TOXICOLOGY POSITION STATEMENT

PARAQUAT

Data summaries
for the WHO/JMPR
Panel of Experts on Pesticide Residues
in 2003

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Explanation

Paraquat is to be re-evaluated by the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues in 2003.

Paraquat was evaluated for acceptable daily intake by the Joint Meetings in 1970, 1972, 1976, 1982, and 1985 (Annex 1, FAO/WHO, 1971a, 1973a, 1977a, 1983a, and 1986a). A toxicological monograph was published after the 1970 Meeting (Annex 1, FAO/WHO, 1971b) and monograph addenda were published after the Meetings in 1972, 1976, and 1982 (Annex 1, FAO/WHO, 1973b, 1977b, and 1983b). In 1970 the Meeting estimated an ADI of 0.001 mg/kg b.w. (as paraquat dichloride). The 1982 Joint Meeting noted that the higher ADI established by the 1972 Meeting (0.002 mg/kg b.w. as paraquat dichloride) was based on long-term studies conducted by Industrial Bio-Test Laboratories (IBT), for which no replacement studies, validations, or additional data had been submitted. Considering the evidence available, the 1982 Meeting recommended that a reduced ADI (0.001 mg/kg b.w. as paraquat dichloride) be retained on a temporary basis, pending receipt of further data.

Data were submitted to the 1985 Meeting which met the 1982 request. These data were reviewed by the 1985 Meeting, but logistical difficulties precluded their full evaluation, especially in the light of the considerable amount of information previously evaluated by the Joint Meeting. The 1985 Joint Meeting was aware that the 2-year study in rats that was submitted had been considered by one national authority to indicate a possible oncogenic potential in the rat. The Meeting also noted differing interpretations of the observed lesions by different pathologists. The Meeting therefore recommended that a complete evaluation of all valid data available should be undertaken by the 1986 Joint Meeting. In addition, it requested submission of full descriptions of the lung lesions seen in the new long-term rat study and of historical control data on all lung lesions in the strain of rats utilized in the study in the laboratory in which it was conducted. The Joint Meeting extended the existing temporary ADI until 1986.

The 1986 monograph incorporated the relevant studies summarized in earlier monographs and monograph addenda, the studies submitted for consideration by the 1985 Joint Meeting, and the studies required by the 1985 Joint Meeting, all of which were reviewed by the 1986 Meeting.

This submission incorporates the relevant studies summarised previously (as described above) and new data (company reports and published literature) produced subsequent to the 1986 evaluation.

Paraquat dichloride is the common name for 1,1'-dimethyl-4,4'-bipyridinium dichloride (CAS Reg # 1910-42-5). Paraquat is a contact non-selective herbicide and is sold predominantly as a liquid formulation (Gramoxone) or in combination with other herbicides (e.g. diquat and diuron).

Also known as methyl viologen; paraquat dichloride is an off-white odourless hygroscopic powder, is freely soluble in water, slightly soluble in alcohols and insoluble in organic solvents.

Dose levels may be quoted as paraquat cation or the salt, paraquat dichloride. The latter would normally be selected for classification purposes. However, much of the published literature does not specify which was used. Where possible the dose has been quoted in both paraquat dichloride and in paraquat cation. The default assumption is that published literature refers to paraquat cation. Technical concentrate contains 45.6% paraquat dichloride (33% paraquat cation).

Evaluation for acceptable daily intake

1. Biochemical aspects
(a) Absorption, distribution, and excretion

The absorption, distribution, and excretion of paraquat in experimental animals has been reviewed by WHO (1984).

Since then a modern data package of both excretion balance (Lythgoe R E and Howard E F, 1995a-c), and biotransformation (Macpherson D, 1995) has been generated and submitted as part of the EU re-registration review.

Five male and five female Alpk:ApfSD rats were each given a single oral dose of 1 or 5 mg [^{14}C]-paraquat/kg bw (methyl-labelled paraquat dichloride). In a further study, five male and five female Alpk:ApfSD rats were each given a single oral dose of 1 mg [^{14}C]-paraquat/kg bw preceded by fourteen daily doses of 1 mg unlabelled paraquat/kg bw. The urinary and faecal excretion of radioactivity was monitored for three days after administration, at which time the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.

The excretion of radioactivity was rapid, with over 90% of the dose eliminated within 72 hours in all three studies. At 1 mg [^{14}C]-paraquat/kg there was a small sex difference in that females excreted a slightly higher proportion of the radioactivity in the faeces, with males excreting a slightly higher proportion of the radioactivity in the urine. At 5 mg [^{14}C]-paraquat/kg bw the principal route of elimination was via faeces, with no differences in excretion noted between the sexes.

Overall very little radioactivity was retained in the tissues of either sex. The highest tissue concentrations of radioactivity in both males and females dosed with 1 mg [^{14}C]-paraquat/kg bw (Lythgoe R E and Howard, 1995 a) were present in lungs (0.023 µg and 0.020 µg equiv/g respectively) and kidneys (0.010 µg and 0.011 µg equiv/g respectively). The residual carcasses of male and female rats contained 0.64% and 0.54% of the dose respectively. The total mean percentage recoveries, including excreta and tissue residues, for male rats was 93.2% and for females was 94.4%.

In the second study dosing with 1 mg [^{14}C]-paraquat/kg bw following 14 daily doses of 1 mg unlabelled paraquat/kg bw (Lythgoe R E and Howard, 1995 b), the highest tissue concentrations of radioactivity in both sexes were present in lungs (0.037 µg and 0.023 µg equiv/g respectively). No single tissue contained >0.02% of the administered dose after 72 hours with <0.8% of the dose in the carcass. The tissue concentrations of radioactivity were generally lower in female rats than in males. The

total mean percentage recoveries, including excreta and tissue residues, for male rats was 93.3% and for females was 94.5%.

In the study with a 50 mg [^{14}C]-paraquat/kg bw dose, (Lythgoe R E and Howard, 1995 c), the highest tissue concentrations of radioactivity in both males and females were present in lungs (0.661 μg and 1.077 μg equiv/g respectively) and carcass (0.401 μg and 0.414 μg equiv/g respectively). The tissue concentrations of radioactivity were generally lower in male rats than in females. The total mean percentage recoveries, including excreta and tissue residues, for male rats was 93.6% and for females was 92.5%. No single tissue contained >0.02% of the administered dose after 72 hours with <0.8% of the dose in the carcass.

At all dose levels tested, radioactivity was rapidly eliminated in faeces and urine. Seventy two hours after dosing only low levels of radioactivity were present in the tissues and carcass.

Table 1: Summary of the absorption, distribution, metabolism and excretion data for rats administered paraquat (values as means of 5 animals, expressed as % of administered dose)

DOSE	1 x 1 mg/kg bw		1 x 50 mg/kg bw		(14 +1) x 1 mg/kg	
	male	female	male	female	male	female
Urine 0-6 h	14	8	6	8	16	8
0-24 h	18	11	9	11	19	10
24-72 h	1	1	2	2	1	1
Metabolites 0-72 h	0.8	0.8	0.6	0.6	0.8	0.5
Faeces 0-6 h	64	74	54	50	68	71
0-24 h	9	6	27	28	3	10
Sum of tissues (72 h)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Carcass (72 h)	0.6	0.5	0.8	0.8	0.7	0.6
Total recovery	93	94	94	93	93	94

The 1986 Monograph included the following data. Following oral single-dose administration of 4 - 6 mg/kg bw [^{14}C]-paraquat dichloride to rats, 99 - 102% of the administered dose was found in the faeces (93 - 96%) and in the urine (6%) within 3 days. This information, together with the absence of significant biliary excretion, provided evidence that paraquat is poorly absorbed from the gut (Daniel & Gage, 1966).

In their review of the literature - Lock and Wilks (2001) confirmed low (<5%) biliary excretion of paraquat in rats, rabbits, guinea pigs and dogs thought to be due to the molecular weight of paraquat (at 186) being below the minimal molecular weight of about 500 for chemicals that are excreted in bile.

The low rate of paraquat absorption by the gut was confirmed in experiments in which rats, guinea pigs, and monkeys, orally administered with LD50 doses of [^{14}C]-paraquat, had low peak serum concentrations (2.1 - 4.8 mg/litre). The radioactivity levels reached a maximum 30 - 60 minutes after administration and then remained relatively constant for 32 hours (Litchfield et al., 1973; Conning et al., 1969).

A dose of 126 mg/kg bw paraquat resulted in a maximum rat serum level of 4.8 mg/litre (Murray & Gibson, 1974).

In fasting dogs, low oral doses of paraquat were rapidly but incompletely absorbed, the peak plasma concentration being attained 75 minutes after dosing. After an oral dose of 0.12 mg/kg bw, 46 - 66% was absorbed in 6 hours. After doses of 2 and 5 mg/kg bw, only 22 - 38% and 25 - 28% of the doses were absorbed, respectively (Bennett et al., 1976).

Dose-dependent data from dogs and whole-body autoradiography seem to suggest that absorption is facilitated in the small intestine (WHO, 1984). The tissue distribution of paraquat following a single oral dose of 20 mg paraquat/kg bw to male mice was studied. Initially there was rapid distribution throughout most tissues. At 24 hours paraquat was selectively retained in the lung and skeletal muscle from where it was subsequently slowly eliminated. Following repeated dosing at dietary levels of 50, 125 and 250 ppm to male and female rats for up to eight weeks, there was no evidence of accumulation of paraquat in any of the tissues examined. No paraquat was detected in any tissue one week after cessation of dosing. (Litchfield et al., 1973).

The pulmonary absorption of $[^{14}C]$-paraquat after an intratracheal injection of 1.86 nmol/lung was investigated in the isolated perfused rat lung. The efflux of $[^{14}C]$-paraquat was diphasic, with a rapid-phase half-life of 2.65 minutes and a slow-phase half-life of 356 minutes. It was suggested that the slow phase represented a storage pool, possibly responsible for the pulmonary toxicity of paraquat (Charles et al., 1978).

Various doses of $[^{3}H]$-paraquat (1 pg - 10 μg) in 0.1 ml saline were introduced directly into the left bronchus of rats. Fifteen minutes after instilling 10 ng of $[^{3}H]$-paraquat, 90% of the ion could be accounted for in the tissues and urine, 50% being present in the lung. With doses at or greater than 10 μg, pathological changes were seen in the lung that were similar to those seen after systemic poisoning (Wyatt et al., 1981).

Paraquat absorption through animal and human skin has been studied using an *in vitro* technique. Human skin was shown to be impermeable to paraquat, having a very low permeability constant of 0.73. Furthermore, human skin was found to be at least 40 times less permeable than that of the animals tested, including rats, rabbits, and guinea pigs (Walker et al., 1983). An *in* vivo human study has demonstrated that only 0.3% of an applied dose (over five days after a single application of 0.64 mg paraquat dichloride) is absorbed. If extensive skin damage occurs systemic toxicity may result, but this is a rare occurrence and is associated with misuse of the product (Wester et al, 1984; Levin et al 1979; WHO, 1984).

Observations of dose-related dermal toxicity in experimental animals and human percutaneous poisoning suggest that paraquat absorption is markedly increased in damaged or occluded skin (WHO, 1984).

High concentrations and retention of paraquat were found in lung tissue, relative to other tissues, following oral, intraveneous (i.v.), intraperitoneally (i.p.), subcutaneously (s.c.), and intrabronchial routes of administration in rats, guinea pigs, rabbits, and monkeys (Sharp et al., 1972; Ilett et al., 1974; Murray & Gibson, 1974; Maling et al., 1978; Kurisaki & Sato, 1979; Waddell & Marlowe, 1980; Wyatt et al., 1981). Some of these data are summarized in Tables 1 and 2.

An association between paraquat concentrations in the lung and degree of toxicity or lung injury has been reported (Sharp et al., 1972; Ilett et al., 1974; Waddell & Marlowe, 1980; Wyatt et al., 1981). In one study toxic doses of $[^{14}C]$-paraquat were administered orally and i.v. to rats. Paraquat concentrations in the whole blood were similar to those in the plasma. The distribution of paraquat in various tissues and plasma was then followed for up to 10 days. The initial and secondary administration were 23 minutes and 56 hours, respectively. The concentration in the kidney, lung, and muscle declined at the same rate as in the plasma initially, but the rapid phase in the lung ended after 20 minutes (compared with 1 - 4 hours in other organs), after which it declined, with a half-life of 50 hours. The lung had the greatest retention and consequently contained the highest concentration 4 hours after dosing. Four to 10 days after dosing, the paraquat concentration in the lung was 30 - 80 times higher than in the plasma (Sharp et al., 1972).

Table 2. Paraquat distribution In Tissues (from WHO 1984)

Route	Dose	Species	Time after treatment	Tissue	Concentration	Reference
intrabronchial	10 ng	rat	60 min	Plasma	0.0092 g/l	Wyatt et al., 1981
				Lung	5.2 ng	
				Kidney	0.052 ng	
				Liver	not measured	
				Heart	not measured	
				Brain	not measured	
iv	20 mg/kg	rat	24 hour	Plasma	0.07 mg/l	Sharp et al., 1972
				Lung	6.00 mg/kg	
				Kidney	1.45 mg/kg	
				Liver	0.48 mg/kg	
				Heart	1.20 mg/kg	
				Brain	not measured	
iv	20 mg/kg	rat	24 hour	Plasma	Not measured	Ilett et al., 1974
				Lung	11.36 mol/kg	
				Kidney	1.93 mol/kg	
				Liver	0.90 mol/kg	
				Heart	1.13 mol/kg	
				Brain	0.87 mol/kg	
iv	20 mg/kg	rabbit	24 hour	Plasma	0.28 mol/l	Ilett et al., 1974
				Lung	7.90 mol/kg	
				Kidney	5.25 mol/kg	
				Liver	1.59 mol/kg	
				Heart	1.52 mol/kg	
				Brain	0.49 mol/kg	
ip	15 mg/kg	Rat	24 hour	Plasma	0.32 mol/kg	Maling et al., 1978
				Lung	26.28 mol/kg	
				Kidney	10.40 mol/kg	
				Liver	5.04 mol/kg	
				Heart	4.59 mol/kg	
				Brain	1.22 mol/kg	
oral	126 mg/kg	rat	16 hour	Plasma	0.9 mg/l	Murray & Gibson, 1974
				Lung	5.0 mg/kg	
				Kidney	7.0 mg/kg	
				Liver	2.1 mg/kg	
				Heart	2.7 mg/kg	
				Brain	not measured	

oral	22 mg/kg	Guinea pig	16 hour	Plasma	0.03 mg/l	Murray & Gibson, 1974
				Lung	1.29 mg/kg	
				Kidney	1.99 mg/kg	
				Liver	0.08 mg/kg	
				Heart	0.31 mg/kg	
				Brain	not measured	

iv   intravenous
ip   intraperitoneal

Table 3. Paraquat Distribution In Tissues In mg/kg Mean Tissue (from WHO 1984)

Route	Dose mg/kg bodyweight	Species	Time after dosing	Lung	Kidney	Liver	Heart	Plasma	Reference
oral	126	rat	1 h	3.3	27.5	2.0	1.8	4.7	Murray &
			4 h	3.7	4.5	4.4	0.9	0.8	Gibson,
			32 h	13.6	9.4	5.7	2.8	1.1	1974
			64 h	1.7	1.0	7.7	0.2	0.1	
iv	20	rat	1 h	9.0	25.0	5.0	-	6.0	Sharp et al,
			4 h	8.0	6.0	2.0	-	0.3	1972
			24 h	6.0	1.0	0.4	-	0.07	
			48 h	4.0	0.8	0.3	-	0.05	

iv   intravenous

The high lung tissue concentrations of paraquat were confirmed in another study in rats and rabbits after i.v. injection of 20 mg [14C]-paraquat/kg bw. Although the herbicide showed a selective localization in the rabbit lung, the concentration decreased far more rapidly in the rabbit lung than in the rat lung. The rabbit, unlike the rat, did not show any histological or biochemical signs of lung damage. No preferential subcellular localization of paraquat was found in the lungs of either species. No evidence of covalent binding of paraquat in lung tissue was found. After thorough washing of tissue precipitate with dilute trichloracetic acid, only insignificant amounts of [14C]-paraquat were detected in protein from the brain, heart, kidney, liver, lung, and plasma (Ilett et al., 1974).

Autoradiographic studies using [14C]-paraquat have been carried out on mice and rats. Paraquat was observed in nearly all organs 10 minutes after i.v. injection of 20 mg/kg bw. (Litchfield et al.,1973).

Autoradiographic results similar to those above were obtained in mice after i.v. injection of 288 - 338 g/kg bw of [3H]-paraquat dichloride. Cellular resolution autoradiography showed that paraquat was confined almost entirely to cells having the distribution of alveolar Type II cells. The authors suggested that it was unlikely that the radioactivity was bound to cellular constituents. The Type II cells were found to be susceptible to the toxicity of paraquat (Waddell & Marlowe, 1980; Kimbrough & Gaines, 1970).

No paraquat was detected in the kidney, brain, liver, or lungs when administered in the diet to rats at a concentration of 50 ppm for a period of 8 weeks. At 120 ppm it was found at low concentrations in the lung, kidney, gastrointestinal system, and brain. When administered at 250 ppm, it was detected in the tissues within 2 weeks. No sex differences or any clear pattern of accumulation were noted throughout the 8-

week study. Within one week of return to a normal diet, no paraquat was detected in any tissue examined. Histological changes were observed in all lungs of animals fed paraquat at 250 ppm in the diet (Litchfield, et al., 1973).

Rose et al. (1974) demonstrated an energy-dependent accumulation of paraquat in slices of rat lung that obeyed saturation kinetics. The same investigators later examined the ability of paraquat to accumulate in tissue slices from other organs in vitro. The uptake of the herbicide in brain, adrenal gland, and kidney slices was less than 10% of that observed in lung slices. The authors established the uptake of paraquat by the lung in various species (rat, rabbit, dog, monkey, and man). The human lung accumulated paraquat as readily as that of the rat. Indeed, the kinetics (Vmax and Km) of the process were found to be very similar in the two species. Moreover, there was a relationship between the concentration of paraquat in the different lung areas and the development of microscopic lung lesions (Rose et al., 1976a; Rose & Smith, 1977).

It has been demonstrated that the rate of paraquat efflux from lung tissue is less than its rate of accumulation in lung slices. Efflux from lung slices, prepared from rats dosed i.v. with the herbicide, was found to be biphasic. There was a fast component (half-life of 20 minutes), followed by a first-order slow component characterised by a half-life of 17 hours. The half-life in vitro was similar to that seen *in vivo* following i.v. administration to rats. (Smith et al., 1981). These results are partially consistent with those obtained by Charles et al. (1978) in the isolated perfused rat lung.

A biphasic elimination of paraquat from the plasma of rats after i.v. injection has been reported. The initial rapid phase had a 20-30 minute half-life, and the slower phase a half-life of 56 hours (Sharp et al., 1972).

Prolonged paraquat disappearance from serum following a rapid initial decline was also found after oral administration to rats, guinea pigs, and monkeys. Both the urinary and faecal routes were important in all species studied. In rats 32 hours after dosing, 52% of the administered paraquat was found in the gastrointestinal tract and 17 and 14% were excreted in the faeces and urine, respectively. No radioactivity was found in the expired air. The paraquat in the faeces was due primarily to elimination of unabsorbed paraquat. The prolonged elimination of paraquat in all animals tested indicated retention of the herbicide in the body (Murray & Gibson, 1974).

Following i.v. administration of paraquat to rats, 75 - 79% of the dose was excreted in the urine within 6 hours. In this study, the plasma disappearance of 5 mg/kg bw paraquat was fitted to a 3-compartment model. Total body clearance was estimated to be 8.39 ± 0.54 ml/kg/minute. The relatively high concentration of paraquat found in the duodenal and jejunal walls suggested biliary secretion of the herbicide. The authors' hypothesis was later supported by the observation of radioactivity in the intestines of mice injected i.v. with [^{14}C]-paraquat in whole-body autoradiographic studies (Maling et al., 1978; Waddell & Marlowe, 1980).

The dog was used as a model to evaluate the influence of paraquat-induced renal failure on the kinetics of paraquat elimination. After i.v. injection of a trace dose of [^{14}C]-paraquat (30-50 g/kg bw), the kinetics of distribution was described by a 3-compartment model. To obtain a good fit of the curve, it was necessary to sample the

central (plasma) compartment for at least 24 hours after dosing. Simulation of paraquat levels in the peripheral compartments suggested the existence of a compartment with rapid uptake and removal (kidney) and another with slow uptake (lung). The renal clearance of paraquat approximated total body clearance, indicating that paraquat elimination occurs through renal excretion. The urinary excretion rate of an i.v. dose was rapid, approximately 80 - 90% of the dose being eliminated during the first 6 hours. Intravenous injection of a large toxic dose of paraquat (20 mg/kg bw), however, brought about a marked decrease in renal clearance, from 73 ml/minute to 18 ml/minute after 2.5 hours and 2 ml/minute after 6 hours. These data suggest that kidney damage could contribute to paraquat accumulation in the lung (Hawksworth et al., 1981).

Lock and Wilks (2001) have recently conducted an extensive review of the published literature and found no new data on absorption, distribution or metabolism. However there is new data on the mechanism of elimination of paraquat from the body, which is almost exclusively via the kidneys.

The renal clearance of paraquat is greater than that of creatinine in the rat (Lock, 1979; Chan et al., 1997); dog (Hawksworth et al.,1981); sheep (Webb, 1983); monkey (Purser and Rose, 1979) and humans (Bismuth et al., 1982); Thus paraquat is actively secreted by the kidney. Renal tubular secretion was completely inhibited by N^1-methylnicotinamide suggesting that paraquat is secreted via a cationic transport system (Hawksworth et al., 1981).

The transport mechanisms for organic cations in renal proximal tubular cells are not fully understood. Recently two membrane proteins, organic cation transporter 1 (Grundemann et al., 1994) and organic cation transporter 2 (Okuda et al., 1996) have been isolated from rat kidney. The organic cation transporter 1 located on the basolateral membrane will transport tetraethylammonium, and this can be inhibited by other organic cations such as quinine. The organic cation transporter 2, which is predominantly expressed in the kidney, stimulates the uptake of tetraethylammonium and this can be markedly inhibited by cimetidine. Studies using freshly isolated renal proximal tubules and renal cell lines have shown that paraquat is transported across the basolateral membrane (from the blood stream into the renal tubular epithelial cell) using an organic cation transport system (Groves et al., 1995; Chan et al., 1996 a and b, 1997,1998). The transport of paraquat can be blocked by the addition of the divalent cation quinine, cimetidine and to a lesser extent tetraethylammonium (Chan et al., 1996b) suggesting that paraquat may be transported by both transport systems.

Exit across the apical membrane into the tubular lumen is also an active process, current evidence suggests that there are two cation transport systems, an electroneutral organic cation /H^+ exchange (Sokol et al., 1988) and P-glycoprotein (Dutt et al., 1992). Studies with rabbit brush-border membrane vesicles have shown that paraquat is a substrate for the cation/H^+ exchange transporter, and further that it can inhibit the transport of other monovalent cations such as tetraethylammonium (Wright and Wunz, 1995).

In the rat in vivo, the fractional excretion of paraquat decreased from 2.1 at a plasma concentration of about 0.4 nmol/ml to 1.2 at a plasma concentration of 21nmol/ml, demonstrating that the excretion of paraquat is greater than the glomerular filtration

rate and that the process is saturable (Chan *et al.*, 1997). Thus, at low plasma concentrations paraquat will be readily cleared from the body, however at higher plasma concentrations the system will become saturated and less paraquat will be cleared. At toxic doses it is well established that paraquat can cause renal functional impairment. In rats, orally dosed with 126 mg ion/kg (Lock, 1979) and mice given 50 mg ion/kg, i.v. (Ecker *et al.*, 1975b) renal impairment was observed 17-24h after dosing. In the cynomologus monkey given 85 mg ion/kg, po the decline in renal clearance was seen 12h after dosing the first time examined (Purser and Rose, 1979). In dogs given 20 mg ion/kg, i.v. (Hawksworth *et al.*, 1981) renal impairment was observed as early as 2.5 hr after dosing. An early report on the renal handling of paraquat by the dog, suggested that paraquat was reabsorbed by the proximal tubules, this study was conducted at high plasma concentrations (54-810 nmol/ml) where the transport system will have been saturated and function impairment will almost certainly have occurred (Ferguson, 1973). The weight of evidence strongly supports the view that paraquat is actively secreted by the kidney of laboratory animals and humans. The implication of impairment of renal excretion is that more paraquat is available in the plasma, to accumulate into the lung.

(b) Biotransformation

Rats, dogs, and guinea pigs
A new biotransformation study (Macpherson D, 1995) was submitted as part of the EU re-registration review and is described below.

Urine and faecal samples collected from male and female Alpk:ApfSD rats administered an oral dose of 1 or 5 mg [^{14}C]-paraquat/kg bw (methyl-labelled paraquat dichloride) were analysed to investigate the metabolic fate of paraquat in the rat. Urinary excretion accounted for group mean values of 10-20% of the administered radioactivity, with the remaining dose being excreted quite rapidly in faeces.

Chromatographic analysis of urine showed that 93-96% of urinary radioactivity co-chromatographed with paraquat. Three very minor radiolabelled metabolites were detected in urine, but together represented less than 1% of the dose.

The chromatographic analysis of faecal samples proved to be more difficult than the analysis of urine, however based upon the development of a sequential extraction procedure, up to 92% of the radioactivity was extracted from faeces. Using a combination of TLC and HPLC chromatographic analyses, the two acidified extracts were shown to contain a single major radiolabelled peak, which co-chromatographed with paraquat, with no evidence of any significant metabolites. The same result was obtained for both sexes and at both dose levels investigated.

It was therefore established that following administration of paraquat at either single or repeated 1 mg/kg bw doses or a single 50 mg/kg bw dose to male and female rats, the bulk of both urinary and faecal radioactivity corresponded to the parent molecule. It was therefore concluded that paraquat dichloride is resistant to metabolic breakdown following oral administration to rats, (Macpherson D, 1995).

This is consistent with data described in the 1986 monograph.

After oral administration of [^{14}C]-paraquat to rats, dogs, and guinea pigs, most of the radioactivity was excreted in 4 days, mainly in the faeces as unchanged paraquat. The remaining label was present in urine, which contained 12% (rats), 45% (dogs), and 9% (guinea pigs) of the dose administered. Paraquat was the main radioactive component of rat (0.4%) and dog (0.4%) urine, with monoquat and paraquat dipyridone accounting for 0.3%, and 0.1% respectively of the administered dose in rat urine, and 0.5%, and 0% respectively of the dose in dog urine. After subcutaneous (s.c.) administration of [^{14}C]-paraquat to rats, over 90% of the administered radioactivity was excreted in the urine in 4 days. While the excretion produce was mainly paraquat, chromatography indicated that monoquat (1.9%), paraquat monopyridone (3.2%), and paraquat dipyridone (1.1%) were also present. Although traces of monoquat and paraquat monopyridone were also found in rat faeces, there was no evidence of extensive metabolism of paraquat by the gut microflora. Intestinal bacteria from rat caecal contents did not degrade paraquat *in vitro* to any measurable extent (Annex 1, FAO/WHO, 1977b).

These conclusions were in contrast with the results of other studies previously evaluated which indicated that when paraquat (50 mg/kg bw of [^{14}C]-labelled dichloride salt) was given to rats, 25% of the radioactivity excreted in the faeces could be attributed to products of metabolism by gut microflora. Examination of extracts indicated the presence of only one metabolite in addition to paraquat. Thirty percent of the paraquat was broken down when incubated anaerobically with rat caecal contents; the metabolites were not identified. Urine from rats injected intra-peritoneally (i.p.) with ^{14}C-methyl-labelled paraquat contained 87% of the administered radioactivity in 24 hours, which was entirely unchanged paraquat (Plant Protection Ltd, 1972).

Hens

When a single oral dose of [^{14}C]-methyl-labelled paraquat was administered to hens at 60 ppm in the diet, all the dose was recovered quantitatively in the faeces within 3 days. At least 98% of the recovered radioactivity was unchanged paraquat. Analysis of the tissues of hens after 18-22 days of continuous dosing with [^{14}C]-paraquat (at 6 ppm in the total diet) indicated that it did not accumulate in the hens (Hemingway & Oliver,1974).

Continuous dosing of hens with radiolabelled paraquat for up to 10 days, at a rate of 30 ppm in the diet, resulted in total radioactive residues in the eggs of up to approximately 0.05 mg/kg paraquat ion equivalent. At least 80% of the radioactivity was due to unchanged paraquat. The residue was almost entirely in the yolk rather than in the albumin (Hendley et al.,1976a).

Pigs

Pigs excreted an oral dose of paraquat principally in the faeces as unchanged paraquat. Two pigs were dosed with [^{14}C]-labelled paraquat for 7 consecutive days at a rate equivalent to 50 ppm in the diet. One was dosed with [^{14}C]-methyl-labelled and

the second with [^{14}C]-ring-labelled paraquat. The pigs were sacrificed 2 hours after receiving the final dose. By this time, 69 - 73% of the administered residue had been recovered in the faeces and approximately 3% had been recovered in the urine. More than 90% of the radioactivity in the faeces was present as unchanged paraquat. Total radioactive residues in the tissues were low. More than 90% of these residues were due to unchanged paraquat, except in liver, where approximately 70% was due to unchanged paraquat and 4 - 7% was due to monoquat ion (Leahey et al., 1976; Spinks et al., 1976).

Goats

[^{14}C]-ring-labelled paraquat was administered to a goat in mid-lactation twice daily for 7 days at a dose equivalent to 100 ppm in the diet. Total radioactive residues in the milk were less than 0.01 mg/kg paraquat cation equivalent; 76% was unchanged paraquat. Total radioactive residues were 0.74 mg/kg, 0.56 mg/kg, and 0.1 mg/kg in kidney, liver, and muscle respectively. There was no significant metabolism of paraquat, except in the liver, where 48% of the residue was paraquat and about 3% was each of the metabolites monoquat ion and monopyridone ion (Hendley et al., 1976b).

Sheep

A single dose of [^{14}C]-methyl-labelled paraquat administered to a sheep via a rumen fistula was recovered quantitatively within 10 days. Approximately 4% of the dose was excreted in the urine and the remainder in the faeces. More than 95% of the radioactivity in urine and faeces was present as unchanged paraquat. Small amounts of monoquat ion (1%) and monopyridone ion (2-3%) were also detected (Hemingway et al., 1972).

When injected subcutaneously (s.c.), paraquat was also excreted rapidly in the urine. Over 80% of the dose was excreted in the urine, 69% within the first day after treatment. Unchanged paraquat accounted for most of the radioactivity (90%); paraquat monopyridone was present at 2-3% of the dose and monoquat was present in trace amounts. This pattern of metabolism was virtually identical to that seen in the urine following dosing via the rumen (Hemingway et al., 1972).

