PARAQUAT

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Explanation

Paraquat is a bipyridilium herbicide that was evaluated by the JMPR in 1970, 1972, 1976, 1985 and 1986 (Annex 1, references *14*, *18*, *26*, *47*), in order to establish an acceptable daily intake (ADI). A toxicological monograph was published after the 1970 JMPR and addenda to the monograph were published after the 1972, 1976 and 1982 Meetings. A toxicological monograph was published after the 1986 JMPR. At the JMPR in 1970, an ADI of 0–0.001 mg/kg bw, as paraquat dichloride, was established. The 1972 JMPR assigned an ADI of 0–0.002 mg/kg bw, while the 1982 JMPR reduced the ADI to 0–0.001 mg/kg bw. The 1986 JMPR established an ADI of 0–0.004 mg/kg bw as paraquat ion (equal to 0–0.006 mg/kg bw as the dichloride).

Paraquat was re-evaluated by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. A considerable amount of data has been generated since 1986 and was submitted for evaluation; these data include studies on the absorption, distribution, metabolism and excretion of paraquat and numerous studies of toxicity (acute, reproductive and developmental). Furthermore, a substantial number of papers in the open literature on, inter alia, the genotoxicity and neurotoxicity of paraquat have been reviewed. In all studies relevant to risk assessment, doses and intakes are expressed as paraquat ion.[1]

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

In a study of the absorption, distribution and excretion of paraquat, a single oral dose of ^{14}C-labelled paraquat ion at 1 mg/kg bw was administered to five male and five female Alpk:ApfSD rats by gavage. Paraquat dichloride was used as the test material; the purity of the ^{14}C-labelled material was 100%, while that of the unlabelled material was >96%. The specific activity of the radiolabelled material was 4.0996 GBq/mmol and that of the dosing solution was 4.12 MBq/g. Urine was collected 6 h after dosing and urine and faeces were collected separately at 12, 24, 36, 48 and 72 h after dosing. The animals were killed after 3 days and selected organs and tissues were removed. The amount of radioactivity remaining in the blood, selected tissues and the carcasses was estimated. Excretion of the radiolabel was rapid: in the first 24 h, in males 17.9% of the dose was excreted in the urine and 63.1% in the faeces. Equivalent figures for females were 11.6% and 74.1%. More than 90% of the radiolabel was eliminated in 72 h in both sexes. More radiolabel was excreted in the faeces of females than males. Only low concentrations of radiolabel were retained in the residual carcasses (0.64% and 0.54% of the administered dose in male and females respectively), the highest concentrations (0.01–0.02%) being found in the liver, lungs and kidneys (Lythgoe & Howard, 1995a).

In a second study of the absorption, distribution and excretion of paraquat, daily oral doses of paraquat (1 mg of paraquat ion/kg bw) were administered by gavage to eight male and eight female Alpk:ApfSD rats for 14 days. Paraquat dichloride was used as the test material; the purity of the ^{14}C-labelled material was 100%, while that of the unlabelled material was >96%. A single oral dose of ^{14}C-labelled paraquat ion at 1 mg/kg bw was subsequently administered by gavage. The specific activity of the radiolabelled material was 4.0996 GBq/mmol and that of the dosing solution was 4.12 MBq/g. Urine was collected 6 h after dosing and urine and faeces were collected separately at 12, 24, 36, 48 and 72 h after dosing. The animals were killed after 3 days and selected organs and tissues were removed. The amount of radioactivity remaining in the blood, selected tissues and the carcasses was estimated. Excretion of the radiolabel was rapid: in the first 24 h, in males, 18.8% of the dose was excreted in the urine and 68.3 % in the faeces. Equivalent figures for females were 10.3% and 70.7%. Of the radiolabel, 92.5% was eliminated within 72 h in the male rats and

[1] The relative molecular mass of paraquat is 186.3; the relative molecular mass of paraquat dichloride is 257.2; therefore 1 g of paraquat dichloride = 0.724 g of paraquat ion.

93.9% in female rats. Tissue concentrations of radiolabel were generally lower in the females than in males. Only low concentrations of radiolabel were retained in the residual carcass (0.70% and 0.55% of the administered dose in males and females, respectively), the highest concentrations being found in the lungs, livers and kidneys (Lythgoe & Howard, 1995b).

In a third study of the absorption, distribution and excretion of paraquat, a single dose of ^{14}C-labelled paraquat (50 mg of paraquat ion/kg bw) was administered by gavage to five male and five female Alpk:ApfSD rats. The specific activity of the dosing solution was 79.83 kBq/g. Urine was collected 6 h after dosing and urine and faeces were collected separately at 12, 24, 36, 48 and 72 h after dosing. The animals were killed after 3 days and selected organs and tissues were removed. The amount of radioactivity remaining in the blood, selected tissues and the carcasses was estimated. Excretion of the radiolabel was rapid: in the first 24 h, in males, 9.2% of the dose was excreted in the urine and 54.5 % in the faeces. Equivalent figures for females were 11.6% and 49.6%. Of the label, 92.7% was eliminated in 72 h in the male rats and 91.7% in female rats. The highest concentrations of radioactivity were retained in the lungs and residual carcass (Lythgoe & Howard, 1995c).

Daniel & Cage (1966) investigated the absorption and excretion of paraquat (and diquat) in albino Wistar rats given ^{14}C-labelled paraquat dichloride (0.94 mCi/mmol) as single oral doses at 4 or 6 mg/kg bw, or paraquat dimethosulfate as oral doses at 2.5–24 mg/kg bw, or subcutaneously at a dose of 21 or 23 mg/kg bw. Paraquat was poorly absorbed from the gut. After administration by either route, most of the radiolabel was found in the excreta within 2 days. After oral administration of paraquat, no radiolabel was detected in the bile (Daniel & Cage, 1966).

Dey et al. (1990) studied the pharmacokinetics of ^{14}C-labelled paraquat (111 mCi/mmol) administered to male Sprague-Dawley rats as a single subcutaneous injection at a dose of 72 µmol/kg bw. This dose was considered to be one that would produce lung damage but avoid kidney damage. Blood was sampled through indwelling cannulae, and urine and faeces were collected at 2, 4, 6, 8, 12, and 24 h and then daily for 7 days. Non-cannulated rats treated in the same way were exsanguinated at intervals from 10 min to 7 days after dosing; tissue concentrations of ^{14}C were measured in selected organs. The right lungs and kidneys were processed for histopathological examination. Histopathological examination showed changes characteristic of paraquat-induced lung pathology, without renal damage. Paraquat was rapidly absorbed, with peak blood concentrations of 58 µmol/l after 20 min. The pharmacokinetics were best characterized as a two-compartment open model, the mean half-life ($t_{½}$) being approximately 40 h. Highest tissue concentrations observed were in the kidney (358 nmol/g of tissue) and lung (64 nmol/g tissue), both at 40 min after administration of paraquat (Dey et al., 1990).

The distribution of paraquat in the brain was examined in male adult Wistar-derived Alderley Park rats after subcutaneous administration of paraquat (containing ^{14}C-labelled paraquat with a specific activity 2 mCi/mmol) at a dose of 20 mg of ion/kg bw. The aim of this study was to determine whether paraquat crosses the blood–brain barrier. After administration, the concentration of radiolabel in the brain reached a maximum (0.05% of administered dose) within the first hour and then rapidly disappeared. Twenty-four hours after administration, however, a residual amount of paraquat still remained in the brain (1.6 nmol/g wet weight) and could not be removed by intracardiac perfusion. Most of the paraquat was associated with five structures, two of which (the pineal gland and linings of the cerebral ventricles) lie outside the blood–brain barrier. The remaining three brain areas

(the anterior portion of the olfactory bulb, hypothalamus and area postrema) do not have a blood–brain barrier. Overall, the distribution of ^{14}C-labelled paraquat in the brain 24 h after systemic administration was highly correlated to the blood volume. The authors concluded that paraquat remaining in the brain 24 h after systemic administration was associated with elements of the cerebral circulatory system, such as the endothelial cells that make up the capillary network, and that there was limited entry of paraquat into brain regions without a blood–brain barrier (Naylor et al., 1995).

The extent to which paraquat entered the brain was compared in groups of neonatal (aged 10 days), adult (aged 3 months) and elderly (aged 18 months) Wistar-derived Alpk:ApfSD rats. Both male and female neonatal rats were studied, while the adult and elderly rats were males. Groups of six to eight rats were given a single dose of [^{14}C]paraquat (103 mCi/mmol) at 20 mg/kg, administered subcutaneously, and killed 30 min or 24 h after injection; blood was taken by cardiac puncture and brains were removed. Groups of four neonatal, adult or elderly rats were similarly injected and killed 24 or 48 h after dosing; the brains of these animlas were subjected to histopathological examination. At all ages, plasma concentrations of paraquat were much higher at 30 min than at 24 h. At 30 min, the concentration of paraquat in the brain was highest in the elderly rats. While at 24 h the concentration in the brains of the adult and elderly rats had decreased, it remained high in the brains of the neonatal rats. Autoradiography showed similar distributions of paraquat in the various brain regions, paraquat being found in areas outside the blood–brain barrier or where the barrier is incomplete, e.g. the dorsal hypothalamus, area postrema and anterior olfactory bulb. There was no evidence for paraquat-induced cell damage in the neonatal brain, although there was increased paraquat entry into the brain in neonates than in older rats (Widdowson et al., 1996a).

In a study of the entry of paraquat into the brain, five male Wistar-derived Alpk:ApfSD rats were given paraquat (labelled with [^{14}C]paraquat; specific activity, 20 µCi/ml) at a dose of 5 mg of ion/kg bw per day) daily for 14 days by oral administration, and another five rats received a single oral dose of paraquat (labelled with [^{14}C]paraquat; specific activity 106 µCi/ml) at a dose of 5 mg ion/kg bw. The rats were killed 24 h after the last of the 14 doses or after the single dose. Concentrations of paraquat in the brain were 10 times higher in rats receiving multiple doses than in those receiving single doses. The same paper described a study of neuropathology, which included behavioural tests (see below) (Widdowson et al., 1996b).

In a study that used a brain microanalysis technique with detection by high-performance liquid chromatography–ultraviolet (HPLC–UV), paraquat, administered subcutaneously at a dose of 5, 10 or 20 mg/kg bw, was found to appear in the dialysate of the striatum in male Wistar rats. It was also found that paraquat did not allow 1,2,3,6-tetrahydropyridinium ion to penetrate the blood–brain barrier. Intraperitoneal injection of L-valine (200 mg/kg) 30 min before administration of paraquat at a dose of 20 mg/kg bw reduced the striatal extracellular concentrations of paraquat. The authors hypothesized that paraquat is taken up into the brain via the neutral amino acid transporter (Shimizu et al., 2001; see also McCormack & Di Monte, 2002).

In a study in anaesthetized male Wistar rats, the excretion of paraquat was found to be greater than the glomerular filtration rate, and to be concentration-dependent and saturable, implying that paraquat is secreted by a process involving active transport (Chan et al., 1997).

Groups of albino Wistar rats were given diets containing paraquat at a concentration of 50, 120 and 250 mg/kg (as paraquat ion) for 8 weeks. Groups comprised 30 animals at the two lower dietary concentrations and 40 animals at the highest concentration. After 2, 4 and 8 weeks, 10 rats per group were killed and selected organs were analysed for paraquat. At 50 mg/kg, paraquat was not detected in the kidneys, liver, brain or lung at any time, but was present in the gastrointestinal tract and, at low concentrations, in muscle. At 120 mg/kg, paraquat was detected in kidneys, lung and gastrointestinal tract. At 250 mg/kg, paraquat was detected in kidneys, lung and gastrointestinal tract (Litchfield et al., 1973).

Mice

The tissue distribution of paraquat was studied using whole body autoradiography in mice treated by intravenous injection. Mice received ^{14}C-labelled paraquat at a dose of 20 mg of paraquat ion/kg. Two mice were killed at each time-point after the paraquat injection (10 min, 1, 5, 24 and 72 h). Paraquat was found to be concentrated in the liver and cartilage, and was not detected in the central nervous system. Paraquat was also present in the lungs, notably so after 24 h. At 72 h, radioabel was only present in the stomach and intestinal contents (Litchfield et al., 1973).

Hens

Three Warren hens were given gelatin capsules containing ^{14}C-ring-labelled paraquat (purity, 99.7%; specific activity, 1.216×105 dpm/µg) at a daily dose of 4.52 mg of paraquat ion (0.247 mCi) for 10 days. One hen was used as the control. The hens were killed 4 h after the last dose. The highest concentration of radiolabel was found in the kidneys, while rather less was found in the gizzard and liver. Very little was found in fat. Paraquat was found at a concentration of 0.052 µg/g in eggs, mostly in the yolk (Hendley et al., 1976b).

Dogs

Greyhound dogs were given ^{14}C-labelled paraquat at a dose of 30–50 µg/kg bw. The authors of this study considered that the kinetics could be described by a three-compartment open linear system (Bennett et al., 1976).

The elimination of paraquat was studied in the female greyhound dog. After intravenous injection of low doses (30–50 µg/kg) of ^{14}C-labelled paraquat, radiolabel was found to be rapidly excreted in the urine, the clearance being greater than the glomerular filtration rate, suggesting a process of active secretion. Secretion could be inhibited by *N*′-nicotinamide. Large doses of paraquat (20 mg/kg bw) produced renal failure, as evidenced by a decrease in both renal creatinine and paraquat clearance. The elimination of paraquat could be described by a three-compartment open model (Hawksworth et al., 1981).

Goats

An English white nanny goat was dosed with ^{14}C-ring-labelled paraquat (purity, 99.7%; specific activity, 2.28×104 dpm/µg) in the diet at a dose equivalent to 100 µg of paraquat ion/g of diet. This was done by adding 206.6 mg of radiolabelled paraquat (as ion) to the diet at the morning and afternoon feeding, daily for 7 days. Another nanny goat was used as the control. The goats were killed 4 h after the last feeding with radiolabelled paraquat. Radioactivity was measured in the urine, faeces, stomach, milk, and in selected tissues. At sacrifice, 2.4% and 50.5% of the administered material had been excreted in the urine and faeces respectively. The stomach contents included 33.2% of the administered

dose. The highest concentration of radiolabel seen in the milk was 0.009 µg/g (on the morning of day 7). The highest tissue concentrations were found in the kidney and liver (Hendley et al., 1976a).

Pigs

A pig (Large White × Welsh boar) was given 100 mg of ^{14}C-methyl-labelled paraquat (purity, 99.3%; specific activity, 4.88 × 104 dpm/µg of paraquat ion) on 7 consecutive days; this was calculated to be equivalent to about 50 µg of paraquat ion/g of diet. A second boar acted as the control. The daily dose was spotted onto the commercial pig diet. The pig was killed 2 h after the final dose. The highest concentrations of paraquat were present in the kidney and liver (Leahey et al., 1976).

In a second study in pigs, ^{14}C-methyl-labelled paraquat dichloride (purity, 99.45%; specific activity, 4.72 dpm/µg) at a daily dose of approximately 100 mg of paraquat ion was administered twice daily for 7 days to a Large White × Welsh boar. The dose contained about 2 mCi of radiolabel and the content of paraquat was calculated to be equivalent to about 50 µg paraquat ion/g of diet. The daily dose was spotted onto commercial porcine diet pellets. A second boar acted as the control. The highest concentrations of radiolabel were found in the kidney, with somewhat less being found in the liver and lung (Spinks et al., 1976).

Monkeys

Purser & Rose (1979) studied the renal handling of paraquat administered orally at a dose of 85 mg of paraquat ion/kg bw (containing 500 µCi of ^{14}C-labelled paraquat) to three male cynomolgus monkeys (*Macaca fascicularis)*. In two monkeys, peak plasma concentrations were observed at 2 h in two monkeys and at 10 h in the third monkey. The renal clearance of paraquat was high during the first 10 h, but fell markedly as renal failure set in at 14 h. The clearance of paraquat was always well in excess of the clearance of creatinine, suggesting an active secretory process.

Studies in more than one species

The disposition of orally-administered ^{14}C-labelled paraquat dichloride was studied in male Sprague-Dawley rats, male and female guinea-pigs, and monkeys (*Macaca fascicularis*). The doses used were: rats, 126 mg/kg bw (0.175 µCi/mg); guinea-pigs, 22 mg/kg bw (1.25 µCi/mg); and monkeys, 50 mg/kg bw (0.4 µCi/mg). In the case of the rats and guinea-pigs, the doses used were LD_{50}s at 7 days. A total of 61 rats, 21 guinea-pigs and three monkeys were used. For the rats and guinea-pigs, urine and faeces were collected and groups were sacrificed at various times up to 21 days after the administration of paraquat. Selected organs were collected at sacrifice. For the monkeys, blood samples were taken at 30 min, 1, 2, 4, 8, 16 and 32 h after administration of paraquat and daily thereafter. In the rats, deaths were seen mainly after 5 days. A large portion of the paraquat was not absorbed from the gastrointestinal tract. Peak serum concentrations of radiolabel were seen at 30–60 min, while concentrations of radiolabel were higher in liver, kidneys and lungs than in serum. Similar results were found in the guinea-pigs. In the monkeys, one of which died on day 8, serum concentrations of radiolabel decreased rapidly after the first time-point (Murray & Gibson, 1974).

There is evidence that paraquat is taken up into the lungs by a process of active uptake, the normal substrate being endogenous diamines, e.g. putrescine and polyamines such as spermine and spermidine (see review by Smith, 1985). Diquat is not a substrate for this system and this fact accounts for the different organ-specific toxicity of these two bipyridilium herbicides (this is discussed further below).

1.2 Biotransformation

Rats

In the Daniel & Cage (1966) study in albino Wistar rats treated with ^{14}C-labelled paraquat dichloride, discussed above, some evidence of metabolism was found. Of the dose of paraquat administered orally, 30% of the radiolabel was present in the gut as metabolic products. Furthermore, a small amount of metabolite was present in the urine after oral but not subcutaneous administration, suggesting that metabolites were absorbed from the gut. Studies in vitro, using faecal homogenates, suggested that microbiological metabolism was responsible for this. In the study of Murray & Gibson (1974) in male Sprague-Dawley rats, male and female (mixed) guinea-pigs and cynomolgus monkeys (*Macaca fascicularis*), metabolites were not observed.

Urine and faeces samples from the studies in rats, described above (Lythgoe & Howard, 1995a, b, c), were pooled separately for the females and males of each study for the whole 72 h of that study. After extraction, samples were analysed by thin-layer chromatography. In all cases, paraquat accounted for the vast majority of the radiolabel in the urine (95.0% of urinary label in the males receiving the lower dose and 93.6% females receiving the lower dose). Three minor metabolites were found in urine; these were not further identified. Faecal material showed that the vast majority of the radioactivity in all cases was unchanged paraquat. It was therefore concluded that paraquat was largely unmetabolized (Macpherson, 1995).

Hens

In the study in hens, residues in tissues were generally unchanged paraquat. A small amount of a metabolite, 1-methyl-(4′-pyridyl), was found in the livers and kidneys (Hendley et al., 1976b).

