* 1981).

To determine its effectiveness as an antidote for paraquat poisoning, triquat (100 mg/kg bw, ip) was injected into rats one hour after administration of paraquat (200 mg/kg bw cation, ip). No antidotal effect as observed. This study was considered to have limited regulatory value due to the absence of methodological and observational details (Henderson, 1982).

A role for N-acetylcysteine (NAC) against oxidative lung damage in paraquat-poisoned male rats was investigated by injecting NAC (50 mg/kg bw, ip) at 8 and 24 h after paraquat (30 mg/kg bw, ip; amount of cation unspecified). NAC had no effect on survival but there was a decrease in the paraquat-induced release of chemoattractants for neutrophils in the bronchoalveolar fluid and a reduction in the infiltration of inflammatory cells into the lungs (Hoffer *et al*, 1993).

Parenteral administration of $MgSO_4$ was investigated as a potential treatment for paraquat poisoning given that previous studies in rats had demonstrated that Mg2+ reduces the absorption of paraquat from the GIT. $MgSO_4$ (300 mg/kg bw, sc) was administered to rats at 0, 1, 2 and 3 h after po dosing with 125 or 150 mg/kg bw paraquat (amount of cation unspecified). Ten days after treatment, the majority of $MgSO_4$-treated rats survived (19/20) regardless of the time at which $MgSO_4$ was administered. This contrasted with the controls (ie those not treated with $MgSO_4$) where only 1/5 rats survived. Plasma, lung and kidney paraquat levels were approximately 3-fold lower than the control following po, sc or po/sc administration of $MgSO_4$ (Heylings & Treblicock, 1994).

Experiments were performed to examine the antidotal potential of aromatic sulphonates and to compare these to the treatment of paraquat poisoning with Fuller's earth and activated charcoal. BeDS (1000 mg/kg bw, po), NDS-2 (1000 mg/kg bw, po & sc) or NTS (1000 mg/kg bw, po) were administered to female rats at 1, 2, 3 and 4 h after dosing with paraquat cation (100, 125, 150, 200 mg/kg bw, po; 10, 20 mg/kg bw, sc). Treatment with BeDS resulted in 100 and 20% survival (after 10 days) when administered one hour and 2 h, respectively, after paraquat (125 mg/kg bw cation, po). NDS-2 and NTS achieved 80-100% survival when given up to 4 h after po administration of 125 mg/kg bw paraquat cation, 40% after 150 mg/kg bw and 0% after 200 mg/kg bw. The survival of the untreated control group at the same dose of paraquat was 40%. Significantly lower paraquat levels were detected in the plasma (4, 24 h), kidney (2, 24, 48 h), lung (24, 48 h) and stomach (24 h) following treatment with NDS-2 (1000 mg/kg bw, po) one hour after paraquat administration (150 mg/kg bw cation, po) relative to the control. Plasma (4 h), kidney (24 h) and large intestine paraquat levels (24 h) were also significantly reduced with NDS-2 (1000 mg/kg bw, po), when administered one hour after a sc injection of paraquat (10

or 20 mg/kg bw cation). This same treatment regime also increased paraquat levels in the large intestine (4 h) and stomach (24 h). Oral dosing with paraquat (150 mg/kg bw) followed one hour later with a sc dose of NDS-2 (100 mg/kg bw) increased the concentration of paraquat in the plasma (24 h), kidney (24 h) and large intestine (24 h), while decreasing the concentration in the stomach (24 h). Fuller's earth was given to rats at 1, 2, 3 and 4 h after dosing with paraquat (125 mg/kg bw cation, po). Ten days after exposure, survival ranged from 40 to 100% in treated-rats compared to 20% in the control group. A significant decrease in plasma, lung and kidney paraquat concentrations occurred 24 h after administration of paraquat (150 mg/kg bw cation, po). A significant reduction in plasma, lung and kidney paraquat concentrations were also reported when rats were treated with 1500 mg/kg bw activated charcoal following po administration of 150 mg/kg bw paraquat cation (Treblicock & Heylings, 1995).