Cows

When cows were given single oral doses of [^{14}C]-methyl paraquat at 8 ppm, 96% of the radioactivity was recovered in the faeces during the following 9 days; 0.7% was recovered in the urine. Unchanged paraquat accounted for most of the radioactivity in the faeces (96%) and urine (62 - 90%), but traces of the monoquat ion and monopyridone ion were also detected in the urine. Only 0.003% of the radioactivity was recovered in milk; the maximum radioactive residue (0.005 mg/kg, paraquat cation equivalent) was observed on the day after dosing. About 15% of this radioactivity was present as unchanged paraquat. Monoquat ion and monopyridone ion (3 - 25%) were also found in the milk. The radioactivity not identified as paraquat, monoquat, or monopyridone was incorporated into natural constituents of milk. The residue of any single compound in the milk was never greater than 0.002 mg/kg. (Leahey et al., 1972).

Cows were fed for 3 months diets containing 254, 80, or 170 ppm paraquat cation (equivalent to 0.8, 2.5, or 5.5 mg/kg bw/day). The paraquat was present as a residue in dried grass obtained from a pasture that had been sprayed with Gramoxone and subsequently weathered. The diet was accepted satisfactorily and no toxicological effects were observed during the trial. Pathological examination of tissues from animals slaughtered within 24 hours of the end of the feeding trial showed no toxic effects attributable to paraquat. The tissue residues, including muscle and liver, determined in cows at the 2 higher dose rates, varied between 0.01 and 0.09 mg/kg except in the kidney, where 0.20 - 0.31 mg/kg was found. These fell to low (0.04 mg/kg in the kidney) or non-detectable levels in an animal fed the high-paraquat diet for 30 days and then maintained on an untreated diet for 12 days before slaughter. Very low residues of paraquat were present in milk samples taken weekly during the trial (121 samples ranging from 0.0001 - 0.0006 mg/kg; 1 sample = 0.001 mg/kg) (Edwards et al., 1974).

Summary of the absorption, distribution, metabolism and excretion of paraquat

The absorption, distribution, metabolism and excretion of paraquat has been studied following single low (1 mg paraquat/kg bw), single high (50 mg paraquat/kg bw) or repeated low dose gavage administration in deionised water to rats. In all studies the absorption as determined by extent of urinary excretion was low (*ca* 10-20%), though what absorption there was occurred rapidly (within 6 hours). Results from single or repeat (14 daily doses) dosing at 1 mg paraquat/kg bw showed excellent consistency, demonstrating that paraquat is unlikely to accumulate in the body. There was evidence of reduced absorption in males receiving 50 mg paraquat/kg bw/day compared with 1 mg paraquat/kg bw/day and an extension of the period of faecal excretion in both sexes at the higher dose. At the low dose, females excreted less of the dose in urine than males, this difference was not evident at the high dose. The organ having the highest concentration of paraquat 72 hours post dosing was the lung (*ca* 0.02 μg equivalents/g after the low doses and *ca* 0.8-1.1 μg equivalents/g after the high dose). Total residues in the carcass and organs at 72 hours post dosing were <1% of the administered doses.

Evidence from published studies supports the above findings and indicates other species handle paraquat dichloride in a similar way to rats.

Analysis of urine and faeces for metabolites showed paraquat dichloride to undergo hardly any metabolism, with less than 1% of the administered dose being excreted in the form of metabolites.

Dermal absorption data show that paraquat is poorly absorbed through human epidermis.

### (c) Biochemical Mechanism of paraquat toxicity

Several reviews or monographs have summarised the biochemical mechanism of paraquat toxicity in plants (Calderbank, 1968), bacteria (Fridovich & Hassan, 1979), and animals (Bus et al., 1976; Autor, 1977; Smith et al., 1979; Smith, 1985). The

mechanism of the toxic action of paraquat has also been extensively reviewed by WHO (1984).

Paraquat can be reduced to form a free radical, which is stable in aqueous solution in the absence of oxygen (Michaelis and Hill, 1933).
$$PQ^{2+} + e^- \rightarrow PQ^{+\cdot}$$
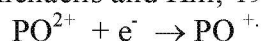
In the presence of oxygen, in biological systems, the radical will rapidly re-oxidise to the cation with the concomitant production of superoxide anion O_2^- (Farrington et al., 1973).
$$PQ^{+\cdot} + O_2 \rightarrow PQ^{2+} + O_2^-$$
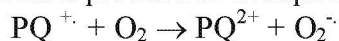
Thus, once paraquat enters a cell it will undergo alternate reduction followed by re-oxidation a process known as redox cycling. Gage (1968b) first reported that the paraquat cation could be reduced by rat liver NADPH-dependent microsomal flavoprotein reductase to form the radical, with the concomitant oxidation of NADPH. Redox cycling of paraquat has also been reported in microsomal preparations of lung, liver and kidney (Baldwin et al., 1975) and in lung microsomal and slice systems (Adam et al., 1990). Studies using antibodies against NADPH-cytochrome c reductase have shown that paraquat radical formation can be blocked, demonstrating a role for this enzyme in the reduction process (Bus et al., 1974; Horton et al., 1986). Further support for a key role for NADPH-cytochrome c reductase comes from the studies of Kelner and Bagnel (1989) using a lymphoblastoid cell line with a specific deficiency in this enzyme which they reported was very resistant to paraquat toxicity.
Thus, provided there is sufficient NADPH, as an electron donor, and O_2 as an electron acceptor, paraquat will redox cycle inside a cell, generating superoxide anion and consuming NADPH. This reaction is believed to be a key step in the mechanism of paraquat toxicity, however the biochemical consequence of this reaction, which leads to lung cell death, are complex and still not fully understood. Recent studies with endothelial cells in culture have indicated that xanthine oxidase can also mediate redox cycling of paraquat to produce superoxide anion (Sakai et al., 1995) indicating that two intracellular enzyme systems are probably involved.

Mammalian cells have many enzyme systems, which provide them with protection against free radical attack, and it is assumed that once these defences have been overwhelmed that cell death occurs. Superoxide dismutase (SOD) is a family of metalloenzymes that can dismutate superoxide anion to hydrogen peroxide and oxygen.
$$O_2^- + O_2^- \rightarrow H_2O_2 + O_2$$
The importance of this enzyme in cellular toxicity comes from studies where cellular SOD activity has been genetically modified either by spontaneous mutation or by the transfection of SOD genes. Bilinski and Litwinska (1987) isolated a mutant yeast deficient in SOD activity, which had a greater sensitivity to paraquat than its isogenic wild type. In contrast, Hela cells which possess a higher content of both manganese and copper/zinc SOD had an increased resistance to paraquat (Krall et al., 1988). Transfection of human copper/zinc SOD into various cell lines also lead to resistance to paraquat toxicity (Elroy-Stein et al., 1986; Krall et al., 1988). Recent studies have shown that mice lacking copper/zinc SOD show a marked increase in sensitivity to paraquat. $Sod^{-/-}$ mice showed a median survival time of about 1.5 days after 10mg/kg ip, while the $Sod^{+/-}$ and $Sod^{+/+}$ mice appeared normal at the end of 7 days of observation (Ho et al., 1998). These studies provide strong evidence for a role for
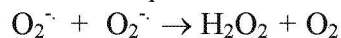
superoxide anion radical in the mechanism of cellular toxicity and for the role of copper/Zn SOD in protecting the lungs against paraquat toxicity.

However, superoxide anion itself is unlikely to be the ultimate toxic species as it has limited reactivity in biological systems (Halliwell and Gutteridge, 1984). Dismutation of superoxide anion leads to hydrogen peroxide formation, which can undergo detoxification by catalase and glutathione peroxidase. Studies with genetically engineered cells have shown that the balance between these two enzymes plays an important role in cellular toxicity of paraquat. Increasing intracellular concentrations of SOD to high levels can alter the balance of metabolism of hydrogen peroxide from two electron addition via catalase and glutathione peroxidase to produce water to allow an increase in one electron metabolism to form hydroxyl radical.

$$H_2O_2 \rightarrow^{e-} \rightarrow OH^- \rightarrow^{e-} \rightarrow H_2O$$
$$\downarrow$$
$$2e-$$

$$\downarrow catalase/GSH \, peroxidase$$
$$H_2O$$
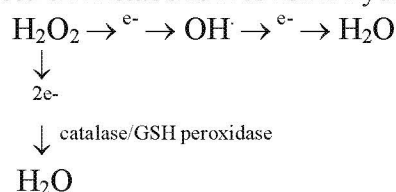
Increasing intracellular SOD content to a very high level, ultimately leads to an increase in toxicity to paraquat in a number of transfected cells or *Escherichia coli* (Elroy-Stein *et al.*, 1986; Bloch and Ausubel, 1986; Scott *et al.*, 1987; Scott and Eaton, 1996). In contrast, cells having an increase in both SOD and catalase exhibited a greater resistance to paraquat than with just SOD alone (Krall *et al.*, 1988).

Generation of hydroxyl radical has been proposed as the critical event in the toxicology of paraquat, this reaction requires the presence of iron and is generated by the Fenton reaction. In this reaction ferrous ions react with hydrogen peroxide to generate hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$$
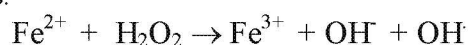
Under physiological conditions free iron predominately exists in the ferric form (Fe^{3+}) as a chelate with ADP, ATP and citrate, the reduction of ferric iron may be achieved directly by the paraquat radical (Winterbourn and Sutton, 1984; Sutton *et al.*, 1987) or indirectly by superoxide anion generated from the redox cycling of paraquat (McCord and Day, 1978).

A role for transition metals such as iron in the toxicity is supported by studies showing that paraquat toxicity is reduced by removal of iron and enhanced by its addition (Kohen and Chevion, 1985; Sion *et al.*, 1989; Van Der Wal *et al.*, 1990). The role of the iron chelator desferrioxamine in affording some protection against paraquat toxicity will be discussed in the section on antidotes.

Many other studies have been conducted both *in vitro* and *in vivo* to explore the effect of altered anti-oxidant status on the toxicology of paraquat. Examples include the role of GSH and GSH reductase (Bus *et al.*, 1976; Keeling, *et al.*, 1982; Hardwick *et al.*, 1990), the role of selenium deficiency, vitamin E and glutathione peroxidase (Bus *et al.*, 1976b; Cagen and Gibson, 1977; Block, 1979; Omaye *et al.*, 1978; Kelner *et al.*, 1995) and the role of metallothionein (Satoh *et al.*, 1992; Lazo *et al.*, 1995). Metallothionein appears to have play a role as a free radical scavenger in addition to its well established role as a heavy metal chelator, metallothionein has been reported

to quench both superoxide anion and hydroxyl radicals, with a significantly higher reactivity towards hydroxyl radicals (Thornalley and Vasak, 1985). Genetically engineered animals have been used as tools to try and elucidate the function of the various antioxidant defence mechanism against paraquat-induced oxidant injury. In addition to the discussion above regarding mice deficient in copper/zinc SOD, Sato *et al.*, (1996) found mice deficient in metallothionein I and II genes to be more susceptible to paraquat toxicity. Glutathione peroxidase deficient mice show a increased susceptibility to paraquat toxicity with a mean survival time of 5hr compared to the wild type of 69hours following an ip dose of 50mg/kg (Cheng *et al.*, 1998). Mice over expressing glutathione peroxidase are more tolerant to paraquat toxicity; wild type mice given a large 125mg/kg ip dose of paraquat died within 5hours while the mice over expressing the enzyme lived until about 54hours (Cheng *et al.*, 1998).

A schematic representation of the key requirements to enable paraquat to enter a cell and the subsequently redox cycling steps believed to lead to cytotoxicity is shown below. Three hypotheses have been proposed to account for the ensuing cytotoxicity, one involving lipid peroxidation, another the oxidation of NADPH and the third mitochondrial toxicity, none of these hypotheses are mutually exclusive.

Schematic representation of the key requirements to enable paraquat to enter a cell
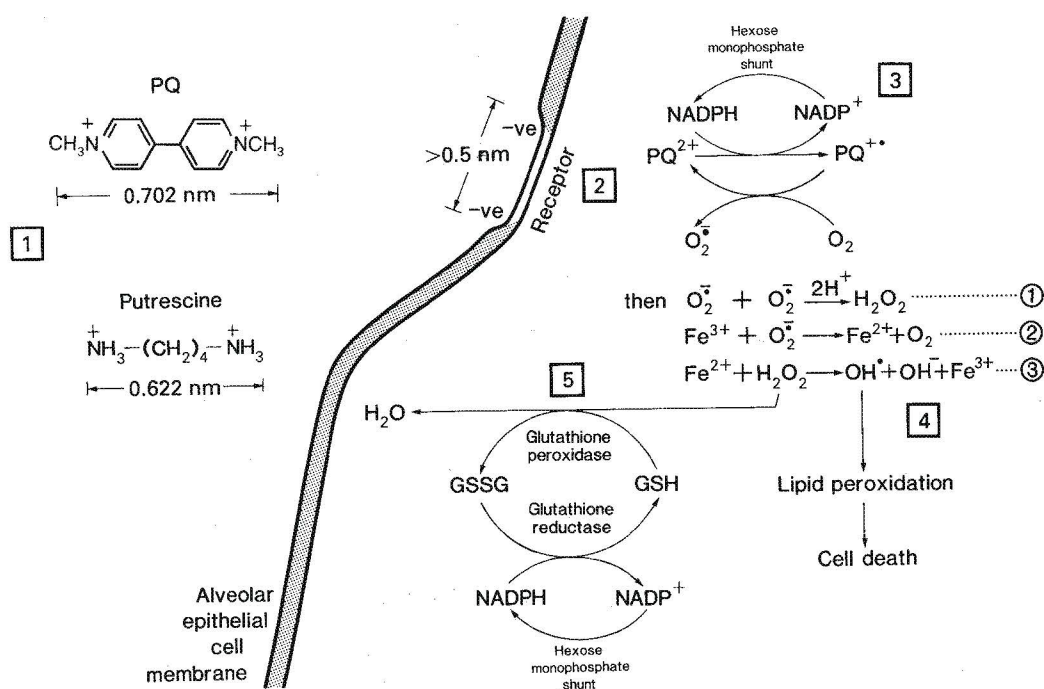
Mechanism of toxicity of paraquat: A schematic representation of the mechanism of toxicity of paraquat. 1 = structure of paraquat and putrescine showing the geometric standards of the distance between the n atoms; 2 = transport system which recognises paraquat, minimum separation of charge of approximately 0.5nm; 3 = redox cycling of paraquat utilising NADPH; 4 = formation of hydroxyl radical leading to lipid peroxidation; 5 = detoxification of H_2O_2 via glutathione reductase/peroxidase couple,

utilising NADPH. From Smith, (1987). Reproduced with permission from *Human Toxicology.*

Lipid peroxidation hypothesis   Bus and co-workers (1974; 1976a) proposed the sequential generation of superoxide anion and hydroxyl radical and the initiation of lipid peroxidation as the mechanism of cellular toxicity of paraquat. However, there is little direct evidence which demonstrates lipid peroxidation occurs in the lung of animals dosed with paraquat before there is morphological evidence of cell damage. Paraquat-induced lipid peroxidation has been demonstrated *in vitro* in broken cell systems and isolated cells from the lung and liver (Bus *et al.*, 1976a; Kornbrust and Mavis, 1980; Trush *et al.*, 1981; Sata *et al.*, 1983; Saito *et al.*, 1985; Sandy *et al.*, 1986; Aldrich *et al.*, 1983) and *in vivo* (Bus *et al.*, 1976b; Reddy *et al.*, 1977; Burk *et al.*, 1980). However, others have questioned its significance in the toxicity, for example Steffen *et al.*, (1980) only found a small increase in the exhalation of ethane (a marker of lipid peroxidation) in rats suffering from respiratory distress following exposure to paraquat and oxygen. Similarly, others have been unable to find evidence of lipid peroxidation in the lungs of mice given large doses of paraquat (Shu *et al.*, 1979; Younes *et al.*, 1985) or it is only detected as a late event in the toxicity (Ogata and Manabe, 1990). So the question remains as to whether lipid peroxidation is a cause, or a consequence, of the toxicity. These contrasting findings *in vivo* may also reflect the difficulty in detecting a small but critical increase in lipid peroxidation in the alveolar type I and II cells and Clara cell that are only a small population of the total cells in the lung.

Oxidation of NADPH hypothesis   Intracellular redox cycling of paraquat results in the oxidation of NADPH leading to cellular depletion such that those cells that selectively accumulate paraquat can no longer function normally. Fisher *et al.*, (1975) first suggested that the redox potential of lung cell may be altered by the redox cycling of paraquat. A marked stimulation of the activity of the pentose phosphate pathway in the lung has been observed following exposure to paraquat (Fisher *et al.*, 1975; Rose *et al.*, 1976b; Bassett and Fisher, 1978; Keeling *et al.*, 1982 and Fisher and Reicherter, 1984). Since this pathway represents the major cellular source of NADPH, it is inferred that this response represents an attempt by lung cells to maintain their levels of reducing equivalents under conditions of oxidative stress. In those cells in which paraquat is accumulated, the concentration may be very high and result in very fast rates of NADPH oxidation. If the rate of consumption exceeds the rate of formation via the pentose phosphate pathway, the concentration of NADPH will fall below that required to maintain cell viability. Witschi *et al.*, (1977) first demonstrated that the $NADPH/NADP^+$ ratio in the lungs of rats dosed iv with paraquat was decreased suggesting that oxidation of the reduced nucleotide had occurred. Later studies by Keeling and Smith, (1982) demonstrated that the shift in $NADPH/NAD^+$ ratio in the lung following subcutaneous administration of paraquat was the result of NADPH loss from the lung. A consequence of depletion of cellular NADPH is that the cell shuts down it synthetic pathways which are dependent on this nucleotide, such as the synthesis of fatty acids (Keeling *et al.*, 1982). A loss of NADPH may also have particular importance for alveolar type II cells which produce pulmonary surfactant (Brigelius *et al.*, 1986).

NADPH is also consumed in an attempt by the lung to detoxify hydrogen peroxide that is formed via the glutathione peroxidase/reductase enzyme system (Figure 5) to

regenerate reduced glutathione (GSH) from its oxidised form (GSSG). In general large changes in lung GSH and GSSG are not seen after paraquat administration (Bus *et al.*, 1976a; Reddy *et al.*, 1977; Shu *et al.*, 1979; Keeling and Smith, 1982) this may explain why lipid peroxidation has not been conclusively demonstrated *in vivo*, as this would not become apparent until both NADPH and GSH were markedly reduced. However, formation of protein mixed disulphides is increased in the lung *in vivo* (Keeling *et al.*, 1982; Keeling and Smith, 1982) and in perfused liver (Brigelius *et al.*, 1982). These changes in protein mixed disulphides in the lung are presumably a response to oxidative stress and may not be critical to the cellular toxicity. This notion is supported by studies with the bipyridyl diquat, which can undergo redox cycling in the lung (Rose *et al.*, 1976b; Witschi *et al.*, 1977). Diquat also produced increases in protein mixed disulphide content in the lung without affecting NADPH content at a dose that did not cause lung injury (Keeling and Smith, 1982). This indicates that NADPH depletion subsequent to redox cycling is a critical step in the mechanism of paraquat toxicity.

The role of mitochondria in the toxicity of paraquat   Another hypothesis that has been proposed is that paraquat toxicity is due to mitochondrial damage, based on morphological findings of early mitochondrial changes in alveolar type II cells (Hirai *et al.*, 1985) Ultrastructural studies of the time course of development of paraquat-induced lung injury have also reported early changes to mitochondria such as swelling and altered staining density (Smith and Heath, 1974a; Sykes *et al.*, 1977; Keeling *et al.*, 1981). These mitochondrial changes were also observed in the lungs of rats exposed to paraquat and 85% oxygen, which enhance paraquat toxicity to the lung (Keeling *et al.*, 1981). However, as discussed with regard to the lipid peroxidation hypothesis, the question is are the effects on mitochondria a cause, or a consequence, of paraquat toxicity. Early studies with isolated liver mitochondria reported only minor changes in mitochondria respiration by paraquat (Gage, 1968b). More recent studies have reported that paraquat cation can be reduced by NADH-ubiquinone oxidoreductase (Complex I) located on the inner mitochondrial membrane (Fukushima *et al.*, 1993; Shimada *et al.*, 1998), these authors also showed that paraquat was able to stimulate lipid peroxidation in submitochondrial particles (Yamada and Fukushima, 1993). These findings show that mitochondria have the potential to generate superoxide anion from paraquat provided it can gain access. In general, studies with intact mitochondria support the orginal findings of Gage, (1968b) showing that little or no effects are seen (Lambert and Bondy 1989, Constantini *et al.*, 1995) unless very high concentrations of paraquat are present (Kopaczyk-Locke, 1977; Yamamoto *et al.*, 1987; Thakar and Hassan, 1988; Palmeira *et al*, 1995). Paraquat has been shown to induce a Ca^{2+}-dependent permeability transition of the inner mitochondrial membrane leading to membrane depolarisation, uncoupling and matrix swelling in isolate rat liver mitochondria (Constantini *et al.*, 1995). This opening of the membrane permeability pore does not occur in the absence of added Ca^{2+} and requires the presence of rotenone, leading one to question the relevance of this observation to the *in vivo* situation. It seems likely that any intracellular increases in Ca^{2+} would only occur once paraquat had entered the lung cell, undergone redox cycling and altered mixed disulphide status. In summary, mitochondrial damage has been observed in the lung prior to cell death, it seems likely that this response is secondary to changes taking place in the cytosol.

The involvement of oxygen   As discussed earlier, the redox cycling of paraquat to form superoxide anion requires oxygen and hence oxygen plays a critical role in the toxic process. It has been known for many years that hyperoxia is toxic to the lung, causing damage to endothelial cells through a mechanism that involves the formation of reactive oxygen species (Frank and Massaro, 1979; Jenkinson, 1982). One of the therapeutic measures for anoxia in human cases of paraquat poisoning was the addition of air supplemented with oxygen. However, it has been shown that increasing the oxygen concentration potentiates the lethality of paraquat to rats (Fisher *et al.*, 1973; Douze and van Heijst, 1977; Kehrer *et al.*, 1979; Keeling *et al.*, 1981) increasing the injury to the lung. While the converse is true, rats exposed to paraquat in a hypoxic environment are protected relative to those exposed to paraquat in air (Rhodes *et al.*, 1976). Detailed histopathology on the lungs of rats exposed to paraquat alone or paraquat in an atmosphere of 85% oxygen, showed that the damage was primarily localised to the alveolar type I and II cells with little evidence of endothelial cell damage, showing that oxygen potentiated paraquat toxicity (Keeling *et al.*, 1981). These findings have recently been reproduced using isolated rat and human alveolar type II cells exposed to either paraquat in air or paraquat and increasing concentrations of oxygen. Increasing the oxygen concentration in the atmosphere potentiated the toxicity of paraquat, while lowering the oxygen concentration to 10% afforded some protection (Hoet *et al.*, 1997). The mechanism underlying this synergistic effect of oxygen on paraquat toxicity is not entirely clear, it seems unlikely that oxygen would normally be rate limiting for paraquat to redox cycle, a more likely explanation is that the cellular defence mechanisms that protect against oxygen and paraquat toxicity are more rapidly overwhelmed.

In summary, the key events leading to cellular toxicity are (1) accumulation of paraquat into the cell and (2) its ability to redox cycle and produce oxidative stress. It seems likely that a combination of depletion of NADPH plus the generation of hydroxyl radical leading to lipid peroxidation and mitochondrial dysfunction is involved but the precise temporal relationships have not as yet been established.

2. Toxicological studies

(a) Acute toxicity

New modern studies were performed to support re-registration in both the US and EU on the acute toxicity of paraquat in the rat, Table 4. Previously reported data from various species are summarized in Table 5.

Table 4. Acute toxicity of paraquat dichloride technical material in the rat

Species	Strain	Sex	Route	LD_{50} /LC_{50} (95% CL or range)	Purity (%)	Reference
Rat	Alpk:AP fSD	Male	Oral	344 (246–457) mg technical paraquat dichloride/kg bw (114 mg paraquat cation/kg bw ≈ 157 mg PDC/kg bw/day)	45.6% PQ dichloride (33% w/w PQ cation)	Duerden L, 1994a
Rat	Alpk:AP fSD	Female	Oral	283 (182–469) mg technical paraquat dichloride/kg bw (93 mg paraquat cation/kg bw ≈ 128 mg PDC/kg bw/day)	45.6% PQ dichloride (33% w/w PQ cation)	Duerden L, 1994a

| Rat | Alpk:AP fSD | Male and Female | Dermal | >2000 mg technical paraquat dichloride/kg bw (660 mg paraquat cation/kg bw ≈ 911 mg PDC/kg bw/day) | 45.6% PQ dichloride (33% w/w PQ cation) | Duerden L, 1994b |
| Rat | Alpk:AP | Male and female | Inhalation (4 hour, nose-only) * | 0.83 – 1.93 mg paraquat dichloride/m^3 (equivalent to 0.6 – 1.4 mg paraquat cation/m^3 ≈ 0.8 – 1.9 mg PDC/kg bw/day) | (21.5% w/v PQ cation) | McLean-Head L K et al, 1985 |

* Paraquat dichloride is non-volatile and formulations containing paraquat are not applied through equipment which would generate a significant proportion (> 1% w/w) of spray droplets of diameter less than 50 μm. Inspirable atmospheres of paraquat dichloride will, therefore, not be produced so the above data is not relevant to a human risk assessment.

PDC paraquat dichloride

Table 5. Acute toxicity of paraquat dichloride in various species.

Most papers do not give specification of the test material, therefore assumed to be the paraquat cation.

Species	Sex	Route	LD_{50} /LC_{50} as paraquat cation (also quoted as PDC)	Reference
Mouse	Male	Oral	260 mg/kg bw (359)	Shirasu & Takahashi, 1977
Mouse	Female	Oral	210 mg/kg bw (290)	Shirasu & Takahashi, 1977
Mouse	Female	Oral	196 mg/kg bw (270)	Bus et al., 1975a
Mouse	Male and Female	ip	29-30 mg/kg bw (40-41)	Shirasu & Takahashi, 1977. Bus et al., 1975a, Bus et al., 1976, Ecker et al., 1976
Mouse	-	iv	50 mg/kg bw (69)	Ecker et al., 1975
Mouse	Male	sc	30 mg/kg bw (41)	Shirasu & Takahashi, 1977
Mouse	Female	sc	27 mg/kg bw (37)	Shirasu & Takahashi, 1977
Mouse	-	dermal	62 mg/kg bw (86)	Bainova, 1971
Rat	Male	Oral	161 mg/kg bw (222)	Shirasu & Takahashi, 1977
Rat	Female	oral	187 mg/kg bw (258)	Shirasu & Takahashi, 1977
Rat	Male	oral	110 mg/kg bw (152)	Kimbrough & Gaines, 1970
Rat	Female	oral	100 mg/kg bw (138)	Kimbrough & Gaines, 1970
Rat	Female	oral	112 – 150 paraquat cation/kg bw (155 – 207)	Clark et al, 1966
Rat	Female	oral	150 mg paraquat cation/kg bw (207)	Mehani, 1972
Rat	-	oral	126 mg/kg bw (174)	Murray & Gibson, 1972
Rat	-	oral	200 mg/kg bw (276)	Howe & Wright, 1965
Rat	Male	ip	18 mg/kg bw (25)	Shirasu & Takahashi, 1977
Rat	Female	ip	19 mg/kg bw (26)	Shirasu & Takahashi, 1977
Rat	Female	ip	19 mg paraquat cation/kg bw (26)	Clark et al., 1966

Rat	Female	ip	16 mg paraquat cation/kg bw (22)	Mehani, 1972
Rat	Male	sc	19 mg/kg bw (26)	Shirasu & Takahashi, 1977
Rat	Female	sc	23 mg/kg bw (32)	Shirasu & Takahashi, 1977
Rat	-	sc	22 mg/kg bw (30)	Makovskii, 1972
Rat	Male	dermal	80 mg paraquat cation/kg bw (110)	Kimbrough & Gaines, 1970
Rat	Female	dermal	90 mg paraquat cation/kg bw (124)	Kimbrough & Gaines, 1970
Rat	-	dermal	350 mg/kg bw (483)	Makovskii, 1972
Rat	Male and Female	inhalation	10 mg/l (13.8)	Bainova & Vulcheva, 1972
Rat	-	inhalation	1 mg/l (1.38)	Gage, 1968
Rat	Male & Female	inhalation	6 μg paraquat cation/l/hour (8)	Gage, 1968a
Rat	-	inhalation	6 mg/l (8)	Makovskii, 1972
Guinea Pig	-	oral	22 - 80 mg/kg bw (30 – 110)	Howe & Wright, 1965; Murray & Gibson, 1972; Makovskii, 1972
Guinea Pig	Male	oral	30 mg paraquat cation/kg bw (41)	Clark et al., 1966
Guinea Pig	Female	ip	3 mg paraquat cation/kg bw (4)	Clark et al., 1966
Guinea Pig	-	sc	5 mg/kg bw (7)	Makovskii, 1972
Guinea Pig	-	dermal	319 mg/kg bw (440)	Makovskii, 1972
Guinea Pig	-	inhalation	4 mg/l (5.5)	Makovskii, 1972
Rabbit	Male	oral	100 mg/kg bw (152)	Kuo & Nanikawa, 1990
Rabbit	Male	oral	50 mg (45-58confidence limits) paraquat cation/kg bw (69)	Mehani,1972
Rabbit	Male	dermal	236 mg paraquat cation/kg bw (collars removed) (326)	Clark et al., 1966
Rabbit	Male	dermal	>480 mg paraquat cation/kg bw (with collars) (662)	McElligott, 1972
Rabbit	Male	ip	25 mg (15-30confidence limits) paraquat cation/kg bw (35)	Mehani, 1972
Rabbit	Male	ip	18 mg (11-31 confidence limits) paraquat cation/kg bw	McElligott, 1972

Cat	Female	oral	35 mg paraquat cation/kg bw (48.3)	Clark et al., 1966
Cat	-	oral	40-50 mg/kg bw (55-69)	Howe & Wright, 1965
Hen	-	oral	300-380 mg/kg bw (414 – 524)	Howe & Wright, 1965
Hen	-	oral	262 mg/kg bw (362)	Clark et al., 1966
Turkey	-	oral	250-280 mg/kg bw (345-386)	Smalley, 1973
Turkey	-	ip	100 mg/kg bw (138)	Smalley, 1973
Turkey	-	iv	20 mg/kg bw (28)	Smalley, 1973
Turkey	-	dermal	375 mg/kg bw (518)	Smalley, 1973
Dog	Male	sc	1.8 (1-6.1 confidence limits) mg/kg bw (2.5)	Nagata et al., 1992
Dog	Female	sc	3.5 (2.4–10.1 confidence limits) mg/kg bw (4.83)	Nagata et al., 1992
Monkey	-	oral	50 mg/kg bw (69)	Murray & Gibson, 1972
Sheep	-	oral	50-75 mg/kg bw (69-104)	Howe & Wright, 1965
Cow	-	oral	50-75 mg/kg bw (69-104)	Howe & Wright, 1965

Key: - sex not given

ip intraperitoneal injection
iv intravenous injection
sc subcutaneous injection
PDC paraquat dichloride

Skin Irritation: The skin irritation potential of paraquat technical material was assessed in a modern study (Duerden L, 1994c). A group of 3 young female adult New Zealand White albino rabbits received a single four-hour application of approximately 0.5 ml technical paraquat dichloride (45.6% paraquat dichloride, 33% w/w paraquat cation) to a 2.5 x 2.5 cm area of intact skin on the shorn flank. The test substance was held in place with an occlusive dressing. The application site was cleansed with water at the end of the 4 hour dosing period.