Goats

In the study in goats, residues in tissues were generally unchanged paraquat. In the liver, small amounts of 4-(1,2-dihydro-1-methyl-2-oxo-4-pyridyl)-1-methyl pyridinium ion and 1-methyl-4-(4′-pyridyl) pyridinium ion were found. The latter compound was also found in peritoneal fat (Hendley et al., 1976a).

Pigs

In the study by Leahey et al. (1976), all the radiolabel in the tissues, except the liver, wasfound to be in the form of paraquat. In the liver, 7% of the radiolabel was accounted for by 1-methyl-4-(4′-pyridyl) pyridinium ion. In the study by Spinks et al. (1976), 4% of the radiolabel was accounted for by 1-methyl-4-(4′-pyridyl) pyridinium and 70% by unchanged paraquat.

2 Toxicological studies

2.1 Acute toxicity

The results of studies on the acute toxicity of paraquat administered by a variety of routes are summarized in Table 1. Clinical signs seen after administration of paraquat by the oral, subcutaneous or intraperitoneal routes included decreased activity, dehydration and breathing irregularity. In animals that died after administration of paraquat by these routes, mottled areas of lung were seen. Scabbing of skin was seen after administration by the dermal route, but no systemic signs of poisoning were present. After rats had inhaled paraquat, clinical signs and appearances post mortem were similar to those seen after oral, subcutaneous or intraperitoneal administration.

(a) Dermal irritation

The dermal irritation potential of paraquat dichloride technical concentrate (paraquat ion, 33% w/w) was assessed in young adult female New Zealand white albino rabbits. Undiluted test material was applied to the depilated left flank of the rabbits, which was then covered by gauze and impermeable rubber. These were left in place for 4 h. After removal of the dressing and cleansing of the application site, the Draize scale was used to assess erythema and oedema, 30–60 min and 1, 2, 3, 4, 7, 14, 17, 20, 21 and 23 days after exposure. Slight erythema was observed, which regressed by 3 days and 4 days in two animals, but still remained after 23 days in the third animal. Very slight transient oedema was seen in one animal (at the 30/60 min observation time), very slight oedema was seen in the second, this still being present at 4 days but not at 7 days, while there was no oedema in the third rabbit (Duerden, 1994c).

(b) Ocular irritation

The potential for paraquat dichloride technical concentrate (paraquat ion, 33% w/w) to produce irritation of the eye was assessed in young adult female New Zealand white albino rabbits. Test material (0.1 ml) was applied to the left eye of each rabbit. Rabbits were dosed sequentially; and mild systemic toxicity was noted in the third rabbit to be dosed. Accordingly, this rabbit was killed. The fourth rabbit was collared to prevent oral ingestion of the test material. The eyes of rabbits 1, 2 and 4 were then examined and the degree of irritation was assessed using the Draize scale from 1 h to up to 28 days after instillation. Initial pain was graded as slight or was absent. Slight or mild corneal opacity was seen in all three animals, this effect resolving within 17 days. Redness and chemosis of the conjunctiva was seen in all animals and resolved by 28 days and 14 days after exposure. No effect was seen on the iris, while erythema of the eyelids and mucoid discharge was observed. Paraquat was considered to be a moderate ocular irritant (Bugg & Duerden, 1994).

In a study of ocular toxicity, paraquat was administered at a concentration of 6.25, 12.5, 25, 50 and 100% of a solution containing 242 mg of paraquat ion/ml. A total of 15 male New Zealand white rabbits were used, nine rabbits receiving different doses in each eye and six rabbits receiving the same dose in both eyes. Control eyes received normal saline. In all cases, 0.2 ml of solution was pipetted into the lower conjunctival sac, and the eyes were examined at 12 h and then daily for 20 days, ocular lesions being scored on the Draize scale. At 6.25 and 12.5%, severe conjunctival reactions were seen, with occasional slight corneal damage at 12.5%. At higher concentrations (25 and 50%), the iris was congested and swollen and there was corneal opacification; a pannus reaction was also seen. Animals to which the 100% solution was administered died within 6 days. The time of

Table 1. Acute toxicity of paraquat

Species	Strain	Sex	Route	LD_{50} (mg/kg bw) (95% confidence interval)	Reference
Mouse	ICR	M	Per os	360 (324–400)[a]	Shirasu & Takahashi (1977)
	ICR	F	Per os	290 (254–331)[a]	Shirasu & Takahashi (1977)
	ICR	M	Subcutaneous	41.0 (36.9–45.5)[a]	Shirasu & Takahashi (1977)
	ICR	F	Subcutaneous	36.8 (32.9–41.2)[a]	Shirasu & Takahashi (1977)
	ICR	M	Intraperitoneal	40.6 (35.6–46.3)[a]	Shirasu & Takahashi (1977)
	ICR	F	Intraperitoneal	39.2 (35.6–43.1)[a]	Shirasu & Takahashi (1977)
	Swiss-Webster	M	Intraperitoneal	39 (32.5–46.8)	Sinow & Wei (1973)
	Swiss-Webster	F	Intraperitoneal	30 (26.3–34.2)	Bus et al. (1976a)
Rat	NS	F	Per os	112 (104–122)[b]	Clark et al. (1966)
	NS	F	Per os	150 (139–162)[b]	Clark et al. (1966)
	Alpk : APfSD	M	Per os	344 (246–457)[c]	Duerden (1994a)
	Alpk : APfSD	F	Per os	283 (182–469)[c]	Duerden (1994a)
	Sprague-Dawley	M	Per os	223 (199–259)[a]	Shirasu & Takahashi (1977)
	Sprague-Dawley	F	Per os	258 (228–292)[a]	Shirasu & Takahashi (1977)
	Sherman	M	Per os	100[d]	Kimbrough & Gaines (1970)
	Sherman	F	Per os	110[d]	Kimbrough & Gaines (1970)
	NS	F	Per os	150 (110–173)	Mehani (1972)
	Sprague-Dawley	M	Per os	126	Murray & Gibson (1972)
	Sprague-Dawley	M	Subcutaneous	26.8 (23.9–30.0)[a]	Shirasu & Takahashi (1977)
	Sprague-Dawley	F	Subcutaneous	32.0 (28.1–36.5)[a]	Shirasu & Takahashi (1977)
	NS	F	Intraperitoneal	19 (16–21)[b]	Clark et al. (1966)
	Sprague-Dawley	M	Intraperitoneal	24.8 (21.8–28.3)[a]	Shirasu & Takahashi (1977)
	Sprague-Dawley	F	Intraperitoneal	26.8 (23.7–30.6)[a]	Shirasu & Takahashi (1977)
	NS	F	Intraperitoneal	16 (10–26)	Mehani (1972)
Rat	Sherman	M	Dermal	80[d]	Kimbrough & Gaines (1970)
	Sherman	F	Dermal	90[d]	Kimbrough & Gaines (1970)
	Alpk : APfSD	M	Dermal	>2000[c]	Duerden (1994b)
	Alpk : APfSD	F	Dermal	>2000[c]	Duerden (1994b)
	Alpk : APfSD	M	Inhalation	0.6–1.4[ef]	McLean et al. (1985)
	Alpk : APfSD	F	Inhalation	0.6–1.4[ef]	McLean et al. (1985)
Rabbit	NS	M	Per os	50 (45–58)	Mehani (1972)
	NS	M	Intraperitoneal	25 (15–30)	Mehani (1972)
Guinea-pigs	NS	M	Per os	30 (22–41)[b]	Clark et al. (1966)
	Sprague-Dawley	M & F	Per os	22	Murray & Gibson (1972)
	NS	F	Intraperitoneal	3[b]	Clark et al. (1966)
Hens	Rhode Island	F	Per os	262 (200–346)[b]	Clark et al. (1966)
Turkeys	White	F	Per os	Approx. 290	Smalley (1973)
	White	F	Intraperitoneal	Approx. 100	Smalley (1973)
	White	F	Intravenous	Approx. 20	Smalley (1973)
	White	F	Dermal	375	Smalley (1973)
Cats	NS	F	Per os	35 (27–46)[b]	Clark et al. (1966)
Dog	Beagles	M	Subcutaneous	1.8 (1.0–6.1)	Nagata et al. (1992)
	Beagles	F	Subcutaneous	3.5 (2.4–10.1)	Nagata et al (1992)
Monkeys	Cynomolgus (*M. fascicularis)*	M & F	Per os	50	Murray & Gibson (1972)
	Cynomolgus (*M. fascicularis)*	M	Per os	70[b]	Purser & Rose (1979)

NS, not stated; M, male; F, female

[a] Paraquat dichloride; purity, >98%

[b] Dose quoted as paraquat ion

[c] Technical paraquat dichloride (33% w/w paraquat ion)

[d] as dimethylsulfate

[e] LC_{50} (at 4 h) (mg of paraquat ion/m^{-3})

[f] Material used was paraquat dichloride, 21.5% w/v, but results were expressed as paraquat ion; aerosol mass median aerodynamic diameter (MMAD), <0.3 µm; rats exposed by nose only

maximal effect was around 9 days and those who received the 25% and weaker solutions showed recovery thereafter (Sinow & Wei, 1973).

(c) Dermal sensitization

A study of the sensitization potential of paraquat dichloride technical concentrate (paraquat ion, 33% w/w) was based on the maximization test of Magnusson & Kligman (1969). Female albino (Hsd/Poc : DH) guinea-pigs were used. The positive control was 2-mercaptobenzothiazole. A preliminary study was carried out to determine the concentrations of test material that gave an acceptable degree of irritancy and no signs of systemic toxicity. In the main study, 30 guinea-pigs were used (20 as test animals and 10 as controls). For induction, each animal received Freund complete adjuvant diluted 1 : 1 with deionized water, 0.03% w/v test material, and 0.03% w/v test material with Freund complete adjuvant 1 : 1 with deionized water, which were injected intradermally at three different sites in the previously depilated scapula region. One week later, the scapula region was again clipped and the test material (10% w/v) was applied topically over the injection sites. Animals serving as negative controls were treated in the same way except that the three inducing injections were Freund complete adjuvant 1 : 1 with deionized water, deionized water, and again Freund complete adjuvant 1 : 1 with deionized water. Animals serving as positive controls (20) were treated in the same way as the test animals except that the test substance administered was 2-mercaptobenzothiazole, and there were 10 negative controls for this group. For these guinea-pigs, the topical applications consisted of deionized water. Two weeks after the topical applications, both flanks of all animals were clipped free of hair and a preparation of 30% test material on an occlusive dressing was applied to one flank and a preparation of 10% test material to the other flank. These were left in place for 24 h. Erythematous reactions were recorded at 24 h and 48 h later. One animal in the test group died, but no erythema was found at either time in this group, nor in the negative control group. In contrast, erythema was seen in 19 of the positive controls, and it was concluded that paraquat had no sensitization potential (Duerden, 1994d).

2.2 Short-term studies of toxicity

(a) Oral administration

Mouse

In a 13-week dietary study, groups of 20 male and 20 female ICR-CRJ SPF mice were given paraquat dichloride (purity, 93.3%) at a dietary concentration of 0, 10, 30, 100 and 300 mg/kg, equal to 1.18, 3.65, 11.5 and 35.8 mg of paraquat dichloride/kg bw per day in males and 1.38, 3.91, 13.8 and 41.9 mg of paraquat dichloride/kg bw per day in females. These doses are equal to 0, 0.85, 2.64, 8.33 and 25.9 mg of paraquat ion/kg bw per day in males and 0, 1.00, 2.83, 9.99 and 30.3 mg of paraquat ion/kg bw per day in females. Mice were observed daily for mortality and daily clinical observations were undertaken. Animals found dead or that were killed in extremis were subjected to immediate autopsy. The mice were weighed weekly and food and water consumption were measured twice per week. On day 91, blood was collected from at least 10 mice from each group for haematological examination and for clinical chemistry. The mice were then examined post mortem. Autopsy was carried out on the remainder of the mice the next day, at which time urine was collected for urine analysis. At necropsy, selected organs were weighed and these and other organs were fixed and sections made for histopathological examination. Mortality was observed at 300 mg/kg, two females dying from pulmonary damage, one in week 2 and one in week 11. The decedents' lungs showed pulmonary oedema, small round cell infiltration with phago-

cytosis, and, in one animal, eosinophilic swelling of the epithelial cells of the alveoli. At 300 mg/kg in both sexes, there was reduced body-weight gain, almost from the inception of the study, however, these values were only significantly different from those of controls at a few time intervals. No intergroup difference in food intake was observed, but a slight reduction in food conversion efficiency was seen at 300 mg/kg in both sexes. No intergroup differences were seen in water intake. No test material-related intergroup differences were seen in haematological parameters (although a reduction in mean corpuscular volume at 300 mg/kg may have been test material-related in males) or in clinical chemistry findings. Terminal body weights were reduced in males at the highest dietary concentration, as were the absolute weights of the heart, liver and muscle. An increase in relative lung weight and a decrease in relative liver weight were also seen. In females at the highest dietary concentration, an increase in absolute pituitary, lung, kidney and spleen weight was observed, accompanied by a decrease in ovarian weight. Relative weights of organs also showed some intergroup differences, those of the pituitary, thyroids, lung, kidneys and spleen being increased while that of the ovaries was decreased. Eosinophilic hypertrophy of alveolar epithelial cells was observed in both sexes (17 out of 20 males, and 12 out of 18 surviving females). Pulmonary oedema and alveolar cell proliferation was also seen in a few males and in one female. Accordingly, the no-observed-adverse-effect level (NOAEL) for the study was 100 mg/kg (equal to 11.5 mg of paraquat dichloride/kg bw per day for males and 13.8 mg of paraquat dichloride/kg bw per day for females), on the basis of decreased body-weight gain and histopathological changes in the lungs at 300 mg/kg. These NOAELs are equal to 8.33 mg of paraquat ion/kg bw per day in males and 9.99 mg of paraquat ion/kg bw per day in females (Maita et al., 1980a).

Rat

In a 13-week study, groups of 20 male and 20 female Fischer CDF (F344) CRJ rats were given diets containing paraquat dichloride (purity, 93.8%) at a concentration of 0, 10, 30, 100 or 300 mg/kg (0, 7, 22, 72 and 217 mg/kg of paraquat ion, equal to 0, 0.49, 1.44, 4.74, 14.2 mg of paraquat ion/kg bw per day for males and 0, 0.52, 1.53, 5.14 and 15.27 mg of paraquat ion/kg bw per day for females). Another group received diet containing no test material and acted as controls. The rats were examined daily for adverse clinical signs, body weight was measured weekly and food and water intake were measured twice per week. Ninety-one days after the start of the study, at least 10 animals of each sex per group were chosen for blood sampling. The samples were used for haematology and clinical chemistry and, after sampling, the animals were examined post mortem. On day 92, urine analysis was carried out on the remaining rats, which were examined post mortem. At necropsy, selected organs were weighed and these and other organs were fixed in 10% formalin; they were then processed for histopathological examination. No rats died during the study and no abnormal clinical signs were seen. At the highest dietary concentration there was markedly reduced body-weight gain and decreased food and water intake in both sexes. Neither reduced body-weight gain nor reduced food intake was seen at lower dietary concentrations. No test material-related abnormalities were found on haematological examination, clinical chemistry or urine analysis. In the males, terminal body weights and absolute weights of brains, pituitaries, thyroids, livers, kidneys, spleens and muscles were decreased at the highest dietary concentration. Also in males, relative weights of brain, pituitary, lung, kidneys, adrenals, testes and muscle were increased. In females, terminal body weights were depressed at the highest dietary concentration, together with the absolute weight of the heart, lung and liver. Relative brain, lung, kidney, ovary and muscle weights were increased at the highest dietary concentration. These changes proba-

bly reflected the reduced food intake at the highest dietary concentration. On histopathological examination, alveolar epithelial hypertrophy was observed in males (6 out of 20) while in females, there was an increased prevalence of brown pigmentation of the spleen, both at the highest dietary concentration. The NOAEL was therefore 100 mg/kg in both sexes, equal to 4.74 mg of paraquat ion/kg bw per day for males and 5.14 mg of paraquat ion/kg bw for females on the basis of reduced body-weight gain and reduced food and water intake at the highest dietary concentration, together with pathological changes in the lungs and spleen (Maita et al., 1980b).

Dog

In a 6-week study, groups of three male and three female beagle dogs received technical-grade paraquat (purity, 32.2%) at a dietary concentration of 35 or 90 mg/kg as paraquat ion (equivalent to 0.875 and 2.25 mg of paraquat ion/kg bw per day) for 6 weeks. An additional group of three males and three females received capsules containing paraquat at a dose of 0.75 mg of paraquat ion/kg bw per day, also for 6 weeks. The controls from Sheppard (1981b) were used (see below) and the results were also compared with the group receiving paraquat at 20 mg/kg in that study, as this is comparable to the dose of 0.75 mg/kg bw per day in capsules. Animals were observed periodically during the working day, and by a veterinarian before the start of the study and preterminally. Ophthalmoscopy and auscultation of the chest were undertaken before the start of the study and before termination. Body weights were recorded weekly and food consumption was measured daily. Blood was taken for clinical pathology before the start of treatment, and after 3 and 5 weeks of treatment. Urine analysis was carried out. Lungs and kidneys were weighed at necropsy, and these organs and portions of other selected organs were processed for histopathological examination. There were no adverse clinical effects, nor were there any paraquat-related effects on ophthalmoscopy. On auscultation, increased respiratory sounds were heard in animals from several groups: of these, the finding in two males and three females at 90 mg/kg may have been test material-related. Body weights decreased in males at a dietary concentration of 90 mg/kg throughout the study and in the females at 90 mg/kg towards the end of the study. Body-weight gain was reduced in those females given paraquat at 0.75 mg/kg bw per day. Food intake was reduced at 90 mg/kg in females towards the end of the study. In the males fed paraquat at a dietary concentration of 90 mg/kg, there was a reduction in erythrocyte volume fraction, haemoglobin and erythrocyte count. No test material-related findings were seen in clinical chemistry investigations or urine analysis. One female fed the diet containing paraquat at 90 mg/kg had a markedly increased lung weight. Changes were seen at 0.75 mg/kg bw per day and at 90 mg/kg in the lungs, both macroscopically and microscopically. The macroscopic changes comprised grey, red or purple depressed areas. In all animals receiving capsules containing paraquat at 0.75 mg/kg bw per day, and in five of the six animals receiving diet containing paraquat at a concentration of 90 mg/kg, there was histopathological evidence of alveolitis, such as intra-alveolar accumulations of mononuclear cells, interstitial hypercellularity and fibrosis and alveolar hyperplasia. Occasional giant cells and pigmented macrophages were seen. It was concluded that for paraquat administered in the diet the NOAEL was 35 mg/kg (equivalent to 0.875 mg of paraquat ion/kg bw per day) and that paraquat was more toxic when administered by capsule than when mixed with the diet (Sheppard, 1981a).