A study was performed to determine whether NG-nitro-arginine methyl ester (L-NAME) (an inhibitor of nitric oxide synthase) could afford protection against paraquat toxicity. L-NAME (50 mg/kg bw, ip) was given to male rats at various times (0, 4, 24, 35 h) after po dosing with 100 mg/kg bw paraquat cation. NAME failed to show any antidotal effect and in fact exacerbated toxicity (Farnworth & Simpson, 1995).

Given that the above study suggested that inhibitors of nitric oxide (NO) synthase exacerbate paraquat toxicity in the rat, a follow-up study examined whether NO generators might reduce paraquat toxicity. Sodium nitroprusside (SNP; 1, 1.5 or 3 mg/kg bw, sc), N-[ethyoxycarbonyl]-3-[4-morpholinyl]-sydnone imine (molsidomine; 100 mg/kg bw, sc), S-nitroso N-acetyl penicillamine (SNAP; 0.5 or 1 mg/kg bw, sc) or isosorbitol dinitrite (32 mg/kg bw, po) showed no antidotal activity when given to male rats immediately or at 0, 3, 6 or 8 h after po dosing with paraquat (100, 125 mg/kg bw cation) (Farnworth & Simpson, 1996).

The ability of the hydroxyl and peroxyl radical scavenger, melatonin, to protect against paraquat-induced oxidative damage in rat lung, liver and serum was examined. Administration of melatonin (50 mg/kg bw, ip) to male rats at 30 minutes before, and at 2, 6, 10 and 14 h after an ip injection of paraquat (50-480 mg/kg bw) increased the LD_{50} from 79 to 251 mg/kg bw. Melatonin also stopped paraquat-induced lipid peroxidation in the lung and liver, and prevented the paraquat-induced reduction in glutathione levels and the concomitant increase in oxidised glutathione in the liver and lungs (Melchiorri *et al,* 1996).

To investigate their effect as potential antidotes for paraquat toxicity, astaxanthin (1, 10, 25, 100 mmol/kg bw, ip), crocetin (2, 8.5 mmol/kg bw, ip), glycine (200, 400 mmol/kg bw, ip) or melatonin (10 mg/kg bw, ip) were administered to rats from 0-24 h after a single po dose of paraquat (100, 125, 150 mg/kg bw cation). Crocetin and glycine exhibited no antidotal activity. Astaxanthin increased the maximum lethal dose (MLD) from approximately 100 to 150 mg/kg bw paraquat cation, however this result was considered to be equivocal due to the small group sizes and the fact that the difference in the number of survivors between treated and control groups was small. Although the study authors concluded that melatonin had no antidotal activity, 5/5, 1/5 and 5/5 rats survived at 100, 125 and 150 mg/kg bw paraquat cation respectively. Survival in the corresponding controls was 3/4, 3/5 and 3/5, respectively (Farnworth & Heylings, 1997).

In an attempt to treat paraquat poisoning in male dogs, haemoperfusion was performed for 2 h using activated carbon or cation exchange resin 2 h after a single iv dose of 7.5 mg/kg bw

paraquat dichloride (equivalent to 5.43 mg/kg bw cation). Both treatments significantly reduced plasma paraquat levels relative to the untreated controls, with the cation exchange resin marginally more effective than the activated charcoal. Paraquat levels were reported to increase following cessation of perfusion. These results were considered to be equivocal as no survival or raw data were provided (Maini & Winchester, 1975; Maini *et al*, date unspecified).

In a study designed to investigate the treatment of paraquat poisoning, dogs were dosed intravenously with 10 mg/kg bw paraquat dichloride (equivalent to 7.24 mg/kg bw paraquat cation) or an equivalent po dose of Gramoxone, and then treated with a combination of 30% w/v Fuller's earth and 5% w/v MgSO4 given at doses of 250 mL every 4 h. Charcoal haemoperfusion was commenced 3 h after paraquat administration and continued for 6-10 h. Survival was markedly improved with this treatment regime (6/7 dogs survived versus 1/8 in the control group) with approximately 6.6-17.5% of the applied dose removed from the plasma (Widdop *et al*, 1975).