The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 1 hour and 1, 2 and 3 days after removal of the dressings and then at intervals up to 34 days until all sites appeared normal. Mean erythema and oedema scores were calculated. Any additional signs of skin irritation were also noted. Bodyweights were recorded at the start of the study.

There was very slight erythema in all 3 rabbits (score 0.7-1.0 on a scale of 0-4); very slight oedema in one rabbit (score 1.0 on a scale of 0-4) and desquamation, thickening and scabbing in one rabbit. Erythema regressed 2-3 days after dosing in two rabbits and after 27 days in one rabbit; oedema regressed 7 days after dosing and all other

signs had regressed after 34 days. There was no indication of full tissue destruction. The primary dermal irritation score at 72 hours was 0.5.

This study shows technical paraquat dichloride to be a slight but persistent skin irritant. (Duerden, L 1994c).

The effects of paraquat on the skin have been reviewed by WHO (1984). Paraquat can provoke local irritation of the skin. Clark et al. (1966) found skin irritation in rabbits only when paraquat was applied beneath occlusive dressings in aqueous solutions (total doses 1.56, 5.0 and 6.25 mg paraquat cation/kg bw). In mice and rats the application of solutions of 5–20 g paraquat/litre (paraquat cation) in single and 21-day repeated dermal toxicity tests provoked dose-related toxic dermatitis with erythema, oedema, desquamation and necrosis (Bainova, 1969). A delayed skin irritant action was reported by Fodri et al. (1977) in guinea pig studies.

Eye Irritation: The eye irritation potential of paraquat dichloride technical material was assessed in a modern study (Bugg, L & Duerden L, 1994). The potential of paraquat to cause primary irritation of the eye was studied in three female New Zealand white rabbits that received a single instillation of 0.1 ml of technical paraquat dichloride (45.6% paraquat dichloride, 33% w/w paraquat cation) into the conjunctival sac of one eye. The eyes were graded for ocular irritation at 1 hour and 1, 2, 3, 4 and 7 days and then periodically until day 28, using the Draize scoring system. The untreated eye served as a control.

Instillation into the eye caused no to slight initial pain (class 1-2 on a 0-5 scale). Corneal effects (slight or mild opacity involving 25 – 50% of the cornea) were seen but had cleared by day 17. Conjunctival effects were seen (slight to severe redness which had disappeared by day 28; discharge which was still present in 2 of the rabbits at the end of the study and slight to mild chemosis which had cleared by day 14). There was no iridial involvement. Additional signs of irritation seen included slight to severe mucoid discharge, slightly convoluted eyelids and slight erythema of the upper/lower eyelids.

This study shows technical paraquat dichloride to be a persistent moderate to severe irritant to the rabbit eye (class 5 on a 1-8 scale) (Bugg, L & Duerden, L., 1994).

The effects of paraquat on the eye have been reviewed by WHO (1984). The instillation of diluted paraquat (up to 500 g/l paraquat dichloride) to the rabbit eye induced inflammation within 24 hours and this persisted for 96 hours (Clark et al., 1966). In another study 62.5, 125, 250, 500 or 1000 g/l of paraquat cation was instilled into rabbit eyes. Concentrations of 62.5 and 125 g/l caused severe conjunctival reactions; higher levels (250-500 g/l) provoked iritis and pannus, and at 500 g/l corneal opacification, iritis and conjunctivitis occurred. All rabbits receiving 0.2 ml at 1000 g/l in one eye or 0.2 ml of 500 g/l in both eyes died within 6 days of application (Sinow & Wei, 1973).

Skin sensitisation: The sensitisation potential of technical paraquat dichloride (45.6% paraquat dichloride, 33% paraquat cation) was assessed in a modern study (Duerden, L 1994d) using a method based on the maximisation test of Magnusson and Kligman.

Groups of 20 test and 10 control young adult female Dunkin Hartley guinea pigs were used for the main study. Two main procedures were involved; (a) the induction of an immune response; (b) a challenge of that response.

In test animals, the induction phase involved 3 intradermal injections (approximately 0.1 ml each) of a Freund's complete adjuvant (FCA) plus corn oil in the ratio 1:1; a 0.03% w/v solution of paraquat dichloride in deionised water; and a 0.03% w/v solution of paraquat dichloride in a 1:1 preparation of Freund's complete adjuvant (FCA) plus deionised water. This was followed 1 week later by a topical induction of paraquat dichloride as a 10% (w/v) solution in deionised water (0.2 – 0.3 ml) applied on filter paper under an occlusive dressing for 48 hours. For control animals the intradermal injections were Freund's complete adjuvant plus deionised water in the ratio 1:1; deionised water alone; and FCA and deionised water in the ratio 1:1. The topical applications were as for the test animals except that deionised water only was applied to the filter paper.

In the challenge phase, two weeks after completion of the induction phase, 0.05 – 0.1 ml of either a 30% (w/v) preparation of paraquat dichloride in deionised water, the maximal non-irritating concentration, or deionised water alone was applied to the shorn flanks under an occlusive dressing for 24 hours. Skin sites were examined approximately 1 and 2 days after removal of the dressings. The animals were assessed daily for any signs of systemic toxicity and their bodyweights were recorded at the beginning and end of the study. The sensitivity of the assay was confirmed in animals exposed to a positive control (30% w/v aqueous mercaptobenzothiazole solution) administered in a similar manner to the test material.

One test animal died following topical induction, showing no signs of toxicity. There was no evidence of erythema in either test or control animals from the paraquat exposed groups. In the positive control segment, 19/20 animals responded, confirming the sensitivity of the test. Technical paraquat dichloride (33% w/w paraquat cation) was negative in the Magnusson and Kligman maximisation study and is not classifiable as a skin sensitiser under the EU criteria. (Duerden, L 1994d).

Summary of acute toxicity, irritancy and sensitisation:

Technical paraquat dichloride (33% w/w paraquat cation) produced by Syngenta is of moderate acute toxicity orally, low acute toxicity dermally and very toxic by inhalation to the rat when generated as respirable particles. Lungs were identified as the key target organ following oral and inhalation exposure. In rabbits technical paraquat dichloride is a slight but persistant skin irritant and a moderate and persistant eye irritant. Technical paraquat dichloride produced no evidence of skin sensitising potential in guinea pigs when tested using a maximisation protocol.

Other studies cited in the literature and tabulated above give the acute oral LD_{50} to rats as 100–200 mg/kg bw, to mice as 196-260 mg/kg bw and lower in the guinea pig, rabbit, cat, monkey, sheep and cow, between 22-100 mg/kg bw. The acute oral LD_{50} to the hen and turkey was between 250 and 380 mg/kg bw. The acute dermal LD_{50} to the rat was 80-350 mg/kg bw, to mice was 62 mg/kg bw, to guinea pig was 319 mg/kg bw, to rabbit was 236 to >480 mg/kg bw and to the turkey was 375 mg/kg bw. The

acute ip LD_{50} was between 3 and 30 mg/kg bw in small mammals and was 100 mg/kg bw in the turkey. These various studies reported in the literature indicate that there may be differences in the acute toxicity profile (particularly dermal toxicity and sensitisation) between different sources of paraquat and different strains of animals. It also highlights the lack of clarity in reporting many of the research studies which were not conducted to regulatory guidelines (eg specification of dose, purity of test material). Further low dermal toxicity may be attributable to secondary oral ingestion.

(b) Short-term studies of toxicity
The sub-chronic toxicity of paraquat has been investigated in a 90 day feeding study in the mouse, a 21 day inhalation study in the rat, a thirteen week feeding study in the rat and the dog, and a 21 day dermal toxicity study in the rabbit.

Mouse
13 week mouse toxicity study: Groups of 20 male and 20 female ICR-CRJ SPF mice were maintained on diets containing 0, 10, 30, 100 or 300 ppm paraquat dichloride for 13 weeks (equivalent to 0, 7.2, 22, 72, or 217 ppm paraquat cation). At the 300 ppm paraquat dichloride dietary level, 2 female mice died from pulmonary damage. Both males and females in this group showed significantly reduced body-weight gain and a slight reduction in efficiency of food utilisation. Food intake and water intake were not affected. No abnormalities considered related to paraquat treatment were seen during haematological, blood biochemistry, or urine analyses. A few statistically-significant changes in absolute and relative organ weights were seen at termination, mainly in males and females of the 300 ppm paraquat dichloride group. However, only an increase in lung weight of females in the 300 ppm paraquat dichloride group was reported by the authors of the study to coincide with histopathological changes of the same organ, namely eosinophilic swelling of the alveolar epithelium walls which was observed in both sexes at this dietary level. The no-effect level in this study with respect to pulmonary damage and other parameters was 100 ppm paraquat dichloride, equivalent to 8.3 (males) and 10 (females) mg paraquat cation/kg bw/day. Equivalent to 11.5 (males) and 13.8 (females) mg paraquat dichloride/kg bw/day. (Maita et al., 1980a).

Rat
3 week inhalation study: In a repeat dose inhalation study, Sprague-Daley rats were exposed (whole body) to respirable aerosols of paraquat dichloride (cation content: 40%; particle size: <2 μm diameter) for 3 weeks (6 hours/day, 5 days/week). The concentrations of paraquat cation in the inhalation chambers were 0, 0.01, 0.1, 0.5 and 1.0 μg/l (nominal) or 0, 0.012, 0.112, 0.487 and 1.280 μg/l, respectively, (analytical). The numbers of rats assigned to these groups were 32 (control), 16 (0.5 μg/L group) and 36 (remaining groups). Clinical condition, bodyweights and food consumption were measured throughout the study. One-half of the rats in each group were examined grossly and microscopically after 15 exposures and the remaining rats were examined 2 weeks after the termination of the exposure (recovery period). These examinations were restricted to the respiratory tract (nasal passages, pharynx, tongue, larynx, trachea and lungs). The 1.0 μg/l group was abandoned after the first exposure because 28 males (78%) and 29 females (80%) died from respiratory failure after that exposure.

Toxic signs were not observed in the 0.01 µg/l group and there was no mortality in this or the other test groups. All rats in the 0.1 µg/l group had nasal discharge and squamous keratinising metaplasia and/or hyperplasia of the epithelium of the larynx. The changes in the epithelium were still observed in 69% of the rats at the end of the recovery period. In the 0.5 µg/l group examined after 3 weeks of treatment the following findings were reported: (1) extensive ulceration, necrosis, inflammation and squamous keratinising metaplasia, and marked/moderate hyperplasia of adjacent epithelia in the larynx of all rats; and (2) aggregations of foamy macrophages in the bronchioles or alveoli, hypertrophy of the epithelium and thickened alveolar walls in the lungs of most or all rats. After a 2 week recovery period, no ulceration or necrosis was observed in the larynx, but changes in the lungs were still seen. In addition, disruption of bronchiolar epithelium, adjacent to the macrophage aggregation, was noted.

The NOEL and LOEL for sub-chronic (3 weeks) inhalation toxicity, for both sexes, are 0.01 µg/l and 0.10 µg/l respectively, expressed as paraquat cation equivalent to 0.014 µg/l and 0.14 µg/l respectively expressed as paraquat dichloride. (Hardy, C et al, 1979).

13 week rat: Groups of 20 male and 20 female Fischer CDF (F344) rats were fed diets containing 0, 10, 30, 100 or 300 ppm paraquat dichloride (93.3% pure) for thirteen weeks (equivalent to 0, 7.2, 22, 72, 217 ppm paraquat cation). The diets were analysed for homogeneity, stability and achieved concentration. Blood for haematological and clinical chemistry analyses was collected at termination and urine was collected prior to termination on day 92 from 10 animals/sex/group. All rats were subjected to a full examination *post mortem*. The weights of 12 organs were recorded and a comprehensive range of tissues taken for histopathological examination from all animals. Appropriate statistical analyses were performed.

There were no mortalities or adverse clinical findings during the study. Bodyweight gain was reduced at 300 ppm paraquat dichloride in both sexes. Food consumption and utilisation were reduced at 300 ppm paraquat dichloride. Water consumption was reduced in top dose males and in all treated females during the first half of the study. Urine parameters were unaffected. Reductions in erythrocyte parameters consistent with microcytosis were seen in 300 ppm paraquat dichloride females. A variety of changes in clinical chemistry parameters were seen at 300 ppm paraquat dichloride, affecting females more than males: decreases in calcium, cholesterol, GPT activity and total protein, with increases in LDH activity, potassium, alkaline phosphatase activity and glucose levels.

Autopsy findings were restricted to the 300 ppm paraquat dichloride dose groups. Organ weights tended to be reduced at this dose, in absolute but not relative terms, though an increase in lung weights in both sexes was seen at 300 ppm paraquat dichloride. Alveolar epithelial hypertrophy was increased in top dose males. Females at 300 ppm paraquat dichloride had increases in brown pigment in the spleen and sub-pleural lymphoid hyperplasia, with a reduction in calcification of the renal tubules, the latter showing a dose-response. There was no evidence of adverse effects at 100 ppm paraquat dichloride or below.

When administered to rats for 13 weeks, paraquat dichloride produced reduced weight gain, erythrocytic changes and lung lesions at 300 ppm. The NOAEL is 100 ppm paraquat dichloride (equivalent to 4.9 mg paraquat cation/kg bw/day, equivalent to 6.8 mg paraquat dichloride/kg bw/day). (Maita, K et al, 1980).

Rabbits

3 week dermal toxicity study: In a repeat dose dermal toxicity study, male and female New Zealand white rabbits, 6/sex/group, were exposed (intact skin) to technical paraquat dichloride (46.3% paraquat dichloride, 33.5% paraquat cation) for 21 consecutive days (6 hours/day). Paraquat dichloride was applied as an aqueous solution (1.0 ml/kg bw) at the following concentrations: 0, 1.5, 3.4, 7.8 or 17.9 mg paraquat dichloride/kg bw/day (0, 0.5, 1.15, 2.6 or 6.0 mg paraquat cation/kg bw/day, respectively).

Treatment-related effects were observed only at the two highest concentrations tested. In the 7.8 mg paraquat dichloride/kg bw/day group, scabbing at the dosing site was seen in two males and one female. The following toxic signs were observed in the 17.9 mg paraquat dichloride/kg bw/day group, in all rabbits, at the dosing site: scabbing; slight to well-defined erythema; minimal to moderately severe inflammation, acanthosis and hyperkeratosis (females only); slight to severe erosin/ulceration and surface exudates; and decreases in absolute weight (18%) and relative weight (organ/body weight and organ/brain weight ratios; 17-22%) of testes. No gross histological changes were found in the lungs, the target organ. Based on dermal irritation, the NOEL and LOEL are respectively, 3.4 and 7.8 mg paraquat dichloride/kg bw/day equivalent to 1.15 mg/kg bw/day and 2.6 mg/kg bw/day, expressed as paraquat cation. (Cox, R, 1986).

Following repeated daily dermal applications, beneath occlusive dressings, to rabbits of 1.56 to 50 mg paraquat cation/kg bw/day gave a MLD of 6.24 (4.6-8.5) mg paraquat cation/kg bw/day over 20 days. At higher doses the skin was reddened and sloughing with local oedema, while at the lowest dose some scab formation was seen after about 7 days. Systemic effects at examination *post mortem* included tubular necrosis, focal hepatocellular necrosis and pulmonary congestion. Where the skin was not occluded (rabbits were fitted with collars), the MLD for 20 days was between 7.25-14.5 mg paraquat cation/kg bw/day for the animals which had collars removed after decontamination and at least 24 mg paraquat cation/kg bw/day for the animals which had collars left on continually, suggesting secondary oral ingestion or poor decontamination in the former group. The rabbits showed marked signs of salivation, which was associated with glossitis and ulceration of the tongue. The animals refused to eat and death occurred in a state of cachexia; the effect was less marked at lower doses. The histological changes consisted of peraleratosis and occasional intra-epidermal pustules (McElligott, 1972).

In mice and rats, the application of solutions of 5-20 g paraquat/l in single and 21-day repeated dermal toxicity tests provoked dose-related toxic dermatitis with erythema, oedema, desquamation and necrosis (Bainova, 1969).

Dogs

6 week dog: Groups of 3 male and 3 female beagle dogs were dosed via the diet (400 g/day) with technical paraquat dichloride (32.2% paraquat cation) to give dose levels of 35 or 90 ppm paraquat cation for six weeks. A further group were capsule dosed with technical paraquat dichloride to give a dose of 0.75 mg paraquat cation/kg bw/day. The diets were analysed for achieved concentration. Clinical condition, bodyweights and food consumption were measured throughout the study. Ophthalmoscopy and auscultation were performed pre-dosing and pre-terminally. Jugular vein blood samples were taken from all dogs (before feeding) pre-experimentally and at weeks 3 and 5 for clinical chemistry and haematological assessment. Urinary parameters were measured pre-experimentally and at weeks 3 and 5.

Distinct toxic effects were seen at 90 ppm paraquat cation. There were adverse effects on bodyweight (both sexes) and food consumption (females), with gross and microscopic pulmonary lesions. There was one animal with increased lung weight. Pulmonary lesions were also seen in the group dosed by capsule with 0.75 mg paraquat cation/kg bw/day. At 35 ppm paraquat cation there were no treatment-related effects. There were no treatment-related effects on clinical chemistry or haematological parameters in any of the groups. Sheppard DB (1981a).

13 week dog: Groups of 3 male and 3 female beagle dogs were dosed via the diet (400 g/day) with technical paraquat dichloride (32.2% paraquat cation) to give dose levels of 0, 7, 20, 60 or 120 ppm paraquat cation for thirteen weeks. The diets were analysed for homogeneity, stability and achieved concentration. Clinical condition, bodyweights and food consumption were measured throughout the study. Ophthalmoscopy and auscultation were performed pre-dosing and at weeks 6 and 12. Jugular vein blood samples were taken from all dogs (before feeding) pre-experimentally and at weeks 6 and 12 for clinical chemistry and haematological assessment. A limited range of clinical chemistry and haematological parameters were measured at week 3. Urinary parameters were measured pre-experimentally and at weeks 3, 6 and 12; at weeks 6 and 12 paraquat levels in urine were also measured. After 13 weeks all dogs were killed and examined *post mortem*. Ten organs were weighed and at least 30 tissues from all dogs were examined histopathologically. Appropriate statistical analyses were performed.

Two males and 2 females in the 120 ppm paraquat cation group showed marked paraquat toxicity and were killed in extremis between days 16 and 23, having shown marked dyspnoea and body-weight loss. Both surviving dogs at 120 ppm paraquat cation also showed body-weight loss. A slight overall reduction in body-weight gain among the females of the other treatment groups was considered not to be treatment-related. There were no treatment-related effects on food intake except for one surviving high-dose female which showed a loss of appetite from week 8 onward. There were no distinct treatment-related changes in any of the haematological, biochemical or urinary parameters examined. Lung weights were increased in all animals in the 120 ppm paraquat cation group and in 2 animals from the 60 ppm paraquat cation group. All other organ weights were in the normal range. Distinct gross and histological treatment-related lung lesions were seen in all dogs in the 60 and 120 ppm paraquat cation groups. Minor renal lesions (swelling of the cortical tubules) were also found histologically in a few of these animals. There were no

discernible gross or histological treatment-related pulmonary lesions in the dogs of the 7 or 20 ppm paraquat cation groups. The focal pulmonary lesions in these animals were of a type and incidence similar to those found in the controls. Microscopic examination of 34 other tissues from each animal showed no treatment-related changes. Slight haemoconcentration was seen in one high-dose dog at termination. The NOEL (on the basis of lung and kidney lesions) is 20 ppm paraquat cation (0.56 mg and 0.71 mg paraquat cation/kg bw/day for males and females respectively equivalent to 0.77 mg and 0.98 mg paraquat dichloride/kg bw/day) and the LOEL is 60 ppm paraquat cation (1.75 mg and 1.92 mg paraquat cation/kg bw/day for males and females respectively equivalent to 2.42 and 2.65 mg paraquat dichloride/kg bw/day), respectively. (Sheppard, 1981b).

Summary of short term toxicity

The short term toxicity of technical paraquat dichloride has been investigated in dietary studies in mice, rats and dogs, in a 3 week inhalation study in the rat and in a dermal study in rabbits, as summarised in the table below.

Table 6: Summary of sub-acute toxicity studies with technical paraquat dichloride

Study type	Dose levels	NOAEL as paraquat ion	Comment
13 week mouse dietary	0, 10, 30, 100, 300 ppm paraquat dichloride ($\cong$ 0, 7.2, 22, 72, 217 ppm paraquat cation)	100 ppm paraquat dichloride (8.3 mg paraquat cation/kg bw/day males; 10.0 mg paraquat cation/kg bw/day females $\cong$ 13.8 mg paraquat dichloride/kg bw/day)	Lung lesions and reduced bodyweight gain at 300 ppm paraquat dichloride
13 week rat dietary	0, 10, 30, 100, 300 ppm technical paraquat dichloride($\cong$ 0, 7.2, 22, 72, 217 ppm paraquat cation)	100 ppm paraquat dichloride ($\cong$ 4.9 mg paraquat cation/kg bw/day $\cong$ 6.8 mg paraquat dichloride/kg bw/day)	Lung lesions, reduced bodyweight gain and microcytosis at 300 ppm paraquat dichloride
6 week preliminary dog study	35, 90 ppm paraquat cation (dietary), 0.75 mg paraquat cation/kg bw/day (capsule)	35 ppm paraquat cation (equivalent to 1 mg paraquat cation/kg bw /day for males and 1.3 mg paraquat cation/kg/day for females $\approx$ 1.4 and 1.8 mg paraquat dichloride/ kg bw/day for males and females respectively).	Bodyweight gain (both sexes) and food consumption (females) reduced at 90 ppm paraquat cation. Lung lesions (alveolitis) seen in both sexes at 90 ppm paraquat cation and 0.75 mg paraquat cation/kg bw/day.
13 week dog dietary	0, 7, 20, 60, 120 ppm paraquat cation)	20 ppm paraquat cation (0.6 mg paraquat cation/kg bw/day $\cong$ 0.77 mg paraquat dichloride/kg bw/day)	Mortality at 120 ppm paraquat cation. Lung lesions at 60 and 120 ppm paraquat cation. (NB only 3 dogs/sex)
3 week rabbit dermal	0, 1.5, 3.4, 7.8, 17.9 mg paraquat dichloride/kg bw/day ($\cong$ 0, 0.5, 1.15, 2.6, 6.0 mg paraquat cation/kg bw/day)	3.4 mg paraquat dichloride/kg bw/day (1.15 mg paraquat cation/kg bw/day $\cong$ 1.59 mg paraquat dichloride/kg bw/day)	Severe irritation at 6 mg paraquat cation/kg bw/day. No gross lesions in lungs.
3 week inhalation, rat, whole body	nominal doses 0, 0.01, 0.1, 0.5 and 1.0 µg/l paraquat cation;	NOEL and LOEL for both sexes was 0.01 µg/l and 0.10 µg/l paraquat cation	No mortality. Lesions in the larynx and lung. After 2 weeks recovery, no

| exposure | (0, 0.012, 0.112, 0.487 and 1.280 µg/l paraquat cation, analysed) | respectively (≅ 0.014 and 0.14 µg paraquat dichloride/l) | ulceration or necrosis in the larynx, but changes still present in the lung. |

Consistent findings were lung lesions (alveolar epithelial hyperplasia; alveolitis; chronic pneumonitis) and reduced bodyweight gain, with dogs the more sensitive species. Variations in haematology and clinical chemistry parameters were not consistent between studies or species.

c) Long-term studies of toxicity and carcinogenicity

Mice

Two carcinogenicity studies in mice are reported; in the first (Sotheran et al., 1981) treatment continued for up to 99 weeks and in the second, a Japanese study (Toyoshima et al., 1982a), treatment continued for 104 weeks.

In a study initiated in 1977, groups of 60 Alderley Park, Swiss-derived mice, 60/sex/dose level, were administered technical paraquat dichloride (32.7% w/w paraquat cation) incorporated into the diet of to give dose levels of 0, 0 (two control groups), 12.5, 37.5 and 100/125 ppm paraquat cation for 97 to 99 weeks (when mortality was approaching 80% in all groups). At week 36 the top dose level was increased from 100 to 125 ppm because no toxic signs had appeared after 35 weeks of dosing. These doses corresponded to 0, 0, 1.87, 5.6 and 15.0/18.7 mg paraquat cation/kg bodyweight/day. The diets were analysed for homogeneity, stability and achieved concentration. A satellite group of 10 per sex per group was designated for interim sacrifice after 52 weeks. All mice had free access to tap water and treated or control diet except when food was removed overnight prior to urine collection. Mice were examined routinely for mortality, clinical abnormalities, masses, body weight and food consumption. Urinary paraquat determinations were performed every 13 weeks on 10 mice/sex/group. Tissue (lung and kidney) and plasma paraquat levels were determined in samples from the satellite group at termination.

After 52 weeks all surviving mice in the satellite groups were killed and given a macroscopic examination *post mortem* – only abnormal tissues were preserved. All surviving mice in the main study were killed after 97-99 weeks of treatment. Each animal was given a detailed macroscopic examination and a comprehensive selection of tissues were taken and examined histopathologically for neoplastic and non-neoplastic changes.

Dietary analyses showed the majority of batches to be within 10% of the nominal values, with adequate homogeneity and stability. Clinical signs were consistent between groups though an increase in genital sores and decreased hair loss were noted in the top dose females. Mortality rates were increased in intermediate dose males (week 43 onwards) and top dose females (week 68 onwards) – the former is probably a chance finding due to the lack of a corresponding finding in top dose males. Survival was >45% in all groups at week 80. Bodyweight gain was increased at 12.5 ppm paraquat cation in both sexes at the beginning of the study; lower bodyweight gains were seen in top dose females after the dose level was increased (week 44

onwards). Food consumption was slightly lower in paraquat treated animals than in controls, with food utilisation efficiency increased in males.

Paraquat levels in urine showed dose related values, though not always in direct proportion to administered levels – a positive result in a control sample from week 13 was investigated but the cause could not be found though mis-dosing was not considered to have occurred. Plasma paraquat levels at 52 weeks were dose-related in males but in females there was only a minimal difference between the groups. Kidney and lung analyses were subject to interference but available data showed levels of paraquat to be proportional to dietary levels. These results indicate absorption of paraquat was not saturated at the levels used in this study.

Histological examination of animals dying during the study or killed at termination showed the kidney to be the major target organ with tubular effects being prevalent at 125 ppm with pelvic dilatation evident at ≥37 ppm in males. There was no evidence of treatment-related effects on the lungs other than a few instances of alveolar wall thickening/hypercellularity. Ocular lesions were similar in controls and treated groups.

Table 7: Non-neoplastic findings in mice treated with paraquat for 2 years

	Dietary Concentration of Paraquat cation (ppm)									
	Males					Females				
	0	0	12.5	37.5	125	0	0	12.5	37.5	125
Week 0-98 number examined	45	35	45	47	48	48	39	41	46	52
Kidney tubule degeneration plus dilation	0	0	0	2	15*	0	0	1	1	11*
Kidney tubule degeneration - no dilation	8	3	9	13	16*	1	5	2	3	15*
Kidney tubule dilation - no degeneration	0	1	2	1	3	0	0	0	0	7*
Kidney hydropic degeneration	0	0	0	1	7	1	1	1	3	8*
Kidney pelvic dilatation – mild/marked	5	3	5	16*	16*	1	1	2	1	3
Kidney tubules pigmented	0	1	1	1	3	0	0	0	1	5*
Alveolar walls – thick/focal hypercellularity	2	2	3	1	6	0	0	0	2	5*
Alveolar macrophages - increased	1	5	4	2	3	1	3	3	1	7
Terminal kill number examined	15	25	15	13	12	12	21	19	14	8
Kidney tubule degeneration - no dilation	0	0	0	1	6	0	2	0	0	5
Kidney tubule degeneration plus dilation	0	3	2	4	2	0	0	0	0	0
Kidney tubule dilation - no degeneration	0	0	1	1	2	0	2	1	4	2
Mammary gland – active secretion						0	0	0	0	2
Pituitary cysts	3	7	4	1	1	0	1	0	0	0

* P<0.05

There were no significant increases in total neoplastic lesions following paraquat administration. Occasional increases in individual tumour incidence were seen (eg.

pituitary adenoma at weeks 53-78 and lung adenoma at weeks 78-98), these were of tumours typical of aged mice and not statistically significant nor consistent with time and dose. There was an increase in kidney adenomas in top dose males but not in females, this was probably secondary to degenerative lesions.

Table 8: Neoplastic findings in mice treated with paraquat for 2 years

| | Dietary Concentration of Paraquat cation (ppm) | | | | | | | | | |
| | Males | | | | | Females | | | | |
	0	0	12.5	37.5	125	0	0	12.5	37.5	125
Week 53-78 number examined	18	13	15	19	17	15	13	10	17	20
Pituitary adenoma	0	0	0	0	0	1	0	3	3	3
Lung adenoma	1	3	3	4	1	0	2	0	1	2
Lung adenocarcinoma	0	1	0	0	2	0	0	0	0	0
Harderian gland adenoma	1	2	2	3	3	1	1	0	1	1
Mammary adenocarcinoma						0	0	1	0	0
Lymphosarcoma	5	2	6	5	6	6	5	3	3	10
Week 78-98 number examined	21	16	20	15	25	24	15	22	19	20
Adrenal phaeochromocytoma	0	0	0	0	0	1	0	0	0	2
Lung adenoma	3	2	2	3	7	4	2	1	1	8
Lung adenocarcinoma	0	1	1	0	1	1	0	1	0	0
Kidney adenoma	0	1	2	0	4	0	0	0	0	0
Harderian gland adenoma	2	0	4	1	0	1	0	3	1	3
Mammary adenocarcinoma						0	0	1	0	1
Total number examined	60	60	60	60	60	60	60	60	60	60
Pituitary adenoma	0	2	4	2	0	11	15	18	16	12
Adrenal phaeochromocytoma - benign	1	0	1	0	0	1	0	3	0	3
Lung adenoma	8	11	7	9	11	8	5	5	4	10
Lung adenocarcinoma	0	2	1	0	3	1	0	2	0	0
Kidney adenoma	2	2	2	0	6	0	0	0	0	0
Harderian gland adenoma	5	7	11	7	8	2	2	8	4	5
Mammary adenocarcinoma						0	0	3	0	1

It is concluded that paraquat is not tumourigenic in mice. An NOAEL of 12.5 ppm paraquat cation (1.5 mg paraquat cation/kg bw/day ≈ 2.1mg paraquat dichloride/kg bw/day) can be derived based on dilation of the renal pelvis in males at 37.5 ppm and a variety of kidney effects at 125 ppm. The NOEL for females is 37.5ppm (4.3 mg paraquat cation/kg bw/day ≈ 5.9 mg paraquat dichloride/kg bw/day) (Sotheran et al., 1981).