In a 13-week study, groups of three male and three female beagle dogs received paraquat (paraquat ion, 32.2% w/w) at a dietary concentration of 0, 7, 20, 60 or 120 mg/kg as paraquat ion. These dietary concentrations are equal to doses of 0, 0.20, 0.55, 1.75 and

3.52 mg of paraquat ion/kg bw per day in males and 0, 0.24, 0.71, 1.92 and 4.26 mg of paraquat ion/kg bw per day in females. Animals were observed more than once daily, and by a veterinarian before the start of treatment and after 6 and 12 weeks of treatment. Ophthalmoscopy was carried out before the start of the study and after 6 weeks of treatment. Auscultation of the chest was carried out before the start of the study, 6 weeks after the start and immediately before the end of the study. The animals were weighed weekly and food consumption was measured daily. Blood samples were taken by jugular venepuncture before the start of treatment and after 6 and 12 weeks of treatment. These samples were used for haematological investigations and for clinical chemistry. At autopsy, selected organs were weighed and these and other selected organs were fixed and processed for histopathological examination. At the highest dietary concentration two males and two females were killed in extremis. These animals exhibited marked dyspnoea as well as increased respiratory sounds (harsh râles) and loss of body weight before they were killed. One female at 200 mg/kg showed pyrexia and malaise at 3 weeks; this was treated successfully with procaine penicillin and dihydrostreptomycin. The same animal showed loss of appetite from week 8; it was again treated with antibiotics. Survivors at the highest dose showed body-weight loss. Slight but significant reductions in weight gain were seen in females at 7, 20 and 60 mg/kg, compared with the controls; there was no clear dose–response relationship. These effects were not considered to be related to treatment. Food consumption was reduced in one female at the highest dietary concentration. Retinal engorgement was seen in one animal each at 7 mg/kg and 20 mg/kg, and two at 120 mg/kg. No intergroup treatment-related haematological or clinical chemistry findings were present, except in one of the decedents where haemoconcentration was seen. No test material-related effects on urinary parameters were seen. Absolute and relative lung weights were increased in all animals at 120 mg/kg and in two animals at 60 mg/kg; while not statistically significant (lungs from only two animals of each sex were weighed at the highest dietary concentration), these findings were considered to be biologically significant. Histopathological changes were seen in the lungs at 60 and 120 mg/kg. These changes consisted of proliferative alveolitis, with interstitial cellular infiltration (eosinophils and polymorphs) and exudate. Some renal (distal tubular) changes were seen in the same groups. The NOAEL was considered to be 20 mg/kg, equal to 0.55 mg/kg of paraquat ion per kg bw per day in males, and 0.71 mg/kg of paraquat ion per kg bw per day in females, on the basis of increases in lung weight and histopathological changes at the next higher dietary concentration (Sheppard, 1981b).

In a 1-year feeding study, groups of six male and six female beagle dogs were given diets containing technical-grade paraquat dichloride (paraquat ion, 32.2%) at a concentration of 0, 15, 30 or 50 mg/kg as paraquat ion for 1 year. Although no overall means were given in the study report, they were quoted in the submission document. Intakes were 0, 0.45, 0.93 and 1.51 mg of paraquat ion/kg bw per day for males and 0, 0.48. 1.00 and 1.58 mg of paraquat ion/kg bw per day for females (see Clapp, 2002). The dogs were observed twice daily, and examined by a veterinarian before the start of the study and after 13, 26 and 39 weeks, and also between weeks 48 and 51 of treatment; the examination by the veterinarian included auscultation and ophthalmoscopy. Body weights were measured weekly and food consumption daily. Haematology and clinical chemistry measurements were carried out during the study on jugular venous blood samples taken before the start of the study and at weeks 4, 8, 12, 16, 20, 39 and 52. Urine for urine analysis was collected over an 18 h period before the start of the study, and at weeks 8, 16, 24, 39 and 50. Urine samples were collected at week 39 for analysis for paraquat. At termination, necropsy was undertaken and selected organs were weighed, and these and other organs were processed for

histopathological examination. Samples of kidney, liver and lung, taken at necropsy, were analysed for paraquat. Respiratory dysfunction was observed at 50 mg/kg (hyperpnea). Increased vesicular sounds were heard in the lungs at auscultation. Erythema of the dorsum of the tongue was seen at 30 and 50 mg/kg in males, and at 50 mg/kg in females. No test material-related effects were seen on ophthalmoscopy. No test material-related effect on body-weight gain was seen. Reduced food consumption was seen at 50 mg/kg. No haematological changes were seen that were attributable to paraquat. Alkaline phosphatase activity was elevated in females at 30 and 50 mg/kg, and plasma concentrations of triglycerides were raised in both sexes at 50 mg/kg. Urinary specific gravity was elevated at 50 mg/kg in males. Lung weights (both absolute and relative) were significantly increased at 50 mg/kg. Spleen weights were elevated at 50 mg/kg in both sexes, but the biological significance was unclear, and the mean in males was influenced by one outlier. At 30 and 50 mg/kg, macroscopically there was yellow discoloration in the lungs. Microscopically, there was peribronchial mononuclear infiltration, peribronchial and interalveolar fibrosis and changes in the alveolar epithelium (alveolar cell hyperplasia and hypertrophy). These changes were accompanied by the presence of haemosiderin-laden macrophages. These changes were more severe at 50 mg/kg than at 30 mg/kg. Erythrophagocytosis in the bronchial lymph nodes was present at 30 mg/kg and 50 mg/kg. A dose-related increase in urinary paraquat was found at week 29. Paraquat was not found in the livers at any dietary concentration, but was found in the kidneys at 30 and 50 mg/kg. Paraquat was detected in the lungs. The NOAEL for the study was 15 mg/kg on the basis of erythema of the tongue at 30 mg/kg in males, elevated alkaline phosphatase in both sexes, and histopathological changes in the lung at ≥ 30 mg/kg. This NOAEL is equal to 0.45 mg of paraquat ion/kg bw per day (Kalinowski et al., 1983).

Cows

Groups of two Friesian cows were fed diets containing paraquat (as residues in dried grass) at a concentration of 25, 80 or 170 mg/kg as paraquat ion for 3 months. These dietary concentrations were equivalent to 0.375, 1.2 and 2.55 mg of paraquat ion/kg bw per day. During the trial, milk was collected from the cows. After they had been slaughtered, autopsy was carried out and organs, inter alia lung, liver and kidney, were examined histopathologically. Concentrations of paraquat were determined in the liver, kidney, renal fat and the pectoralis and adductor muscles. No adverse clinical effects were noted during the study, although the milk yield decreased (this was attributed to poor nutrition). No histopathological change attributable to paraquat was found. The concentration of paraquat in the milk was very low (in one sample, 0.001 mg/kg; in the remainder, <0.0006 mg/kg). The highest tissue residues were in the kidney (0.20–0.31) and liver (<0.01–0.09). Concentration in cardiac and skeletal muscle and renal fat were much lower. The NOAEL was the highest dietary concentration, 170 mg/kg, equivalent to 2.55 mg of paraquat ion/kg bw per day (Edwards et al., 1974).

(b) Dermal application

Rabbits

In a 21-day study of dermal toxicity, groups of six male and six female New Zealand white albino rabbits were given technical-grade paraquat (purity, 33.5%), at a dose of 0, 1.5, 3.4, 7.8 or 17.9 mg/kg bw per day (equal to 0, 0.5, 1.15, 2.6 and 6.0 mg of paraquat ion/kg bw per day), applied in distilled water under an occlusive dressing to the clipped dorsal thorax. Distilled water without paraquat was applied to the control animals. The

period of exposure was 6 h per day. Animals were observed twice daily. They were more thoroughly examined and dermal irritation was assessed on days 1, 2, 4, 8, 11, 15, 18 and 21. Animals were weighed twice weekly and food consumption was measured weekly. Blood samples for haematology and clinical chemistry were collected before the start of the study and at termination. After 21 days, the animals were weighed and killed, and designated organs were weighed. These and further selected tissues were fixed and processed for microscopic pathological examination. No mortality was observed and all animals appeared to be clinically normal throughout the study. Body weights and food consumption were similar in all groups. No differences between the groups were seen in haematological measurements or clinical chemistry. Neither organ weight data nor histopathological examination showed evidence of test material-related systemic toxicity. Evidence of skin irritation was seen at the two highest doses. Microscopic evidence of skin irritation was seen in most animals at the highest dose and in some animals at a dose of 2.6 mg of paraquat ion/kg bw per day. Findings included erythema, erosion, ulceration, exudate, acanthosis and chronic inflammatory change. Accordingly, the NOAEL was 1.15 mg of paraquat ion/kg bw per day on the basis of skin changes at higher doses (Cox, 1986).

(c) Exposure by inhalation

Rats

In a 3-week inhalation study, an aerosol of technical-grade paraquat (paraquat ion, about 40%) was administered to groups of 36 male and 32 female albino Sprague-Dawley CD rats The rats were exposed for 6 h per day, 5 days per week, for 3 weeks (i.e. 15 exposures). There were two control groups, one of which received no exposure to aerosol and the other received a saline aerosol. There were two test groups, one of which received aerosolized paraquat at a concentration of 0.01 µg of paraquat ion/l and the other 0.1 µg of paraquat ion/l. Particles had aerodynamic diameters of <0.7 µm. The rats were examined twice daily and, more thoroughly, once a week. Animals were weighed daily for the first week and then twice per week. Food consumption was measured weekly. Water consumption was measured daily, 5 days per week. Interim kills were carried out as follows: 3 days after the first exposure, four males and four females in each group were killed for histopathological examination of the nasal passages, pharynx, larynx and lungs (i.e. the rats were given a single exposure, left for 2 days, and then sacrificed). Additionally, 1 day after the third exposure, four males in each group were killed for examination of the nasal turbinates only. Eight animals of each sex per group were killed after the last exposure and the remainder (16 animals of each sex per group) were killed after a 3-week recovery period. Macroscopic examination was carried out post mortem but no microscopic pathology was performed. No treatment-related clinical signs were seen. No treatment had any effect on body-weight gain or food or water consumption. Aerosol containing paraquat ion at a concentration of 0.01 µg/l did not produce histopathological changes in the larynx, while exposure to aerosol containing paraquat ion at a concentration of 0.1 µg/l did produce such changes. In the animals examined 3 days after exposure at 0.1 µg of paraquat ion/l, there was squamous metaplasia at the base of the epiglottis. One day after the third exposure, there was ulceration, necrosis, acute inflammatory change and squamous metaplasia and/or hyperplasia especially at the base of the epiglottis and arytenoid processes. In those animals sacrificed in the interim for examination of the turbinates, no abnormalities were seen. Accordingly, the NOAEL for the study was 0.01 µg of paraquat ion/l on the basis of histopathological changes in the upper respiratory tract at the higher dose (Grimshaw et al., 1979).

2.3 Long-term studies of toxicity and carcinogenicity

Rats

In a 104-week study, groups of 80 male and 80 female Fischer (F344/DuCrj) rats were given diets containing paraquat dichloride (purity, >98%) at a concentration of 0, 10, 30, 100 or 300 mg/kg. Intakes of paraquat dichloride were 0, 0.35, 1.06, 3.52 and 10.6 mg/kg bw per day for males, and 0, 0.43, 1.34, 4.32 and 11.7 mg/kg bw per day for females. These intakes of paraquat dichloride represented intakes of 0, 0.26, 0.77, 2.55 and 7.67 mg of paraquat ion/kg bw per day in males, and 0, 0.31, 0.97, 3.13 and 8.47 mg of paraquat ion/kg bw per day in females. Eight rats of each sex were killed at 26, 52 and 78 weeks, while the survivors were sacrificed at 104 weeks. During the study, animals were observed daily and clinical findings, including mortality, were recorded. Animals that died during the study were subjected to necropsy followed by histopathological examination, as were those that were sacrificed in extremis. Body weight was measured weekly until week 26 of the study, and fortnightly thereafter. Food and water consumption was measured twice per week. At termination, haematological and clinical chemistry studies were carried out on 10 males and 10 females per group. At necropsy, selected organs were weighed and portions of these and of other organs were fixed and processed for histopathological examination. No clinical effect attributable to the test material was seen, but there was some indication of increased mortality between weeks 66 and 74 of the study in females at the highest dose. There was a reduction in body-weight gain and food and water consumption in both sexes at 300 mg/kg (the highest dietary concentration). The effect on body-weight gain was greater in the males. Some effects on haematology were observed. At 26 weeks, there was a decrease in white blood cell count at 300 mg/kg in males, but no differences between groups were seen in females. At 52 weeks, there were minor changes in mean corpuscular haemoglobin and haemoglobin concentration, and a decrease in white blood cell count at 300 mg/kg in males, but no differences between groups were seen in females. At 78 weeks, there was a decrease in white blood cell count at 300 mg/kg in males, but no test material-related differences between groups were seen in females. At 104 weeks, in males, there were minor changes in mean corpuscular volume and mean corpuscular haemoglobin. In females, at 104 weeks, there were minor changes in mean corpuscular haemoglobin concentration (a reduction) at 300 mg/kg. At 26 weeks, in males, a reduction in aspartate aminotransferase activity and globulin was observed at 300 mg/kg, as well as a rise in blood concentrations of glucose at 100 and 300 mg/kg. At 26 weeks, in females, an increase was seen in γ-glutamyl transpeptidase at 300 mg/kg, and a decrease at 10 mg/kg. At 26 weeks, total protein, albumin and globulin concentrations were all decreased in females at 300 mg/kg. At 52 weeks, in males, a reduction in aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase activity, and in cholesterol and calcium concentrations was seen at 300 mg/kg. At 52 weeks, females showed no test material-related changes in clinical chemistry. At 78 weeks, males showed reductions in alkaline phosphatase, alanine aminotransferase and γ-glutamyl transpeptidase activity were seen accompanied by an increase in albumin and a decrease in globulin at 300 mg/kg, whereas females showed no test material-related changes in clinical chemistry. At termination at 104 weeks, a decrease in globulin was seen in males at 300 mg/kg, while no noteworthy changes in clinical chemistry parameters were seen in females. In males at the highest dietary concentration, body weight at necropsy was decreased at 26, 52 and 78 weeks and at termination. Although some changes in organ weights were observed, many of these did not appear to be test material-related. At 26 weeks in males at 300 mg/kg, however, relative but not absolute lung weight was increased, as it was at 52 weeks. At 300 mg/kg, at 78 weeks, absolute lung weight was decreased and relative lung weight in males did not differ from those of controls, while at

termination, neither value was different from that of controls. In females, at 26 weeks, a reduction in body weight was not seen at any concentration. At 26 weeks, an increase in relative lung weight was seen at the two higher dietary concentrations, and this was accompanied by an increase in absolute lung weight at 100 mg/kg only. At 52 weeks and 78 weeks, there were no differences between groups in body weight or absolute or relative lung weight in females. At termination, in females, decreased body weight and an increased relative but not absolute lung weight was observed at 300 mg/kg. A reduction in absolute and relative ovarian weight was observed at 26 weeks at the highest dietary concentration. On histopathological examination, there were changes in the lungs at 300 mg/kg in both sexes and at 100 mg/kg in males. The changes consisted of proliferation of interalveolar septum cells and hyperplasia of the alveolar epithelium. The frequency of pulmonary adenoma was increased in females at 300 mg/kg (see Table 2). Histopathological evidence of cataract was found in both sexes at 300 mg/kg (see Table 3). The NOAEL for the study was 30 mg/kg (1.06 mg of paraquat dichloride/kg bw per day and 1.34 mg of paraquat dichloride/kg bw per day in males and females respectively) on the basis of clinical chemistry changes in males, increased lung weight in females and histopathological changes in the lungs of males at ≥100 mg/kg. These NOAELs are equal to 0.77 and 0.97 mg of paraquat ion/kg bw per day in males and females respectively (Yoshida et al., 1982).

In a study of chronic toxicity, groups of 62 male and 62 female JCL:Wistar rats were fed diets containing paraquat (purity, 98%) at a concentration of 0, 6, 30, 100 or 300 mg/kg for up to 104 weeks. These dietary concentrations provided intakes of paraquat dichloride equal to 0, 0.25, 1.26, 4.15 and 12.25 mg/kg bw per day in males, and 0, 0.3, 1.5, 5.12, 15.29 mg/kg bw per day in females. These intakes are equal to 0, 0.18, 0.91, 3.00 and 8.87 mg of paraquat ion/kg bw per day in males, and 0, 0.22, 1.09, 3.71 amd 11.1 mg of paraquat ion/kg bw per day in females. Six rats of each sex per group were killed at 26 weeks and 52 weeks; the survivors were killed at 104 weeks. The rats were examined twice per day, deaths were recorded and clinical findings noted. Ophthalmoscopy was carried out before treatment, and before sacrifice in those killed at 26 and 52 weeks, and at termination. Body

Table 2. Incidence of lung tumours in rats fed diets containing paraquat (survivors + decedents)

Sex	Lung tumour	Dietary concentration (mg/kg)				
		0	10	30	100	300
Males	Adenoma	1	2	3	4	3
	Adenocarcinoma	0	0	2	1	3
Females	Adenoma	1	2	0	1	7
	Adenocarcinoma	0	0	0	0	0

From Yoshida et al., (1982)

Table 3. Incidence of cataract in rats fed diets containing paraquat (decedents + survivors)

	Dietary concentration (mg/kg)				
	0	10	30	100	300
Males	8	4	7	9	46
Females	7	7	8	11	42

From Yoshida et al. (1982)

weight and food consumption were measured weekly until week 26 and thence fortnightly. Haematological and clinical chemistry end-points were measured in blood samples taken from animals killed at 26 weeks, at 52 weeks, and from the survivors at termination. Included among the clinical chemical parameters measured were activities of plasma, erythrocyte and brain cholinesterases. Urine analysis was performed on the animals killed at 26 and 52 weeks and on the survivors at termination. Animals killed at 26 and 52 weeks and survivors to termination were subjected to necropsy, as were decedents. Selected organs were weighed and these and other organs were fixed and processed for histopathological examination. No clinical effects were observed. At the highest dietary concentration in females, there was a decrease in weight gain in the middle of the study (weeks 43, 42–48 and 54), otherwise body-weight gain was unaffected. No substantial intergroup differences in food consumption or in water intake were noted. At week 26, at the highest dietary concentration, there was a decrease in erythrocyte count, erythrocyte volume fraction and haemoglobin and a reticulocytosis in males and in the erythrocyte count and haemoglobin in females. At 300 mg/kg, at week 52, decreased erythrocyte count and increased numbers of polymorphs were seen in males, and lowered erythrocyte count, haemoglobin concentration and leukocytes were seen in females. At week 104, both sexes showed decreases in erythrocyte count, erythrocyte volume fraction and haemoglobin, and an increase in reticulocytes was observed in males. At 26 weeks, a decrease in total protein was seen in both sexes at 300 mg/kg, with a decrease in alkaline phosphatase activity in females at this dietary concentration. At week 52, decreased total protein was found in both sexes, as well as reduced blood concentrations of glucose in males and reduced aspartate aminotransferase and alanine aminotransferase activites in females. There were no differences between the groups in urine analysis at any time-point. In the rats sacrificed at week 26, there were increases in absolute kidney weights (right kidney only) in males and in both absolute kidney weights in females and absolute ovarian weights in females. At week 52, at the highest dietary concentration, in males there was an increase in both absolute thyroid and kidney weights, while females showed an increase in absolute ovarian weights and a decrease in the relative weights of the brain, heart and liver. At termination, at 300 mg/kg, males showed reductions in the absolute and relative heart weights, while females showed lowered absolute and relative liver weights and decreased absolute heart weight. At necropsy and histopathological examination, no findings could be attributed to the test material. The NOAEL was therefore 100 mg/kg in both sexes (equal to 4.15 and 5.12 mg of paraquat dichloride/kg bw per day in males and females respectively), on the basis of haematological observations and lowered plasma concentration of total protein at the highest dietary concentration. These NOAELs are equal 3.00 and 3.71 mg of paraquat ion/kg bw per day in males and females, respectively (Toyoshima et al., 1982).