The positive effect of charcoal haemoperfusion for the treatment of paraquat poisoning in dogs was confirmed in a subsequent study. Animals were haemoperfused for 10-12 h starting at 3, 6, 12 or 18 h after a single po gavage with Gramoxone (10 mg/kg bw paraquat; cation unspecified). No control dogs survived while 7/10 perfused dogs survived. Haemoperfusion removed 1-19% of the applied dose from the plasma (Widdop *et al*, 1977).

A subsequent study in dogs that compared the effect of haemoperfusion using cation exchange resin or activated charcoal was considered to have limited regulatory value due to the absence of an untreated paraquat control group and the small group sizes. Dogs were given a po dose of Gramoxone containing 40 or 50 mg/kg bw paraquat cation, under anaesthesia, and 6 h later haemoperfused for a period of 10 h. No animals survived beyond 4 days. Marginally higher levels of paraquat were removed from the plasma using activated charcoal (Harling *et al*, 1978).

The effectiveness of chlorpromazine as a therapeutic agent to treat paraquat toxicity in dogs was investigated by administering chlorpromazine (3.0 or 5.0 mg/kg bw/d x 5, im) 30 minutes after administration of paraquat (20 mg/kg bw, sc; amount of paraquat cation unspecified). No antidotal effects were observed including any improvement of survival rates (Yamada *et al*, 1993).

A study was undertaken to determine whether forced diuresis could improve survival and protect the kidneys of paraquat-poisoned male cynomolgus monkeys. Forced diuresis was commenced 4 h after a po dose of 85 mg/kg bw paraquat dichloride (equivalent to 61.54 mg/kg bw paraquat cation) and continued for 24 h. Animals were kept under anaesthesia for the duration of the study. Forced diuresis did not prolong survival and had no effect on the rate of paraquat excretion, however, diuresed animals did retain some renal function (shown by the measurement of plasma urea and creatinine) up until death (Purser, 1976).

Three male patients who had deliberately ingested between 20-100 g paraquat (200-300 mL concentrate; equivalent to 14.48-72.4 g paraquat cation) were given Fuller's earth/MgSO4 immediately on admission to hospital. Haemoperfusion was commenced from 7-30 h after ingestion, however none of the patients survived (Widdop *et al*, 1975).

A review of 96 poisoning cases indicated that a variety of treatments such as iv fluid, mannitol, antibiotics, hydrocortisone, cortisone, prednisone, fluorouracil, diuresis, lung transplant, furosamide and/or O2, parenteral fluids, stomach wash, charcoal, dialysis, haemodialysis, thioctic acid, heparin, gastric lavage, enemas, peritoneal dialysis, exchange transfusion, steroids, Fuller's earth, bentonite, azathioprine and cyclophosphamide failed to provide any real benefit. The main determinates of survival during these cases appeared to be the amount of paraquat ingested and how quickly after ingestion treatment was initiated. At doses greater than 50 mL (amount of paraquat cation unspecified) no patients survived if left untreated, while 50% survival was reported following treatment. Doses less than 50 mL (amount of paraquat cation unspecified) resulted in 13% survival in untreated patients and 73% in treated patients (Fletcher & Cavalli, 1976).

Following deliberate ingestion of 60-80 mL Gramoxone S (200 g paraquat cation/L) by a male patient, gastric lavage, instillation of bentonite, haemodialysis and haemoperfusion failed to prevent death, although haemoperfusion cleared approximately 0.22 ppm paraquat cation from the serum (Okoneck *et al*, 1976 a & b).

A combination of gastric lavage, Fuller's earth/$MgSO_4$ and haemoperfusion were used to treat 10 patients who had ingested between 30-300 mL paraquat concentrate (amount of paraquat cation unspecified). Gastric lavage was performed in 9 patients between 10 minutes and 3 h after ingestion. Patient's then received Fuller's earth/$MgSO_4$ (30%/5%) at 4 hourly intervals over 24-48 h. Charcoal haemoperfusion was commenced 3-30 h after ingestion and lasted between 3 and 21.5 h. Haemoperfusion removed between 18-560 mg paraquat cation (mean 184 mg). Nine patients died from 12 h to 19 days after paraquat ingestion; 7 died from multiple organ failure and 2 from pulmonary fibrosis. The one patient that recovered was reported to have ingested the smallest amount of paraquat (Vale *et al* 1977).

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