In a 1979 study groups of 80 JCL:ICR mice (Japan Clea Laboratories Co., Tokyo) per sex per dose were fed diets containing 0, 2, 10, 30 or 100 ppm paraquat dichloride equivalent to 0, 1.4, 7.2, 22 or 72 ppm paraquat cation (nominal concentrations) equivalent to 0, 0.26, 1.31, 3.92 or 13.09 mg paraquat dichloride/kg bw/day for males and 0, 0.26, 1.32, 3.82 or 13.03 mg paraquat dichloride/kg bw/day for females respectively (expressed as analytical concentrations) for 104 weeks. There were two interim kills in this study, further groups of 10 male and 10 female mice received the same dose and were sacrificed after 26 or 52 weeks of treatment.

Treatment-related findings were observed only in the 100 ppm paraquat dichloride group and included increased mortality in the females; decreased total protein, erythrocytes, leucocytes, haemoglobin and haematocrit in males and females; decreased polymorphonucleocytes in the males and decreased GPT and alkaline phosphatase activities in the females; decreased absolute and/or relative weights of adrenals, thyroid, liver and urinary bladder in the males; decreased absolute weight of brain in the females and increased absolute and/or relative weights of kidneys, lungs and heart in the males.

There was no evidence of carcinogenicity in this study. Gross, non-neoplastic and neoplastic lesions were observed in various organs of the male and female mice, but were not treatment-related. The most frequent lesions were observed in the following organs: lungs (congestion, nodes, pneumonia, thickening of alveolar walls and adenocarcinoma in both sexes); liver (dilatation in the females and tumours in the males); kidneys (discolouration and coarse surface in both sexes, dilatation of renal pelvis and cell infiltration in the males, nephropathy in the females); spleen (swelling in both sexes); thymus (atrophy in both sexes and hypertrophy in the females); mesenteric lymph node (swelling and cell infiltration in both sexes) and eyes (corneal cell proliferation in both sexes and corneal calcification in the females). Leukemia, amyloid degeneration and leukaemia cell infiltration were also observed frequently in the males and females.

It is concluded that paraquat is not tumourigenic in mice. Based on the haematological and blood chemistry changes a NOEL of 30 ppm paraquat dichloride (equivalent to 2.9 and 2.8 mg paraquat cation/kg bw/day for males and females respectively ≈ 4.0 and 3.9 mg paraquat dichloride/kg bw/day) was determined (Toyoshima et al., 1982a).

Rats

Three long-term toxicity/carcinogenicity studies are reported. All three were ongoing during the same time period. The earliest study was a lifetime study, initiated in April

1978 and conducted in the UK where Fischer 344 rats were maintained on diets containing paraquat dichloride for 113-117 weeks for males and 122-124 weeks for females at levels of 0, 0, 25, 75 and 150 ppm paraquat cation (Woolsgrove, BW et al., 1983). The other two studies were both conducted in Japan. In one, initiated in February 1979, Wistar rats were maintained on diets containing paraquat dichloride for 104 weeks at levels of 0, 6, 30, 100 or 300 ppm paraquat dichloride (Toyoshima, S et al., 1982) and in the second, initiated in September 1979, Fischer SPF rats were maintained on diets containing paraquat dichloride for 104 weeks at levels of 0, 10, 30, 100 and 300 ppm paraquat dichloride (Yoshida et al., 1982).

In a chronic feeding/carcinogenicity study, initiated in April 1978, Fischer 344 rats, 80/sex/group, were fed diets containing paraquat dichloride (cation content: 32.69%) for 113-117 weeks (males) and 122-124 weeks (females). Based on the results of a preliminary study, the doses selected for this study were 0, 0, 25, 75 or 150 ppm paraquat cation, (0, 0, 1.25, 3.75 or 7.5 mg paraquat cation/kg bw/day). Starting with the test week 6, the female rats at all dose levels ingested more paraquat (15-33%) per kilogram of body weight than did the male rats at the same dose levels.

Twenty rats from each group (10 of each sex) were sacrificed after one year of treatment: 5 of each sex/group, for the usual interim sacrifice and another 5 of each sex/group, to determine paraquat concentration in tissues. Gross necropsy was performed on all rats, with the exception of the rats used for the determination of paraquat concentration in tissues, all rats were also examined microscopically.

The distribution of mortality was unaffected by treatment. There was approximately 50% mortality in all groups at the end of the study. At 150 ppm paraquat cation, statistically-significant reductions in body-weight gain (10-34% during weeks 11-68 for males and 11-34% during weeks 27-78 for females), food consumption (3-8%), and efficiency of food utilisation in both sexes were observed (12-21% in males and 11-13% in females during weeks 13-52). There was a statistically-significant depression of body-weight gain in the first year of the study in males receiving 75 ppm paraquat. Water consumption was not significantly affected at any dietary level tested. Paraquat accelerated, in a dosage-dependent manner, the onset and progression of cataract changes, ranging from minor opacity to total cataract in both males and females. Treatment-related ocular lesions were first seen at 52 weeks. Thereafter, ophthalmoscopy revealed a statistically-significant dosage-related increase in the incidence, progression, and severity of lenticular cataract in the 150 ppm group and, toward the end of the study (103 weeks), in the 75 ppm group. Syngenta considers that the appropriate end point to use for risk assessment purposes is the incidence of cataracts at the end of the 2 year dosing period which is generally accepted as the period for chronic toxicity. Beyond this time point, the picture becomes unreliable because of the increasing geriatric changes. However 25 ppm was the NOEL for lenticular cataracts in both males and females at termination (week 112/113 for males and week 118/119 for females).

Table 9: Incidence of total cataracts in a rat chronic toxicity/carcinogenicity study

Sex/week		Dose level (ppm)				
		0	0	25	75	150
Males	102/103	4	1	4	16	56
	112/113	5	3	5	19	52

Females 102/103	3	6	5	14	51
118/119	8	9	11	33	37

A statistically-significant higher incidence of secondary eye lesions was found at termination in females receiving 75 or 150 ppm paraquat when compared to controls. Haematological investigation (RBC counts, total and differential leucocyte counts, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin concentration, platelet and reticulocyte counts, and prothrombin and partial thromboplastin times) and blood biochemistry (urea, glucose, ALT, and AST) indicated no significant effects attributable to paraquat administration. Urinalysis did not reveal any treatment related changes. Reductions in liver and testicular weights were noted at termination in the 150 ppm dietary group.

Macroscopic examination at necropsy revealed a treatment-related increase in the incidence of focal subpleural changes in animals killed at termination in all dietary groups. This effect was most marked in females receiving 75 ppm and in both sexes receiving 150 ppm paraquat. Microscopic examination of lung tissues indicated that treatment with paraquat at 150 ppm, in both sexes, and possibly at 75 ppm in males, was associated with proliferative lesions of the alveolar epithelium. These lesions were not easily classified into non-neoplastic or neoplastic, nor into adenoma or carcinoma. This study provided strong evidence for the induction by paraquat of a proliferative lesion of the alveolar epithelium and some controversial evidence for the induction of lung adenomas in female Fischer 344 rats. There was no treatment-related increase in the incidence of lung adenocarcinoma at any dose level in either sex. At 25 ppm, significant increases in the incidence of proliferative lung lesions, compared to the controls, were not observed. Increased relative weight of the lungs (lung/body weight ratio) in the males (16%) and the females (14%) was seen in animals sacrificed at the termination of the study.

Slight dilation of the fourth ventricle of the brain was evident in females receiving 150 (36%) or 75 ppm paraquat, but not in males at these dosages or in either sex at 25 ppm. A statistically significant increase in the incidence of apparent degeneration of occasional/several sciatic nerve fibers was noted in decedent males receiving 75 or 150 ppm paraquat. Both hydrocephalus and nervous tissue changes were considered by the authors of the study possibly to be associated with paraquat treatment. Pathology summaries indicate that atrophy of the testes was recorded in the high-dietary group (5/33) but not in controls at termination, and moderate lymphoid hyperplasia was observed in the respiratory epithelium of males receiving 75 and 150 ppm paraquat and dying between 52 weeks and termination.

The above findings show that paraquat enhanced the development of ocular lesions in all of the treated groups. The predominant lesions detected ophthalmoscopically were lenticular opacities and cataracts. These lesions were either not observed or were observed infrequently before week 103. At week 103, dose related statistically significant increases in the incidence of ocular lesions were observed only in the mid-dose and high-dose male and female groups. Based on these findings, the NOEL and the LOEL for systemic toxicity, for both sexes, are 25 ppm paraquat cation (1.25 mg paraquat cation/kg bw/day) and 75 ppm paraquat cation (3.75 mg paraquat cation/kg bw/day), respectively. (Woolsgrove B W et al., 1983).

During the initial review of this study, there were concerns that paraquat might be carcinogenic in the rat. There was a dose-related increase in the incidence of pulmonary neoplasms (adenomas and carcinomas), but especially adenomas, in the male and female rats. The incidence of pulmonary neoplasms was 2.9, 5.7, 8.6 and 10.1% in the control, low-dose, mid-dose and high-dose male groups, respectively. The corresponding values for the female groups were 0, 2.9, 4.3 and 14.3%, respectively. A statistically significant increase was observed only in the pulmonary adenomas and only in the high-dose female rats. Most of the neoplasms were observed at the termination of the study. However, there were difficulties in characterising pulmonary lesions as non-neoplastic or neoplastic, or as adenomas or carcinomas and lung tissue was, therefore, examined by four independent pathologists (Busey, 1986). The findings of these pathologists were very similar and were within the historical incidence of adenomas and carcinomas. According to one pathologist, the incidence of pulmonary adenomas and carcinomas in the control, low-dose, mid-dose and high-dose male rats was 2.9, 5.7, 2.8 and 5.8%, respectively. The corresponding incidences for the female groups were 0, 1.4, 2.8 and 0%, respectively. According to another pathologist, the incidence of pulmonary adenomas and carcinomas in these groups was 2.9, 5.7, 4.3 and 5.8%, respectively (males) and 0, 1.4, 2.8 and 2.8%, respectively (females). There was no evidence of treatment-related carcinogenicity in the lungs.

There was a higher incidence of squamous cell carcinomas in the head region (middle ear, hard palate, head tissue and skin), an uncommon tumor, but the incidence was generally low and not dose related. The incidence of these carcinomas (combined) was 2.1, 4.3, 0 and 8.6% in the control, low-dose, mid-dose and high-dose male groups respectively. The corresponding incidences for the female groups were 0, 0, 4.3 and 2.9%, respectively. All of these carcinomas occurred during the second year of study. The incidence of benign pheochromocytomas in the adrenal medulla and perifollicular adenomas and carcinomas in the thyroid was also increased in the high-dose male rats. However, the relationship of these neoplasms to treatment was unclear and additional data were, therefore, requested to evaluate adequately the carcinogenic potential of paraquat in tissues other than the lungs.

An independent pathologist (Ishmael, J. 1987), reviewed slides from the head region and concluded that there was no justification for combining squamous cell carcinomas (which occurred in four different sites of the head) for assessment purposes. According to the pathologists (US EPA's and the registrant's), the skin and oral and nasal cavities have different morphology and physiology and separate biological functions, and cannot be considered as a single organ in terms of assessment of carcinogenic effects. When each of these sites was considered independently, in the accepted manner for assessing tumor incidence, in no instance was there a statistically significant difference between the treated and control rats. Paraquat was, therefore, not carcinogenic in the head region of the rat.

The incidence of pheochromocytoma in the adrenal gland of the control, low-dose, mid-dose and high-dose male rats was 8.6, 12.6, 11.4 and 17.4%, respectively. The incidence of pheochromocytoma in the historical control male rats ranged from 6.7% to 38.8% and this tumor occurred in each of the eight studies from which historical data were obtained. Considering the historical incidence, the incidence of

pheochromocytoma in this study (11.4- 17.4%) did not appear to be paraquat-related because it fell within the range reported for the historical control male rats.

The incidence of parafollicular adenomas and carcinomas in the thyroid gland of the concurrent control, low-dose, mid-dose and high dose male rats was 11.9, 16.9, 13.6 and 18.8%, respectively. The incidence of these neoplasms in the historical control male rats ranged from 13.3% to 35.4%. Considering the historical incidence, the incidence of parafollicular adenoma and carcinoma in the high-dose male rats (18.8%), in this study, did not appear to be treatment-related. (Woolsgrove et al (1983); Chevron Chemical Co. (1985); Willis, G.A. (1987); Ishmael, J. (1987); ICI Americal, Inc. (1989); Ashby et al., 1983; Busey, 1986; Ishmael & Godley, (1983)

In another chronic feeding/carcinogenicity study, initiated in February 1979, Wistar strain rats, 62/sex/group, were fed diets containing technical grade paraquat dichloride (purity: 98%) for 104 weeks. Based on the results of a preliminary study, the doses of paraquat selected for this study were 0, 6, 30, 100 or 300 ppm paraquat dichloride (nominal concentrations). These values were equivalent to 0, 0.25, 1.26, 4.15 or 12.25 mg paraquat dichloride/kg bw/day (males) and 0, 0.30, 1.50, 5.12 or 15.29 mg paraquat dichloride/kg bw/day (females) (analytical concentrations). The interim sacrifices, using 6 rats/sex/group, took place after 26 and 52 weeks of treatment.

Findings different from those in the controls were observed only in the 300 ppm paraquat dichloride group and included increased mortality in males and females; decreased erythrocytes, hemoglobin, and serum protein in males and females; decreased hematocrit, glucose and corpuscular cholinesterase activity in males; decreased leucocytes, albumin/globulin ratio and alkaline phosphatase, GOT (glutamic-oxaloacetic transaminase) and GPT (glutamic-pyruvic transaminase) activities in females; increased polymorphonucleocytes in males; increased potassium and glucose in females; decreased absolute and/or relative weights of heart in males and females; and liver and brain in females; and decreased absolute weights of kidneys in males and females, and ovaries.

Ocular changes were not detected before the initiation of treatment. After the treatment was started, cataracts were observed during each examination (weeks 26, 52 and 104) in 1 to 4 rats per group, including controls. At test weeks 52 and 104, corneitis (keratitis) and conjunctivitis were also observed in all groups, including controls. In this study, the incidence of ocular changes was low and dose unrelated. Considering the test weeks 26-104, the incidence of ocular changes in the male and female treated rats was as follows: cataracts, 1.6-17.6% (controls 1.6-13.8%); corneitis, 0-5.9% (controls, 0-6.7%); and conjunctivitis, 0-5.4% (controls, 0-6.7%). There was no evidence of any ocular changes caused by treatment with paraquat in this study.

Paraquat was not carcinogenic in this study. Gross, non-neoplastic and neoplastic lesions were observed in various organs of males and females, but did not appear to be treatment-related (either a dose-relationship was lacking or the incidence was similar in the controls and the paraquat-treated groups). The most frequent lesions were observed in the following organs: lungs (congestion, nodes, peribronchiolitis, pneumonia and thickening of alveolar walls); liver (bile duct proliferation and fibrosis); kidneys (rough surface and nephritis); pituitary (hypertrophy, haematoma

and benign tumors); thyroid (benign tumors); adrenals (cysts); spleen (swelling); mesenteric lymph node (swelling and inflammation); testes and ovaries (atrophy); uterus (cysts and polyps); and mammary glands (cysts, adenomas, fibromas, fibroadenomas and adenocarcinomas).

Based on the above findings, the systemic NOEL is 100 ppm of paraquat dichloride (equivalent to 3.0 mg paraquat cation/kg bw/day and 3.7 mg paraquat cation/kg bw/day for males and females respectively ≈ 4.1 and 5.1 mg paraquat dichloride/kg bw/day). The systemic LOEL is 300 ppm of paraquat dichloride (equivalent to 9.0 mg paraquat cation/kg bw/day and 11.2 mg paraquat cation/kg bw/day for males and females respectively ≈ 12.4 and 15.5 mg paraquat dichloride/kg bw/day). (Toyoshima, S et al. 1982).

In a study initiated in September 1979, groups of 80 male and 80 female Fisher SPF rats were fed diets containing 0, 10, 30, 100 or 300 ppm paraquat dichloride for 104 weeks. Eight rats/sex/group were sacrificed after urinalysis at 26, 52, and 78 weeks and were subjected to haematological examination. All surviving animals were sacrificed at 104 weeks and, among these, 10 rats/sex/group were subjected to haematological and biochemical examination. All animals, including those killed on schedule and those found moribund and killed during the study, were autopsied and subjected to gross necropsy and histopathological examination of approximately 30 tissues.

Mortality was increased in female rats of the 300 ppm paraquat dichloride group from week 66 to week 74 when compared with that of other groups, including controls. Both male and female rats at the 300 ppm paraquat dichloride dietary level showed a marked statistically-significant reduction in body-weight gain when compared to control groups. Food consumption, efficiency of food utilisation, and water consumption were also statistically significantly lower in these rats when compared to control animals.

Haematological examination showed a statistically-significant reduction in total white cell count in male rats of the 300 ppm paraquat dichloride group, when compared to controls, at 26, 52, and 78 weeks, but not at 104 weeks. This change was not considered by the authors of the study to be attributable to the administration of paraquat dichloride. Biochemical examination indicated a statistically-significant reduction in globulin in male rats of the 300 ppm paraquat dichloride group at 26, 78, and 104 weeks when compared to controls. Clinical observations, RBC counts, haemoglobin, mean red-cell volume (MCV), mean cell hamoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet counts, differential WBC counts, plasma alkaline phosphatase, lactic acid dehydrogenase, blood urea nitrogen, glucose, total cholesterol, GOT, GPT, total and direct bilirubin, GGPT, calcium, total protein, albumin, and urinalysis indicated no significant effects attributable to the administration of paraquat at any dose levels.

Throughout the entire administration period, a statistically significant reduction was found in the absolute weights of various organs of male and female rats of the 300 ppm paraquat dichloride group at interim sacrifices. This change was considered by the authors of the study to be related to the reduction in body weight observed in these animals. Histological examination of the lung at termination showed a marked,

treatment-related, statistically-significant increase in the incidence of proliferation of interalveolar septum cells and of hyperplasia of alveolar epithelium in both male and female rats at 300 ppm paraquat dichloride and in male rats at 100 ppm paraquat dichloride, when compared to controls. There was a marked, statistically-significant increase in the incidence of cataract in male and female rats of the 300 ppm paraquat dichloride group killed or found dead after week 79. This treatment-related change was reported to be the same microscopically as that observed in the tissues collected from those control rats which had spontaneous, age-related cataracts.

Male rats of the 300 ppm paraquat dichloride group also showed a statistically-significant increase in the incidence of local atrophy of renal tubules when compared to controls. Females of the same dietary group had a statistically-significant increase in the overall incidence of diffusive fatty changes of the liver and pulmonary fibrosis when compared to controls. Kidney and liver lesions were not considered by the authors of the study to be attributable to the administration of paraquat. A significant increase in the incidence of pulmonary adenoma (7/80) was found in female rats of the 300 ppm paraquat dichloride group when compared to controls (1/80). There was no significant increase in the incidence of lung adenoma in male rats, but a few of them had lung adenocarcinoma (1 in each of the 30 and 100 ppm paraquat dichloride groups, 3 in the 300 ppm paraquat dichloride group, and none in the controls). The authors noted that, although the historical incidence of pulmonary adenoma in rats of this strain is reportedly rather low (about 2%), 6/80 (7.5%) of the control rats developed pulmonary adenoma in a 24-month chronic toxicity study carried out separately in their laboratory. Based on these considerations, the authors of the study concluded that the incidence of pulmonary adenoma found in the present paraquat study in female rats in the 300 ppm paraquat dichloride group did not exceed the background incidence of pulmonary adenoma in rats of this strain.

On the basis of the lung and eye lesions the NOEL of paraquat dichloride determined in this study for Fisher SPF rats after 104-week treatment was 30 ppm paraquat dichloride (equivalent to 0.77 mg paraquat cation/kg bw/day in male rats $\approx$ 1.1 mg paraquat dichloride/kg bw/day) and 100 ppm paraquat dichloride (equivalent to 3.12 mg paraquat cation/kg bw/day in female rats ($\approx$ 4.3 mg paraquat dichloride/kg bw/day) (Yoshida et al., 1982).

Dogs

Groups of 6 male and 6 female beagle dogs were fed diets (400 g daily) containing technical grade paraquat dichloride (cation content 32.3%) which contained 0, 15, 30, or 50 ppm paraquat cation for 1 year. Based on actual group mean bodyweights and food consumption, these doses corresponded to 0, 0.45, 0.93 or 1.51 mg paraquat cation/kg bw/day for males and 0, 0.48, 1.00 or 1.58 mg paraquat cation/kg bw/day for females.

During the study there were no deaths. No effects due to paraquat were observed on body weight. The reduced food consumption of 1 male and 1 female dog, both in the 50 ppm paraquat cation group, was considered to be treatment-related. There was clinical evidence of respiratory dysfunction (hyperpnoea) in some dogs fed 50 ppm paraquat cation. Mean lung weights of male and female dogs fed 50 ppm paraquat

cation were 35 and 60% higher than those of controls, respectively. Histopathological examination of the lungs showed a statistically-significant increase in the incidence of chronic pneumonitis in both sexes at the 30 and 50 ppm paraquat cation dietary levels when compared to controls. This lesion consisted of interstitial fibrosis, alveolar epithelialization, and mononuclear cell infiltration. No other toxicologically-significant treatment-related effects were seen during clinical observations, haematological or biochemical investigations, or during gross and microscopic examination of approximately 40 tissues from each animal at termination. On the basis of the pulmonary changes, the authors of this study concluded that the dietary NOEL for paraquat in dogs over 1 year of treatment was 15 ppm paraquat cation, equivalent to 0.45 (males) and 0.48 (females) mg paraquat cation/kg bw/day ($\approx$ 0.62 and 0.66 mg paraquat dichloride/kg bw/day). (Kalinowski et al., 1983).

Summary of chronic toxicity/carcinogenicity

Dietary, chronic toxicity/carcinogenicity studies have been performed in mice and rats and a 1 year dietary chronic toxicity study has been conducted in dogs. They are summarised in the table below. Paraquat did not produce significant increases in tumour incidences in any species.

In the Alderley Park, Swiss mouse, the kidney was found to be the target organ with tubular lesions evident at 125 ppm paraquat cation and pelvic dilatation seen in males at 37 ppm paraquat cation and above. Alveolar hypercellularity present in females at 125 ppm paraquat cation was the only sign of pulmonary toxicity. There were no ocular lesions and no evidence of oncogenicity. A NOAEL of 12.5 ppm paraquat cation (ca 1.5 mg paraquat cation/kg bw/day $\approx$ 2.1 mg paraquat dichloride/ kg bw/day) was established in this study. (Sotheran et al, 1981).

Paraquat was not tumourigenic in the JCL:ICR mouse. Based on the haematological and blood chemistry changes an NOEL of 30 ppm paraquat dichloride (3.92 and 3.82 mg paraquat dichloride/kg bw/day for males and females respectively) or 2.9 and 2.8 mg paraquat cation/kg bw/day for males and females respectively was determined. (Toyoshima et al., 1982a).

In a chronic life time rat study, Fischer 344 rats were administered dietary dose levels of 0, 0, 25, 75 or 150 ppm paraquat cation for at least 110 weeks. Ocular opacities were evident clinically at 75 and 150 ppm paraquat cation and confirmed by ophthalmoscopy and histology; similar lesions were also present at the very end of the study (week 110) in the 25 ppm paraquat cation groups. Other findings considered to be of low toxicological significance included testicular lesions, peripheral nerve degeneration, hydrocephalus (slight dilation of the 4th ventricle of the brain) and bile duct hyperplasia observed at 75 and 150 ppm paraquat cation. In this study the ocular lesions present at the lowest dose level (25 ppm paraquat cation) were consistent with an acceleration of typical aging lesions, but were not seen in any other studies and were clearly not evident at the time when chronic studies are normally terminated (103 weeks). Paraquat dichloride was not oncogenic in this study. Syngenta considers 25 ppm paraquat cation ($\approx$ 1.2 mg paraquat cation/kg bw/day $\approx$ 1.7 mg paraquat dichloride/kg bw/day) to be a NOAEL at 2 years (Woolsgrove et al, 1983).

In a chronic feeding/carcinogenicity study, Wistar strain rats, 62/sex/group, were fed diets containing 0, 6, 30, 100 or 300 ppm paraquat dichloride. There was no evidence of carcinogenicity and the incidence of ocular changes was low and not dose-related. The systemic NOEL is 100 ppm of paraquat dichloride (4.15 and 5.12 mg paraquat dichloride/kg bw/day, for males and females, respectively); or 3.0 mg/kg/day (males) and 3.7 mg/kg/day (females), expressed as paraquat cation. The systemic LOEL is 300 ppm of paraquat dichloride (12.25 and 15.29 mg/kg/day, for males and females, respectively); or 9.0 mg/kg/day (males) and 11.2 mg/kg/day (females), expressed as paraquat cation. (Toyoshima, S et al. 1982).

In a chronic Fischer SPF rat study (using diets containing 0, 10, 30, 100 or 300ppm paraquat dichloride for 104 weeks), it was concluded that the incidence of pulmonary adenoma found in female rats in the 300 ppm group did not exceed the background incidence of pulmonary adenoma in rats of this strain. On the basis of the lung and eye lesions the no-effect level (NOEL) determined in this study for Fisher SPF rats after 104-week treatment was 30 ppm paraquat dichloride, equivalent to 0.77 mg paraquat cation/kg bw/day in male rats and 100 ppm paraquat dichloride, equivalent to 3.12 mg paraquat cation/kg bw/day in female rats ($\approx$ 1.1 and 4.3 mg paraquat dichloride/kg bw/day. (Yoshida et al., 1982).

In the 1 year dog feeding study, the systemic NOEL (based on the observed pulmonary changes) is 15 ppm (males: 0.45 mg/kg/day and females: 0.48 mg/kg/day, expressed as paraquat cation). The systemic LOEL is 30 ppm (males:0.93 mg/kg/day and females: 1.00 mg/kg/day, expressed as paraquat cation $\approx$ 1.3 and 1.4 mg paraquat dichloride/kg bw/day). (Kalinowski et al 1983).

The US EPA place paraquat dichloride in Category E for carcinogenicity (evidence of non-carcinogenicity for humans) (EPA RED, 1997).

Table 10: Summary of chronic toxicity/carcinogenicity of paraquat

Study	NOAEL (as paraquat cation)	Comment	Ref.
2 year mouse dietary (0, 0, 12.5, 37.5, 100/125 ppm paraquat cation $\cong$ 0, 0, 1.87, 5.6, 15.0/18.7 mg paraquat cation/kg bw/day)	12.5 ppm paraquat cation (1.5 mg paraquat cation/kg bw/day $\approx$ 2.1 mg paraquat dichloride/ kg bw/day)	No significant increases in tumour incidences. Key effect was renal lesions at $\geq$37 ppm. No ocular lesions.	Sotheran et al, 1981
2 year mouse dietary (0, 2, 10, 30, 100 ppm paraquat dichloride $\cong$ 0, 1.4, 7.2, 22, 72 ppm paraquat cation)	30 ppm paraquat dichloride ($\cong$ 2.9 and 2.8 mg paraquat cation/kg bw/day for males and females respectively $\approx$ 4.0 and 3.9 mg paraquat dichloride/kg bw/day)	No evidence of carcinogenicity. Increased mortality in females at 100 ppm paraquat dichloride and haematological and blood chemistry changes.	Toyoshima et al, 1982
2 year rat dietary (0, 10, 30, 100, 300 ppm paraquat dichloride $\cong$ 0, 7.2, 22, 72, 217 ppm paraquat cation)	30 ppm paraquat dichloride ($\cong$ 0.77 mg paraquat cation/kg bw/day for males; 100 ppm paraquat dichloride ($\cong$ 3.12 mg paraquat cation/kg bw/day for females) ($\approx$ 1.1 and 4.3	Reduced body weight gain, lung lesions, cataracts. No evidence of carcinogenicity.	Yoshida et al, 1982

	mg paraquat dichloride/kg bw/day)		
2 year rat dietary (0, 6, 30, 100 or 300 ppm paraquat dichloride, corresponding to 0, 0.25, 1.26, 4.15 or 12.25 and 0, 0.30, 1.50, 5.12 or 15.29 mg paraquat dichloride/kg bw/day for males and females respectively.	100 ppm paraquat dichloride (4.15 and 5.12 mg paraquat dichloride/kg bw/day for males and females respectively; or, 3.0 and 3.7 mg/kg bw/day as paraquat cation for males and females respectively	Not carcinogenic. The incidence of ocular changes was low and not related to dose.	Toyoshima et al, 1982
Lifetime rat dietary study. Up to 117 weeks (males) and 124 weeks (females), 0, 0, 25, 75 or 150 ppm paraquat cation; corresponding to 0, 0, 1.25, 3.75 and 7.5 mg paraquat cation/kg bw/day respectively.	25 ppm paraquat cation (1.25 mg paraquat cation/kg bw/day $\approx$ 1.7 mg paraquat dichloride/kg bw/day). (Based on ocular lesions) at 2 years.	There was no evidence of carcinogenicity. Key effect was ocular lesions at 75 and 150 ppm paraquat cation – dose and time related severity.	Woolsgrove et al, 1983
1 year dog dietary (0, 15, 30 and 150 ppm paraquat cation), corresponding to 0, 0.45, 0.93 and 1.51 mg paraquat cation/kg bw/day in males and 0, 0.48, 1.00, 1.58 mg paraquat cation/kg bw/day in females	15 ppm paraquat cation (0.45 mg paraquat cation/kg bw/day $\approx$ 0.62 mg paraquat dichloride/kg bw/day)	Lung lesions and signs of respiratory abnormalities at 30 and 50 ppm paraquat cation.	Kalinowski et al, 1983

(d) Genotoxicity

Paraquat has been extensively examined in a wide range of assays both *in vitro* and *in vivo*, including end-points for gene mutation, chromosomal damage and DNA repair. The *in vitro* assays allow an assessment of the intrinsic ability of the compound to induce genotoxicity whilst the *in vivo* assays determine whether any such activity will be expressed in the whole animal.

In vitro, paraquat has been found overall to be negative in the most widely conducted genotoxicity assay, the Ames test. It does, however, induce chromosomal aberrations in mammalian cells *in vitro* at high or cytotoxic concentrations. The clastogenic action of paraquat has been investigated and it is reported that it is not paraquat itself, but a reactive oxygen species that is responsible for the effects observed. Following from these observations, a number of other *in vitro* assays have been conducted using paraquat as a material that can generate reactive oxygen species. These have produced mixed results, but overall provide further support for the conclusion that paraquat can cause DNA damage in vitro through formation of reactive oxygen species, especially at high and/or cytotoxic doses of paraquat.