Groups of 70 male and 70 female Fischer 344 rats were given diets containing paraquat (technical grade, 32.69%) at a dietary concentration of 25, 75 or 150 mg/kg as paraquat ion (equivalent to a dose of 1.25, 3.75 or 7.5 mg of paraquat ion/kg bw per day) for a period of at least 113 weeks (males) and 122 weeks (females). Two additional groups of rats served as controls. There were also additional satellite groups of five males and five females from one control group and all three test groups which were sacrificed at 1 year for estimation of paraquat concentrations in certain tissues. Ten male and ten female rats from each group were sacrificed for histopathological examination at 1 year. Rats were inspected once or twice daily, mortality was recorded and rats in extremis were sacrificed and necropsied (see below). Ophthalmoscopic examination of both eyes was carried out before the start of the study and after 4, 14, 26, 52 and 79 weeks of treatment for 20 males and 20 females from each control group; the test groups were examined in a similar manner.

Surviving males were examined ophthalmoscopically at 110 weeks and 112/113 weeks (termination) and surviving females at 110 and 118/119 (termination). Food consumption was recorded weekly and water consumption was recorded over 3-day periods during each of the first four weeks, and during weeks 13, 26, 41, 52, 65, 78, 92 and 101. Body weight was recorded weekly for the first 12 weeks, then fortnightly until week 68, and then weekly until termination. Before the start of treatment and after 14, 26, 40, 53, 66, 79, 92 and 102 weeks, blood was taken for measurement of haematological and clinical chemistry parameters, and additionally in males at 111/112 weeks and in females at 118/119 weeks. Urine samples were collected periodically for urine analysis and for analysis of paraquat in the urine. Five animals of each sex per group were sacrificed at 52 weeks for estimation of concentrations of paraquat in the liver, lungs, kidneys, skin, plasma and urine. Necropsy was performed on all decedents, the 10 animals of each sex per group sacrificed at 52 weeks and those surviving to termination, and selected organs were weighed. These and other selected organs were preserved and processed for histopathological examination. Mortality was not affected by treatment and survival to termination was 38–55% in males and 47–50% in females. No clinical adverse effect was seen, except corneal opacity, which was seen at 150 mg/kg in males and at 75 mg/kg in females. At ophthalmoscopy, cataracts were seen at 150 mg/kg in both sexes and, from 103 weeks, at 75 mg/kg in both sexes. In the males, the prevalence of cataracts was not unequivocally increased at 25 mg/kg; however, a statistical analysis of the eye changes revealed an increase in posterior capsular changes at week 110 in the males at 25 mg/kg. Food intake at 150 mg/kg was reduced in both sexes, in the males for the first year of the study and in the females during the first 6 weeks; these changes were small. Depression of body-weight gain was seen at 150 mg/kg in both sexes, but was more severe in males and was also present in males at 75 mg/kg. Body-weight gain in males at 25 mg/kg and in females at 75 and 25 mg/kg was not different from that in the controls. Test material-related effects were not seen on haematological or clinical chemistry parameters, or on urine analysis. At 52 weeks, the concentration of paraquat in the urine was dose-related. In rats sacrificed at 52 weeks, paraquat was detected in the plasma and kidneys at all dietary concentrations, while paraquat was present in the lungs of animals at 75 and 150 mg/kg; only at 150 mg/kg and in females was paraquat found in the liver. Paraquat was found in some skin samples taken from males at 75 mg/kg and from both sexes at 150 mg/kg. No test material-related effects were seen on organ weights, other than those attributable to changes in body weight. Macroscopically, there was an increase in corneal opacity and focal subpleural changes at 75 and 150 mg/kg. Proliferative alveolar changes were also seen at these dietary concentrations. Lung histopathology was examined by two groups (Tables 4–6). An

Table 4. Initial assessment of lung histopathology in rats given diets containing paraquat

	Dietary concentration (mg/kg)									
	0 (Control group 1)		0 (Control group 2)		25		75		150	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Number of rats examined	70	70	69	69	70	70	70	70	69	70
Adenoma	1	0	2	0	3	1	5	2	4	8[a]
Carcinoma[b]	1	0	0	0	1	1	1	1	3	2
Total neoplasia	2	0	2	0	4	2	6	3	7	10[a]
Alveolar epithelialization	2	3	2	7	2	5	7	8	8	3

From Woolsgrove (1983)

[a] $p < 0.001$

[b] Bronchiolar-alveolar or squamous cell carcinomas

Table 5. Second assessment of lung histopathology in rats given diets containing paraquat

	Dietary concentration (mg/kg)									
	0 (Control group 1)		0 (Control group 2)		25		75		150	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Number of rats examined	70	70	69	69	70	70	70	70	69	70
Adenoma	0	0	0	0	2	0	1	1	1	0
Carcinoma	1	0	1	0	2	1	1	1	3	0
Adenomatosis	2	4	4	4	5	5	8	4	11[a]	13[a]

From Ishmael & Godley (1983)
[a] $p < 0.01$

Table 6. Final assessment of lung histopathology in rats given diets containing paraquat

	Dietary concentration (mg/kg)									
	0 (Control group 1)		0 (Control group 2)		25		75		150	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Number of rats examined	70	70	69	69	70	70	70	70	69	70
Bronchioalveolar adenoma	2	0	0	0	2	0	0	1	0	1
Bronchioalveolar carcinoma	1	0	1	0	2	1	2	1	2	1
Squamous cell carcinoma	0	0	0	0	0	0	1	0	2	0
Focal adenomatous hyperplasia	2	4	3	5	7	5	9	7	15	7
Diffuse adenomatous hyperplasia	0	1	0	0	0	0	0	0	1	3
Focal alveolar wall fibrosis	1	8	4	5	4	8	6	13	3	12
Diffuse alveolar wall fibrosis	0	3	0	5	2	3	3	4	8	3

From Busey (1986)
[a] $p < 0.01$

initial assessment of lung histopathology was made based on that of Life Sciences Research's own staff pathologists and of two consultant pathologists from the USA (Table 4). The other assessment was by Ishmael (Table 5), at that time Head of Pathology at Imperial Chemical Industries. There were some clear differences. Finally, the slides were examined by four independent pathologists and the results were reported by Busey (1986) (Table 6). It was concluded that the differentiation of bronchiolar adenomas and carcinomas from the non-neoplastic lesions typical of paraquat was difficult. However it was also concluded that the incidence of lung neoplasms in the test groups was comparable to that in the control groups (Ishmael & Godley, 1983; Woolsgrove, 1983; Sotheran et al., 1981; Woolsgrove, 1985; Busey, 1986; Ishmael, 1987).

It was concluded from the data summarized in Table 6 that there was no association between the incidence of adenomas, carcinomas or the two combined, and exposure to paraquat. In contrast, there was a significantly increased incidence of adenomatosis at 150 mg/kg when all animals were included in the analysis (i.e. those sacrificed at 52 weeks, decedents and those killed at termination).

Ishmael (1987) reviewed the slides of the head region, in which squamous cell carcinomas of the skin and subcutis had been reported. In males, 11 such tumours were seen in the study (1, 2, 2, 0 and 6 in the two control groups and at the lowest, intermediate and

highest doses, respectively) as originally reported and in Ishmael (1987). The site of origin of these tumours, however, differed and Ishmael (1987) suggested they should not be considered as a single phenomenon for statistical purposes. Other changes seen included dilatation of the fourth ventricle of the brain (hydrocephalus) in females at 75 and 150 mg/kg. Cysts and cystic spaces were seen in the spinal cords and, in males, prevalence was significantly greater than that in the controls in all test groups, although there was no clear dose–response relationship. This pathological change was found in females, but the frequency in test groups and control groups was similar (and similar to the frequency in the males in test groups). Degeneration of the sciatic nerve was found in males at 75 and 150 mg/kg. Changes were present in the eyes. At the highest doses, peripheral lenticular degeneration, more severe in females, and pear-shaped posterior peripheral lenticular change was seen. Mid-zonal lenticular degeneration, lens capsular fibrosis and/or lens ruptures were all seen. At 75 mg/kg, changes were milder. These changes were seen in both decedents and those rats surviving to termination. At the highest concentration, in the decedents, peripheral retinal degeneration was observed in females and proteinaceous vitreous humour was seen in males. Some changes were seen at the lowest dietary concentration; in male survivors these were moderate peripheral morgagnian corpuscles, slight peripheral lenticular degeneration, moderate mid-zonal lenticular degeneration and loss of outer nuclear layer of the retina. The last was unlikely to be a compound-related effect as the prevalence was lower in both the controls and at higher doses. In female survivors to termination, at the lowest dietary concentration, changes observed were moderate peripheral morgagnian corpuscles, slight peripheral lenticular degeneration and moderate mid-zonal lenticular degeneration.

At termination, there was no clear evidence of an effect on the retina at the lowest dose, although in males at the two higher dietary concentrations there may have been an effect on the periphery of the retina. This study was continued for a longer duration than that recommended by the OECD (104 weeks for long-term studies in rats). The NOAEL was 25 mg/kg for lenticular lesions after 103 weeks at 25 mg/kg in males and likewise in females at 103 and 110 weeks (see Table 7 for ophthalmoscopy findings at 103 weeks and Table 8 for lens findings at necropsy). This NOAEL is equivalent to 1.25 mg of paraquat ion/kg bw per day. This interpretation is supported by the findings from the other long-term studies in rats.

Table 7. Frequency of effects on the lens (in life) at 103 weeks in rats given diets containing paraquat

Finding	Dietary concentration (mg/kg)									
	0 (Control group 1)		0 (Control group 2)		25		75		150	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Opacity	1	0	4	0	0	0	0	1	0	0
Vacuolation	0	0	1	0	0	0	0	0	1	1
Suture line opacity	0	1	1	0	1	0	14*	9*	1	1
Posterior polar opacity/cataract	3	0	0	0	1	0	8	5	19	30
Posterior capsular opacity/cataract	0	2	0	5	0	4	3*	6*	24*	12*
Radial cataract	0	0	0	0	1	0	2	2*	8*	5*
Total cataract	1	1	1	1	2	1	3	1	5*	4

From Ishmael (1987)

* Greater incidence than combined control groups, statistically significant at $p = 0.05$ or less

Table 8. Frequency of effects on the lens at necropsy in rats given diets containing paraquat (all animals, regardless of time of death)

Finding	Dietary concentration (mg/kg)							
	0 (Control groups 1 and 2)		25		75		150	
	Males	Females	Males	Females	Males	Females	Males	Females
Number of eyes examined	219	226	112	112	114	107	115	114
Peripheral morgagnian corpuscles								
Slight	64	50	29	14	26	8*	7*	8*
Moderate	38	70	31	39	19	27	25	16
Marked	12	34	19*	31*	35*	52*	69*	84*
Peripheral lenticular degeneration								
Slight	18	60	25*	29	32*	23	26*	10*
Moderate	8	33	13*	30*	39*	31*	34*	43*
Marked	1	7	4	4	6*	10*	22*	32*
Pear-shaped posterior lenticular change	6	42	11*	32*	51*	48*	73*	74*
Midzonal lenticular degeneration								
Slight	7	27	5	20	18*	14	14*	19
Moderate	0	12	4*	13	19*	27*	39*	37*
Marked	0	0	0	3	3*	23*	29*	27*
Heart-shaped	0	2	0	0	1	18*	18*	15*

From Ishmael (1987)
* Greater incidence than combined control groups, statistically significant at $p = 0.05$ or less

Mice

In a 104-week study, groups of 80 male and 80 female JCL:ICR mice were given diets containing paraquat (paraquat dichloride; purity, 98%) at a dietary concentration of 0, 2, 10, 30 or 100 mg/kg, providing intakes of paraquat dichloride equal to 0, 0.26, 1.31, 3.92 and 13.09 mg/kg bw per day in males, and 0, 0.26, 1.32, 3.82 and 13.03 mg/kg bw per day in females. These intakes are equal to 0, 0.19, 0.95, 2.84 and 9.48 mg of paraquat ion/kg bw per day in males, and 0, 0.19, 0.96, 2.77 and 9.43 mg of paraquat ion/kg bw per day in females. At weeks 26 and 52, 10 male and 10 female per group were sacrificed. The mice were examined twice daily and adverse clinical effects, including mortality, were noted. Body weight and food consumption were measured weekly until week 26, and fortnightly thereafter. Blood was taken for haematology and clinical chemistry (including determination of plasma, erythrocyte and brain cholinesterase activities) from the animals killed at 26 and 52 weeks and from those that survived to termination. Urine analysis was performed on animals killed at 26, 52 and 104 weeks. Survivors were sacrificed at 104 weeks. Necropsy was carried out on the animals killed at 26 and 52 weeks and on those that survived to termination, as well as the decedents. Selected organs were weighed and tissue from these and further selected organs was fixed and processed for histopathological examination. There were no effects of the test material on mortality. No clinical effects attributable to the test material were noted. The test material had no effect on body-weight gain or food consumption. Falls in the erythrocyte count, erythrocyte volume fraction, haemoglobin, white blood cell count and lymphocyte count were noted in males and in the haemoglobin concentration and white blood cell count in females at 100 mg/kg in week 26. At week 52, also at 100 mg/kg, a decreased erythrocyte count, and decreases in erythrocyte volume fraction and white blood cell count were observed in males and lowered erythrocyte count and

haemoglobin concentration in females. At week 104, lowered erythrocyte count, erythrocyte volume fraction and polymorphonuclear leukocytes (%) were observed in males, and decreases in the erythrocyte volume fraction and haemoglobin concentration in females. Clinical chemistry findings included lowered total plasma protein in both sexes at the highest dietary concentration in week 26. At week 52, lowered total protein was seen in males and decreases in aspartate aminotransferase and alkaline phosphatase activites, with increased blood concentration of glucose, were seen in females. At week 104, reductions in total protein and increases in blood glucose were observed in both sexes. Urine analysis showed no abnormality at any time in either sex. The absolute and relative weights of the (left) adrenal at 30 mg/kg in males killed at 26 weeks was decreased in comparison with those of the controls. In male at 100 mg/kg, at 26 weeks, adrenal and thyroid absolute and relative weights were decreased in comparison with those of controls, and absolute and relative lung weights were elevated. At week 52, there was an increase in absolute heart weight in males, while at week 104, decreases in absolute thyroid, liver and bladder weight were noted in males, together with an increase in relative (left) kidney weight. A drop in the absolute brain weight was noted at week 104 in females. No macroscopic or microscopic changes that could be attributed to the test material were found in the decedents or sacrificed animals. The NOAEL was therefore 30 mg/kg on the basis of haematological and clinical chemistry changes in both sexes, at the next highest dietary concentration. This was equal to 3.92 and 3.82 mg of paraquat dichloride/kg bw per day in males and females, respectively (2.84 and 2.77 mg of paraquat ion/kg bw per day in males and females, respectively) (Toyoshima et al., 1982).

A lifetime feeding study in mice was carried out; termination was at 97–99 weeks, at which time mortality was approaching 80%. Groups of 60 male and 60 female Swiss mice were fed diets containing paraquat at a dietary concentration of 0, 12.5, 37.5 or 100 mg/kg (technical grade dichloride; paraquat ion, 32.7%) for up to 99 weeks. The dietary concentration of paraquat received by groups of mice at 100 mg/kg was increased to 125 mg/kg from week 36, as few adverse effects had been noted, other than a decrease in food consumption, up to that time. The intakes of test material were equivalent to 0, 1.88, 5.62 and 15.0/18.7 mg of paraquat ion/kg bw per day. The control groups were duplicated. Satellite groups of 10 male and 10 female mice received the diet for 52 weeks and were used for measurement of paraquat concentrations in plasma, kidney and lung; in the case of the satellite groups, the control groups were not duplicated. Further satellite groups of 15 mice of each sex were fed the diet and acted as microbiological sentinels. The mice were observed daily for clinical effects, while body-weight determinations were recorded weekly for 12 weeks, fortnightly from week 12–36, weekly from week 36–40 and thence fortnightly. Food consumption measurements were undertaken weekly for the first 12 weeks of the study, and during weeks 36–40; at all other times, it was measured for 1 week during each 4-week period. Urine samples for measurement of concentration of paraquat were collected at 3-month intervals. Mice in extremis were sacrificed, as were those that survived to termination. These mice and decedents were subjected to necropsy, after which histopathological examination was carried out. Tissues from the satellite group were not subjected to autopsy. The main clinical findings were swellings and sores in the genital area of the male and, to a lesser extent, female mice, accompanied by incontinence. Mortality was greater than in the combined control groups in male mice receiving paraquat at a dietary concentration of 37.5 mg/kg and in female mice receiving paraquat at a dietary concentration of 125 mg/kg. The former is unlikely to be a compound-related effect, as an increase in the mortality at the highest dietary concentration was not observed in males. Body-weight gain was unaffected in males, while body-weight gain was decreased in females at the highest dietary

concentration (but not until this dietary concentration had been raised from 100 mg/kg to 125 mg/kg) and, after week 68, at a dietary concentration of 37.5 mg/kg. Food consumption was affected: in males, food consumption was reduced at all dietary concentrations to some extent, particularly early in the study; however, the effect did not appear to be dose-related. In females, decreased food consumption at all dietary concentrations was found, but this was more consistent and severe at the highest concentration. Concentrations of paraquat in the urine were found to be dose-related. On one occasion, paraquat was found in the urine of female controls, in trace amounts. In the satellite group, concentrations of paraquat in plasma were related to the dietary concentration that the animals had received for males, but less clearly so for females. In the case of the liver and lung tissue samples, some difficulties were encountered with some samples in analysis, but where analysis was possible, the results appeared to be related to dose. Changes in the proximal tubules of the kidneys (hydropic degeneration, eosinophilia, degeneration and/or dilatation) were seen at the highest dietary concentration, and there was evidence that, in the decedents, the predominant change was hydropic degeneration, eosinophilia, while degeneration and/or dilatation were seen in survivors to termination. Some very mild renal changes were seen in males at 37.5 mg/kg. In the lungs, alveolar focal hypercellularity was found at a higher frequency at the highest dietary concentration than at the lower concentrations or in controls in both sexes. There was no evidence of differences in cataract formation between the groups. There was an increase in adenomas in males and females receiving paraquat at the highest dietary concentration and dying after 52 weeks and before termination than in controls, but this was not dose-related. Moreover, at termination, a lower incidence of these tumours was observed in these animals than in controls. The NOAEL was 12.5 mg/kg (equivalent to 1.88 mg of paraquat ion/kg bw per day) on the basis of decreased body-weight gain in females and renal changes in males at the next highest dietary concentration. Paraquat was not considered to be tumorigenic (Sotheran et al., 1981).

2.4 Genotoxicity

Paraquat has been the subject of many tests for genotoxicity (see Table 9). Paraquat consistently gave negative results in well-established assays for reverse mutation in strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100). There was one positive result in *S. typhimurium* TA102, a strain that is particularly responsive to reactive oxygen species. More variable results were obtained in the less well-established assays for forward mutation in *S. typhimurium* and in assays for DNA damage in bacteria, for example the *umu* test, SOS test, tests for DNA repair and the rec assay) and in an assay for gene mutation in *Aspergillus nidulans*. In comparison with the assays for reverse mutation in *S. typhimurium*, these assays are not well validated. The assays for mutation in plants are not well validated and no conclusions could be drawn from them. Paraquat gave fairly consistently positive results in assays for chromosomal damage in mammalian cells. Positive results were consistently obtained in assays for DNA damage (sister chromatid exchange, unscheduled DNA synthesis and the comet assay) in mammalian cells. These data suggest that paraquat has mutagenic potential in vitro.

The results of tests for genotoxicity with paraquat in *Drosophila melanogaster* were conflicting, and are in any case irrelevant to the situation in mammals in vivo. In studies of DNA damage (unscheduled DNA synthesis) in mammalian systems in vivo and of chromosome damage in germ cells (dominant lethal test), paraquat gave negative results. The results of the majority of assays for clastogenicity (metaphase analysis to investigate chromosomal aberrations or tests for micronucleus formation) in bone marrow were negative.

Table 9. Results of studies of genotoxicity with paraquat

End-point	Test-object	Concentration/Dose	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1–1000 µg/plate	>99.9%	Negative ± S9	McGregor (1977)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	0.5–500 µg/plate	100%	Negative ± S9	Shirasu et al. (1978)
Reverse mutation	*S. typhimurium*, TA1535, TA1538, TA98, TA100	0.16–5000 µg/plate	>99%	Negative ± S9	Anderson (1977)
Reverse mutation	*E. coli*, WP2 *hcr*	0.5–500 µg/plate	100%	Negative ± S9	Shirasu et al. (1978)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1–50 µg/plate	NS	Negative	Benigni et al. (1979)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	Not clear	NS	Negative	Eisenbeis et al. (1981)
Reverse mutation	*S. typhimurium* TA98, TA100	0–1 mmol/l	NS	Negative	Moody & Hassan (1982)
Reverse mutation	*S. typhimurium* TA98, TA100 (not clear what other strains were used), *E coli* WP2 *hcr*	Not clear	NS	Negative, but full results not given	Shirasu et al. (1982)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100, *E coli* WP2 *hcr*	5000 µg/plate	NS	Negative	Moriya et al. (1983)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA97, TA98, TA100	0–20 µg/plate	NS	Negative	Lin et al. (1988)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA97, TA98, TA100	0–50 µg/plate	NS	Negative	Lin et al. (1989)
Reverse mutation	*S. typhimurium* TA102	10 ng/plate	NS	Negative	Levin et al. (1984)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 *E. coli* WP2 *hcr*	0.5–500 µg/plate	NS	Negative	Shirasu et al. (1978)
Reverse mutation	*E. coli* IC203, *oxyR* deficient and WP2 *uvrA*/Pkm101	1 µg/plate	NS	Negative	Martínez et al. (2000)
Reverse mutation	*S. typhimurium* TA100, TA98	0.06 µmol/plate	NS	Negative	Nishimura et al. (1982)
Reverse mutation	*S. typhimurium* TA102, TA2638, *E. coli* WP2/Pkm101 and WP2 *uvrA*/Pkm101	0–10 µg/plate	NS	Positive	Yamaguchi (1981)
Reverse mutation	*S. typhimurium* TA100	20 µg/plate	NS	Negative	Watanabe et al. (1998)
Forward mutation	Mouse lymphoma L5178Y cells $Tk^{+/-}$	31.3–1000 µg/plate	45.66% w/w technical-grade paraquat dichloride	Negative ± S9	Clay & Thomas (1985)
Forward mutation at the *Hprt* locus	Chinese hamster V79 cells	1–5 mmol/l	NS	Negative	Speit et al. (1998)
Forward mutation to azaguanine resistance	*S. typhimurium* His G46, TA92, TA1535	0.1–1 µg/plate	NS	Positive	Benigni et al. (1979)

Table 9. Continued

End-point	Test-object	Concentration/Dose	Purity	Results	Reference
Forward mutation to azaguanine resistance	*S. typhimurium* His G46, TA92, TA1535, TA1538, TA100	0.1–2.5 µg/plate	NS	Positive	Bignami & Crebelli (1979)
Umu test	*S. typhimurium* TA1535/Psk1002	1000–3333 µg/ml	NS	Positive	Oda et al. (1985)
Umu test	*S. typhimurium* TA1535/Psk1002	1000 µg/ml	NS	Positive	Nakamura et al. (1987)
Umu test	*S. typhimurium* TA1535/Psk1002	0.1 ml/tube	NS	Negative	Degirmenci et al. (2000)
Umu test	*E. coli* K12 AB1157, AB2463 H/r30, H/s30, NG30, R15, B/r, B_{S-1}	4 mg/ml	NS	Positive	Degirmenci et al. (2000)
SOS-induced DNA damage	*E. coli* $WP2_s(\lambda)$	0.02–67.11 µmol/l	NS	Positive ± S9	DeMarini & Lawrence (1992)
SOS-induced DNA damage	*E. coli* PQ300	Not clear	99	Negative	Eder et al. (1989)
SOS-induced DNA damage	*E. coli* PQ37, PM21, GC4798	Not clear	NS	Negative	Müller & Janz (1992)
DNA repair	*S. typhimurium* TA1538, TA1978	100 µg/plate	NS	Positive	Benigni et al. (1979)
Rec assay	*B. subtilis* recombination wild-type H17 and deficient M45	1–500 µg/disc	100%	Negative	Shirasu et al. (1978)
Gene mutation	*Aspergillus nidulans* (plate assay)	0–1000 µg/plate	NS	Positive	Benigni et al. (1979)
Gene mutation	*A. nidulans* (liquid assay)	20 mg/ml	NS	Negative	Benigni et al. (1979)
Lethal recessive	*A. nidulans* (liquid assay on quiescent conidia)	20 mg/ml	NS	Positive	Benigni et al. (1979)
Intrachromosomal recombination	*Saccharomyces cerevisiae*	0–35 mg/ml	NS	Negative	Brennan et al. (1994)
DNA damage	*S. cerevisiae*	1–20 mmol/l	NS	Negative	Paesi-Toresan et al. (1998)
Gene conversion	*S. cerevisiae*	100–900 mg/kg	NS	Positive	Parry (1973)
Gene conversion	*S. cerevisiae*	1000 mg/kg	NS	Negative	Siebert & Lemperle (1974)
Reverse and forward mutation	*Nostoc muscorum* (blue-green alga)	50 and 75 mg/kg	NS	Positive	Vaishampayan (1984a)
Reverse and forward mutation	*N. muscorum* (blue-green alga)	25–75 mg/kg	NS	Positive	Vaishampayan (1984b)
Cytogenetics	*Vicia fava* (broad/fava bean)	NS	NS	Negative	Gopalan & Njagi (1979)
Somatic mutation (*Drosophila* wing spot test)	*Drosophila melanogaster*	2–8 mmol/l	99%	Negative	Torres et al. (1992)
SMART assay	*D. melanogaster*	NS	NS	Negative	Ramel & Magnusson (1992)
SMART assay	*D. melanogaster*	0–10 mmol/l	NS	Negative	Gaivao & Comendador (1996)
SMART assay	*D. melanogaster*	0–10 mmol/l	NS	Positive	Gaivao et al. (1999)
SMART assay	*D. melanogaster*	0–16 mmol/l	NS	Negative	Vontas et al. (2001)

Table 9. Continued

End-point	Test-object	Concentration/Dose	Purity	Results	Reference
Chromosome test	*D. melanogaster*, *mus* 302 repair-defective females	200 mg/kg	NS	Negative	Woodruff et al. (1983)
Forward mutation at *Tk* locus	Mouse lymphoma L5178Y cells	0–200 µg/ml	NS	Positive	McGregor et al. (1988)
Mutation to thioguanine resistance	Chinese hamster V79 cell lines transfected with bacterial *gpt* (G12, G10)	200–300 µmol/l	NS	Negative	Kitahara et al. (1996)
Chromosomal aberration	Chinese hamster cells	≤200 µg/ml	45% technical grade	Positive	Lin et al. (1987)
Chromosomal aberration	Chinese hamster cells resistant to hydrogen peroxided (H_2O_2)	50–400 µg/ml	NS	Positive	Sawada et al. (1988)
Chromosomal aberrations and sister chromatid exchange	Chinese hamster fibroblast cells	3–10 mmol/l for chromosomal aberrations, and 0–0.75 mmol/l for sister chromatid exchanges	NS	Positive	Nicotera et al. (1985)
Chromosomal aberrations and sister chromatid exchange	Chinese hamster lung cells	0.08–20 µmol/l	NS	Positive	Tanaka & Amano (1989)
Chromosomal aberration	Human peripheral blood lymphocyte culture	1–50 µg/ml, chromosomal aberrations	99%	Negative	Ribas et al. (1997/8)
Sister chromatid exchanges	Chinese hamster ovary cells	0.625–100 µg/ml	45% technical grade	Negative	Wang et al. (1987)
Sister chromatid exchanges	Rat tracheal epithelial cells	0.625–2.5 µg/ml	45% technical grade	Positive	Wang et al. (1987)
Sister chromatid exchanges	Human peripheral blood lymphocyte culture	1–4000 µg/ml for sister	99%	Positive	Ribas et al. (1997/8)
Sister chromatid exchange	Chinese hamster lung fibroblasts	1.2–245 µg/ml	99.4% dichloride	Positive –S9, effect less +S9	Howard et al. (1985)
Cytogenetics	Human lymphocytes	250–2500 µg/ml	99.6% w/w dichloride	Clastogenic at toxic doses only	Sheldon et al. (1985a)
Micronucleus formation	Human peripheral blood lymphocyte culture	1–4000 µg/ml	99%	Negative	Ribas et al. (1997/8)
Micronucleus formation, optimized to detect excision repair	Human peripheral blood lymphocyte culture	25–100 µg/ml	99%	Negative	Surrallés et al. (1995)
Unscheduled DNA synthesis	Human epithelial-like cells	20–2000 µg/plate	NS	Positive, without dose–response relationship	Benigni et al. (1979)
Unscheduled DNA synthesis	Rat thymocytes and human peripheral blood lymphocytes	Rat thymocytes: 180–1800 µg/ml, human lymphocytes: 900 µg/ml	95%	Equivocal	Rocchi et al. (1980)
Unscheduled DNA synthesis	Rat primary hepatocytes	10^{-9}–10^{-2} mol/l	Paraquat dichloride 99.6%	Negative	Trueman et al. (1985)
Comet assay for DNA damage	Human peripheral blood lymphocytes	≤2000 µg/ml for 4 h	99%	Positive (+S9) Positive (–S9)	Ribas et al. (1995)

Table 9. Continued

End-point	Test-object	Concentration/Dose	Purity	Results	Reference
Comet assay for DNA damage	Rat alveolar macrophages and epithelial type II cells	0–10 µmol/l	NS	Positive	Dušinská et al. (1998)
Comet assay for DNA damage	Chinese hamster cells	1–5 mmol/l	NS	Negative	Speit et al. (1998)
Comet assay for DNA damage	Rat astroglial cells	20–80 µmol/l	NS	Positive	Frederiksen & Clausen (1999)
Comet assay for DNA damage	Human cell line A549 and THP-1	10–100 µmol/l	NS	Positive	Don Porto Carera et al. (2001)
Comet assay for DNA damage	Human cell lines HeLa and Hep G2 and human peripheral lymphocytes	0–350 µmol/l	NS	Positive	Petrovská & Dušinská (1999)
Chromosomal damage	Chinese hamster fibroblasts	0.2–0.8 mg/ml 3 h	NS	Positive	Sofuni & Ishidate (1988)
Chromosomal damage	Chinese hamster cells	0.8 mg/ml 3 h	NS	Positive	Sofuni et al. (1988)
Chromosomal damage	Chinese hamster V79 cells	1–5 mmol/l	NS	Negative	Speit et al. (1998)
Chromosomal damage	Mouse (male and female BALB/C) bone-marrow and germ cells	Bone marrow: 7–23 mg/kg bw (single intraperitoneal dose) or 1.5, 3.0 and 5.0 mg/kg bw per day intraperitoneally for 10 days. Germ cells: 1.5, 3.0 and 5.0 mg/kg bw per day intraperitoneally for 5 days	NS	Equivocal (repeat doses); Negative (single dose)	Rios et al. (1995)
In vivo					
Micronucleus formation	Mouse (C57 Bl/6J/Alpk)	51.75, 82.8 mg of paraquat ion/kg bw (single dose by gavage)	Paraquat dichloride, 33.07% w/w paraquat ion	Negative	Sheldon et al. (1985b)
Micronucleus formation	Mouse (male Swiss albino)	83 mg/kg bw per os	NS	Positive	Prabakaran & Moorthy (1998)
Micronucleus formation	Mouse (male ICR)	2 × 15 mg/kg bw intraperitoneally	98%	Positive	Melchiorri et al. (1998)
Micronucleus formation	Mouse (pregnant female Swiss)	10 or 20 mg/kg bw subcutaneously	99%	Negative	Pena et al. (1999)
Micronucleus formation	Mouse (male Swiss)	2 × 20 mg/kg bw ip	NS	Positive[a]	Ortiz et al. (2000)
Cytogenetics	Rat (outbred Wistar-derived)	6.5–19.0 mg/kg bw, daily for 5 days (as paraquat ion, by gavage)	Paraquat dichloride, 100%	Negative (fuzzy banded cells were seen)[b]	Anderson et al. (1978)
Cytogenetics	Rat (Alpk : AP Wistar-derived)	15–150 mg/kg bw single dose by gavage	Paraquat dichloride, 33.07% w/w paraquat ion	Negative	Howard et al. (1987)
Chromosomal damage	Mouse (male CFLD)	Single dose 60 mg/kg bw per os; 2.4 mg/kg bw per os twice per week for 6 weeks; single dose 15 mg/kg bw intraperitoneally; 0.55–5.5 mg/kg bw × 5 intraperitoneally	25% paraquat ion	Negative	Selypes et al. (1978)

Table 9. Continued

End-point	Test-object	Concentration/Dose	Purity	Results	Reference
Unscheduled DNA synthesis	Rat (Alpk : AP Wistar-derived)	45–120 mg/kg bw single dose by gavage	Paraquat dichloride (technical) 33.07% paraquat ion	Negative	Trueman & Barber (1987)
DNA damage	Rat (male Wistar)	20 mg/kg bw intraperitoneally	NS	Negative	Sørensen & Loft (1999)
Dominant lethal mutation	Mouse (male Swiss-Webster)	66 mmol/kg bw per day	NS	Negative	Pasi et al. (1974)
Dominant lethal mutation	Mouse (male CD-1)	0.04–4 mg ion/kg bw per day	23.8% paraquat ion	Negative	Anderson et al. (1976)

In three tests for micronucleus formation in vivo (two using intraperitoneal administration and one using administration per os), paraquat gave positive results. In these three tests, the doses used were high; it is thus possible to conclude that paraquat may induce chromosome damage at high doses in assays in bone marrow in vivo.

The hypothesis that these effects are caused by the well-established ability of paraquat to generate reactive oxygen species, which are not detoxified at high doses owing to saturation of cellular defensive mechanisms, is likely to be the explanation for the results discussed above. For such an effect it is likely there would be a threshold as, except at high doses, reactive oxygen species are rapidly detoxified.

A mechanistic study was carried out into the ability of paraquat to produce "fuzzy-banded" chromosomes from rat bone-marrow cells (Anderson et al., 1979). From this study, it was concluded that paraquat was interfering with staining performed by the Giemsa method.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a three-generation study of reproductive toxicity, Wistar-derived Alderley Park rats were given diets containing technical-grade paraquat (25.8% paraquat ion) at a concentration of 0, 30 or 100 mg/kg. These dietary concentrations were equivalent to intakes of 0, 2.0 and 6.67 mg of paraquat ion/kg bw per day. The F_0 animals started on the test diets when aged 35 days, and they and their progeny remained on the diet throughout the study. The animals were examined daily and body weight and food consumption were recorded weekly. For the first mating, one male and two females receiving paraquat at the same dietary concentration were housed together. This was done at 105 days and produced the F_{1a} generation. The litters were examined after parturition and the number of live-born and stillborn pups recorded, together with the clinical state of the former. Offspring were examined daily for the number of live or dead offspring, and at 21 days, each litter was counted, weighed, sexed and autopsied. After 10 days, the second mating was carried out as described above. From the F_{1b} pups produced, 12 male and 24 female weanlings were selected to become the F_1 parents. The remainder of the offspring were killed and examined. At 100 days, one male and two females receiving paraquat at the same dietary concentration were housed together.

This mating produced the F_{2a} generation. The litters were examined after parturition and the number of live-born and stillborn pups was recorded, together with the clinical state of the former. Offspring were examined daily for the number of live or dead offspring, and at 21 days each litter was counted, weighed, sexed and autopsied. After 10 days, the second mating was carried out as described above. From these F_{2b} pups, 12 male and 24 female weanlings were selected to be the F_2 parents. Again two litters were produced (F_{3a} and F_{3b}), but this time all the offspring were killed and examined at 5–7 weeks. Tissues from 10 progeny of each sex per dietary concentration were examined histopathologically. No test material-related clinical effects were seen. Increased body-weight gain was seen in the groups of male rats receiving paraquat. This was particularly marked in the F_0 rats and was noted from 6 weeks onwards at both 30 and 100 mg/kg; it also occurred in the F_{1a} and F_{1b} rats, for which the body weights of females were also increased; this finding was not noted in the F_2 rats. Paraquat had no significant effect on food consumption. No adverse treatment-related effects were seen on reproductive performance (number of pregnancies to term, mean litter size, pup sex distribution and body weight at weaning). In the F_{1b} litters, the mean litter size was smaller at 30 mg/kg than in the controls or at 100 mg/kg. In the F_2 generation, litter size was increased at 100 mg/kg, but was within the range for historical controls. On histopathological examination, hydropic change was found in the renal tubules of weanlings that had been fed paraquat at a dietary concentration of 100 mg/kg. The NOAEL for the offspring was therefore 30 mg/kg (equivalent to 2.0 mg of paraquat ion/kg bw per day) on the basis of renal tubular changes in the weanlings at the highest dietary concentration. The NOAEL for the parents and for reproductive toxicity was 100 mg/kg (equivalent to 6.67 mg of paraquat ion/kg bw per day), the highest dietary concentration administered (Fletcher et al., 1972).