In vivo, paraquat has been examined in both the rat and the mouse using the oral route of administration and shown to be non-genotoxic when tested to maximum tolerated doses in the bone marrow micronucleus assay, the bone marrow cytogenetic assay, the liver DNA repair assay and the germ cell dominant lethal assay. Conflicting results have been reported for some assays using other routes of administration (eg

intraperitoneal administration) or endpoints examining for the presence of oxidised DNA bases in animals or for mutation in Drosophila. It is concluded that whilst paraquat has been shown to induce a genotoxic response in some in vitro systems, often at high or cytotoxic dose levels, it has no significant genotoxicity *in vivo*.

Table 11: Results of assays for the genotoxicity of paraquat

There are numerous genotoxicity studies reported, many of which are research studies and may not be compliant with modern guidelines. The packages of regulatory studies conducted / commissioned by Syngenta (or its former companies Zeneca and ICI) are highlighted in the table below by emboldening the study details (end point and test system):

End-point	Test system	Concentration	Purity (%)	Results	Reference
A. IN VITRO ASSAYS					
Bacterial gene mutation assays					
Ames test	S.typhimurium TA1535, TA1537, TA1538, TA98 and TA100	Dose not given	Formulation assumed to be 200g/l	**Negative**	Eisenbeis SJ et al, 1981
Ames test	S.typhimurium TA1535, TA1537, TA1538, TA98 and TA100 and E.coli WP2 hcr	Up to 5000 µg/plate	Not quoted	**Negative**	Morija M et al, 1983
Ames test	S.typhimurium TA1535, TA1537, TA1538, TA98 and TA100	1.0- 50 µg/plate	Not quoted	**Negative** toxic effect at 10 µg/plate and higher in some strains	Benigni R et al, 1979
Ames test	S.typhimurium TA98 and TA100	0-1.0 mM	Not quoted	**Positive**	Moody CS & Hanan HM, 1982
Ames test	S.typhimurium TA97, TA98, TA100, TA1535, TA1537 and TA1538		Not quoted	**Equivocal**	Lin J-K et al, 1989
Ames test	E.coli WP2 hcr	Dose not given	Not quoted	Full results not given	Shirasu Y et al, 1982
Ames test	S.typhimurium TA98 and TA100	10^{-4}M	Not quoted	Superoxide anion promotes Benzo(a)pyrene positive	Wei CI et al, 1985
Ames test	S.typhimurium TA102	10 ng / plate	Not quoted	**Negative**	Levin DE et al, 1984
Ames test	S.typhimurium TA98, TA100, TA1535, TA1538	0.4 -360 µg/plate	100	**Negative**	Shirasu Y et al., 1978

Ames test	S.typhimurium TA98, TA100, TA1535, TA1538	0.12-1807 µg/plate	99	**Negative**	Anderson D, 1977
Ames Test	S.typhimurium TA98, TA100, TA1535, TA1538, TA 1535	1µg-1mg/ plate	99.9	**Negative** (cytotoxic at 1mg/plate)	McGregor DB, 1977
Ames test	S.typhimurium TA97, TA98, TA100, TA1535, TA1537 and TA1538	0-20 µg/plate	Not quoted	**Equivocal**	Lin J-K et al, 1988
Ames test	S.typhimurium TA98 and TA100	0.06 µmol/plate	Not quoted	**Negative**	Nishimura N et al, 1982
Ames test	S.typhimurium TA100	20 µg/plate	Not quoted	**Positive** (without activation)	Yamaguchi T, 1981
Forward mutation assay	S.typhimurium His G46, TA92, TA1535, TA1538 and TA100	0.1-2.5 µg/plate	Not quoted	**Positive**	Bignami M and Crebelli R, 1979
Ames test	S.typhimurium TA100 and TA104	0-0.1 mg/plate	Not quoted	**Positive**	Ueno H et al, 1991
Ames test	S.typhimurium TA102 and TA2638; E.coli WP2/pKM101 and WP2 uvrA/pKM101	0-10 µg/plate	Not quoted	**Negative**	Watanabe K et al, 1998
Ames test	E.coli IC203 (deficient in OxyR) and WP2 uvrA/pKM101	1 µg/plate (dissolved in H_2O) 1000 µg/disc	Not quoted	**Negative**	Martinez A et al, 2000

Bacterial SOS-induction

SOS chromotest	E. coli PQ37, PM21 and GC4798	40µl/1.2ml medium (dissolved in H_2O)	99%	**Negative**	Eder E et al 1989
Umu test	E. coli; K12 AB1157, AB2463 H/r30, Hs30, NG30, R15, B/r and B$_{S-1}$	4mg/ml	Not quoted	**Positive**	Yonei S et al, 1986
Umu test	S.typhimurium TA1535/pSK1002	1000-3333 µg/ml	Not quoted	**Positive**	Oda Y et al, 1985
Umu test	S.typhimurium TA1535/pSK1002	1000 µg/ml	Not quoted	**Positive**	Nakamura S et al, 1987
Umu test	*umu*-test S.typhimurium TA1535	0.1ml/tube	Not quoted	**Negative**	Degirmenci E et al 2000
Microscreen assay	E.coli WP2s (λ)	0.02-0.26µM	Not quoted	**Positive** + S9 (More toxic - S9).	DeMarini DM and Lawrence BK, 1992
SOS chromotest	E.coli K-12 and PQ300	Not reported	Not quoted	**Negative**	Müller J and Tanz S, 1992

Plant assays

Assay	Test system	Dose	Purity	Result	Reference
Mutation assay	Nitrogen-fixing blue-green alga	50 and 75 ppm	Not quoted	**Positive**	Vaishampayan A 1984 (a)
Mutation assay	Nitrogen-fixing blue-green alga	25, 50 and 75 ppm	Not quoted	**Positive**	Vaishampayan A 1984 (b)
Cytogenetic test	Vicia faba root (meristematic cells)		Not quoted	**Negative**	Gopalan HNB & Njagi GDE, 1979
Cytogenetic test	Allium fistulosum	1, 10 and $\geq$100 µg/ml	Not quoted	**Positive**	Alekperov UK, 1967
Cytogenetic test	Barley seed (root cells)	10^{-4}, 5.0×10^{-5} and 2.5×10^{-5}M	Not quoted	**Positive**	Stroev VS, 1970
Sub-mammalian eukaryotic assays					
Gene conversion	Sacchyromyces cerevisiae	100-900 ppm	Not quoted	**Positive** (reduced survival).	Parry JM, 1973
Gene conversion	Saccharomyces cerevisiae	1000 ppm	Not quoted	**Negative**. (reduced survival).	Siebert D and Lemperle E, 1974
Gene mutation and lethal recessive damage	Aspergillus nidulans	20-1000 µg (plate incorporation assay) 20 mg/ml (liquid test)	Not quoted	**Positive** (plate assay); **negative** (liquid test). **Positive** for lethal recessive damage.	Benigni R et al, 1979
Chromosome test	Drosophila (mus-302 repair defective females)	200 ppm	Not quoted	**Negative**	Woodruff RC et al, 1983
Somatic mutation	Drosophila wing spot test	2-8 mM	99%	**Equivocal** .	Torres C et al, 1992
Chromosome test	Saccharomyces cerevisiae	0-35 mg/ml	Not quoted	**Negative** (Reduced survival at 15-35 mg/ml).	Brennan RT et al, 1994
SMART assay	Drosophila (strains OK, 91-C, LS, H79 91-R and HR)	0-10 mM	Not quoted	**Positive**	Gaivão I & Comendador MA, 1996
SMART assay	Drosophila	Dose not given	Not quoted	**Negative**	Ramel C & Magnusson J, 1992
SMART assay	Drosophila	0-10 mM	Not quoted	**Positive** (toxicity at high doses)	Gaivão I et al, 1999
SMART assay	Drosophila	0-16mM	Not quoted	**Negative**	Vontas JG et al 2001
DNA damage	Saccharomyces cerevisiae	1-20mM	Not quoted	**Negative**	Paesi-Toresan SO et al 1998
Mammalian gene mutation assays					
Mouse lymphoma cells	L 5178 Y tk$^+$/tk$^-$ mouse lymphoma cells	10-200 µg/ml	Not quoted	**Positive** (-S9)	McGregor et al 1988
Mouse lymphoma cells	**L 5178 Y mouse lymphoma cells**	23 – 361 µg/ml	99%	**Negative**	Clay & Thomas, 1985

Chinese hamster cells	*Transgenic gpt*[+] Chinese hamster cell lines (G10 and G12)	200 and 300μM	Not quoted	**Negative**	Kitahara et al 1996
Chinese hamster ovary cells	Chinese hamster ovary K1-BH4, AS52 and XRS-5 cell lines	0-2mM	Not quoted	**Negative**	Salazar JJ and Hsie AW 1999
Mammalian cells	V79 Chinese hamster cells (HPRT)	1-5mM	Not quoted	**Negative**	Speit G et al 1998

DNA damage assays

DNA unwinding	Erlich Ascites Tumour (EAT) cells in vitro	Up to 2mM	Not quoted	**Negative**	Peter B et al 1992
Comet assay	Human peripheral blood lymphocytes in vitro	Up to 2000 μg/ml for 4hrs	99%	**Positive** (+S9), **Negative** (–S9)	Ribas G et al 1995
Micronucleus assay	Human lymphocytes in vitro	Up to 100 μg/ml	99%	**Negative**	Surralles J et al 1995
Comet assay	Cultured astroglial cells in vitro	20-80μM	Not quoted	**Positive**	Frederikson CM and Clausen J 1999
Comet assay	Human peripheral lymphocytes (HeLa and Hep G2)	0-300μM	Not quoted	**Positive**	Petrovska H and Dusinska M 1999
Comet assay	V79 Chinese hamster cells	1-10mM	Not quoted	**Negative**	Speit G et al 1998
Comet assay	Rat alveolar macrophages and epithelial type II pneumocytes	0-10 μM	Not quoted	**Positive**	Dušinská M et al 1998
Comet assay	human alveolar epithelial cell line A549 and human monocytic cell line TITP-1	10^{-2}-10^{-7}M (cytotoxicity); 10-100 μM (genotoxicity)	Not quoted	**Positive**	Don Porto Carero et al 2001
DNA damage	8-OH-dG and 8-OH-G in blood, liver, lung, kidney, heart and brain Wistar rats	20mg paraquat dichloride/kg ip	Not quoted	**Positive**	Tokunaga I and Takeichi S 1996
DNA damage	Human lung cancer cells HCC-15 and NCI-H2009	0-2mM	Not given	**Positive**	Zienolddiny S et al 2000
DNA damage	Calf thymus DNA incubated with mouse liver, lung or brain homogenate fractions	0-1.0mM	Not given	**Positive** (without melatonin) **Negative** (with melatonin)	Yamamoto H and Mohanan PV 2001

DNA repair assays

Bacterial	S.Typhimurium strains TA1538 and	100 μg/plate	Not quoted	**Weakly positive**	Benigni R et al 1979

TA 1978

Bacterial	B.subtilis H17/M45	1-500 µg/disk	100%	**Negative**	Shirasu Y et al 1978
Mammalian cells	Human epithelial-like cells (EUE)	20-2000 µg/ml	Not quoted	**Equivocal** (no dose response)	Benigni R et al 1979
Mammalian cells	Human lymphocytes in vitro	900 µg/ml	95%	**Equivocal**	Rocchi P et al 1980
Mammalian cells	**Hepatocyte cultures from male Alderley Park rats**	1 nM - 10 mM (0.19 ng/ml - 1.86 mg/ml)	99.6%	**Negative**	Trueman RW et al., 1985

Cytogenetic Assays

Chromosomal damage	Chinese hamster lung fibroblasts in vitro	3 –10 mM	Not quoted	**Positive** (at high doses)	Nicotera TM et al 1985
Chromosomal damage	Chinese hamster lung cells in vitro	0.08-20.0µM	Not quoted	**Positive**	Tanaka R and Amano Y 1989
Chromosomal damage	Chinese hamster lung fibroblasts in vitro	0.1 – 0.8 mg/ml	Not quoted	**Positive**	Sofuni T and Ishidate M 1988
Chromosomal damage	Chinese hamster lung fibroblasts in vitro	50 – 400 µg/ml	Not quoted	**Positive**	Sawada M et al 1988
Chromosomal damage	Chinese hamster ovary cell in vitro	Up to 200 µg/ml	45 (technical grade)	**Positive** (at cytotoxic doses, +S9 significantly reduced response)	Lin MF et al 1987
Chromosomal damage	Unspecified Chinese hamster cells in vitro	0.8mg/ml for3 hours	Not quoted	**Positive**	Sofuni T et al 1985
Chromosomal damage	Human lymphocyte cells in vitro	75 and 150 µg/ml	Not quoted	**Positive**	Joenje H et al 1987
Chromosomal damage	**Human lymphocytes in vitro**	90, 903, & 1807µg/ml	99.6	**Positive** (at cytotoxic doses)	Sheldon et al., 1985a
Chromosomal damage	Human lymphocytes in vitro	15 –60 µl/ml	Gramoxone formulation containing 20% paraquat dichloride	**Positive** (at cytotoxic levels)	Salam AZE-A et al 1993
Sister chromatid exchange	**Chinese hamster lung fibroblasts in vitro**	0.9, 1.8, 9, 18, 90 & 177 µg/ml	99.4	**Positive** (reduced with metabolic activation)	Howard et al., 1985
Sister chromatid exchange	Chinese hamster lung cells in vitro	Up to 30 µM	Not quoted	**Positive**	Tanaka R 1992
Sister chromatid exchange	Chinese hamster lung cells in vitro	4µM	Not quoted	**Positive** (reduced by pre-treatment with pentoxifylline).	Tanaka R 1995
Sister chromatid exchange	Primary rat tracheal epithelial cells	0.625 – 2.5µg/ml	45% technical grade	**Positive**	Wang TC et al 1987

Assay	System	Dose	Purity	Result	Reference
Sister chromatid exchange	Chinese hamster ovary cells	6.25 - 100µg/ml	45% technical grade	**Negative**	Wang TC et al 1987
Sister chromatid exchange	Cultured human lymphocytes	1-4000µg/ml	99	**Positive**	Ribas G et al 1997/98
Sister chromatid exchange	Chinese hamster lung cells	0-10µM	Not quoted	**Positive**	Tanaka R 2000
Micronucleus assay	Cultured human lymphocytes	1-4000µg/ml	99	**Negative**	Ribas G et al 1997/98
Chromosomal damage	Cultured human lymphocytes	1-50µg/ml	99	**Negative**	Ribas G et al 1997/98
Chromosomal damage	V79 Chinese hamster cells	1-5mM	Not quoted	**Positive** (at cytotoxic doses)	Speit G et al 1998

B. IN VIVO ASSAYS

In vivo assays Cytogenetic assays

Assay	System	Dose	Purity	Result	Reference
Cytogenetic assay	**Bone marrow. Alderley Park wistar male rat**	6.5 – 19 mg paraquat cation/kg orally for 5 days	100	**Negative**	Anderson D et al 1978
Cytogenetic assay	**Bone marrow. Alderley Park Wistar male rat**	19 mg paraquat cation/kg orally for 1 or 5 days	100	**Negative**	Anderson D et al 1979
Cytogenetic assay	**Bone marrow. Alderley Park Wistar male and female rat**	15, 75 and 150mg paraquat cation/kg orally x1	33.07	**Negative**	Howard CA et al 1987
Micronucleus assay	**Bone marrow Male and female C57Bl/6J/Alpk mice**	52 and 83mg paraquat cation/kg x1	99.4	**Negative**	Sheldon T et al 1985 (b)
Micronucleus assay	Peripheral blood erythrocytes Female Swiss mice	10 and 20mg/kg sc	99	**Negative** (20mg/kg toxic) **Positive** (10mg/kg)	Pena LFM et al 1999
Micronucleus assay	Bone marrow and peripheral blood male Swiss mice	20mg/kg ip x2 ,48 hrs apart	Not given	**Positive** (melatonin significantly reduces the effect)	Ortiz G et al 2000
Micronucleus assay	Bone marrow Male Swiss albino mice evaluated at 6 time points between 12 and 168 hrs	83mg/kg orally	Not given	**Positive** (48 and 72 hrs only)	Prabakaran P and Moorthy MV 1998
Chromosomal damage	Bone marrow CFLD male mice	60 or 2.5 twice/wk x 6wks orally; inhalation; 15 or 0.55 – 5.5mg ip every other day for	Gramoxone formulation containing 25% paraquat cation	**Negative**	Selypes A et al 1977

Chromosomal damage	Bone marrow BALB/c male and female mice	7-23mg/kg ip or 1.5 to 5.0 mg/kg ip for 10 days	Gramoxone 200	**Negative** (single dose) **Equivocal** (after repeat toxic doses).	Rios ACC et al 1995
Dominant Lethal assay	Swiss-Webster male mice	66 mmol/kg ip	Not quoted	**Negative**	Pasi A et al 1974
Dominant Lethal assay	**Male CD-1 mice**	0.04, 0.4 and 4.0 mg/kg orally for 5 days	23.8 (w/v)	**Negative**	Anderson D et al 1976
Chromosomal damage	Peripheral blood and bone marrow cells Male ICR mice	15mg/kg ip x2 ,24hrs apart	98	**Positive** (but melatonin greatly reduces the effect)	Melchiorri D et al 1998
DNA damage / repair assay					
DNA repair	**Unscheduled DNA synthesis in livers of Alderley Park rats**	45 – 120 mg paraquat cation /kg x1	33.07 technical grade	**Negative**	Truman RW and Barber G 1987
DNA damage	Liver and lung Male Wistar rats	20mg/kg ip	Not quoted	**Negative**	Sorensen M and Loft S 1999

(e) Reproductive toxicity

(i) Multigeneration studies

Mice: Three non-guideline studies in the mouse are reported in the literature and have been included for completeness (Dial and Dial, 1987; Dial and Dial, 1989; Bus and Gibson, 1975)

Groups of 24 male and female ICR albino mice were maintained on dietary levels of 0, 45, 90 or 125 ppm paraquat cation in a two-generation reproduction study. The date of parturition and number of young born were recorded for each paired female. In the first generation phase females were allowed eight weeks from pairing to produce a litter and cages were checked daily for adult and F_1 mortality. All litters were weaned on day 30 postnatally and either segregated by sex or paired, without inbreeding, for use in the second generation study. Exposure of the F_1 mice to the various dose levels was continuous to 49 days postnatally except that parental mice were exposed until the F_1 were weaned. The lungs were excised and examined histopathologically from sucklings, weanlings and adults of control and paraquat groups in which mortality occurred.

In the second generation phase the F_1 males and females were randomly paired within the various treatment groups at 30 days of age. Siblings were not paired. The control group consisted of 24 pairs. Offspring of parents fed paraquat were divided into two groups of 12 pairs each per respective parent treatment group. One group of 12 pairs was removed from the parents' diet and placed on control diet when the mice were weaned at 30 days of age; the remaining 12 pairs were continued on the respective

parents' paraquat diet. The dates of the first and second parturitions and number of young born per litter were recorded for each paired female for 16 weeks. Mortality data was collected for adults in all groups and sucklings and weanlings in the control and 125 ppm groups. The lungs were excised and examined histopathologically from sucklings, weanlings and adults of control and paraquat groups in which mortality occurred.

There were no effects on age to parturition, number born or abnormalities in the pups in the first generation following administration of 45 or 90 ppm paraquat cation. At 125 ppm paraquat cation, however, an increase in mortality was seen in the dams and pups during the first weeks of life. The second generation mice were more resistant to the effects of paraquat, the only effect being a statistically significant increase (P<0.05) in the age of the mothers at second parturition when fed 125 ppm paraquat cation. (Dial & Dial, 1987).

Subsequent studies to explore the basis for the high mortality in the first generation dams and pups exposed to 125 ppm paraquat cation in diet, showed that they almost certainly died from lung damage. This only occurred in pups exposed prenatally via the placenta, not in pups exposed post-natally (Dial & Dial, 1989). Bus & Gibson (1975) also reported that paraquat given to mice in their drinking water at either 50 or 100 ppm paraquat cation from day 8 of gestation and to the young until 42 days of age increased pup mortality at 100 ppm but not at 50 ppm. The lungs of mice killed 42 days after 100 ppm showed extensive alveolar consolidation and collapse, and areas of thickening of intra-alveolar septa, supporting the view that the deaths at this dose were probably due to lung damage. No significant pathological changes were seen in the lungs of the 50 ppm or control mice, nor in the liver or kidneys of mice of any treatment group. There were no treatment-related effects on the number of live foetuses or on the postnatal growth rate at either treatment level.

Rats: Three multigeneration studies are reported. A modern study (Lindsay et al, 1982); a study conducted to a Japanese protocol involving a teratology sub group Suzuki et al, 1983) and an earlier study (Fletcher et al, 1972).

The potential reproductive effects of technical paraquat dichloride (32.7% w/v paraquat cation) was investigated in a 3-generation (2 litters per generation) study. Groups of 15 male and 30 female (F0 parents) weanling Alpk Wistar-derived rats were fed diets containing 0, 25, 75, or 150 ppm paraquat cation (corresponding to 0, 1.25, 3.75 or 7.5 mg paraquat cation/kg bw/day respectively $\approx$ 0, 1.7, 5.2 or 10.4 mg paraquat dichloride/kg bw/day). Test diets were fed continuously throughout the study. Samples of diet were analysed for achieved concentration and homogeneity.

After 12 weeks pre-mating administration of paraquat diets, the F_0 parents were mated to produce the first litters, F_{1a} and F_{1b}. These litters were reared to weaning. The breeding programme was repeated with F_1 parents selected from the F_{1b} litters which were mated at least 11 weeks after selection giving F_{2a} and F_{2b} litters. The F_2 to F_3 mating was identical to the F_1 to F_2 mating. During mating two females were housed with one male. Daily vaginal smear examinations were performed to determine when mating occurred – designated Day 1 of gestation. After 21 days if there was no evidence of mating, the first male was removed and after a 3 day interval was replaced by a male of proven fertility.

During the study all rats were observed daily for mortality, abnormalities in clinical condition and behaviour. Bodyweight and food consumption were determined regularly. Urine samples were collected pre-mating from 3 parental animals/sex/group for paraquat determinations. Moribund or dead rats were examined *post mortem*. Parental animals were given a full examination *post mortem* at termination of the generation and selected tissues (lung, testes and abnormalities) were examined histopathologically. Twenty five female and 10 male F_1 parents were given a full examination *post mortem* and a wide range of tissues were examined histopathologically. All pups found dead or considered abnormal were given an extensive examination. Litters were examined at least once daily and grossly abnormal pups or those dying before day 18 were removed for teratological examination by Wilson sectioning. Approximately 50% of pups were killed post weaning and given a gross *post mortem* examination with abnormal tissues taken for histopathological examination. Five pups/sex/group from the F_{1b} and F_{2b} litters and 10/sex/group from the F_{3b} litters received a full examination *post mortem* with a wide range of tissues examined histopathologically. A count of all live and still-born pups was made within 24 hours of parturition (day 0) and thereafter at days 4, 10 and 21 *post partum*. The sexes and any clinical abnormalities of the pups were also recorded at these times. Individual pup bodyweights were recorded within 24 hours of birth (day 0) and at days 4, 10, 21 and 28 *post partum*.. The mean gestation period, bodyweight gain during pregnancy, propoirtion of pups born alive, survival to day 21 and proportion of litters viable at day 10 were determined.

Dietary analyses showed achieved concentration, homogeneity and stability to be satisfactory. Urine analyses for paraquat showed a good dose relationship in F_0 animals but in F_1 males and F_2 males and females the results for 75 ppm animals were similar to those from 150 ppm animals indicating saturation may have been approached.

There were no adverse effects on parental body weights or food consumption. However, there was a high incidence of mortality (27-43%) in the high dose F_0, F_1 and F_2 females, due mostly to severe lung damage caused by paraquat. No treatment-related changes were found in the reproductive performance (male and female fertility, live-born and survival indexes, and litter size) or in the reproductive tract of parents or offspring. Body weight gain during pregnancy was reduced in the second mating of each generation in animals from the 150 ppm group although litter weight gain was satisfactory by day 28 in all instances. Development of the reproductive tract in all treated offspring was substantially comparable to that in controls.

Post mortem findings related to treatment were seen at 150 ppm and occasionally at 75 ppm. A red/purple discolouration of the lung and fibrosis were seen in both pups and parents from the 150 ppm groups. Alveolar histiocytosis was more pronounced at 150 and 75 ppm in all male parents and in F_1 and F_2 females. A high incidence of renal pelvis dilatation was evident in all groups including controls. An increased incidence of internal hydrocephalus was seen in F_{2b} males. There was no evidence of eye lesions associated with paraquat administration.

It was concluded that paraquat had no effect on reproductive performance or development of the reproductive organs of Alderley Park rats when administered at dietary levels up to 150 ppm over 3 generations. Increased incidences of lung lesions at 150 and 75 ppm indicate that the overall NOAEL is 25 ppm paraquat cation, equivalent to approximately 2.5 mg paraquat cation/kg bw/day (Lindsay et al., 1982).

Groups of 12 male and 24 female rats were fed diets containing 0, 30, or 100 ppm paraquat cation from 35 days of age. Three generations bred from these animals received the same diets during the whole period under test. Two litters were bred from each generation, and the effects on growth, food intake, fertility, fecundity, neonatal morbidity, and mortality were noted. No evidence was seen of damage to germ-cell production or of structural or functional damage in the animals. In this study, pregnant and young animals did not appear to be more vulnerable to paraquat than did adults. However, the incidence of renal hydropic degeneration in 3 - 4 week-old offspring was slightly increased in the 100 ppm paraquat cation group (Fletcher et al., 1972).

In a 3-generation study with 2 litters per generation, groups of 30 male and 30 female Sprague-Dawley rats (F_0 parental generation) were given diets containing 0, 72, 145, or 290 ppm paraquat cation from 5 weeks of age (13 weeks prior to mating to obtain the first litter, F_{1a}) until the end of the second lactation (lactation of F_{1b} litters). In the second generation (F_{1b}), 30 males and 30 females per group were treated from immediately after weaning until the end of the second lactation (lactation of F_{2b} litters). In the third generation (F_{2b}), the same number of rats were treated immediately after weaning for at least 13 weeks. In a teratology study sub-group 5 pregnant females of the parental generation (F_0) and 10 pregnant females of the second generation (F_{1b}) were killed on day 20 of pregnancy and examined macroscopically. Fetuses were examined for number, sex, weight, external and internal abnormalities, and progress of ossification. Another subgroup was used as a postnatal investigation group, where natural parturition of pregnant females was permitted to occur. In this group the duration of gestation, parturition conditions, the number of live and still-born pups, sex, and external abnormalities were recorded. Live pups were investigated until weaning. Five male and 5 female rats per group of the first (F_0) and second (F_{1b}) generations and 10 male and 10 female rats per group of the F_{2b} litters were subjected to histopathological examination of approximately 25 tissues.

In the parental generation there were significant increases in mortality and clinical signs attributable to paraquat (asthmatoid wheezing) in several rats of the 290 ppm group of each generation from the early stage of the dosing period. There were 29 treatment-related deaths/moribund animals, all from the 290 ppm dietary level (5 among F_{1b} animals and 24 among F_{2b} animals), but only 4 deaths among control rats (1 among F_0 female rats and 2 among F_{1b} female rats at parturition and 1 among F_{2b} rats during the dosing period). Histopathological examination of these dead or moribund F_{2b} rats showed, in some cases, hyperplasia of the alveolar epithelium and, in most cases, diffuse thickening and fibrosis of the alveolar walls.

There was a decrease in body-weight gain in both male and female rat of the F_0 and F_{2b} generations at 290 ppm during the early stage of the dosing period. Body-weight gain was also reduced in F_{1b} females at 290 ppm during the gestation and lactation

periods. Reductions in food consumption and efficiency of food utilisation were seen in F_0 and F_{2b} females. There was a significant decrease in water consumption in F_0 and F_{1b} females during lactation. No effect of the compound was observed on the reproductive performance of parental rats. Macroscopic examination revealed an apparently higher incidence of white spots in the lungs of both male and female rats of the 290 ppm group in all 3 generations. Treatment-related changes of the lung were confirmed by histopathology in rats of each generation. These lesions were dose-dependent and included zonal thickening and fibrosis of the alveolar walls, zonal atelectasis, and accumulation of foam cells. There were no treatment-related changes in organ weights. A treatment-related statistically-significant reduction in the lactation index was found in F_{1a} and F_{1b} litters of the 290 ppm group. A statistically-significant reduction in the lactation index was also observed in F_{2a} litters of the 290 ppm group, but it was not clear to the authors of the report whether this change was attributable to treatment. There were no statistically-significant differences in lactation index between other treatment groups and controls nor in the number of still-births and live births, sex ratios, or viability indexes in any of the treated groups of both generations, when compared to controls. The prolonged duration of gestation in F_0 rats of the parental group at 145 and 290 ppm was considered by the authors of the report to be accidental. The teratology phase showed a statistically-significant delay in ossification in F_{1b} fetuses from F_0 parents treated with 290 ppm paraquat and in F_{2b} fetuses from F_{1b} parents of all treated groups. It was not clear to the authors of the report whether the retarded growth was due to the treatment. There was a treatment- related statistically-significant higher incidence of female pups with retarded opening of the vagina in both F_{1b} and F_{2b} litters at 290 ppm. There was a statistically-significant decrease in body weight in male, but not in female, fetuses at 72 and 290 ppm. There were no statistically-significant differences between fetuses from treated rats and control fetuses in the number of corpora lutea or implantations, percentage of implantations, number of dead or live fetuses, sex ratios, or placental weights. No external or internal malformations were detected in fetuses of any treatment group.

The authors of the report concluded that there was no evidence suggesting that paraquat was teratogenic and that the only treatment-related change which was enhanced by treatment of successive generations of Sprague-Dawley rats with paraquat was "an increase in death" at 290 ppm paraquat cation. A no-effect level for paraquat was not found in this study due to delayed ossification in F_{2b} fetuses in all treated groups (Suzuki et al., 1983).

(ii) Developmental toxicity

Mice: Three developmental studies in the mouse are reported; a modern study (Palmer K, 1992); an earlier study which did not achieve a maximum tolerated dose (Hodge et al, 1978a) and a research study reported in the literature (Bus et al, 1975b).

In a modern developmental toxicity study, technical paraquat dichloride (38.2% paraquat cation) was administered to 26 female Crl:CD1 (ICR) BR mice/dose by gavage in deionised water at dose levels of 0, 7.5, 15 or 25 mg paraquat cation/kg bw/day from days 6 through 15 of gestation.

At 25 mg paraquat cation/kg bw/day, four dams were killed *in extremis*, having shown clinical signs of toxicity (piloerection, labored respiration, hunched posture, hypothermia, hypoactivity and/or pale extremities and eyes). Decreases in body weight and body weight gain (p < 0.01) were seen at 25 mg paraquat cation/kg bw/day from day 12 to termination. At study termination, nine animals from the 25 mg paraquat cation/kg bw/day group had dark red lung lobes. Increases in lung with trachea and kidney weights and a possible decrease in pregnancy rate were noted at 25 mg paraquat cation/kg bw/day. No maternal effects were observed at either 7.5 or 15 mg paraquat cation/kg bw/day.