In another multigeneration study, Wistar-derived Alderley Park rats were fed diets containing technical-grade paraquat dichloride (32.7% w/w paraquat ion) at a concentration of 0, 25, 75 or 150 mg/kg as paraquat ion. These diets provided intakes equivalent to 0, 1.67, 5.0 and 10 mg of paraquat ion/kg bw per day. The F_0 parents comprised 15 male and 30 female rats per group; these rats were mated 12 weeks after the start of the study to produce the F_{1a} litters and 7 days after the last F_{1a} litter had been weaned (at 21 days) the F_0 parents were remated to produce the F_{1b} litters. The F_{1b} litters were weaned at 28 days. F_1 parents (30 females and 15 males per group) were selected from the F_{1b} litters and mated 11 weeks later to produce the F_{2a} and 7 days after the last F_{2a} litter had been weaned (at 28 days), remated to produce the F_{2b} litters (these were also weaned at 28 days). The F_2 parents were selected from the F_{2b} litters and mated 11 weeks later. All male parents were killed after mating to produce the F_{1b} or F_{2b} litters and the females were also killed, but after weaning of the F_{1b} or F_{2b} litters. The F_0 and F_2 parents were subjected to gross examination post mortem and the testes of all the males were fixed and processed for histopathological examination. Lungs from eight males and eight females of each group, and any abnormal tissues from all the animals were also fixed and processed for histopathological examination. Of the F_1 parents, 25 females and 10 males per group were subjected to a full autopsy and a wide range of tissues were processed for histopathological examination. Litters were examined at least daily and dead or abnormal pups removed for examination. Live and stillborn pups were counted and sexed at 24 h and 4, 10 and 21 days post partum. Pup weights were measured at 24 h and at 4, 10, 21 and 28 days post partum. All grossly abnormal pups and those found dead up to 18 days post partum were taken for teratological examination. Those aged > 18 days were taken for histopathological examination. Of the pups of the F_{1a}, F_{2a} and F_{3a} litters, about 50% were discarded, the remainder being subjected to gross necropsy; any abnormal tissues were processed for histopathological examination. After selection of

the parents for the next generation, five males and five females from the F_{1b} and F_{2b} litters and 10 males and 10 females from the F_{3b} litters were subjected to detailed histopathological examination. Test diets were fed throughout the study. The rats were observed daily, with a more detailed observation once per week, clinical observations and mortality were recorded. Body weights and food consumption were recorded weekly throughout the premating period. During the premating period, urine was taken from three males and three females per group for analysis for paraquat. After the premating period, the male rats were weighed at 4-weekly intervals. No adverse effects were noted on parental clinical status, body weights or food consumption. Mortality was seen in female F_0 and F_1 parents receiving paraquat at the highest dietary concentration, mostly during or just after suckling a litter, no such effect being seen with the F_2 parents. No effect on body weight attributable to the test material was seen in the parents. There was some indication of an increase in food consumption in the F_0 parents and a decrease in food consumption in the F_1 parents. As these effects were not clearly dose-related, it is difficult to attribute them to paraquat: moreover, no effect on food consumption was noted with the F_2 parents. Measurements of urinary paraquat showed that dose-related absorption of paraquat occurred during the study. There were no adverse effects on fertility of the F_0 parents, male or female, during production of either F_1 litter. At 25 mg/kg (F_{1b}) and 75 mg/kg (F_{1b}), there was a reduction in the duration of gestation; in view of the lack of any such finding at higher dietary concentrations, this is unlikely to be related to the diet. There were no treatment-related effects on live-born offspring, maternal neglect or survival indices. In production of the F_2 litters by the F_1 parents, no effect of the paraquat was seen on fertility, body-weight gain of the pregnant dams, duration of gestation or live-born offspring, survival indices or litter size. No adverse effects on male or female fertility were noted in the F_2 parents on male or female fertility during production of the F_3 litters. Body-weight gain of the F_2 females when pregnant with F_{3a} or F_{3b} litters was increased at 75 mg/kg. The offspring of all three generations were healthy during lactation, although mortality in the F_{1b} litters was higher than that in the other litters. There were some differences in F_1 and F_3 litter weights between the groups, but they were not dose-related. Three of the F_0 female parents receiving paraquat at the highest dietary concentration died, and the lungs of these animals showed alveolar oedema, perivascular oedema and inflammatory cell infiltration (mainly macrophages, with a few neutrophils); profibroblasts and early fibrosis was also observed. Four lactating females receiving paraquat at the highest dietary concentration and suckling the F_{1b} litters died or were killed in extremis and their lungs showed similar changes. At termination, significant histopathological changes were confined to animals receiving paraquat at 150 mg/kg. These changes comprised consolidation, with alveolar fibrosis, epithelialization and infiltration with a few macrophages and profibroblasts. There was also hypertrophy and hyperplasia of the bronchial epithelium, with perivascular oedema and mixed inflammatory cell infiltration. No other treatment-related findings were seen in the F_0 female rats. In the F_0 male rats, there was an increase in focal histiocytosis at 75 and 150 mg/kg. In the F_1 parents, 13 females dying during late lactation were from the groups receiving 150 mg/kg and had lung changes similar to those described above. At termination of the F_1 females, five of the 17 surviving rats at 150 mg/kg had mild to severe lung changes. There was an increase in focal alveolar histiocytosis in the lungs at 75 and 150 mg/kg. This change was also present in the lungs of the male survivors at termination at 75 and 150 mg/kg. In the F_2 parental females, six rats at 150 mg/kg, which died or were killed in extremis, lung changes were observed at histopathological examination. At termination of the F_2 parental survivors, a proportion of both males and females at 150 mg/kg had some lung changes, as described above, and there was an increase in focal alveolar histiocytosis in the lungs at 75 and 150 mg/kg. Histopathological changes in the reproductive system were not seen in the parental animals of either

sex. In the offspring, mild perivascular inflammatory cell infiltration was seen in lungs of four out of five male and two out of six female F_{1b} offspring at the highest dietary concentration. Otherwise, there were no pathological changes in the F_1, F_2 or F_3 offspring that could be directly attributed to the test material, although one F_{3b} litter starved (and died or were sacrificed in extremis) as a result as a result of the death of the mother. The NOAEL was 25 mg/kg, equivalent to 1.67 mg of paraquat ion/kg bw per day, on the basis of lung changes at 75 and 150 mg/kg in adult rats. The NOAEL for reproductive toxicity was 150 mg/kg, the highest dietary concentration administered, this being equivalent to 10 mg of paraquation/kg bw per day. The NOAEL for toxicity in the offspring was 75 mg/kg, equivalent to 5.0 mg of paraquat ion/kg bw per day (Lindsey et al., 1982).

In another three-generation study, groups of 30 male and 30 female Sprague-Dawley (CRJ:CD) rats were fed diets containing paraquat dichloride (purity, 98.6%) at a concentration of 0, 100, 200 or 400 mg/kg. The intakes of test material achieved are given in Table 10.

The F_0 parents received the diets from week 5 weeks until after weaning of the second (F_{1b}) litters. There was a 13-week premating period after which the males and females were mated to produce the F_{1a} litters. The pups were examined for number of live born, stillbirths, sex ratio and external abnormalities. Eight pups per litter were investigated until weaning at 21 days after birth, and the pups were then examined post mortem. Two weeks after the F_{1a} litters had been weaned, the F_0 females that were successful breeders were housed with their previous mates to produce the F_{1b} litters. The F_0 females bearing the F_{1b} litters were divided into three groups, five females being used for teratology, five for postnatal investigations and 10 to produce the F_1 parents as follows. Five pregnant females of each group were killed on day 20 of gestation. After macroscopic examination, the uteruses were removed and examinations carried out to determine number of live fetuses, fetal deaths and resorptions. Live fetuses were weighed, sexed and examined for external abnormalities. One-third of all live fetuses were fixed in Bouin solution, examined in detail and dissected. The remainder were fixed in 95% alcohol, stained with alizarin red S and examined for skeletal abnormalities. Five pregnant females underwent parturition and the duration of gestation and numbers of live pups and stillbirths were recorded, together with sex ratio and any external abnormalities of the pups. Where there were more than eight pups per litter, the excess were stained with alizarin red S and examined for skeletal abnormalities. The remaining live pups were retained until weaning at 28 days, when they underwent exami-

Table 10. Measured intake of paraquat dichloride (mg/kg bw per day) in a three-generation study

Group	Dietary concentration (mg/kg)			
	0	100	200	400
F_0 males	0	6.6	13.0	25.1
F_0 females	0	7.2	13.8	29.3
F_{1b} males	0	9.6	19.8	38.7
F_{1b} females	0	10.2	20.8	32.9
F_{2b} males	0	8.5	16.9	40.9
F_{2b} females	0	9.6	19.6	48.7
Mean for males*	0 (0)	8.2 (5.9)	16.6 (12.0)	34.9 (25.3)
Mean for females*	0 (0)	9.0 (6.5)	18.1 (13.1)	37.0 (26.8)

From Suzuki et al. (1983)
* Intake of paraquat ion in parentheses

nation post mortem. Ten pregnant females underwent parturition and the duration of gestation and numbers of live pups and stillbirths were recorded, together with sex ratio and any external abnormalities of the pups. Where there were more than eight pups per litter, 4 days after birth, the excess were killed, stained with alizarin red S and examined for skeletal abnormalities. The remaining live pups were retained until weaning at 21 days. Mortality, viability and growth status were recorded, and 30 males and 30 females at each dietary concentration were selected to be the next generation, the remainder being autopsied with their (F_0) dams. The F_1 rats, which produced the F_{2a} and F_{2b} litters, were treated similarly to the F_0 rats (see above), being fed the diets from the time they were weaned until the weaning of their second (F_{2b}) litters. Ten pregnant females were, however, used for teratology studies and 10 for postnatal investigation, any remaining being allowed to give birth, after which the dams and pups were sacrificed. The third generation (from the F_{2b} litters) were fed the diets from the time they were weaned until at least 13 weeks later.

Throughout the study, all animals were examined daily and the F_0 and F_{1b} females were weighed on days 0, 7, 14 and 20 day of gestation and on days 0, 7, 14 and 21 post partum (during lactation). The F_{1b} litters to be used for postnatal investigation were weighed 0, 4, 7, 14, 21 and 28 days after birth, and those that were used to produce the next generation were weighed 0, 4, 7, 14 and 21 days after birth. Food consumption was measured weekly for each cage, but not during dosing of the F_0 generation and not in the mating period that produced the F_{1b} litters. Water consumption by 10 males and 10 females per dietary concentration was measured weekly, except for the F_0 mating period and the mating period that produced the F_{1b} litters. At autopsy of the parental rats, selected organs were weighed, and these and other selected organs were fixed and processed for histopathological examination.

No deaths were seen in the F_0 parents, but excess deaths were seen in the subsequent generations. At 400 mg/kg, five F_{1b} females died (compared with two of the controls). There were 14 deaths or animals killed in extremis in F_{2b} males at 400 mg/kg, and 10 deaths or rats killed in extremis in F_{2b} females at 400 mg/kg. At 400 mg/kg, wheezing was heard in rats of each generation and this was often accompanied by weight loss. At 400 mg/kg, there was a reduction in body-weight gain in male and female F_0 and F_{2b} rats at an early stage during dosing, and in female F_{1b} rats during gestation and lactation. There was a reduction in food consumption in F_0 and F_{2b} rats at 400 mg/kg early in the dosing period. No treatment-related changes were seen in reproductive parameters (corpora lutea, implantation number, implantation (%), number of dead and live fetuses, sex ratio or placental weight), nor were any teratogenic effects seen. Retarded ossification was noted in fetuses from the F_0 dams at 100 mg/kg, and in those from the F_{1b} dams at 100 mg/kg and 400 mg/kg. Furthermore, there were reductions in body weights of male fetuses from the F_{1b} females at 100 and 400 mg/kg. Retardation of ossification was also noted in fetuses from all test groups of F_{1b} dams. There were reductions in body-weight gain in F_{2b} pups at 400 mg/kg. There was retarded opening of the vagina in both the F_{1b} and F_{2b} female pups at 400 mg/kg. No effects on organ weights were seen that were clearly attributable to the test material. However, F_0 animals at 400 mg/kg showed a reduction in brain weight, both absolute and relative. Histopathologically, alveolar hyperplasia and fibrosis was found in F_0 males at 400 mg/kg, in F_{1b} females at 100, 200 and 400 mg/kg, and in F_{2b} rats of both sexes at 400 mg/kg. At 400 mg/kg, F_{1b} rats also showed atelectasis, congestion and haemorrhage, while in the decedents from F_{2b} rats at 400 mg/kg, alveolar wall hyperplasia and fibrosis, atelectasis, congestion, haemorrhage and oedema were found. The LOAEL for maternal toxicity was 100 mg/kg on the basis of lung changes seen in female F_{1b} rats (this dietary

concentration is equal to 9.0 mg of paraquat dichloride/kg bw per day and 6.5 mg of paraquat ion/kg bw per day). No NOAEL for fetal toxicity was seen because of retarded ossification and decreased body weight, the LOAEL for fetal toxicity being 100 mg/kg (equal to 9.0 mg of paraquat dichloride/kg bw per day and 6.5 mg of paraquat ion/kg bw per day in the dams.[2] The NOAEL for pup toxicity was 200 mg/kg on the basis of decreased body weight in F_{2b} pups at 400 mg/kg and retarded opening of the vagina in F_{1b} and F_{2b} female pups at 400 mg/kg. This NOAEL is equal to 16.6 mg of paraquat dichloride/kg bw per day in males and 18.1 mg of paraquat dichloride/kg bw per day in females (12.0 and 13.1 mg of paraquat ion/kg bw per day in males and females, respectively). The NOAEL for reproductive toxicity was 400 mg/kg, (the highest dietary concentration). This is equal to 34.9 mg of paraquat dichloride/kg bw per day and 25.3 mg of paraquat ion/kg bw per day in males, and 37.0 mg of paraquat dichloride/kg bw per day and 26.8 mg of paraquat ion/kg bw per day in females. An overall NOAEL for the study was not elicited, as histopathological evidence of lung damage was found at all dietary concentrations in F_{1b} female rats, and delayed ossification and reductions in body weight in fetuses were seen at ≤100 mg/kg. The overall LOAEL for the study was 6.5 mg of paraquat ion/kg bw per day (Suzuki et al., 1983).

Mice

In a two-generation study of reproductive toxicity, groups of 24 pairs of ICR albino mice (paired at age 30 days) and given diets containing paraquat at a concentration of 0, 45, 90, or 125 mg/kg (equal to 0, 45, 90 or 125 mg of paraquat ion/kg feed and equivalent to 0, 6.75, 13.5 or 18.75 mg of paraquat ion/kg bw per day). Females were allowed 8 weeks from pairing to produce a litter and cages were checked daily for parental and pup mortality. The pups were weaned after 30 days and either segregated or paired for use in producing the second generation. Exposure of the parental (F_0) mice continued until the weaning of the F_1 mice, which were exposed to the diet for 49 days postnatally. Lungs were excised from sucklings, weanlings and adults in groups in which mortality had been observed, and were processed for histopathological examination. At age 30 days, randomly selected F_1 mice were paired (not siblings) to produce the next generation. The control group comprised 24 pairs and the groups receiving paraquat comprised two groups of 12 pairs at each dietary concentration. One group of 12 pairs at each dietary concentration was removed from the test diet and placed on control diet on weaning, whereas the other remained on the same diet as their parents. No differences were observed in the age of females at first parturition, pups borne/litter or in pup abnormalities; at the highest dietary concentration, however, the number of pairs of mice producing litters was reduced because of maternal deaths. Furthermore, effects on the mortality of F_1 offspring were observed at the highest dietary concentration. The age of F_1 females at second parturition was increased, and mortality in the F_2 generation at 7 weeks was increased at 125 mg/kg. Excess mortality was not observed in the F_1 parents. Maternal and offspring lungs were histopathologically abnormal, with extensive fibrosis at the highest dietary concentration and in a few instances, in the dams, at the intermediate concentration. The NOAEL for the study was 45 mg/kg, equivalent to 6.75 mg of paraquat ion/kg bw per day. The NOAEL for pup toxicity was 90 mg/kg (equivalent to 13.5 mg of paraquat ion/kg bw per day) on the basis of excess mortality and histopathological changes in the lungs. Specific reproductive toxicity was not seen (Dial & Dial, 1987).

[2] Assuming no paternal effect.

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 29 or 30 rats (strain not stated) were given paraquat dichloride (purity, 100%) at a dose of 0, 1, 5, or 10 mg of paraquat ion/kg bw per day by oral gavage on days 6–15 of gestation. Animals were examined daily and maternal body weight was measured on days 0, 3, 6, 8, 12, 16 and 21. Food consumption was not measured. On day 21 of gestation the animals were killed and their uteri were examined for live fetuses and resorptions; corpora lutea were counted. Fetuses were removed, weighed, sexed and observed for gross malformations, then preserved before examination for soft tissue or skeletal abnormalities. Alternate fetuses were examined for soft tissue or skeletal abnormalities. Maternal lungs and kidneys from at least 11 surviving rats per group were fixed and processed for histopathological examination. Observed mortality in the group receiving the highest dose was attributed to paraquat. Clinical signs of maternal toxicity occurred in many animals at 5 mg/kg bw per day and in most animals at 10 mg/kg bw per day. These signs were piloerection, weight loss, hunched appearance and, sometimes, respiratory distress. Reduced maternal body-weight gain was seen at 5 and 10 mg/kg bw per day, the effect being greater at the higher dose. The decedent dams at the highest dose showed, grossly, patchy red areas in the lungs, while microscopically there was alveolar oedema with polymorphonuclear infiltration. Proximal tubular degeneration in the kidneys was also found. These changes were not present in the groups receiving a dose of 5 mg/kg bw per day or the survivors to 21 days in any group. Slightly reduced mean fetal weights were seen at 5 and 10 mg/kg bw per day (the significance at $p < 0.05$ at 5 mg/kg bw per day depended on one female who had 12 resorptions out of 14 implants, and the two fetuses were very small). Significant intergroup differences in fetal survival, number of viable fetuses, proportion of females with resorptions, numbers of corpora lutea and sex ratios were not seen. If, however, the female receiving a dose of 5 mg/kg bw per day that had 12 resorptions out of 14 implants, and whose two fetuses were very small was included, a difference was apparent in viable fetuses as a proportion of implant numbers between the control group and the group receiving a dose of 5 mg/kg bw per day. No intergroup differences in skeletal abnormalities were found, but retarded ossification (caudal vertebrae and forelimb and hindlimb digits) was seen at 5 and 10 mg/kg bw per day. No fetal soft-limb abnormality was found that was attributable to treatment. The NOAEL for maternal and fetal toxicity was 1 mg/kg bw per day on the basis of clinical signs, and reduced body-weight gain in the dams and reduced mean fetal weights and retarded ossification in the fetuses. Teratogenicity was not observed (Hodge et al., 1978a).

In a study of developmental toxicity, groups of 24 female Alpk:ApfSD Wistar-derived rats were given technical-grade paraquat dichloride (paraquat ion, 38.2% w/v) at a dose of 0, 1, 3 or 8 mg of paraquat ion/kg bw per day by gavage on days 7–16 of gestation. Clinical observations were recorded daily and body weight was recorded on days 1, 4, 7–16, 19 and 22 of gestation. Food consumption was recorded over 3-day periods: days 1–4, 4–7, 7–10, 10–13, 13–16, 16–19 and 19–22. On day 22 of gestation, the rats were killed and their uteri weighed and examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination. No compound-related adverse clinical finding was recorded. There was a small amount of weight loss at the highest dose between days 1 and 2 of dosing (days 7–8 of gestation) and the difference in weight between the group receiving the highest dose and the controls was significant on days 8–14 and 16 of gestation. The effect on body weight of the dams at the highest dose (8 mg of paraquat ion/kg bw per day) was considered to be

test material-related. Developmental toxicity was not seen. Paraquat was not teratogenic The NOAEL for maternal toxicity was 3 mg of paraquat ion/kg bw per day on the basis of effects on body weight at 8 mg of paraquat ion/kg bw per day, and the NOAEL for developmental toxicity was 8 mg of paraquat ion/kg bw per day, the highest dose tested (Hodge, 1992).