At 25 mg paraquat cation/kg bw/day, there were significant decreases in mean foetal weights and reductions in implantations, live foetuses and uterus weight. In addition, skeletal effects were observed which included statistically significant increases in the number of litters with retarded ossification of the occipital, the number of foetuses and litters with 6 caudal centra , the number of litters with uni- or bilateral extra 14th ribs and the number of foetuses and litters with non-ossified astragalus in the hindlimb. No other developmental effects were observed at this dose level and no developmental effects were observed at either 7.5 or 15 mg paraquat cation/kg bw/day.

No developmental effects were observed at either 7.5 or 15 mg paraquat cation/kg bw/day. The maternal NOEL is 15 mg paraquat cation/kg bw/day, based on clinical signs, death, decreases in body weight and body weight gain, dark red lung lobes, increases in lung with trachea and kidney weights and a possible decrease in pregnancy rate seen at 25 mg paraquat cation/kg bw/day.

The developmental NOEL is 15 mg paraquat cation/kg bw/day, based on decreases in mean foetal weights and retarded ossification of the occipital, increases in the number with 6 caudal centra, increases in the number with uni- or bilateral extra 14th ribs and increases in the number with non-ossified astragalus in the hindlimb seen at 25 mg paraquat cation/kg bw/day.   (Palmer K 1992)

Four groups of at least 20 pregnant SPF Alderley Park mice were gavage dosed with paraquat dichloride (purity 100%) in 0.5% aqueous Tween at doses of 0, 1, 5, or 10 mg paraquat cation/kg bw/day during days 6 to 15 of pregnancy, inclusive. Doses were selected for this study based on a range-finding study in which doses of 5, 10, 20 or 40 mg paraquat cation/kg/day were used; all mice in the 40 mg/kg/day group and one mouse in the 10 mg/kg/day group, died. The decedents all had dark red lungs or dark red patches on the lungs . On day 18 the animals were killed, their uteri were examined, and the foetuses were removed, weighed, sexed, and observed for gross abnormalities. There was some evidence of maternal toxicity in the form of slight reductions in body-weight gain at 5 and 10 mg paraquat cation/kg bw/day, although only that of the middle-dose group was statistically significant. There were no clinical signs or pathological changes in maternal animals attributable to paraquat administration. Water and food consumption were not quantified in this study. Numbers of implantations, viable foetuses and resorptions, sex ratios, and foetal and litter weights showed no significant differences between treated and control groups. There were no increases in foetal external or soft-tissue abnormalities which could be associated with paraquat treatment. There were occasional statistically-significant

differences in ossification of individual bones between treated and control groups, but no dose-related trend indicating either retardation of ossification or increased abnormalities was observed. The authors of the study concluded that paraquat was not teratogenic and had no significant influence on embryonic or foetal development of the mouse at levels up to and including 10 mg paraquat cation/kg bw/day ($\approx$ 13.8 mg paraquat dichloride/kg bw/day). (Hodge et al., 1978a).

The teratogenicity and foetal toxicity of paraquat were examined after oral (20 mg/kg bw/day) or intraperitoneal (1.67 or 3.35 mg/kg bw/day) administration of paraquat to pregnant mice during the period of organogenesis (days 8 - 16 of gestation). The oral dose (which was equal to 1/10 of the oral LD_{50}/day) did not produce significant maternal toxicity, but at the higher of the 2 intraperitoneal doses (which was equal to 1/10 of the i.p. LD_{50}/day) a significant maternal mortality (5/7) and a statistically-significant increase of resorption rate, when compared to controls, were observed. Paraquat did not significantly increase the incidence of gross, soft-tissue, or skeletal abnormalities. At the lower i.p. dose and after oral administration of paraquat, there was a slight but non-significant increase in the number of foetuses with absent or non-ossified sternebrae. However, a significant difference was observed in the incidence of abnormal sternebrae between the 2 control groups (6.9 $\pm$ 3.2% in the i.p. control group and 13.2 $\pm$ 5.8% in the oral control group). The authors of the study concluded that the potential for paraquat as a teratogen appeared to be minimal (Bus et al., 1975b).

Paraquat does not rapidly cross the placenta and enter the embryo of mice when given orally or by ip administration (Bus et al., 1975). In contrast, paraquat appears to readily cross the placenta of rats, being detected in foetuses within 30 minutes of an iv injection to 20 day pregnant rats (Ingebrigtsen et al, 1984).

Rats: Two developmental studies in the rat are reported; a modern study (Hodge 1992) and an earlier study (Hodge, 1978b).

In a modern rat developmental toxicity study, paraquat dichloride (38.2% paraquat cation) was administered to 24 female Alderley Park, Wistar-derived (Alpk:APfSD) rats/dose by gavage in deionised water at dose levels of 0, 1, 3, or 8 mg paraquat cation/kg bw/day from days 7 through 16 of gestation. Throughout the study the rats were observed for abnormalities and bodyweight and food consumption recorded. On day 22 the animals were killed.

There were no signs of maternal toxicity other than a minor reduction in bodyweight gain in the 8 mg/kg bw/day group on days 7-10. There were no treatment related macroscopic findings in the dams. At the 8 mg/kg bw/day there was a reduction in foetal weight and a reduction in the number of live fetuses due to increased pre-implantation losses and early deaths. There was no increase in the overall numbers of fetuses with defects, anomalies or major skeletal effects. Hydrocephaly was seen in 3 foetuses from 2 litters from the 1mg/kg bw/day group, an incidence outside the historical control range but not repeated at higher doses. Dilated ureter was increased in the 8 mg/kg bw/day group (2.9% with 0.4% in control). The incidence of short 14th

ribs was increased, without a dose-response, in all treated groups compared to concurrent controls, but were within the historical control values. There were no other significant dose related findings.

Technical paraquat dichloride (38% w/v) was not teratogenic to rats when given at 8 mg paraquat cation/kg bw/day or less. Maternal toxicity and foetotoxicity were seen at 8 mg paraquat cation/kg bw/day. Maternal and developmental NOAELs of 3 mg paraquat cation/kg bw/day can be set based on increased incidence of dilated ureter, increased early foetal deaths and reduced foetal weight at 8 mg paraquat cation/kg bw/day ($\approx$11.0 mg paraquat dichloride/kg/bw/day) (Hodge MCE 1992).

The teratogenic effects of paraquat were studied in 4 groups of at least 20 pregnant SPF Alderley Park rats after oral administration of paraquat dichloride (100% pure) at dose levels of 0, 1, 5, or 10 mg paraquat cation/kg bw/day during days 6 to15 of pregnancy, inclusive. The test substance was dosed in 0.5% Tween 80. The mean achieved concentrations of paraquat in the dosing solutions were acceptable at 1 and 10 mg paraquat cation/kg bw/day, but the nominal 5 mg paraquat cation/kg bw/day level was found to be nearer to 4 mg/kg bw/day. Throughout the study the rats were observed for abnormalities and bodyweight and food consumption recorded. On day 21 the animals were killed.

There were clear clinical signs of maternal toxicity at 5 and 10 mg paraquat cation bw/day. Six rats at the highest-dose level and 2 at the middle-dose level died or became moribund during the experiment. Histological changes found in the lungs and kidneys of the animals receiving 10 mg paraquat cation which died or became moribund were those known to be associated with oral paraquat poisoning. Slight foetotoxicity was seen at 5 and 10 mg paraquat cation, as shown by a statistically-significant reduction in foetal weight (*ca* 5% at 10 mg/kg bw/day) and retardation in ossification, and by a decrease in the number of viable foetuses per number of implants. According to the authors of the study, these effects were probably associated with maternal toxicity. There were no effects on embryonic or foetal survival and increases in foetal abnormalities were not observed. The authors of the study concluded that paraquat was not teratogenic when administered orally to rats, even when there was clear evidence of maternal toxicity. However, it did cause slight foetotoxicity at the 2 highest-dose levels.

Reduced ossification of the 7th and 8th caudal vertebrae was seen at 10 mg/kg bw/day with slight dose related reductions in ossification of the digits of both the fore and hind limbs at 5 and 10 mg/kg bw/day. There was no evidence of teratogenicity . Purified paraquat dichloride (100% pure) was not teratogenic in rats at maternally toxic dose levels. NOAELs for maternal and developmental toxicity are 1 mg paraquat cation/kg bw/day based on maternal toxicity (reduced bodyweight gain) and foetotoxicity (reduced ossification) at 5 mg paraquat cation/kg bw/day ($\approx$ 6.9 mg paraquat dichloride/kg bw/day) and above. (Hodge et al., 1978b).

Summary of reproductive toxicity

Paraquat has been tested for reproductive effects in multi-generation studies in the mouse and rat and for developmental effects in mice and rats, the results of which are summarised in the table below.

In mouse reproduction studies, no effects on reproduction were seen at doses up to 125ppm (highest dose tested), although this dose resulted in some mortality in dams and pups. The overall NOAEL was 90 ppm. It was not possible to calculate the intake of paraquat cation/kg bw/day because of lack of data published (Dial and Dial 1987; Bus and Gibson, 1975).

In a modern rat multigeneration study with Alpk Wistar-derived rats there were no adverse effects on reproduction at dose levels up to 150 ppm. Maternal toxicity was evident at this dose (mortality, reduced weight gain in pregnancy, alveolar histiocytosis, lung discolouration and fibrosis) and 75 ppm (alveolar histiocytosis). The NOAEL was 25 ppm (*ca* 2.5 mg paraquat cation/kg bw/day $\approx$ 3.5 mg paraquat dichloride/ kg bw/day). (Lindsay et al 1982).

In a Japanese 3-generation study with 2 litters per generation and a developmental toxicity phase, groups of 30 male and 30 female Sprague-Dawley rats (F_0 parental generation) were given diets containing 0, 72, 145, or 290 ppm paraquat cation. It was concluded that there was no evidence suggesting that paraquat was teratogenic and that the only treatment-related change which was enhanced by treatment of successive generations of Sprague-Dawley rats with paraquat was "an increase in death" at 290 ppm paraquat cation. A no-effect level for paraquat was not found in this study due to delayed ossification in F_{2b} fetuses in all treated groups (Suzuki et al., 1983).

In an earlier rat multigeneration study where rats were fed diets containing 0, 30, or 100 ppm paraquat cation from 35 days of age, pregnant and young animals did not appear to be more vulnerable to paraquat than did adults. However, the incidence of renal hydropic degeneration in 3-4 week-old offspring was slightly increased in the 100 ppm paraquat cation group (Fletcher et al., 1972).

In the mouse developmental studies there was no overt teratogenicity at maternally toxic doses. A number of effects were seen at low or intermediate doses but with no dose response and the relationship to paraquat administration is unclear. An increase in umbilical hernia seen in one study at the mid-dose was not reproduced in the second study and is considered to be a sporadic finding. Alterations in ossification (particularly the astragalus) and increases in minor abnormalities were seen at 25 mg paraquat cation/kg bw/day, indicating delayed development or mild foetotoxicity associated with maternal toxicity. An overall NOAEL is 7.5 mg paraquat cation/kg bw/day (equivalent to 10.4 mg paraquat dichloride/kg bw/day). (Hodge, 1978; Palmer, 1992). This is consistent with ossification effects at 20 mg paraquat cation/kg bw/day reported by Bus et al, 1975.

In the rat developmental studies paraquat did not exhibit teratogenicity at maternally toxic dose levels (up to 10 mg paraquat cation/kg bw/day) but mild foetotoxicity was evident at $\geq$4 mg/kg bw/day. A NOAEL from the rat developmental studies is 3 mg paraquat cation/kg bw/day ($\approx$ 4.1 mg paraquat dichloride/kg bw/day). (Hodge, 1978; Palmer, 1992).

Table 12: Summary of reproductive toxicity

Study type	NOAEL	Comments	Ref
2 gen repro study in mice, dietary 0, 45, 90 or 125 ppm paraquat cation	90 ppm paraquat cation*	No effects on reproduction. In the first generation only, at 125 ppm paraquat cation an increase in mortality was seen in the dams and young pups.	Dial & Dial, 1987
Mouse repro. Dosed in drinking water at 0, 50 or 100 ppm paraquat cation from day 8 of gestation till 42 days post partum.	50 ppm paraquat cation*	No effects on reproduction. An increase in postnatal mortality at 100 ppm paraquat cation. Lungs showed alveolar consolidation and collapse.	Bus & Gibson, 1975
Rat multigen. 0, 30 or 100 ppm paraquat cation	30 ppm paraquat cation ($\cong 3.0$ mg paraquat cation/kg bw/day $\cong 4.1$ mg paraquat dichloride/kg bw/day)	No effects on reproduction. The incidence of renal hydropic degeneration in 3-4 week old offspring was slightly increased in the 100 ppm paraquat cation group.	Fletcher et al, 1972
Rat multigen. 0, 25, 75 or 150 ppm paraquat cation ($\cong 0$, 2.5, 7.5 or 15 mg paraquat cation/kg bw/day)	25 ppm (2.5mg paraquat cation/kg bw/day $\cong 3.5$ mg paraquat dichloride/kg bw/day)	No effects on reproduction. Maternal toxicity at 7.5 and 15 mg paraquat cation/kg bw/day.	Lindsay et al, 1982
Rat multigen (3 gen)with teratology phase (0, 72, 145 or 290 ppm paraquat cation)	NOEL not achieved (<72 ppm paraquat cation)due to delayed ossification in F_{2B} litters in all groups.	Mortality at the top dose; lung damage apparent. No effects on reproduction. Delayed ossification in top dose in teratology phase.	Suzuki et al, 1983
Mouse teratology (20 mg/kg bw/day) or ip (1.67 or 3.35 mg/kg bw/day). (*Unclear from the paper whether paraquat dichloride or paraquat cation values are used*)	Not established ip is an inappropriate route	3.35 mg/kg bw/day ip resulted in death (5/7) and increased resorption rate. At 1.67 mg/kg bw/day ip and oral dose, a slight (not stat sig) increase in the number of foetuses with absent or non-ossified sternebrae. Concluded that potential of paraquat as a teratogen appeared to be minimal.	Bus et al, 1975
Mouse teratology. 0, 1, 5 or 10 mg paraquat cation/kg bw/day in 0.5% Tween 80	10 mg paraquat cation/kg bw/day ($\cong$ 13.8 mg paraquat dichloride/kg bw/day) for developmental and maternal toxicity	Not teratogenic. No effect on embryonic or foetal development.	Hodge et al, 1978
Mouse teratology 0, 7.5, 15 or 25 mg PQ ion/kg bw/day in	25 mg paraquat cation/kg bw/day ($\cong$ 34.5 mg paraquat	No overt teratogenicity. Maternal death at 25 mg/kg bw/day and toxicity at 15 and 25 mg/kg bw/	Palmer et al, 1992

deionised water	dichloride/kg bw/day)-teratogenicity 7.5 mg paraquat cation/kg bw/day ($\cong$ 10.4 mg paraquat dichloride/kg bw/day)-maternal and developmental toxicity	day. Minor abnormalities increased at 15 and 25 mg/kg bw/day generally within historical control range. Non dose related effects at 7.5 mg/kg bw/day of unknown aetiology.	
Rat teratology 0, 1, 5 or 10 mg/kg bw/day paraquat cation	10 mg paraquat cation/kg bw/day ($\cong$ 1.38 mg paraquat dichloride/ kg bw/day)-teratogenicity 5 mg paraquat cation/kg bw/day ($\cong$ 6.9 mg paraquat dichloride/kg bw/day)-maternal and developmental toxicity	No overt teratogenicity. Mild maternal toxicity at 10 mg/kg bw day..	Hodge et al, 1978
Rat teratology 0, 1, 3 or 8 mg paraquat cation/kg bw/day	8 mg paraquat cation/kg bw/day ($\cong$11.0 mg paraquat dichloride/kg bw/day) - teratogenicity 3 mg paraquat cation/kg bw/day ($\cong$ 4.1 mg paraquat dichloride/kg bw/day)-maternal and developmental toxicity	No teratogenicity. Mild maternal toxicity and foetotoxicity at 8 mg paraquat cation/kg bw/day.	Hodge et al, 1992
* insufficient data given to calculate paraquat dose in mg cation/kg bw/day			

(f) Special studies:

(i) Studies on the mechanism of paraquat induced proliferative lung changes

The toxic effects of paraquat were first described by Clark *et al.*, (1966) who reported that the histological effects of paraquat in rats, mice and dogs are similar. The lung, liver, kidney and thymus were affected; the lung being the major target. The effect of paraquat in the cynomologus monkey is similar to that in rats (Murray and Gibson, 1972). In contrast, rabbits do not develop lung lesions following acute oral or ip administration (Butler and Kleinerman, 1971; Mehani, 1972; Ilett *et al.*, 1974; Zavala and Rhodes, 1978). There is one report of daily administration of paraquat in the drinking water to rabbits over several days leading to lung damage that resembles that seen in rats (Restuccia *et al.*, 1974). Inhalation exposure to paraquat produces lung damage in the rabbit (Seidenfeld *et al.,* 1978). The hamster responds in a similar way being refractory to a single subcutaneous dose of paraquat, but lung fibrosis is produced by repeated sc injections (Butler, 1975).

The most extensive studies on the pathogenesis of lung damage produced by paraquat have been conducted in rats. The time course of development of the injury in rats

given a single MLD ip dose was reported by Vijeyaratnam and Corrin (1971) and Smith and Heath, (1974a). Damage to the type I and II alveolar epithelial cells was seen within a day of dosing. This damage was more marked by days 2-4 with large areas of the alveolar epithelium being completely lost; alveolar oedema developed and in some areas haemorrhage into the air spaces occurred. At this time there was extensive infiltration of inflammatory cells into the alveolar interstitium, air spaces and perivascular areas, although the alveolar endothelial capillaries were mainly spared. The animals died as a consequence of severe anoxia usually within the first few days after dosing and this has been confirmed by others (Clark *et al.*, 1966; Sharp *et al.*, 1972; Smith and Rose, 1977). This phase has been called the destructive phase (Smith and Heath, 1976). Similar early pathological changes have been reported by Kimbrough and Gaines, (1970); Brooks, (1971); Modee *et al.*, (1972); Wasan and McElligott, (1972); Smith *et al.*, (1974); Sykes *et al.*, (1977) and Smith and Heath, (1976). Some rats that survive for up to 10-12 days after dosing develop an extensive hypercellular lesion in the lung, which is dominated by proliferation of fibroblasts. This phase of the lesion is called the proliferative phase and is characterised by attempts by the epithelium to regenerate and restore normal architecture of the alveolar epithelium (Kimbrough and Gaines, 1970; Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974a). The findings in these animals are typically extensive intra-alveolar and inter-alveolar fibrosis, which in association with residual oedema reduces gaseous exchange, which results in death from anoxia. It appears that the initial damage to the alveolar epithelium, produced by paraquat, is the primary event in the development of the lung injury, with the proliferative fibrosis being a consequence of the extensive damage produced. For a more detailed review on pulmonary injury see the review by Smith and Heath, (1976).

Pathological findings upon autopsy in human fatalities from paraquat poisoning are similar to those seen in experimental animals, in particular the rat (for a detailed review see Smith and Heath, 1976). The lung is the organ showing the most severe changes in paraquat poisoning. Pulmonary pathology has been divided into two phases, which correspond with the early and late stages of the clinical signs and symptoms (Smith and Heath, 1975).

The destructive phase. This occurs during the first few days after paraquat poisoning and is rarely seen in human autopsy cases, but has been described in a case where an early biopsy was performed (Toner *et al.*, 1970). It is characterised by swelling of the alveolar epithelium, which sloughs off and is thought to be related to early development of pulmonary oedema with congestion and fibrin exudate (Smith and Heath, 1974a). Death due to this pulmonary pathology is rare.

The proliferative phase. This phase is usually seen in patients who survive for longer than 1 week. Pulmonary congestion with interstitial and alveolar oedema continues, sometimes associated with haemorrhage. There is lymphocytic and other inflammatory cell infiltration and occasional proliferation of cells lining the alveolar wall (Bullivant, 1966). The most specific feature is the presence of large quantities of fibroblastic tissue, which is perivascular and peribronchial early on, but later more diffuse (Smith and Heath, 1974b). The pulmonary fibrosis is sometimes associated with an early honeycomb appearance of the lung parenchyma, however, in contrast to a true honeycomb lung the cystic air spaces are dilated respiratory bronchioles and their walls consist of fibrosed, collapsed alveoli.

(ii) Effects on the nervous system and suggested link to Parkinson's disease

Effects on the nervous system
The neurotoxic potential of paraquat has been extensively studied in laboratory animals. No clinical signs of neurotoxicity or consistent neuropathological changes have been reported following long term exposures (1-2+ years) of dietary administration of paraquat to rodents or dogs in regulatory compliant studies (US EPA "RED" 1997). In acute dosing studies in the rat, using high parenteral doses of paraquat at or above the MLD (20-100 mg paraquat cation/kg bw, i.p.) produced signs of neurotoxicity with muscle fasciculation, some tremors and "wet-dog" shakes, typically within 30 min of dosing (Bagetta et al., 1992; Corasaniti et al. 1992; Hara et al., 1993), which is the time of peak blood and brain concentrations. These authors also reported neuronal cell necrosis in the pyriform cortex of these animals' 24h after dosing (Bagetta et al., 1992; Corasaniti et al., 1992). The neuronal cell necrosis could be reduced by administration of atropine but not methylatropine (Bagetta et al., 1992), suggesting some involvement of central muscarinic receptors. No effects were observed after 5 mg/kg i.p. paraquat. The basis for the selective injury to the pyriform cortex is currently not known, but it does not reflect the brain region with the highest concentration of paraquat (Corasaniti and Nistico, 1993; Naylor et al., 1995a and b). Others have reported that paraquat (20 mg/kg, s.c.) does not produce neuronal cell necrosis in the pyriform cortex of perfused-fixed material from rats 24 and 48h after dosing (Naylor et al., 1995b; Widdowson et al., 1996a), and have suggested the effect reported by the Italian group may be a fixation artefact. The precise basis for this variance is currently not understood. Similarly, daily oral dosing of paraquat at 5 mg/kg/day for 14 days to rats produced no evidence of neuronal cell necrosis, despite particular emphasis on the pathology of the pyriform cortex, nigro-striatal region and hypothalamus, or behavioural changes indicative of neurotoxicity (Widdowson et al., 1996b). The behavioural effects observed in rats appear to only occur at peri-lethal parenteral doses.

The lack of any consistent neurotoxicity observed in these long term and acute studies may reflect the fact that paraquat, being a di-cation, does not readily cross the mammalian blood-brain barrier and enter the brain after either oral or parenteral administration (Rose et al., 1976; Dey et al., 1990; Corasaniti et al., 1991; Corasaniti and Nistico, 1993; Naylor et al., 1995b; Widdowson et al., 1996a and b). The paraquat concentration associated with the rat brain is always lower than that in the plasma and decreases with time, with the initial concentration detected in the brain being largely associated with blood (Rose et al., 1976; Dey et al., 1990; Naylor et al., 1995b). Paraquat can however be detected in rat brain regions such as the olfactory bulb, area postrema and hypothalamus, which do not possess an effective blood-brain barrier, thus allowing paraquat direct access to these brain regions. Autoradiographic studies have detected paraquat in these regions and in the associated cerebrospinal fluid (ventricles and choroid plexus), but the concentrations were low and only represented a very small percentage of the administered dose, approximately 0.05% at the time of maximal blood concentration 1 hr after dosing (Naylor et al., 1995b; Waddell and Marlowe 1980). Immunohistochemical localisation of paraquat in rat brain has shown it is present in capillary walls and glial cells, but was not detected in neurones (Nagao et al., 1991). A very recent report by Shimizu *et al.*, (2001) however claims that

paraquat is able to traverse the rat blood-brain barrier via a valine sensitive neutral amino-acid transporter, although these findings have yet to be repeated by other researchers.

Direct administration of paraquat into the ventricles, or infusion into certain brain regions, produces signs of neurotoxicity in rats, which is associated with neuronal cell damage (De Gori et al., 1988; Calo et al., 1990; Bagetta et al., 1992, 1994; Corasaniti et al., 1992; Yoshimura et al., 1993; Liu et al., 1995; Liou et al., 1996). These effects were seen at low (2-20 µg) doses of injected paraquat. These observations lend support to the view that little paraquat enters the brain following parenteral administration (20 mg/kg, sc or 4,000 µg /200 g rat) or oral administration (126 mg/kg or 25,200 µg/200 g rat) as no neuronal cell toxicity was seen at these doses.

Possible link with Parkinson's disease
A possible association between paraquat exposure and the development of Parkinson's disease has been the subject of much speculation. The reason for this, is the apparent (and superficial) structural similarity between paraquat and 1-methyl-4-phenylpyridinium ion (MPP$^+$), the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which can induce a Parkinson-like syndrome in monkeys, humans and a particular strain ($C_{57}Bl_6$) of mice (Langston et al., 1983; Lewin, 1984). Administration of MPTP to susceptible animal species produces selective damage to dopaminergic neurons in the substantia nigra, leading to a marked loss of dopamine and clear signs of neurotoxicity. The mechanism for MPTP toxicity (see Markey et al., 1986; Tipton and Singer, 1993) involves its ability to cross the blood-brain barrier and enter glial cells where it can undergo oxidative metabolism by the enzyme monoamine oxidase B to form MPP$^+$. This metabolite then accumulates selectively into dopaminergic neurons via the dopamine transport system, leading to inhibition of mitochondrial respiration, which ultimately leads to the demise of the neurone. Structure-activity relationships suggest that, despite their apparent similarity, paraquat and MPTP are two very different chemicals (Koller, 1986). MPTP is uncharged and lipophilic, and is thereby able to cross the blood-brain barrier, whereas paraquat is charged and hydrophilic, and consequently does not readily enter the brain. In addition, MPTP is a monoamine whose toxic metabolite, MPP$^+$, is able to use a specific monoamine uptake system to gain access to neurones, particularly in the substantia nigra, whereas paraquat is a diamine. It is also very relevant that administration of MPP$^+$ to experimental animals did not produce neurotoxicity, due to its poor entry across the blood-brain barrier (Tipton and Singer, 1993). Thus, like paraquat, MPP$^+$ does not readily enter the brain. Consistent with this, systemic administration of paraquat to $C_{57}Bl_6$ mice or rats did not lead to dopamine depletion or neuronal cell death in the striatum, like that seen with MPTP (Perry et al., 1986; Widdowson et al., 1996b).

Despite these observations, other researchers have reported changes in brain dopamine content following paraquat administration to mice (Endo et al., 1988; Fredriksson et al., 1993). In the latter case, paraquat (0.07 or 0.36 mg/kg) was administered orally to pups on days 10 and 11 after birth at a time when the brain is undergoing rapid growth, and hence might be a more vulnerable to chemical insult. The authors reported a small loss of dopamine and its metabolites, and a decrease in behavioural activity when measured at 120 days of age (Fredriksson et al., 1993). These data are in contrast to the findings of Perry et al., (1986) where a total paraquat

dose of 44 mg/kg s.c. to the same mouse strain, produced no significant striatal dopamine depletion. Attempts to reproduce the findings of Fredriksson et al., (1993) in $C_{57}Bl_6$ mice, in another laboratory, proved inconclusive (Dr. David Ray, University of Nottingham, UK, personal communication), however, a very recent publication by Ding et al., (2001) claims to have observed similar findings to Fredriksson et al using a similar dosing regimen.

A report by Brooks et al., (1999), where paraquat was administered by intraperitoneal injection (i.p.) to $C_{57}Bl_6$ mice at a weekly dose of 5 or 10 mg/kg over 3 weeks, initially suggested that paraquat may produce a reduction in the number of neurones in the nigro-striatal brain region, and a reduction in ambulatory activity in this mouse strain. However, the experimental protocol used by these authors involving the intra-cerebral injection (prior to paraquat dosing) of the neuronal marker, fluorogold, to permit investigation of the dopaminergic neurones in the substantia nigra, may have compromised the blood-brain barrier and allowed paraquat direct access to the brain. Furthermore, the high dose of paraquat (approximately 25-50% of the single dose LD_{50}) used by the authors, suggests that their findings may not have any relevance to normal exposure scenarios in the human population. Subsequent reports from the same group of researchers using similar doses of paraquat administered to the same animal model, have failed to reproduce the same extent of the changes reported in this initial publication (Thiruchelvam et al., 2000a & b).

Thiruchelvam et al., (2000a) investigated the interaction of the two compounds paraquat and the fungicide, maneb (manganese ethylenebisdithiocarbamate), on the nigro-striatal dopamine system in the $C_{57}Bl_6$ mouse model. In this study mice were dosed with saline, paraquat (5-10 mg/kg) and / or maneb (15-30 mg/kg) by i.p. injection, each week for 4 weeks. End point markers of toxicity used were locomotor activity, striatal dopamine (and metabolites) levels and tyrosine hydroxylase immunoreactivity in the striatum. The results showed that paraquat and maneb in combination produced more marked effects than either in isolation. In fact, paraquat (5 or 10 mg/kg) alone produced no significant change in the endpoints measured when compared to control. This is in contrast to the data presented by the same group in the Brooks et al., (1999) report. Though some of the synergistic effects of paraquat and maneb combined were significant, they were small in magnitude, with reductions in tyrosine hydroxylase immunoreactivity in the striatum being reduced by only 10%. In a similar study involving twice weekly i.p. administration of paraquat (10 mg/kg) and / or maneb (30 mg/kg) for 6 weeks (Thiruchelvam et al., 2000b), these same researchers demonstrated a similar increased effect following combined administration of paraquat and maneb involving the additional endpoint marker of cell counts in the substantia nigra and ventral tegmental area. Interestingly paraquat and maneb administration individually produced no significant reduction in cell counts, but the combined administration produced a 20% reduction of cell counts in the substantia nigra with no effect in the ventral tegmental area. The toxicological significance to man of these findings is unclear, since the route of exposure used in these studies is not relevant to human exposure scenarios, and when considered in the context of occupational risk, the scenario of a farm operator being exposed to such very high doses of either chemical does not arise. Furthermore, since these chemicals are not used together in combination in the field, simultaneous exposure to these high doses is inconceivable.

Epidemiological studies

A number of epidemiological studies have been performed that have investigated the factors associated with an increased risk of Parkinson's disease. The first epidemiological work to draw attention to a possible role of pesticides in Parkinson's disease was published by Barbeau et al., (1986) who showed that the regional incidence of the illness in Canada was non-uniform and correlated with a genetically determined enzyme deficiency. While there was certainly a strong correlation between disease incidence and pesticide use, such a correlation was also found for industrial areas and wood processing regions. Since then a number of case-control studies have been published, with varying methodologies and conflicting results. Some studies suggested that the use of herbicides was significantly associated with the development of Parkinson's disease (Ho et al., 1989, Golbe et al., 1990, Semchuk et al., 1991); Others have found no such association (Ohlson and Hogstedt, 1981, Tanner et al., 1989, Koller et al., 1990, Zayed et al., 1990, Tanner et al., 1990). In the case of paraquat; two studies have linked paraquat exposure to Parkinson's disease (Hertzman et al., 1990, Liou et al., 1997), while a further 6 studies have not (Rajput and Ryan 1987; Zilket et al 1988; Bella et al 1991; Vanacore 1991; Hertzman et al 1994 and Kelly et al 1995). The difficulty in interpreting all such studies lies in accurately assessing the exposure of individuals and in the assessment of confounding factors in multiple exposure situations. In the paraquat epidemiology studies sited above non have quantified exposure. Indeed, the reliability of self and proxy derived data for exposure in Parkinson's disease patients has been questioned by Semchuk & Love (1995) who found a considerable degree of misclassification of exposure for some variables. Overall, the epidemiology studies do not show a consistent link between paraquat and Parkinson's disease.

Given that the age corrected incidence of Parkinson's disease has been consistent over time in a given population since the widespread introduction of modern agrochemicals in the 1950's (Ben-Shlomo, 1997) it would seem unlikely that exposure to these chemicals are the cause of Parkinson's disease. Additional evidence against paraquat as a causative factor in Parkinson's disease, however, comes from the many published case reports of paraquat poisoning. There is no evidence of a specific effect of paraquat on the nervous system, nor have neurological sequelae been noticed in survivors of paraquat poisoning (Vieregge et al., 1988). Zilker et al., (1988) carried out detailed neurological follow-up examinations in 4 survivors of paraquat poisoning (latency period between ingestion and follow-up 5 - 10 years) and 3 patients who had had skin contact with paraquat. It was possible to exclude Parkinsonism in all patients. Furthermore, in health surveys conducted in areas of high usage and long-term exposures to paraquat such as Sri Lanka, Malaysia and the Philippines, clinical observations have revealed no neurological deficits in humans, let alone Parkinson's disease (reviewed in Lock & Wilks, 2001).

In conclusion, although the findings from the animal model systems are interesting and warrant further study to determine their human toxicological significance, there is little consistent evidence to date linking paraquat to Parkinson's disease.

3. Observations in humans

a) Case reports - Accidental and Intentional Poisoning

The first cases of fatalities described involved accidental ingestion of the 20% paraquat concentrate (Bullivant, 1966, Swan, 1967, Oreopoulos et al., 1968, Campbell, 1968). A major source of poisoning was the decanting into unlabelled drinks bottles and other containers (Malone et al., 1971). Throughout the 1970's the number of reported cases continued to rise, however, there was a noticeable shift in the circumstances. For example, in the Republic of Ireland the number of accidents due to decanting decreased between 1967 and 1977 from 45% to 4% of total cases (Fitzgerald et al., 1978b). Further analysis of the circumstances of poisoning showed that before 1975 there was an approximately equal proportion of accidental and suicidal cases, whereas after that date suicides accounted for over 90% of cases and all fatalities. A similar pattern was described in Northern Ireland (Carson and Carson, 1976) and the United Kingdom (Howard, 1979a, Bramley and Hart, 1983). A review of deaths from pesticide poisoning in the United Kingdom between 1945 and 1989 showed that the number of paraquat-associated deaths rose continuously from 1973 onwards and peaked in 1981. Since then, the number has steadily declined to pre-1973 levels (Casey and Vale, 1994).

With the increasing use of paraquat throughout the world during the 1970's and 1980's it became apparent that the problem of accidental and intentional poisoning had shifted away from the British Isles and Europe (Onyon and Volans, 1987). A high incidence was reported in particular from Asian countries such as Japan (Naito and Yamashita, 1987), Malaysia (Amarasingham and Lee, unpublished report), Sri Lanka (Hettiarachi and Kodithuwakku, 1989) and Fiji (Goundar, 1984). Paraquat was also the most widely used chemical suicidal agent in Trinidad (Hutchinson et al., 1991) and Surinam (Perriens et al., 1989).

In Costa Rica, Wesseling et al., (1993) examined records of the Forensic Medical Department which showed that over the seven year period from 1980 to 1986 a total of 169 fatalities had occurred from paraquat poisoning. The pathologists had classified the overwhelming majority as suicide-related, although the authors suggested that misclassification occurred in some cases. However, a detailed examination of case records of the Forensic Medical Department between 1990 and 1992 showed that 74 out of 76 paraquat related fatalities were due to suicide from oral ingestion, with 2 fatalities occurring from accidental ingestion (Vargas and Sabapathy, 1995). Government statistics for 1995 and 1996 showed that 62 out of a total of 72 pesticide related fatalities (no compound mentioned) were due to suicide and 2 due to homicide, 8 fatalities were classified as non-occupational, and there were no occupationally related fatalities (Ministerio de Salud, 1997).

Paraquat poisoning is uncommon in the USA, the world's largest market for paraquat-containing products. A ten year survey of calls to US poison centres showed that paraquat (and diquat)-related enquiries accounted for only around 0.01% of the total (Hall, 1995). Most cases showed either no or minor symptoms, with less than 2 fatalities occurring annually, almost all of them related to suicides.

Data on mortality from paraquat poisoning are difficult to compare because of differences in circumstances, treatment and reporting systems. In a collection of data from 14 publications compiled by the International Programme on Chemical Safety (IPCS, 1984), mortality ranged from 36% to 100%, with an overall mortality of 48% (446 of 925 cases). A difference in mortality between ingestion of the liquid

concentrate (20% paraquat ion) and a granular product (2.5% paraquat, 2.5% diquat) has been described by some authors. Park et al., (1975) found that the fatality rate was 15 of 23 (65%) in patients who had ingested liquid concentrate and 3 of 8 (38%) in patients ingesting the granular product. Fitzgerald and Barniville (1978) reported no deaths in 14 patients ingesting the granular product compared to a mortality of 74% in 118 cases of ingestion of the liquid concentrate. In the series published by Howard (1979a) there were 36 deaths from 41 cases (88%) where liquid concentrate was ingested, and 5 deaths from 27 cases (19%) involving the granular product. These differences are largely a reflection of the size of dose ingested.

While suicidal ingestion of paraquat concentrate accounts for most of the recorded fatalities, the problem of accidental ingestion prompted the principle manufacturer of paraquat to introduce formulation changes to the liquid concentrate in the late 1970's and early 1980's (Sabapathy, 1995). A blue colour was added to prevent confusion with drinks, a stenching agent was introduced to alert users, and an emetic was included. In addition, packaging and labelling was improved to deter decanting of the product, and education and training efforts were directed in particular towards smallholder farmers in developing countries, where the majority of incidents occurred. The effect of these efforts is believed to have made a significant contribution to the decrease of accidental paraquat ingestion in many countries (Sabapathy, 1995, Wesseling et al., 1997).

Although ingestion is the route of entry into the body for the overwhelming majority of poisoning cases, there are a few reports of systemic effects from inhalation and dermal exposure (localised skin, eye and upper respiratory effects will be discussed under 'Use Experience'). Inhalation exposure is not a prominent feature in paraquat poisoning cases because of the extremely low ($<10^{-8}$ mm Hg) vapor pressure of paraquat. Respiratory exposure to paraquat during spray applications is very low because the large droplet size prevents the material from going beyond the nasal cavity. Concerns about oral exposure to spray droplets as a result of drainage into the oral cavity and swallowing appear unwarranted because the typical spray concentration of paraquat for hand-held spray applications is 0.1 - 0.2%; and would thus require a dose of 1 - 2 litres of spray solution directly into the nose and into the oral cavity to achieve an lethal dose (Howard, 1980). It is therefore not surprising that there are no reports in the published literature of deaths arising from inhalation exposure. A review of 30 cases of presumed inhalation exposure found no evidence for systemic poisoning (Vlachos and Kontoes, 1987). Where paraquat was measured it was undetectable or at the limit of detection. Patients were either asymptomatic or had non-specific symptoms such as headache, nausea or feeling unwell. Two patients described nose bleeds. In two patients who presented with cough and fever, pneumonia was established as clinical diagnosis. A recent review (Garnier, 1995) concluded that there was only one convincing reported case of possible systemic poisoning following inhalational exposure to paraquat and signs of toxicity were very mild and the patient made a full recovery (Fitzgerald et al., 1978a). In this case, a 43 year old market gardener sprayed a 'stronger than usual' solution (no details of spray concentration available) in a greenhouse and complained of a burning sensation in throat and mouth and weakness. There was biochemical evidence of mild renal failure, but liver function tests and chest x-ray were normal. Paraquat tested positive in urine. Renal function parameters returned to normal within 10 days after exposure.

Furthermore, the US EPA concluded that inhalation was not a relevant route of exposure for human risk (US EPA RED, 1997).

It has already been mentioned that paraquat absorption across intact human skin is extremely low both *in vitro* (Walker et al., 1983) and *in vivo* (Wester et al., 1984). In fifteen cases of single exposures of the skin and eyes during work with paraquat solutions only localised lesions (dermatitis, vesicles, burns, conjunctivitis) were found (Hoffer and Taitelman, 1989). Paraquat was undetectable in plasma except for three cases where it was at the limit of detection. There were no manifestations of systemic toxicity. A small number of case reports describe systemic paraquat poisoning and fatalities from dermal exposure. In six cases there was deliberate or accidental application of paraquat concentrate to the skin, usually in the unfortunate mistaken belief that it could act against parasitic disease (Ongom et al.,1974, Binns, 1976, Wohlfahrt, 1982 (2 cases), Tungsanga, et al.,1983, Garnier, et al., 1994). Three cases (Waight, 1979, Okonek, et al.,1983, Wesseling, et al.,1997) involved widespread accidental contamination of the lower abdomen and legs with the 20% concentrate.

In two cases (Jaros et al., 1978, Levin et al., 1979) it was evident that a far too concentrated paraquat dilution (28 g/l; 2.8% and 40 g/l; 4%, respectively) was applied combined with faulty leaking spray equipment and lack of skin decontamination. In a further case (Athanaselis et al., 1983) it is explicitly claimed that a correct dilution of 0.5% paraquat was used (the maximum recommended rate for knapsack). However, subsequent investigation (Hart, 1984) led to the conclusion that, in fact, a more concentrated paraquat solution, probably in excess of 1.5%, was used.

In one case (Fitzgerald, et al.,1978a) the combination of paraquat exposure and pre-existing skin disease caused the death of the person involved, although very few details are given. Another case (Garnier, et al.,1994) involved the application of multiple herbicidal mixtures, including paraquat, over several days by a man with a history of psoriasis. This man suffered a febrile lung disease but made a complete recovery.

Four cases involve prolonged skin contact with 'diluted' paraquat without pre-existing skin lesions being mentioned. The two cases described by Wohlfahrt (1982) give very few data, which would be useful in this context. In the third case (Papiris et al., 1995), a farmer was exposed for 5 - 6 hours to diluted paraquat from a leaking sprayer, which caused burning, blisters and erosions in his scrotal area. This patient survived after hospital treatment. In the fourth case (Wesseling et al., 1997), a plantation worker experienced chemical burns on his back, scrotum and inner parts of both thighs after spraying paraquat with a leaking knapsack sprayer for three consecutive days. He subsequently died from interstitial fibrosis of the lung.

Thus, there is no indication that paraquat has caused fatal poisoning through skin contact in normal occupational use. The few cases described in the literature occurred as a result of a combination of factors such as misuse (wrong dilution), pre-existing extensive skin disease, faulty equipment, prolonged extensive skin contact and disregard of safety procedures (no decontamination following significant exposure).

b) Experimental Exposure   The percutaneous absorption of radio labelled paraquat has been determined in humans (Wester et al., 1984). Following application of 9

$\mu g/cm^2$ the amount absorbed was 0.29% for the leg, 0.23% for the hand, and 0.29% for the forearm. This gave a calculated *in vivo* absorption rate of 0.03 $\mu g/cm^2$ for the 24 hr exposure period. Paraquat was thus only minimally absorbed, especially in comparison with other commonly available pesticides (Wester and Maibach, 1985).

c) Case reports - Use Experience
Exposure to paraquat under actual field conditions has been assessed in studies with hand held (knapsack), vehicle mounted and aerial applications. Dermal exposure was measured either in patches placed on different body regions or, more recently, using whole body exposure assessments. Inhalation exposure (including oral exposure) was determined using personal air sampling and the air concentration of different particle sizes was measured. Internal dose was assessed using biological monitoring, for which paraquat is an ideal candidate: it is not metabolised, rapidly and completely excreted via the kidneys, stable in urine, and there are sensitive analytical techniques available.

Table 13: Summary of worker exposure and absorption of paraquat

Reference	Country	Application Method	Spray Dilution (%w/v)	Dermal Exposure (mg/hr)	Inhalation Exposure (mg/hr)	Urine level (mg/l)
Swan, 1969	Malaysia	Hand held	0.05	-	-	<0.01-0.32
Hogarty, 1976	Ireland	Hand held	-	-	<0.003	ND
Staiff et al.,1975	USA	Vehicle mounted	0.1	0.01- 3.4[a]	0-0.002	<0.02
		Hand held	0.2	0.01-0.57[a]	<0.001	<0.02
Chester & Woollen, 1981	Malaysia	Hand held	0.1-0.2	<0.01-12[a] 12-170[b]	0-0.005	<0.05-0.76
Wojeck et al., 1983	USA	Vehicle mounted	0.05-0.1	7.0-42[a] 12-169[b]	0-0.07	<0.02-0.03
Chester & Ward,1984	USA	Aerial	0.3	0.1-2.4[b+] 0.05-0.26[b#]	0-0.047[+] 0-0.06[#]	-
Chester et al., 1993	Sri Lanka	Hand held	0.03-0.04	0.94-2.71[b$]	-	<0.03
Van Wendel de Joode et al., 1996	Costa Rica	Hand held	0.1-0.2	0.2-5.7[a]	0-0.043	<0.03-0.24
Singmaster & Liu,1998	Puerto Rico	Hand held	0.1	-	<0.007	-

ND   Not detected

a	Exposure to uncovered skin
b	Total Dermal exposure
+	Aerial - flagger
#	Aerial - pilot
$	mg/g paraquat sprayed
&	Extrapolation from indirect measurement using copper as marker

There is an enormous variation in dermal exposure evident in the studies found in the literature. This is not surprising given the differences in spray strength, volume applied, application technique, environmental conditions, use of personal protective equipment, and differences in study design. Nevertheless, some patterns emerge across the variety of study conditions encountered. It is evident that skin exposure represents by far the most significant route of exposure for paraquat both for hand held applications (Chester and Woollen, 1981, Van Wendel de Joode et al., 1996), and for vehicle-mounted spray applications (Staiff et al., 1975, Wojeck et al., 1983). The lowest dermal exposure was seen for pilots applying paraquat (Chester and Ward, 1984), whereas the total dermal exposure of flaggers is comparable to exposure of uncovered body parts in other spray applications.

Inhalation exposure was approximately three orders of magnitude lower than skin exposure (Staiff et al., 1975, Chester and Woollen, 1981, Wojeck et al., 1983, Chester and Ward, 1984, Van Wendel de Joode et al., 1996, Singmaster and Liu, 1998). Paraquat proved to be below the limit of detection in most samples. Furthermore, the inhalation potential of respirable droplets was found to be negligible since no respirable paraquat could be measured in the breathing zone of exposed workers (Chester and Ward, 1984). The most recent study (Singmaster and Liu, 1998) showed that even under difficult spraying conditions (heavy exertion while spraying on hillsides) paraquat was below the limit of detection.

Paraquat is an ideal candidate for biological monitoring because it is excreted unchanged in urine, where it is comparatively stable. Most of the worker exposure studies mentioned above included measurement of paraquat in urine. Overall, the paraquat concentration in urine was low with the majority of samples being below the limit of detection. None of the samples contained paraquat at levels, which would be indicative of a risk of poisoning (see below).

Topical effects from contact with paraquat during spray operations can occur due to a delayed caustic action of paraquat as a result of poor working practice and hygiene (Howard, 1980). Discoloration (white bands), paronychia and partial or complete loss of nails has been described following contact with concentrated (Samman and Johnston, 1969) and prolonged exposure to diluted paraquat solutions (Hearn and Keir, 1971). Upon cessation of exposure, normal nail growth resumes. Irritant dermatitis, burns and blistering can occur from skin exposure to paraquat concentrate or as a result of prolonged skin contact with contaminated clothing or from leaking spray equipment (Swan, 1969, Van Wendel de Joode, et al., 1996). Nose bleeds have been described (Swan, 1969, Van Wendel de Joode, et al., 1996), most likely from breathing in spray mist or contact with contaminated fingers. No serious or long-term effects have been described. There are a number of case reports of eye damage resulting from splashes with paraquat concentrate (Cant and Lewis, 1968, Joyce, 1969, Peyresblanque, 1969, Watanabe et al., 1979, Deveckova and Mydlik, 1980). Apart from eye irritation and blepharitis, more serious, delayed ocular damage may

occur such as destruction of the bulbar and tarsal conjunctiva and erosion of the corneal epithelium. Anterior uveitis has also been noted. Progressive keratitis and decreased visual acuity may occur and persist for several weeks; however, complete restoration of vision is the norm.

Attempts have been made to establish the frequency of topical effects from paraquat exposure, particularly for hand held applications in developing countries. Surveys have been carried out interviewing 400 smallholder farmers using paraquat in Malaysia (Whitaker, 1989a), 365 smallholders in Central America (Whitaker, 1989b) and 732 smallholders in Thailand (Whitaker et al., 1993). These surveys showed that, in general, farmers were aware of the potentially fatal consequences of swallowing small quantities of the concentrate. Spray practices and standards of personal hygiene were generally adequate, although the wider use of gloves and eye protection when handling the concentrate needed to be encouraged. In all three surveys, approximately 10% of respondents had experienced health effects attributed to the use of paraquat. These were predominantly skin irritation (mainly on hands and feet), nausea and headaches associated with the smell of the product (due to the added stenching agent) and, to a lesser extent, eye irritation, nail damage and epistaxis. Ramasamy and Nursiah (1988) interviewed 1219 Malaysian estate workers, rice farmers, vegetable growers and smallholders about health effects from pesticide use. They found that exposure to organophosphorous insecticides was associated with giddiness and nausea, whereas the main effects associated with paraquat exposure were eye irritation, nail damage and nasal bleeding. However, their survey did not establish cause effect relationships with exposure to specific products. Only three cases of hospitalisation were described among their study population.

The State of California has probably the most comprehensive surveillance system of pesticide-related illness in the world. Between 1971 and 1985 a total of 231 cases of ill health attributed to paraquat were notified to the Worker Health and Safety Branch, California Department of Food and Agriculture (Weinbaum et al., 1995). Of these, 38.5% were listed as systemic effects (mainly dizziness, nausea, lightheadedness, headache, chest pain, vomiting and tiredness), 32% were eye effects (burning, itching, redness), 26% were skin effects (rash and irritation, itching) and 3.5% were local respiratory irritant effects (epistaxis, sore throat). There were no cases of pulmonary fibrosis. Analysis of data from 1981 to 1985 showed that the overall incidence of illness was low at 0.6 per 1000 paraquat applications.

Detailed medical surveys have been carried out to determine whether the long term exposure to paraquat leads to chronic health effects in workers and spray applicators. Swan (1969) found no abnormalities in chest radiographs of groups of Malaysian rubber plantation workers during paraquat applications over several weeks. Howard (1979b) studied two groups of paraquat formulation workers in the United Kingdom and Malaysia. Mean exposure duration for the UK workers was 5 years, and 2.3 years for the Malaysian workers. A history of skin rashes was found in half of the Malaysian workers, but not in the UK workers where the most common finding was epistaxis and nail damage. Eye irritation was more common in the Malaysian than in the UK workers. There was no evidence of any long-term or permanent skin or eye damage.

The most comprehensive medical surveys in paraquat-exposed spray operators were carried out in Malaysia (Howard et al., 1981) and Sri Lanka (Senanayake et al., 1993). In both studies there were detailed clinical examinations, lung function measurements (including CO diffusion capacity), haematological and biochemical investigations, and, in the Sri Lankan study, a chest radiograph was taken. In the Malaysian survey, 27 paraquat spraymen (mean spraying time 5.3 years; mean individual annual quantity of paraquat handled 67.2 kg as paraquat ion) were compared with two control groups comprising 24 general plantation workers and 23 latex factory workers, respectively. In the Sri Lankan survey, 85 paraquat spraymen (mean spraying time 12 years) were compared with two groups of 76 factory workers and 79 general workers, respectively. In both studies there were no clinically significant differences in any of the parameters studies, in particular, the results of the lung function tests showed similar results for exposed and control groups. It was concluded that the long term spraying of paraquat was not associated with any measurable adverse health effects.

A recently published study was carried out in Nicaragua (Castro-Gutierrez et al., 1997), although the investigation dates back to 1987/88. A population of 134 spray workers with at least 2 years spraying experience with paraquat from 15 banana plantations was interviewed, 63 out of which had not experienced skin irritation, and 71 who had a history of skin rash or burn (used as a surrogate measure of intensity of exposure). A questionnaire was used to check for symptoms of respiratory illness and Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV_1) were measured. The results were compared with a control population of 152 unexposed workers. There was a difference in male:female ratio between the exposed and unexposed groups (100:34 and 88:64, respectively). Paraquat-exposed workers gave a significantly more frequent history of Grade 3 dyspnea, but not Grade 1 or 2 dyspnea. There was no difference in the occurrence of chronic bronchitis, and episodic dyspnea with wheezing was more frequent in the group with topical effects only. However, there were no differences between exposed and control workers with regard to restrictive (FVC <80% of predicted value) or obstructive (FEV1:FVC <70% of predicted value) spirometry parameters. In fact, the lowest incidence of restrictive changes was found in the 'intensive exposure' group.

d) Case reports - Atypical Cases of Various Origins
In a case described by Newhouse et al., (1978), a farmer's wife had been spraying paraquat in an orchard for many days. This case is unique in that her complaints started with scratches on arms and legs, which proved non-healing over four weeks. She was then hospitalised for two weeks and discharged without diagnosis. Two and a half weeks later she was readmitted to the hospital because of increased dyspnoea and wheeziness. She was diagnosed as suffering from systemic arteritis and died 12 days after final admission, some 8 weeks after initial exposure. Although the paper links her disease to paraquat exposure, it is doubtful if paraquat was the cause. Firstly, at no time was paraquat measured in blood or urine. Secondly, the time from exposure to her death was more than eight weeks, which is highly unusual for paraquat poisoning. Thirdly, she had a clinical diagnosis (systemic arteritis), which did not include any reference to paraquat poisoning.

George and Hedworth-Whitty (1980) attributed a case of non-fatal lung disease to the inhalation of nebulised paraquat. A 64-year-old woman noticed spray mist drifting into her garden from a spraying operation in an adjacent field. After some 10 minutes

she noticed a chest tightening, and over the next week she became gradually more breathless. She was initially treated with a short course of steroids without much effect. Pulmonary function evaluation some two months later showed severe restriction, but there were no abnormalities in the chest radiograph. She was kept on systemic steroids and her lung function had markedly improved some 7 months after the original incident. Hart (1980) commented that the diagnosis of paraquat-induced lung injury was doubtful. The woman had a history of allergic rhinitis and chronic sinusitis. No previous lung function recording was available and no transfer factor was measured at the time of assessment. The chest radiograph was clear and the description of exposure did not provide convincing arguments for a significant inhalation exposure.

In the case described by Katopodis et al (1993), a 31 year old woman was admitted 4 days after ingestion of 2 g paraquat. The urine test for paraquat was still positive, but her plasma concentration was only 10 µg/l. Charcoal haemoperfusion was carried out over the next 5 days, paraquat levels became undetectable in plasma on day 6 and in urine on day 8. The patient survived without evidence of pulmonary involvement. The authors attributed the favourable outcome to the haemoperfusion therapy even at such a late stage after ingestion. However, the low paraquat plasma concentration at the time of admission would have suggested a good chance of survival anyway (see below and Table 5).

Ragoucy-Sengler and Pileire (1996b) reported a case of paraquat poisoning in an HIV positive patient. Indices of severity of the poisoning suggested a survival probability of 30% on admission, and 3% after 72 hours. The clinical course included acute renal failure and severe hypoxia, however pulmonary fibrosis did not develop. The patient was discharged with normal pulmonary function 18 days after admission. The authors suggested that the immune deficiency on the basis of the patient's HIV infection may have prevented the development of pulmonary fibrosis.

In a case described by Ernouf et al (1998) a 47-year-old man, while under the influence of alcohol, ingested paraquat, which had been decanted into an unmarked a red wine bottle. The patient was a chronic alcoholic. He was admitted to hospital within 3 hours and treated with gastric elimination and antioxidant therapy. Evolution of plasma paraquat concentrations pointed towards a prognosis of delayed death from pulmonary fibrosis, however, the patient died on the fourth day after admission from persistent haemodynamic shock and hypoxaemia. The authors speculated that con-ingestion of ethanol may have enhanced the toxicity of paraquat through increased absorption from the gastrointestinal tract and/or decreased renal clearance. However, it has also been suggested that alcoholism may have a protective effect against paraquat toxicity on the basis of increased synthesis of superoxide dismutase (Ragoucy-Sengler et al 1991).

Methaemoglobinaemia was described in a patient who ingested 'Gramonol', a formulation containing 100g/l paraquat and 140g/l monolinuron (Ng et al 1982). The authors speculated that the superoxide anion and hydrogen peroxide generated by paraquat could oxidise haemoglobin to methaemoglobin. However, in response, Proudfoot (1982) pointed out that monolinuron, along with other substituted urea herbicides, is metabolised to aniline derivatives, which are well-known methaemoglinaemia and haemolysis causing agents. Furthermore, administration of

monolinuron alone had produced methaemoglobinaemia in experimental animals. Instead of a new feature of paraquat poisoning, it appeared therefore that Ng et al. had reported the first human case of monolinuron toxicity. Since then, a further case of paraquat-monolinuron poisoning has been described (Casey et al., 1994) in which the severe methaemoglobinaemia (52%) was successfully treated with methylene blue. However, the patient died after 10 days from the consequences of paraquat poisoning.

In 1975 the Government of Mexico began an aerial spraying programme, financed by the United States, to destroy marijuana fields with paraquat. In 1978 analyses showed that 21% of 61 marijuana samples confiscated in California, Arizona and Texas contained paraquat residues between 3 and >2000 ppm (Turner et al., 1978). Further work demonstrated that, nationally, 0.63% of over 100,000 kg marijuana seized contained detectable paraquat levels with a median of 52 ppm (Liddle et al., 1980). Over 70% of the contaminated samples were found in the South West region of the United States, originating almost exclusively from Mexico. Combustion testing suggested that around 0.2% of the paraquat residue would pass unchanged into marijuana smoke (Brine et al., 1981). On the basis of a worst-case epidemiological risk assessment it was suggested that some marijuana smokers in the South West region might have been at risk of health effects from paraquat inhalation (Landrigan et al., 1983). However, no clinical cases were identified during these studies.

e) Case reports - Clinical Findings and Dosage Response
Information on the clinical course of paraquat poisoning is mainly based on case reports of patients who swallowed paraquat concentrate with suicidal intent. However, the systemic toxic effects are similar regardless of the route of absorption. Paraquat causes nausea which may be prolonged especially following ingestion of emeticised formulations (Meredith and Vale, 1987), as well as vomiting and diarrhoea as a result of its local irritant effect on the gastrointestinal tract. Patients may develop a burning sensation, soreness and pain in the mouth, throat, chest and abdomen (Vale et al., 1987). Ulceration in the mouth and throat, an inability to swallow saliva, dysphagia and aphonia are common. The presence of buccopharyngeal lesions has no prognostic value (Bismuth et al., 1995), in contrast to oesophageal and, in particular, gastric ulcerations which indicate a poor prognosis (Bismuth et al., 1982). Prominent pharyngeal membranes ('pseudodiphtheria') have been reported (Stephens et al., 1981) and perforation of the oesophagus may result in mediastinitis, surgical emphysema and pneumothorax (Ackrill et al., 1978).

The further clinical course is dependent on the amount of paraquat absorbed into the body (usually following ingestion). Attempts have been made to quantify the toxic dose from estimates based on the information given by patients. Although such estimates are often unreliable, a consensus has emerged which is based on experience with many patients. This has allowed the identification of three degrees of intoxication, which are summarised below (for further details see Vale et al., 1987, and Bismuth et al., 1995).

Mild or subacute poisoning. The smallest fatal dose has been quoted as 16.7 mg paraquat cation/kg/bw $\cong$ 23.0 mg paraquat dichloride/kg/bw (Stevens and Sumner, 1991), however, the original reference (FAO/WHO, 1973) makes clear that this value is erroneously low, since the formulation ('Weedol') also contained an equal amount of diquat, so that the total bipyridyl ingestion was approximately 35 mg/kg. This is in

line with clinical experience, which shows that ingestion of less than 20-30 mg paraquat cation/kg has rarely serious consequences. Patients are either asymptomatic or develop nausea and vomiting. Renal and hepatic lesions are minimal or absent. An initial decrease of the diffusing capacity may be apparent in lung function measurements, but full recovery is the norm.

Moderate to severe acute poisoning. This occurs following ingestion of more than 20-30 mg paraquat cation ($\cong$27.6-41.4 mg paraquat dichloride/kg bw), but less than 40 to 50 mg paraquat cation/kg bw ($\cong$ 55-69 mg paraquat dichloride/kg bw). Apart from the localised lesions described above, patients in this group develop renal failure, usually between the second and fifth day after ingestion. Hepatocellular necrosis may occur. Both these lesions are fully reversible. Delayed development of pulmonary fibrosis is responsible for the generally poor prognosis in this group. Clinically and radiologically this appears around seven days after ingestion, but subtle abnormalities are present much earlier, such as a decreased diffusing capacity. The x-ray often shows patchy infiltration, which may progress to opacification in one or both lungs. In thin section computerised tomography, the most common pattern on initial scans is ground-glass attenuation, followed by consolidation with bronchiectasis (Lee et al., 1995). In most cases, pulmonary fibrosis leads to development of refractory hypoxaemia, resulting in death over a period of 5 days to several weeks.

Fulminate or hyperacute poisoning. In cases of massive ingestion (usually well above 40 - 55 mg/kg paraquat cation $\cong$ 55-76 mg paraquat dichloride/kg bw) patients survive less than 4 days and die in cardiogenic shock and multiorgan failure. Apart from renal and hepatic failure, alveolitis and noncardiogenic pulmonary oedema are observed. Other organ systems (adrenal glands, pancreas, heart) are affected and mortality in this group has been suggested to approach 100%.

While this categorisation reflects experience with a large number of cases, it has to be emphasised that there are a significant number of cases reported in the literature where there was survival following the ingestion of alleged doses well above of what is usually considered to be fatal. There are 52 case reports in the literature where a dose apparently in excess of 55 mg paraquat cation/kg ($\approx$ 76 mg paraquat dichloride/kg bw) has been survived. While inaccuracies in estimating the dose may have led to exaggeration of the dose in some cases this appears unlikely in many others.