Mice

In a study of developmental toxicity, groups of 26–37 pregnant mice were given paraquat dichloride (purity, stated to be 100%) at a dose of 0, 1, 5 or 10 mg of paraquat ion/kg bw per day by gavage on days 6–15 of gestation. The animals were observed daily and weighed on days 0, 3, 6, 9, 12, 15 and 18 of gestation. Food consumption was not measured. On day 18, the mice were killed and their uteri were examined for resorptions. Fetuses were removed, weighed, sexed and observed for gross abnormalities, and preserved for examination for soft tissue or skeletal changes. In the mothers, for at least eight animals per group, lungs and kidneys were fixed and processed for histopathological examination. No adverse clinical signs were noted. Maternal body-weight gain was decreased during gestation at 5 and 10 mg/kg bw per day, but only at 5 mg/kg bw per day was the difference from that of controls significant. There were no test material-related effects on maternal pathology. The numbers of implantations, viable fetuses and resorptions, sex ratio and fetal and litter weights were not different between treated and control groups. There was a higher incidence of fetal umbilical hernia at 5 mg of paraquat ion/kg bw per day, but this was considered to be unrelated to dosing. There was no increase in skeletal or soft tissue abnormalities and ossification was not retarded. The NOAEL for maternal toxicity was therefore 10 mg of paraquat ion/kg bw per day, the highest dose tested (since the effects based on reduced weight gain in pregnancy were not dose-related), while the NOAEL for fetal toxicity was also 10 mg of paraquat ion/kg bw per day, the highest dose tested (Hodge et al., 1978b).

In a study of developmental toxicity, groups of 26 Crl:CD1 (ICR) BR mice were given using technical-grade paraquat dichloride (purity, 38.2%) at a dose of 0, 7.5, 15 and 25 mg of paraquat ion/kg bw per day by gavage on days 6–15 of gestation. Maternal mortality (mice that died or were killed in extremis) and clinical signs were recorded daily from the start of gestation. Body weights were recorded on days 0, 6–15 and 18 of gestation. Food consumption was recorded over days 0–6, 6–9, 12–15 and 15–18 of gestation. The remaining females were killed on day 18 of gestation. Females were examined post mortem, when the lungs (with the trachea) and kidneys were weighed. Gestation status was assessed and the gravid uterine weight was recorded. The number of live and dead implantations was recorded. Live fetuses were weighed, examined for external abnormalities and sexed. One-half of the fetuses were examined for visceral abnormalities, and then for skeletal abnormalities, the other half being examined for visceral abnormalities. At the highest dose, there were five decedents at 15–17 days (four killed in extremis and one found dead). In the four killed in extremis, piloerection, laboured breathing, hunched posture, hypothermia, hypoactivity and pallor of the extremities and eyes were observed. No other treatment-related clinical effects were observed. Also at the highest dose, there was a decrease in body-weight gain over days 12–15 and 15–18, and over the whole period of dosing (days 6–15 of gestation); furthermore, body weight in the group receiving the highest dose was lower than that in the controls on day 15 and day 18 of gestation. Body weight and weight gain were unaffected at the lower doses. Significant differences in food consumption were seen on analysis of variance. Although food consumption between days 12 and 15 was reduced in the group receiving the highest dose compared with that in the controls, the difference was

not significant. Food consumption was not reduced in mice at the lower dose. Despite the lack of statistical significance, the present reviewer considered that the reduction in food consumption between days 12 and 15 in the group receiving the highest dose compared with that in the controls may be biologically significant. At necropsy of the mice killed in extremis, dark red patches were found in the lungs. In all mice, absolute and relative lung weights were increased at the highest dose. The difference in absolute but not relative lung weights between the groups disappeared, if the decedents were excluded. The number of implantations, live fetuses, postimplantation loss and fetal sex ratio were not affected by treatment. At the highest dose, retardation of fetal growth was seen and mean fetal weight was decreased. No treatment-related effect on the prevalence of major abnormalities was seen. At 7.5 mg/kg bw per day and 15 mg/kg bw per day, but not 25 mg/kg bw per day, there were more fetuses and litters with minor external/visceral abnormalities, but as this did not appear to be dose-related, the effect was not considered to be treatment-related; this effect was due to an increase in the number of fetuses with renal pelvic cavitation. At the highest dose, there was retardation of ossification of the caudal vertebrae and the occipital and astragalus bone, with misshapen sternebrae. No treatment-related effect was seen at the lower doses. The NOAEL for maternal and fetal toxicity was 15 mg of paraquat ion/kg bw per day on the basis of effects on body weight, reduced food consumption and lung changes in the dams and retardation of ossification in the fetuses at the highest dose tested. Teratogenicity was not seen at any dose (Palmer, 1992).

Groups of Swiss-Webster mice were given paraquat at a dose of 1.67 or 3.35 mg/kg bw per day intraperitoneally or 20 mg/kg bw per day by gavage on days 6–16 of gestation. Gravid mice were sacrificed on day 19 of gestation. The number of live and dead fetuses and resorptions was recorded and the fetuses were removed, dried and examined for gross defects. Equal numbers of pups from each litter were fixed for examination of soft tissue or skeletal anomalies. No teratogenic effect was observed, although a slight degree of non-ossification of sternebrae was seen at all doses. Fetotoxicity, as evidenced by increased resorption (%), was seen at only 3.35 mg/kg bw per day intraperitoneally. At no dose was the number of fetuses, or their mean body weight affected by treatment. The amount of radiolabel reaching the mouse embryo when ^{14}C-labelled paraquat at a dose of 3.35 mg/kg bw administered intraperitoneally or 20 mg/kg bw administered orally on day 11 of gestation was small (Bus et al., 1975).

The developmental toxicity of paraquat was determined in Sprague-Dawley rats treated intravenously with paraquat at a single dose of 15 mg/kg bw on a single day, one of days 7–21 of gestation. The number of live and dead fetuses and resorptions was counted at day 22 (or before for decedent dams). Excess maternal deaths occurred with paraquat compared with controls receiving saline only, and there was an increase in the number of dead and resorbed fetuses (Bus et al., 1975).

Groups of pregnant Swiss-Webster mice were given drinking-water containing paraquat (purity unstated) at a concentration of 50 or 100 mg/l (and 150 mg/l) from day 8 of gestation until postnatal day 42. Pregnant mice receiving paraquat at 150 mg/l died during gestation (at about day 16). Treatment with paraquat at 100 mg/l and 50 mg/l did not alter the postnatal growth rate, nor was postnatal mortality increased at 50 mg/l. Administration of drinking-water containing paraquat at 100 mg/l caused an increase in postnatal mortality, and an increase in the sensitivity of pups to oxygen toxicity on postnatal days 1 and 28, while drinking-water containing paraquat at 50 mg/l did not. At both 50 and 100 mg/l, the sensitivity to oxygen toxicity and to bromobenzene at postnatal day 42 was increased. The

authors considered that the effect of bromobenzene could be caused by depletion of reduced glutathione (Bus & Gibson, 1975).

2.6 Special studies

(a) Mechanistic studies

(i) Histopathological studies on the lung

Small groups of A/He mice were given drinking-water containing paraquat at a concentration of 50–300 mg/l, and retained for 1 to 16 weeks (further details of the material used are not given in the paper). Detailed light and electron microscopical studies were carried out on the mice post mortem. The main findings on light microscopy were vascular dilatation and veins filled with platelets and erythocyte aggregates. At the higher doses, interaveolar septal thickening was seen. At ≥100 mg/l, focal or, sometimes, lobar pneumonitis was observed, with small mononuclear cells, macrophages and neutrophils. In those mice receiving paraquat for 4 weeks or more, fibroblasts were seen in the septal walls. Obliteration of air spaces was seen. Type II cells were observed to be undamaged on electron microscopy in this study, but type I cells were swollen and there was evidence of oedema of interalveolar septa. The alveolar air spaces were filled with a clear exudate and where there was consolidation, fibroblasts and collagen were observed. Lymphocytes and plasma cells were noted (Brooks, 1971).

In a study of the ultrastructure of the rat lung after administration of paraquat, 51 female Wistar albino rats were divided into 17 groups, each group comprising two test animals and one control. On day 1, animals in 15 groups received paraquat at a dose of 40 mg of paraquat ion/kg bw administered intraperitoneally, while groups 16 and 17 received paraquat at a dose of 30 mg of paraquat ion/kg bw. At intervals of between 10 min and 4 h after injection, the animals were killed and the left lungs were fixed with glutaraldehyde via the main bronchus, and processed for electron microscopy. The right lungs were processed for light microscopy. Using light microscopy, changes were not seen until 24 h. After 2 days, microscopy revealed interstitial oedema and a fibrinous exudate, with a polymorph infiltration, which was more widespread after 4 days. Pro-fibroblasts were seen in the vicinity of bronchioles and major blood vessels. Using electron microscopy, after 4 days there was an increase in the quantity of rough endoplasmic reticulum and numbers of mitochondria and free ribosomes in alveolar type I cells. The cells were also thicker. These changes were followed by swelling of mitochondria, fragmentation of the rough endoplasmic reticulum and a reduction in cellular density. Later the cells disintegrated. Changes in the type II alveolar cells did not occur until 8 h and were not pronounced until 18 h after administration of paraquat. The changes consisted of swelling and rupture of the mitochondria, fusion and vacuolation of lamellar bodies and disruption of the cytoplasm. Three days after administration of paraquat, pro-fibroblasts were seen in the alveolar spaces (Smith & Heath, 1974).

In other species, such as rats and dogs, histopathological appearances after treatment with paraquat are generally similar to those in mice (Clark et al., 1966), although Butler (1975) found that the Syrian hamster relatively resistant to interstitial fibrosis. Butler & Kleinerman (1971) found that the New Zealand white rabbit did not develop pulmonary changes typical of paraquat poisoning in other species, despite intraperitoneal administration of paraquat at a dose of 2–100 mg/kg bw and sacrifice of animals being delayed up to 1 month. The only findings in the lungs were occasional small interstitial infiltrates of lymphocytes and plasma cells, minimal alveolar hyperplasia and some alveolar macrophages.

(ii) Mechanism of uptake by pneumocytes

A considerable amount of work has been done on the mechanisms that underlie the toxicity of paraquat. The fact that paraquat is concentrated by the lungs has been discussed above. Rose et al. (1976) showed that lung slices from rats Wistar-derived Alderley Park rats, beagle dogs, New Zealand white rabbits and cynomolgus monkeys (*Macaca fascicularis*) could concentrate paraquat via the polyamine active uptake system. This is the system by which paraquat and the structurally similar polyamines, such as putrescine and spermidine, are accumulated by type II alveolar cells (see reviews by Smith, 1985, Smith et al., 1990 and Lock & Wilks, 2001).

The uptake kinetics of paraquat and putrescine and their mutual inhibition in freshly isolated rat type II cell suspensions was reported. The uptake of paraquat by type II cells exhibited saturation kinetics and could be inhibited in a concentration-dependent manner by putrescine. The authors postulated that the polyamine uptake pathway in type II cells for paraquat and putrescine possessed two separate sites, one for each substrate, and that binding at one site leads to a conformational change in the other (Chen et al., 1992).

(iii) Production of cell damage in the lung

A study in which drinking-water containing paraquat at a concentration of 50 or 100 mg/l was administered to Swiss-Webster mice has already been discussed (see section on developmental toxicity). Drinking-water containing paraquat at a concentration of 100 mg/l increased postnatal mortality, and increased pups' sensitivity to oxygen toxicity at 1 and 28 days after birth, while drinking-water containing paraquat at a concentration of at 50 mg/l did not. At both 50 and 100 mg/l, drinking-water containing paraquat increased the sensitivity to oxygen toxicity and to bromobenzene at 42 days after birth (Bus & Gibson, 1975).

In a study of the hypothesis that the pulmonary toxicity of paraquat is caused by cyclic reduction–oxidation, with generation of superoxide radicals and singlet oxygen, and the production of lipid peroxidation, mouse lung microsomes in vitro were found to catalyse the nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-dependent reduction of paraquat. Incubation of paraquat with NADPH, NADPH-cytochrome reductase and purified microsomal lipid increased the production of malondialdehyde (MDA) production. Addition of superoxide dismutase or 1,3-diphenylisobenzofuran (a singlet oxygen trapper) inhibited paraquat-induced lipid peroxidation. Toxicity caused by paraquat (purity unstated) in mice (strain unstated) was decreased by phenobarbital and increased by selenium, vitamin E or reduced glutathione deficiency. The toxicity of paraquat was increased by exposure to 100% oxygen (Bus et al., 1976b).

In similar studies in rats and mice, Bus et al. (1976a) showed that pretreatment with phenobarbital increased the LD_{50} for paraquat in Swiss-Webster mice, but only when administration of the phenobarbital was continued after the administration of paraquat. Paraquat, administered intraperitoneally at a dose of 30 mg/kg bw, decreased liver concentrations of reduced glutathione and lung concentrations of lipid-soluble antioxidants. After receiving paraquat at a dose of 45 mg/kg bw, Sprague-Dawley rats habituated to 85% oxygen were found to have a longer median time to death than rats exposed to air. These rats were believed to have greater activity of lung enzymes that combat lipid peroxidation.

The effect of paraquat on oxidative radical reactions in the lung was evaluated by studying malondialdehyde production and chemiluminescence (spontaneous and induced

by tertiary butyl hydroperoxide) in the isolated rat lung. After 2 h of perfusion with paraquat at 3.0 mmol/l, malondialdehyde content in lung homogenates was 16 ± 7 nmol/g of dry weight higher than in control lungs; during 30 min of perfusion, malondialdehyde efflux was 33 ± 15 nmol/g of dry weight higher than in control perfusates. Spontaneous chemiluminescence was not increased by 2 h of perfusion with paraquat at concentrations ranging from 0.75 to 6.0 mmol/l. Chemiluminescence induced by tertiary butyl hydroperoxide, however, was 17 ± 3% higher immediately after the addition of hydroperoxide and reached a 16 ± 6% higher plateau for lungs perfused with paraquat than for control lungs. Spectral analysis of the light emitted during induced chemiluminescence demonstrated peak intensity between 630 and 730 nm for controls and for lungs treated with paraquat. Increased production of malondialdehyde and increased induced chemiluminescence indicated that perfusion with paraquat enhances lipid peroxidation in the isolated rat lung (Aldrich et al., 1983).

The redox cycling abilities of paraquat and nitrofurantoin, compared with those of the potent redox cyclers diquat and menadione, was studied in lung and liver microsomes using the oxidation of NADPH and consumption of oxygen. In terms of relative potencies of these compounds to undergo redox cycling, diquat and menadione were similar and much greater than paraquat, which was similar to nitrofurantoin. This was partly attributed to the much lower affinity (K_m) of lung and liver microsomes for paraquat and nitrofurantoin than for diquat and menadione. These data were considered to have important implications in assessing the risk of exposure to paraquat. Low concentrations of paraquat would not be expected to cause lung damage because insufficient compound would be present in the lung to exert toxicity by redox cycling (Adam et al., 1990).

There has been some disagreement over which cell type in the lungs is primarily affected by paraquat. Hirai et al. (1985) injected male Sprague-Dawley rats with paraquat dichloride at 40 mg/kg bw and observed mitochondrial swelling and loss of granules in alveolar type II cells at 6 h.

In a study of the effect on the lungs of paraquat applied to the skin over/next to the lungs of male Long-Evans rats, paraquat (as 1 ml of solution containing 8 g of paraquat) was applied weekly to the back of 18 rats. There were seven control rats. From week 4, two rats were killed per week. After 6 weeks, the concentration of the test solution was increased to 28.5 mg/ml. Lungs, kidneys, livers and the application site were removed at autopsy and processed for histopathological examination. In some of the rats receiving paraquat, there was evidence of intra-alveolar haemorrhage. The medial thickness of large and small pulmonary arteries in the test groups was greater than in the controls. No histopathological change was present in the livers and kidneys. There was necrosis and ulceration of the application site, with acute and chronic inflammatory cell infiltration (Levin et al., 1979).

(b) Liver toxicity

Liver toxicity, as revealed by elevated liver enzymes, jaundice, and histopathological changes in the liver at examination post mortem, is sometimes seen in cases of poisoning with paraquat in humans. A number of studies examining this phenomenon (e.g. Cagen & Gibson, 1977; Burk et al., 1980). Cagen & Gibson (1977) have found that, in Swiss-Webster mice, paraquat was not hepatotoxic, unless the mice were deficient in selenium.

(c) Kidney toxicity

In cases of poisoning in humans, renal tubular damage has been noted at autopsy. In a study of the nephrotoxicity of paraquat in vitro and in vivo, proximal tubular function was monitored in vitro by measuring the accumulation of *p*-aminohippurate and *N*-methylnicotinamide using renal cortical slices from Swiss-Webster mice poisoned with paraquat at the LD_{50} for intraperitoneal administration (50 mg/kg bw). Tubular function in intact Swiss-Webster mice was estimated using disappearance of phenolsulfthalein and [^{14}C] paraquat from plasma in vivo. Glomerular function was estimated using disappearance of iothalamate from plasma in animals injected intravenously with paraquat at a dose of 50 mg/kg bw. Accumulation of *p*-aminohippurate and *N*-methylnicotinamide by renal cortical slices in vitro was not greatly altered. Disappearance in vivo of phenolsulfthalein and [^{14}C] paraquat from plasma was greatly reduced, but iothalamate disappearance was little affected. The authors concluded that nephrotoxicity attributable to paraquat affects primarily the proximal tubule (Ecker et al., 1975).

It has been noted that the uptake of paraquat by rat renal tubular cells in culture is saturable (Chan et al., 1996a). Of two renal tubular cell lines, one resembling proximal tubular cells and the other resembling distal tubular cells, the latter was found to be more resistant to the effects of paraquat (Chan et al., 1996b).