Table 14: Summary of case reports with doses above 55 mg/kg taken by survivors of paraquat poisoning by ingestion

Calculated ingested dose - mg paraquat cation/kg	Dose stated[1]	Number of cases	Body weight (kg)[2]	Age Range (years)	References

55 – 75 (≅ 76-104 mg paraquat dichloride/kg)	15 - 20 ml (9 cases) 1 - 2 mouthful (2 cases) 1 sachet (2 cases)	13	25 - 70	3 - 75	Addo et al., 1984 Iff et al., 1971 Lloyd 1969 (cited in Cavalli, 1977) Mahieu et al., 1977 Ming et al., 1980 Mirchev, 1977 Taki et al., 1996 Talbot et al., 1988
76 – 100 (≅ 105-138 mg paraquat dichloride/kg)	10 - 40 ml (14 cases) 1 - 2 mouthful (4 cases)	18	25 - 70	3 - 65	Addo et al., 1984 Douze et al., 1977 Malone et al., 1971 McKean, 1968 Ragoucy-Sengler et al., 1991 Shahar et al., 1980 Tabei, 1982 Taki et al., 1996 Thomas et al., 1977 Tsatsakis et al., 1996
101 – 200 (≅ 139-276 mg paraquat dichloride/kg)	40 - >50 ml (7 cases) 3 mouthful (2 cases)	9	70	17 - 59	Addo et al., 1984 Douze et al., 1974, 1977 Florkowski et al., 1992 Grundies et al., 1971 Lheureux et al., 1995 Okonek et al., 1980
> 201 (≅ 277 mg paraquat dichloride/kg)	50 - 150 ml (8 cases) 3 - 4 mouthful (2 cases) 1 glass or cup (2 cases)	12	40 - 70	10 - 50	Addo et al., 1984 Douze et al., 1974, 1977 Malone et al., 1971 Okonek et al., 1979, 1980 Tabei et al., 1982 Tsatsakis et al., 1996

[1] Volumes (ml) refer to the 20% liquid concentrate. A volume of 17.5 ml has been used for 'a mouthful'. 'Sachet' refers to a granular formulation containing 2.5% paraquat and 2.5 g diquat.
[2] The following assumptions were used where the body weight was not explicitly stated,:

3 - 6 years	25 kg	7-11 years	40 kg
12 - 16 years	50 kg	17 years and above	70 kg

Talbot et al., (1988b) reported a series of nine cases of suicidal paraquat poisoning in pregnant women. In the cases where the outcome was known, one foetus died probably unrelated to paraquat, three died in utero or after delivery but associated with respiratory distress in the mothers, two died in utero (one mother survived and subsequently had a normal pregnancy with no evidence of teratogenicity from the previous paraquat intoxication). One foetus was aborted. Previously, Fennelly et al., (1968) had reported the case of a woman who was 28 weeks pregnant and died 20 days after paraquat ingestion. Upon autopsy the foetus showed no abnormalities. A 20-week pregnant patient survived the ingestion of a small dose of paraquat and subsequently delivered a normal child (Musson and Porter, 1982).

There are now sufficient case reports in the literature to demonstrate that the development of pulmonary lesions is not inevitably fatal. Fitzgerald et al., (1979b) examined 13 survivors of acute paraquat poisoning after a minimum of 1 year. In two children, no clinical, functional or radiological abnormalities were seen. Of the 11 adults, five non-smokers did also show no evidence of pulmonary disease. Four smokers were considered normal on clinical and radiological criteria, but had a mild deficit in pulmonary function, which could reasonably be attributed to smoking. Two patients had pronounced arterial hypoxaemia, both having had pre-existing pulmonary disease. In one of these two patients new and persistent infiltrates were seen in radiography, which could be ascribed to paraquat lung damage. Hudson et al., (1991) described persistent radiological changes in three survivors of paraquat poisoning. In one case the patient died a year after her first intoxication from a second massive dose of paraquat. Upon autopsy pulmonary changes from the first as well as the second intoxication were present. Lin et al., (1995) studied 16 survivors of moderate to severe paraquat poisoning after 3 months. Detailed lung function showed significant improvements over time. This was confirmed by improvements in chest radiographs, which showed some residual interstitial fibrosis, especially in the lower lobes. Bismuth and Hall (1995) reported five cases, all of which had developed a restrictive pulmonary lesion, but who survived. Two patients were followed up for 4 and 10 years, respectively. In the first patient there was an obstructive component to his pulmonary insufficiency (from smoking), which persisted over time. However, the restrictive component gradually improved over several years, with eventual return to near baseline state. In the second patient (a 13 year old adolescent at the time of intoxication) pulmonary function tests were completely normal 10 years after the poisoning. He had also been able to actively participate in sports.

The measurement of paraquat plasma concentration has proved to be a reliable indicator of the prognosis of the intoxication. Levitt (1979) was the first to demonstrate a relationship between plasma concentration of paraquat, the estimated time after ingestion and the eventual outcome. Based on results from 79 patients with a reasonably well established time of ingestion, Proudfoot et al., (1979) found that those patients whose plasma paraquat concentration did not exceed 2.0, 0.6, 0.3, 0.16, and 0.1 mg/l at 4, 6, 10, 16, and 24 hours after ingestion survived. This semi-logarithmic plot has become known as the predictive line, or 'Proudfoot's curve'. Because of the rapidly decreasing plasma concentration in the first few hours following ingestion no accurate prognosis could be given prior to 4 hours. The authors emphasised that the line to separate survivors and non-survivors was meant to be an approximate guide, and the main use should be to help clinicians in the decision which patients needed urgent aggressive treatment. Subsequently, several other methods have been described to establish the prognosis from plasma paraquat concentrations. None of those methods have been found to invalidate the original estimate by Proudfoot et al., but they have added other dimensions, which may be of help to clinicians. Scherrmann et al., (1987) used data from 30 patients to extrapolate the predictive line beyond 24 hours up to 15 days after intoxication, this was later modified (Scherrmann, 1995) with data from a total of 52 patients.

Table 15: Predictive plasma paraquat concentrations beyond 24 hours separating surviving and non-surviving patients (from Scherrmann, 1995)

Time (hours)	Plasma paraquat

	concentration (ng/ml)
24	100
48	86
72	74
96	63
120	54
144	48
168	42
192	37
216	32
240	27
264	23.5
288	20
312	18

The same authors evaluated the relationship between early urine concentrations and clinical prognosis. They also attempted to correlate urine results obtained by radioimmunoassay with those given by the simple colorimetric dithionite test. Data from 75 patients showed a wide variation in urine concentrations within 24 hours of ingestion. All 17 patients with concentrations of less than 1 μg/ml survived, whereas 51 out of 58 patients with urine paraquat concentrations of more than 1 μg/ml died. No colour was observed in the dithionite test at paraquat concentrations below 0.5 μg/ml (Scherrmann et al., 1987, Scherrmann, 1995).

Using a sample size of 219 patients, Hart et al (1984) were able to calculate the probability of survival of the patient from the initial paraquat plasma concentration. It was noted that the line denoting a 50% probability of survival correlated well with Proudfoot's curve. Sawada et al (1988) categorised their patients into three groups: survivors (n=10), non-survivors who died from respiratory failure (n=9), and non-survivors who died from circulatory failure (n=11). They calculated a severity index of paraquat poisoning (SIPP) from time to treatment since ingestion of paraquat multiplied by the serum level at admission (μg/ml). A boundary SIPP of 10 separated survival from death by either cause, whereas a SIPP of 50 separated deaths from respiratory failure and deaths from circulatory failure. Using data from 128 patients, Ikebuchi et al., (1993) separated survivors and fatal cases by multivariate analysis and established a discriminate function D.

Figure 1: Relationship between the concentration of paraquat in the plasma and the survival of the patient from Hart *et al.*, 1984
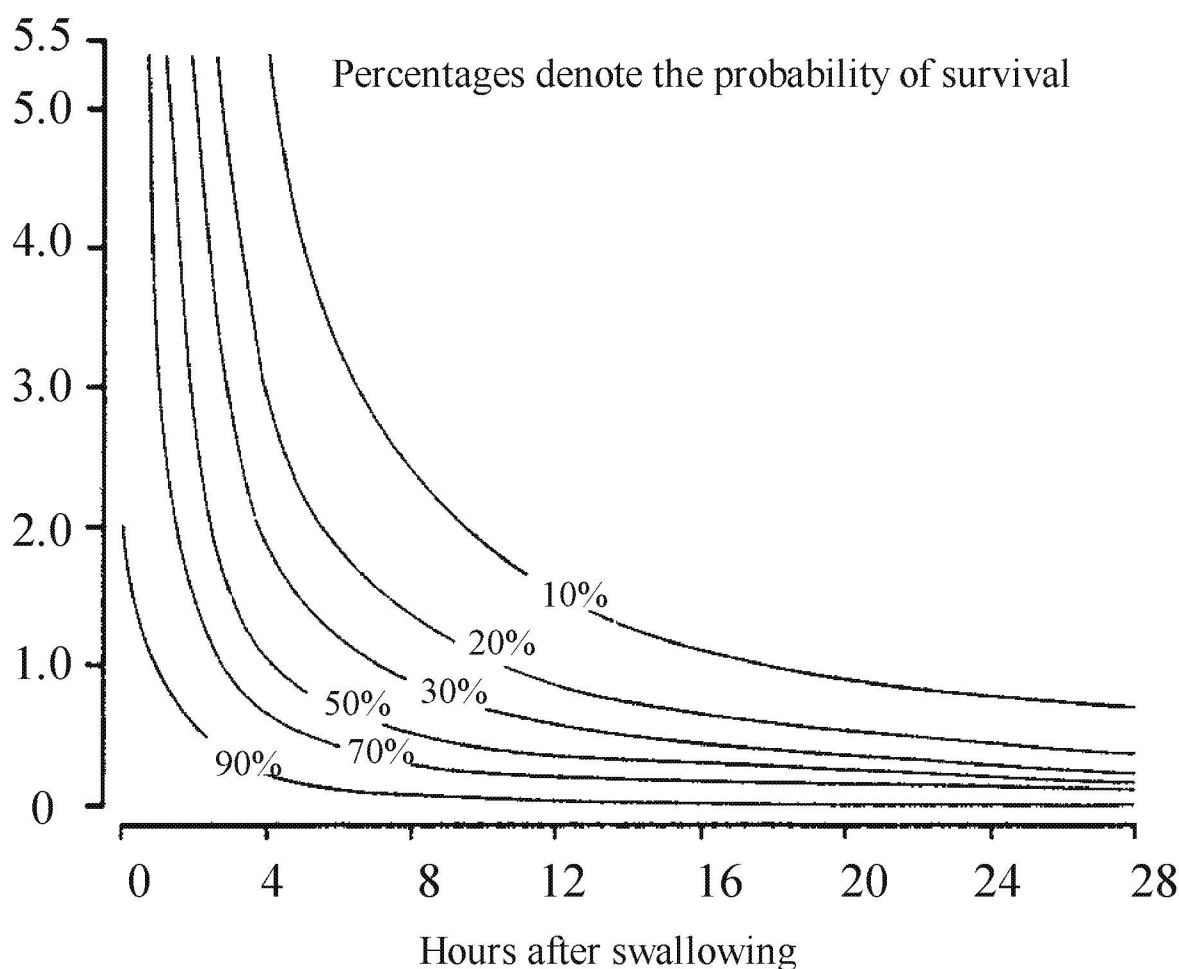
Their toxicological index of paraquat (TIP) could then be divided into three types: TIP 1 is characterised by D > 0.1 (100% survival probability); TIP 2 has the characteristic -0.1 < D < 0.1 and here urgent treatment may influence the outcome; in TIP 3 the discriminate function D < -0.1, and the probability of a fatal outcome is 100%.

All these methods depend on the availability of paraquat analysis, and this is often not the case, or at least not in a timely fashion. Investigators have therefore attempted to predict the outcome of the intoxication using biological indices rather than plasma paraquat concentrations. Suzuki et al., (1989) measured the respiratory index (RI) from blood gas analysis and used it as an index of lung oxygenation in 51 patients. Progressive deterioration of the RI above 1.5 was found in 43 non-survivors, whereas the RI remained below 1.5 in the 8 survivors. Furthermore, the time taken from ingestion for the RI to exceed 1.5 was found to be a good indicator for predicting the survival period in fatal cases. The major weakness of this method is that it cannot predict the outcome at the point of first contact with the patient, unlike the methods relying on plasma paraquat analysis. Also, conditions, which may influence the RI such as pneumothorax, cardio-pulmonary resuscitation, septic shock, pulmonary oedema and pneumonia, limit the usefulness of this method. On the other hand, it can be used at any time after the intoxication, and it is independent from an estimate of time of ingestion. Yamaguchi et al., (1990) reviewed the medical records of 160 patients who had ingested paraquat and calculated an equation derived from serum

creatinine and potassium concentrations and arterial blood bicarbonate level. When plotted against time of ingestion they were able to estimate the probability of survival in three categories (90%, 38% and 3%). Most recently, a different biological index using creatinine measurement from 18 patients has been proposed by Ragoucy-Sengler and Pileire (1996a). They found that the time evolution of blood creatinine in intoxicated patients was linear during the first 24 hours after admission. The rate of increase of creatinine in the patients with fatal outcome was equal to a constant (zero order kinetics). A rate of creatinine increase over 5 hours (dCreat/dt) of > 3 μmol/l/h was found in the 12 fatal cases whereas this value remained < 1.26 for the survivors. As with the method of Suzuki et al., (1989) this biological index is independent from an estimate of time elapsed since ingestion. It has the advantage that a prognosis can be established within a few hours after admission of the patient using a standard biochemical analysis. However, it is currently based on data from a relatively small number of patients and will thus require further confirmation from a larger dataset.

The literature on laboratory findings, and the absorption, distribution, metabolism and excretion following poisoning in man as well as treatments for poisoning have recently been reviewed in Lock and Wilks (2001).

Summary

A small amount of paraquat is rapidly absorbed from the gastrointestinal tract of rats, guinea pigs, dogs, monkeys, and humans , most of the absorbed dose being excreted as unchanged paraquat via the kidneys. Paraquat is rapidly distributed in most tissues, the highest concentrations being found in the lungs and kidneys. The compound accumulates slowly in the lung by an energy-dependent process, which is also responsible for the uptake of putrescine. Saturation kinetics for the accumulation of paraquat by the lung has been shown to be similar in rats and humans. Excretion of absorbed paraquat is biphasic, owing to lung accumulation, and occurs largely in the urine as unchanged paraquat, but also to a limited extent in the bile. The weight of evidence strongly supports the view that paraquat is actively secreted by the kidney in both laboratory animals and humans. Biotransformation of absorbed paraquat is, in general, remarkably poor in all species studied (rats, guinea pigs, dogs, hens, pigs, goats, and sheep) although there is some controversy as to the possibility and extent of its metabolism by the gut microflora. Metabolism occurs via demethylation forming monomethyl dipyridone ion or oxidation to form paraquat pyridone ion and paraquat dipyridone ion. A recent study in the rat has shown this to be minimal.

The mechanism of paraquat toxicity has been investigated extensively, but it has not yet been elucidated completely. The available evidence indicates that paraquat toxicity is due to the ability of the compound to undergo redox cycling in biological systems, resulting in the production of superoxide anion radicals and in the oxidation of cellular NADPH. These effects may lead to the formation of other highly toxic oxygen species and to depletion of important defence mechanisms, both events that are potentially capable of switching on further pathological processes, resulting in damage to Type I and Type II pneumocytes.

In previous *in vitro* studies both positive and negative mutagenicity results were obtained, usually associated with high cytotoxicity. *In vivo*, paraquat has been examined in both the rat and the mouse using the oral route of administration and

shown to be non-genotoxic when tested to maximum tolerated doses in the bone marrow micronucleus assay, the bone marrow cytogenetic assay, the liver DNA repair assay and the germ cell dominant lethal assay. Conflicting results have been reported for some assays using other routes of administration (eg intraperitoneal administration) or endpoints examining for the presence of oxidised DNA bases in animals or for mutation in Drosophila. It is concluded that whilst paraquat has been shown to induce a genotoxic response in some *in vitro* systems at high or cytotoxic dose levels, it has no significant genotoxicity *in vivo*.

The acute oral toxicity of paraquat is higher in guinea pigs, monkeys, cattle, and humans than in rats and birds. No significant sex difference in acute toxicity has been seen via oral, subcutaneous or intraperitoneal administration of paraquat rats or mice. In rabbits, technical paraquat dichloride is a slight but persistant skin irritant and a moderate and persistant eye irritant. Technical paraquat dichloride produced no evidence of skin sensitising potential in guinea pigs when tested using a maximisation protocol. Lungs were identified as the key target organ following oral and inhalation exposure. Paraquat induces characteristic dose-related fibrotic changes in the lungs of mice, rats, dogs, and monkeys, but not in rabbits, guinea pigs, or hamsters. The acute pulmonary toxicity of paraquat in rats and humans is biphasic. In the early, destructive phase, the alveolar epithelial cells are extensively damaged. Death may occur within a few days due to pulmonary oedema. In the later, proliferative phase, fibroblasts and collagen accumulate in the lungs of surviving animals and humans, possibly resulting in fibrosis.

In short-term studies in mice, rats and dogs, consistent treatment-related findings were lung lesions (alveolar epithelial hyperplasia; alveolitis; chronic pneumonitis) and reduced bodyweight gain, with dogs the more sensitive species. Dogs also showed kidney lesions. Variations in haematology and clinical chemistry parameters were not consistent between studies or species.

Dietary chronic toxicity/carcinogenicity studies have been performed in mice, rats and dogs. It has been concluded that paraquat was not oncogenic in any of the 3 rat and 2 mouse chronic/lifetime studies

In a 1-year feeding study in dogs, paraquat caused lung changes at the 2 highest dietary levels; the no-effect level in this study was15 ppm (as paraquat cation), equal to 0.45 mg paraquat cation/kg bw/day in males and 0.48 mg paraquat cation/kg bw/day in females ($\approx$ 0.6 and 0.7 mg paraquat dichloride/kg bw/day).

In five long-term feeding studies, two in mice and three in rats, haematological and blood biochemical changes were observed in both species but were considered to be of little toxicological significance. A lifetime feeding study in the Alderley Park Swiss mouse showed no oncogenic potential for paraquat. Based on renal lesions observed in males, the no-effect level in this study was 12.5 ppm (as paraquat cation), equal to 1.5 mg paraquat cation/kg bw/day ($\approx$ 2.1 mg paraquat dichloride/kg bw/day). In a second study in the JCL:ICR mouse paraquat was not oncogenic and the NOAEL of 30ppm paraquat dichloride was established based on haematological and biochemical changes; equal to 2.8mg paraquat cation/kg bw/day ($\approx$3.9 mg paraquat dichloride/kg bw/day).

In a chronic feeding/carcinogenicity study in Wistar rats fed diets containing up to 300ppm paraquat dichloride, there was no evidence of carcinogenicity and the incidence of ocular lesions was low and not dose related. The systemic NOEL is 100ppm paraquat dichloride equal to 3.0 and 3.7mg paraquat cation/kg bw/day for males and females respectively ($\approx$ 4.1 and 5.1 mg paraquat dichloride/kg bw/day).

A long-term feeding (2 years) study in F344 rats, it was concluded that the incidence of pulmonary adenoma found in female rats in the 300 ppm paraquat dichloride group did not exceed the background incidence of pulmonary adenoma in this strain of rats. On the basis of lung and eye lesions (cataract), the NOAEL in this study is 30ppm paraquat dichloride equal to 0.77 mg paraquat cation/kg bw/day ($\approx$1.1 mg paraquat dichloride/kg bw/day).

In a lifetime study F344 rats were fed diets containing 150ppm paraquat cation for up to 117weeks the initial findings of the study suggested there were treatment related tumours in the lung and head region. However an independent pathology peer review concluded that paraquat was not oncogenic in this study. Ocular opacities were evident clinically at 75 and 150ppm and confirmed by ophthalmoscopy and histology. It was concluded that the NOAEL in this study was 25ppm equal to 1.25mg paraquat cation/kg bw/day ($\approx$ 1.8 mg paraquat dichloride/kg bw/day).

In teratogenicity studies in mice (2) and rats (2), paraquat was shown to be non-teratogenic at doses up to 25 mg paraquat cation/kg bw/day ($\approx$ 34.5 mg paraquat dichloride/ kg bw/day). Maternal toxicity was observed in mice at 15 and 25 mg doses and maternal toxicity and some foetal toxicity were observed in rats at 5 and 10 mg paraquat ion/kg bw/day. No foetal toxicity was seen at doses below those causing maternal toxicity. In a teratology sub-group in a 3-generation reproduction study in rats, paraquat was not teratogenic, but delays in ossification were found at all 3 dose levels tested (72, 145, and 290 ppm paraquat cation equal to approximately 7mg paraquat cation/kg bw/day and above).

In three rat 3-generation reproduction studies paraquat had no effects on the reproductive performance or development of the reproductive organs of rats. The overall NOAEL is 25ppm equal to 2.5mg paraquat cation/kg bw/day ($\approx$ 3.5 mg paraquat dichloride/kg bw/day).

Paraquat produced no consistent neurological findings following dietary administered to rodents or dogs in chronic regulatory studies. However recent literature has indicated that paraquat when administered at high parenteral doses can cause Parkinsonian like symptoms in the C57Bl mouse model. The findings from the animal model systems are interesting and may warrant further study, but their human toxicological significance is yet to be determined. From epidemiological studies there is no consistent evidence to link paraquat to Parkinson's disease.

Observations in humans, including reports on accidental or suicidal poisonings, confirm the type of acute and subacute toxicity observed in some experimental animals. Death occurred after oral and, in some cases, dermal absorption of high doses of paraquat. The organs primarily involved are the lung and kidney and, to a lower extent, the intestinal tract, liver, pancreas, adrenals, and CNS. The minimal lethal dose of paraquat in man is estimated to be about 35 mg paraquat cation/kg bw.

Skin, nail, and eye lesions were found in subjects with prolonged occupational exposure to paraquat, but these are reversible. Significant paraquat concentrations (up to 10 mg/1) were detected in the urine of workers using high spray concentrations, but not in protected workers. Dermal absorption in humans is low (<0.3%) and in operator exposure studies inhalation exposure was shown to be more than 3 orders of magnitude less than dermal exposure, due to large particle sizes used in application of paraquat formulations (gramoxone). The use of protective measures has proved to be effective in preventing lesions due to skin contact.

Table 16: Levels relevant for risk assessment (mg paraquat cation/kg bw/day)

Species	Study	Effect	NOAEL	LOAEL
Mouse	2 year dietary	Toxicity	1.5	5.6
		Carcinogenicity	15.0/18.7	Not oncogenic
	Multigeneration study of reproductive toxicity	Maternal toxicity	90ppm (approx 9 mg paraquat cation/kg bw/day)	125 ppm (approx 12.5 mg paraquat cation/kg/day)
		Pup toxicity	90ppm (approx 9 mg paraquat cation/kg bw/day)	125 ppm (approx 12.5 mg paraquat cation/kg/day)
	Developmental toxicity	Maternal toxicity	7.5 mg paraquat cation/kg bw/day	15 mg parquat cation/kg bw/day
		Embryo- and feto-toxicity	7.5 mg paraquat cation/kg bw/day	15 mg parquat cation/kg bw/day
Rat

Rat cont. | 2 year dietary | Toxicity | 25 ppm (1.25 mg paraquat cation/kg bw/day) | 75ppm(3.75 mg paraquat cation/kg bw/day) |
		Carcinogenicity	10.8 mg paraquat cation/kg bw/day	Not oncogenic
	Two-generation study of reproductive toxicity	Maternal toxicity	2.5 mg paraquat cation/kg bw/day	7.5 mg paraquat cation/kg bw/day
		Pup toxicity	2.5 mg paraquat cation/kg bw/day	7.5 mg paraquat cation/kg bw/day
	Developmental toxicity	Maternal toxicity	3.0 mg paraquat cation/kg bw/day	8.0 mg paraquat cation/kg bw/day
		Embryo- and feto-toxicity	3.0 mg paraquat cation/kg bw/day	8.0 mg paraquat cation/kg bw/day
Dog	1-year study of toxicity	Toxicity	0.45 mg paraquat cation/kg bw/day	0.93 mg paraquat cation/kg bw/day

Estimate of acceptable daily intake for humans

Paraquat was not carcinogenic or directly toxic to reproduction. The dog was found to be more sensitive to the repeat dose effect of paraquat than either mice or rats. The NOAEL of 0.45 mg paraquat cation/kg bw/day from the 1-year dog study is the appropriate value to use in deriving the ADI. This gives a margin of 2.5 with respect to the chronic rat study (25 ppm; 1.25 mg paraquat cation/kg bw/day) and a margin of 2 with respect to the LOEL in the 1-year dog study (lung lesions). A safety factor of 100 is considered appropriate. An ADI of 0.0045 mg paraquat cation/kg bw is proposed.

Acute reference dose

In man the most significant toxicological effect is associated with pulmonary fibrosis. However there is no acute study available where the NOAEL for lung lesions following a single oral or dietary dose have been established. Therefore consideration needs to be given to the NOAEL from repeat dose studies, from rat, dog or mouse. As stated previously the rabbit is refractory to the development of the lung lesion. The relevant studies are summarised below.

Table 17: Summary of studies considered for acute reference dose

Study type	Dose levels	NOAEL as paraquat ion	Comment
13 week mouse dietary	0, 10, 30, 100, 300 ppm paraquat dichloride ($\cong$ 0, 7.2, 22, 72, 217 ppm paraquat cation)	100 ppm paraquat dichloride (8.3 mg paraquat cation/kg bw/day males; 10.0 mg paraquat cation/kg bw/day females $\cong$ 13.8 mg paraquat dichloride/kg bw/day)	Lung lesions and reduced bodyweight gain at 300 ppm paraquat dichloride
13 week rat dietary	0, 10, 30, 100, 300 ppm technical paraquat dichloride($\cong$ 0, 7.2, 22, 72, 217 ppm paraquat cation)	100 ppm paraquat dichloride ($\cong$ 4.9 mg paraquat cation/kg bw/day $\cong$ 6.8 mg paraquat dichloride/kg bw/day)	Lung lesions, reduced bodyweight gain and microcytosis at 300 ppm paraquat dichloride
6 week dog study	35, 90 ppm paraquat cation (dietary), 0.75 mg paraquat cation/kg bw/day (capsule)	35 ppm paraquat cation (equivalent to 1 mg paraquat cation/kg bw /day for males and 1.3mg paraquat cation/kg bw/day for females $\approx$ 1.4 and 1.8 mg paraquat dichloride/kg bw/day for males and females respectively).	Bodyweight gain (both sexes) and food consumption (females) reduced at 90 ppm paraquat cation. Lung lesions (alveolitis) seen in both sexes at 90 ppm paraquat cation and 0.75 mg paraquat cation/kg bw/day.
13 week dog dietary	0, 7, 20, 60, 120 ppm paraquat cation)	20 ppm paraquat cation (0.56 mg paraquat cation/kg bw/day $\cong$ 0.77 mg paraquat dichloride/kg bw/day)	Mortality at 120 ppm paraquat cation. Lung lesions at 60 and 120 ppm paraquat cation. (NB only 3 dogs/sex)
Rat	0, 25, 75 or 150 ppm	25 ppm (2.5mg paraquat	No effects on

| multigeneration | paraquat cation | | cation/kg bw/day ≅ 3.5 mg paraquat dichloride/kg bw/day) | reproduction.Maternal toxicity at 75 and 150ppm |

Since the exposure being considered is to the dietary residue; the dietary route of exposure is the most relevant. Also from subchronic and chronic studies the dog is the most sensitive species. Developmental studies were considered not to be appropriate as they did not include an evaluation of lung pathology. However, in the multigeneration study in the rat lung pathology was examined in both parents and pups. The NOAEL in this study was determined to be 2.5 mg paraquat cation/kg bw/day.

In a combined 6 and 13 week oral toxicity study in the dog, three of the eight groups of 3 males and 3 females were killed after 6 weeks. Dogs receiving a dietary dose of 90 ppm paraquat cation or thoise dosed by capsule at 0.75 mg paraquat cation/kg bw/day developed pulmonary lesions. The dietary NOAEL following 6 weeks dosing was 35 ppm paraquat cation equivalent to 1 mg paraquat cation/kg bw/day for males and 1.3 mg paraquat cation/kg bw/day for females (equaivalent to 1.4 and 1.8 mg paraquat dichloride/kg bw/day for males and females respectively).

Acute reference dose with 100 fold uncertainty factor is 0.01mg/kg/d.

Table 18: Summary of critical end-points

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid (within 6 hours); limited absorption (5-20% in rats)
Distribution	Minimal; highest concentration in lung (after 72 hours)
Potential for accumulation	Low
Rate and extent of excretion	Rapid: extensive excretion (approx 99% after 72 hours)
Metabolism in animals	Minimal metabolism; <1% of administered dose excreted as metabolites
Toxicologically significant compounds	Parent compound
Acute toxicity	
Rats, LD_{50}, oral	93 -116 mg paraquat cation/kg bw
Rats, LD_{50}, intraperitoneal	16 – 19 mg paraquat cation/kg bw
Rats, LC_{50}, inhalation	1 µg/l (not relevant for human risk essessment)
Mice, LD_{50}, oral	196 – 210 mg paraquat cation/kg bw
Rabbit, dermal irritation	Slight but persistant skin irritant
Rabbit, eye irritation	Moderate and persistant eye irritant
Dermal sensitisation (test method used)	Not a sensitiser (Magnusson and Kligman)

Short-term toxicity

Target/critical effect	Lung lesions (alveolar epithelial hyperplasia, alveolitis, chronic pneumonitis); reduced bodyweight gain
Lowest relevant oral NOAEL	0.56 mgparaquat cation/kg bw/day (90day dog study)

Genotoxicity

	Non-mutagenic in most in vitro assays and in all in vivo assays by the oral route.

Long-term toxicity and carcinogenicity

Target/critical effect	pathological changes in kidney (mice); pathological changes in lungs and ocular lesions (rats); pathological lesions in lungs (dogs)
Lowest relevant NOAEL	0.45 mg paraquat cation/kg bw per day (1 year dog)
Carcinogenicity	Not carcinogenic

Reproduction/developmental

Reproduction target/critical effect	No effects on reproduction
Lowest relevant reproductive NOAEL	2.5 mg paraquat cation/kg bw per day
Developmental target/critical effect	Not teratogenic; fetotoxicity only seen with maternal toxicity
Lowest relevant developmental NOAEL	3.0 mg paraquat cation/kg bw per day for maternal and foetal toxicity (rat)

Medical data

	Low levels of operator exposure predominantly via the dermal route. Negligible inhalation exposure. Human health studies have shown no longer term adverse effects following recommended use of the product.

Summary	Value	Study	Safety factor
ADI	0–0.0045 mg/kg bw	1 year study in dogs	100
ARfD	0.01 mg paraquat cation/kg bw/day	6 week study in dogs	100

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