(d) Neurotoxicology

Paraquat is structurally similar to the known dopaminergic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). As a result, paraquat has been considered as a possible etiological factor in Parkinson disease. Paraquat is, however, a dication and does not readily cross biological membranes and the blood–brain barrier, whereas MPTP readily crosses the blood–brain barrier and is oxidized to the dihydropyridinium ion and then the neurotoxic methylphenylpyridinium ion. The methylphenylpyridinium ion is taken up into dopaminergic neurones by the same uptake mechanism as dopamine itself (Fonnum, 1999). Moreover, it was reported that, in a study using an inducible system in neuroblastoma cells (described only in an abstract), the toxicity of paraquat was not mediated by the dopamine transporter (Miller & Quan, 2002). Furthermore, in another abstract it was reported that, while the methylphenylpyridinium ion inhibited dopamine re-uptake in rat and mouse synaptosomes, paraquat did not, and that paraquat had no binding affinity for the dopamine transporter and the D_1 and D_2 receptors (Foster et al., 2003). Shimizu et al. (2003) examined the mechanism by which paraquat is toxic to dopamine neurons in male Wistar rats in vivo, using GBR-12909, a selective inhibitor of the dopamine transporter. GBR-12909 reduced the uptake of paraquat into the striatal tissue, including dopaminergic terminals. Subcutaneous treatment with paraquat at 10 mg/kg bw for 5 days significantly decreased concentrations of brain dopamine and dopamine metabolites in the striatum. When paraquat was administered through a microdialysis probe stereotaxically implanted into the striatum, a transitory increase in extracellular concentrations of glutamate, followed by long-lasting elevations of the extracellular concentrations of nitrite and nitrate and dopamine, were detected in the striatum of rats. This lasted for more than 24 h after treatment with paraquat and could be inhibited by N^G-nitro-L-arginine methyl ester, dizocilpine, 6,7-dinitroquinoxaline-2,3-dione and L-deprenyl.

The behavioural and neuropathological effects in rats of both systemic and intrahippocampal injections of paraquat dichloride were studied by Bagetta et al. (1992). Paraquat (0.1–1.0 µmol), injected into the dorsal hippocampus, produced seizures within a few

minutes of injection, and caused neuronal damage in the CA1 and CA3 pyramidal cell layers, pyriform cortex, dentate granule cell layer and in the hilus fascia dentata at 24 h (*n* = 9 rats). A smaller dose of paraquat (10 nmol) was ineffective. The effects of intrahippocampal injections of paraquat (1 µmol) were prevented by coadministration with atropine (50 nmol). Systemic injections of paraquat (20–100 mg/kg bw) produced forelimb clonus and rearing in 10 out of 15 animals. Neuronal cell death was found 24 h later in nine of these rats and was restricted to the pyriform cortex, this being the region of the brain with the highest concentrations of paraquat. Atropine (at a dose of 150 mg/kg bw given intraperitoneally 60 min previously) completely prevented the motor seizures, but cell death still occurred in two of the six animals tested. The use of certain experimental treatments for intrahippocampal toxicity of paraquat has been studied by the same group of authors (Bagetta et al., 1994).

The effects of paraquat (1–5 µg) on behaviour, morphology and neurochemistry were investigated in male Wistar rats treated by unilateral injection into the substantia nigra. There was vigorous contralateral rotational behaviour in response to administration of apomorphine. The animals were killed 2 weeks after dosing. Morphologically, there was loss of Nissl substance, glial reaction and loss of neurones in the substantia nigra, and neurochemically, there was dopamine depletion (Liou et al., 1996).

In a study of the behavioural and electrocortical effects of paraquat, Wistar-Morini rats were given paraquat administered by cannula into the substantia nigra, pars compacta, an area where dopamine-containing cell bodies are present, and into the caudate nucleus, where dopamine-containing nerve endings of the dopamine nigro-striatal system project. Paraquat was also administered into the locus coeruleus, an area containing noradrenaline cell bodies and into the nucleus raphe dorsalis or into the nucleus raphe medianus, two nuclei containing cell bodies of serotoninergic neurones. Intraventricular administration of paraquat at a dose of 10 and 50 µg caused intense behavioural stimulation and an increase in locomotor activity, circling and the wet-dog syndrome. This was accompanied by desynchronization of the electrocorticogram and the appearance of bilateral high voltage epileptiform spikes, and finally clonic convulsions occurred. The infusion of paraquat into the substantia nigra pars compacta (1 µg) produced contralateral head and neck deviation, rigidity and kyphosis as well as behavioural and motor stimulation. The electrocorticogram activity was desynchronized and characterized by high voltage spike discharges. A similar behavioural, postural and electrocorticogram pattern was seen after infusion of paraquat into the caudate nucleus (10, 25 and 50 µg). In addition, paraquat, infused into the locus coeruleus or into the raphe nuclei (5 and 10 µg), produced circling, escape responses, jumping and clonic convulsions accompanied by electrocorticogram desynchronization and epileptic phenomena. The authors concluded that paraquat was able to produce central neurotoxicological effects that did not seem to be specific, at least for the doses used, for the dopamine nigro-striatal system (Gori et al., 1988).

In a study of the pathological effects of paraquat when administered directly into different parts of the rat brain, the microinfusion of paraquat (3.2, 16, 32 or 160 nmol) into the pars compacta of the substantia nigra produced neuropathological changes culminating in neuronal necrosis. A particular feature of paraquat neurotoxicity after its microinfusion into the substantia nigra (3.2 mmol/1 at 1 µl/min for 1 min) or into the ventral tegmental area (1.6 mmol/1 at 1 µl/min for 1 min), but not into other areas of the brain, was selective vulnerability of hippocampal CA3 neurones. This initially comprised a decrease in dendritic spines, which was followed by neuronal degeneration and cell loss. No damage occurred

after microinfusion of paraquat into other areas of the brain near or distant from the infusion sites. In addition, similar neuropathological alterations occurred in other non-dopaminergic areas. The authors considered that the study showed that paraquat possesses marked neurotoxicity that is not selective for dopaminergic neurones (Calò et al., 1990).

In a study of the effects of injected MPTP and analogues of MPTP inter alia, paraquat and reduced paraquat, C57 black mice were given paraquat in three subcutaneous injections of 14.5 mg/kg bw at an interval of 3 days, each injection being at a maximum tolerated dose. Reduced paraquat was administered in six daily doses increasing from 7.3 to 116.3 mg/kg bw, with a total dose of 342 mg/kg bw; this dose was well tolerated. One month after the last injection with paraquat or reduced paraquat, striatal dopamine was not depleted, while it was severely reduced with MPTP (Perry et al., 1986).

In a study investigating the possible role of paraquat in Parkinson disease, paraquat or MPTP were administered intraperitoneally to groups of six adult C57 Bl/6 mice. The dosing regimen for paraquat was 5 or 10 mg/kg bw given as three injections at weekly intervals, while that for MPTP was 10 or 30 mg/kg administered at 7 days and 16 h later and at 15 days and 16 h later (i.e. four doses). Saline was administered to a control group of six mice. Ambulatory behaviour was monitored. Substantia nigra dopamine neurone number and striatal dopamine terminal density were quantified after death. The data indicated that paraquat elicited a dose-dependent decrease in substantia nigra dopaminergic neurones (assessed by a fluoro-gold prelabelling method), a decline in striatal dopamine nerve terminal density (assessed by measurement of tyrosine hydroxylase immunoreactivity), and a neurobehavioural syndrome characterized by reduced ambulatory activity. Similar findings were seen with MPTP. The authors suggested that systemically absorbed paraquat crossed the blood–brain barrier to cause destruction of dopamine neurones in the substantia nigra and reduction of dopaminergic innervation of the striatum. The use of a parenteral route of administration, however, means that these data are of questionable relevance for risk assessment of paraquat residues (Brooks et al., 1999).

In a study of neurotoxic effects after neonatal exposure to paraquat and MPTP, groups of mice (aged 10 or 11 days) were given vehicle (water), paraquat, or MPTP by mouth; MPTP was administered at a dose of 0.3 or 20 mg/kg bw, and paraquat at a dose of 0.07 or 0.36 mg/kg bw. Neonatal spontaneous motor activity was tested on day 18 in mice given paraquat at 0.36 mg/kg bw. Adult spontaneous motor activity was tested at ages 60 and 120 days. On day 125, the mice were decapitated and the contents of dopamine and serotonin and metabolites in striatum were analysed. Acute toxicity was not observed in any of the groups. No respiratory distress or motor performance dysfunction was seen on day 18 in mice given paraquat at 0.36 mg/kg bw. The results of behavioural tests carried out at age 60 days showed a marked hypoactive condition in the mice given paraquat (at both doses) and MPTP (at both doses). At age 120 days, the hypoactivity persisted and appeared even more pronounced. Reduced striatal content of dopamine and metabolites was seen in the striatum with both compounds, but concentrations of serotonin were unaffected. The effect was greater at the higher doses (Fredriksson et al., 1993).

In a study in two strains of mice, one (C57 black) being the same as that used by Fredriksson et al. (1993), paraquat was administered as single daily doses at 0.36 or 3.6 mg/kg bw to pups aged 10 or 11 days, and appropriate controls were used (Ray, personal communication, 2003). Testing for spontaneous behaviour was carried out at 4 months, and approximately 1 week later the mice were killed and analysed for neurotrans-

mitters in the brain, as well as muscarinic receptor density. In the C57 black mice at 4 months, there was hyperactivity at 0.36 mg/kg bw compared with the controls, while at 3.6 mg/kg bw and in the other strain of mice used (NMRI) at both doses there were no significant differences from the controls. There were no significant intergroup differences in muscarinic receptor density nor in striatum or hippocampus dopamine, metabolites of dopamine or 5-hydroxyindoleacetic acid. The authors concluded that, using similar conditions, they could not replicate the results of the Fredriksson et al. (1993) study.

In the study by Widdowson et al. (1996b) on the entry of paraquat into the brains of male Wistar-derived Alpk:Apfsd rats, discussed above, groups of four rats were dosed daily for 14 days with water (controls) or 5 mg of paraquat ion/kg bw, orally. The rats were killed 24 h after the last of the 14 doses or after the single dose. On days 4 and 12, open field testing was carried out. On day 15, activity was measured over 50 min using an animal activity monitor, while animal grip strength and coordination was tested on days 4, 8 and 15 of the study. The brains were processed for histopathological examination after fixation by intracardiac perfusion. Brain catecholamines were measured by high-performance liquid chromotography using electrochemical detection, while dopamine D_1 and D_2 receptors were labelled using ^{3}H-labelled SCH23390 and spiperone respectively. The density of muscarinic acetylcholine receptors was estimated with ^{3}H-*N*-methyl scopolamine, and of *N*-methyl-D-aspartate (NMDA) receptors by ^{3}H MK-801 binding. The density of benzodiazepine sites on $GABA_A$ receptors was measured by ^{3}H-labelled Ro15-1788 binding. Body-weight gain was decreased in the test animals in comparison with the controls. No differences between the groups were seen in the results of behavioural tests. There was no sign of neuronal cell damage in the test group, in particular in the substantia nigra. The concentration of dopamine was significantly higher in the striatum of rats treated with paraquat than in controls, but this was not the case in the hypothalamus. Differences in D_1, D_2, muscarinic, *N*-methyl-D-aspartate and benzodiazepine sensitive $GABA_A$ receptors was not seen. The authors concluded that paraquat did not behave in the same way as MPTP in the tests used (Widdowson et al., 1996b).

(e) Possible neurotoxic interactions

Thiruchelvam et al. (2000a, 2000b) carried out studies to assess the potential involvement of combined exposure to the herbicide paraquat and to maneb, a manganese-containing ethylene*bis*dithiocarbamate fungicide, in the etiology of idiopathic Parkinson disease.

Male C57 Bl/6 mice were given paraquat dichloride at a dose of 5 or 10 mg/kg bw and/or maneb at a dose of 15 or 30 mg/kg bw, once weekly for a total of 4 weeks, by intraperitoneal injection. End-points assessed were: effects on locomotor activity, density of tyrosine hydroxylase positive neurones, concentrations of dopamine and metabolites, and dopamine turnover. The authors noted that decreases in motor activity immediately after injections were observed more consistently with combined exposures to maneb and paraquat. Concentrations of dopamine and metabolites and dopamine turnover were slightly increased immediately after injection of combined maneb and paraquat, compared with injection of maneb alone. In addition, significant reductions in tyrosine hydroxylase immunoreactivity, measured 3 days after the last injection, were detected in the dorsal striatum of animals given combined treatments, but not those treated with single compounds. The authors concluded that these results demonstrated potentiating effects of combined exposures to paraquat and maneb on nigrostriatal dopamine systems (Thiruchelvam et al., 2000a).

In similar experiments, male C57 Bl/6 mice were given single compounds (paraquat at a dose of 10 mg/kg bw or maneb at a dose of 30 mg/kg) or a combination (paraquat at 10 mg/kg bw paraquat plus maneb at 30 mg/kg bw), twice weekly by intraperitoneal injection for 6 weeks. It was reported that maneb, but not paraquat, reduced motor activity immediately after treatment, and that this effect was potentiated by combined treatment with paraquat and maneb. As treatments progressed, only the groups receiving combined paraquat and maneb showed a failure of motor activity recovery within 24 h. Paraquat and maneb in combination, but not alone, reduced tyrosine hydroxylase and dopamine transporter immunoreactivity in the dorsal striatum, but not in the nucleus accumbens. Reactive gliosis occurred only in response to combined paraquat and maneb in dorsal-medial but not ventral striatum. Tyrosine hydroxylase immunoreactivity and cell counts were significantly reduced only by the mixture of paraquat and maneb, and not by the pesticides alone, in the substantia nigra, while no treatment produced significant effects on tyrosine hydroxylase immunoreactivity and cell counts in the ventral tegmental area. The authors suggested that the combination of paraquat and maneb showed synergistic effects, preferentially expressed in the nigrostriatal dopamine system, and suggested that such mixtures could play a role in the etiology of Parkinson disease. The study was not designed appropriately to investigate potentiation and the results could have reflected dose-additivity (Thiruchelvam et al., 2000b).

(f) Experimental therapies

Among treatments for poisoning with paraquat that have been studied in experimental animals is the injection of the enzyme superoxide dismutase. Steroids have also been studied (Kitazawa et al., 1988; Chen et al., 2003), without apparent benefit. This appeared to be beneficial in rats that had been given paraquat administered by gavage (Autor, 1974). The results of studies in rats suggested that paraquat might potentiate the toxic effects of oxygen (Fisher et al., 1973; Keeling et al., 1981). Prolonged (6 h) haemoperfusion was reportedly successful in saving three out of four pigs (Landrace × Yorkshire gilts) to whom paraquat at a dose of 70 mg/kg bw had been administered by stomach tube. This dose was fatal in untreated pigs and 2 h of haemoperfusion was ineffective. The purity of the paraquat used was not stated, nor is it clear whether the dose was expressed as paraquat ion or dichloride (Yang et al., 1997).

(g) Poisoning of animals

Paraquat poisoning in animals is rare (Blood et al., 1983). Nevertheless, from time to time paraquat is reported as the causative agent in animal poisoning, Longstaffe et al. (1981), for example, reported malicious and accidental poisoning of cats and dogs, and Aleksic-Kovacevic et al. (2003) reported the accidental poisoning by paraquat of five German shepherd dogs.

3. Observations in humans

3.1 Poisoning incidents

Intentional ingestion of paraquat is a major cause of death from poisoning. Casey & Vale (1994) tabulated deaths from pesticide poisoning from 1945–1989 in England and Wales and found that paraquat was responsible for 570 deaths, or 56.3% of all deaths caused by pesticides. From 1982, however, there has been a progressive decline in the annual number of poisonings after the inclusion of emetic, stench and dye into gramoxone formulations.

There are numerous case reports and case series of poisonings with paraquat (e.g. Bullivant, 1966; Campbell, 1968; Malone et al., 1971; Douze et al., 1974; Carson & Carson, 1976; Bismuth et al., 1982; Bramley & Hart, 1983; Naito & Yamashita, 1987; Wesseling et al., 1993; Hall, 1995; Tsatsakis et al., 1996; van Wendel de Joode et al., 1996; Wesseling et al., 1997; Papanikolaou et al., 2001). The effects can be divided into local and systemic effects. Local effects may comprise damage to the skin, nails, and nose (Samman & Johnston, 1969; Hearn & Keir, 1971; Vale et al., 1987; Bismuth et al., 1995), and sore throat, dysphagia and epigastric pain may also occur. Local effects to the eye may heal only slowly and with scarring (Peyresblanques, 1969; Devečková et al., 1980). After ingestion of formulation concentrate, ulceration of the upper gastrointestinal tract is often observed. Although these effects are unpleasant, the findings from those poisonings with a fatal outcome are generally referable to the respiratory system, death being preceded by dyspnoea and cyanosis. Crepitations may be heard. Radiology initially reveals diffuse fine mottling of the lungs. Renal dysfunction may partly be a direct effect of paraquat and partly be caused by hypovolemia; often mild, renal dysfunction impairs the only route of elimination available (Marrs & Proudfoot, 2003). Lung function tests are commonly abnormal (Bismuth et al., 1982). At autopsy, there may be a pleural effusion, and damage to the upper respiratory tract. Grossly, the lungs appear solid, with haemorrhages, including subpleural ones. Histologically, there is oedema and the alveoli are airless with fibroblastic proliferation in the alveolar walls. Infiltration with mononuclear cells, polymorphs, macrophages and eosinophils has been reported. The longer the survival time, the greater the proliferation of epithelium and fibroblasts in the alveoli (Carson & Carson, 1976). Tubular damage in the kidney has been reported as well as mid-zonal and centrilobular degeneration in the liver. Proudfoot et al. (1979) reported that the plasma concentration of paraquat was a good predictor of the outcome in that persons whose plasma concentrations were below 2.0, 0.6, 0.3, 0.16 and 0.1 mg/l at 4, 6, 10, 16 and 24 h respectively after ingestion survived. Scherrmann et al. (1987) reported that plasma concentrations of paraquat in persons admitted more than 24 h after poisoning were predictive of the outcome of the poisoning in most patients. Furthermore, they concluded, on the basis of study of 53 patients, that persons with urinary concentrations of paraquat of <1 mg/l within 24 h of exposure would survive, while a fatal outcome could be anticipated in most persons in whom the urinary concentration of paraquat was >1 mg/l.

In a fatal case of paraquat poisoning in a pregnant woman, who developed the typical symptoms and signs of paraquat poisoning and at postmortem had the typical lung pathology of paraquat poisoning, the fetal lungs were normal (Fennelly et al., 1968). Talbot & Fu (1988), however, who reported the details of nine pregnant women who deliberately ingested paraquat, stated that paraquat in one case was concentrated 4–6 times in the fetus. In another of the cases, the amniotic fluid contained paraquat at twice the concentration of that in the maternal blood. All the fetuses died, whether or not caesarian section was carried out. A case of paraquat poisoning in early pregnancy was reported from French Guiana. A woman who was 10 weeks pregnant took Grammoxone®, in a suicide attempt. She developed oliguria and underwent dialysis. The blood concentration of paraquat was 0.22 mg/l. No pulmonary symptoms or signs were noted and renal function progressively returned to normal. The woman gave birth normally at 39 weeks and both mother and baby remained well during 4 years of follow-up (Raynal et al., 2003).

Although most patients who have radiological lung changes go on to develop progressive and ultimately fatal lung damage, there are a few case reports in which patients have developed persistent radiological changes but have survived (e.g. Hudson et al., 1991).

There is also evidence that, in such patients, some recovery may occur over time (Ming et al., 1980; Lin et al., 1995; Papiris et al., 1995).

It has been reported that alcohol may increase the severity of paraquat poisoning (Ernouf et al., 1998), but the reverse has also been suggested (Ragoucy-Sengier et al., 1991).

The vast majority of paraquat intoxications are by ingestion. Athanaselis et al. (1983), however, reported the poisoning via the skin of a 64-year-old spray operator. Fluid had leaked down his back for several hours, causing irritation of the skin. Two days later, the spray operator visited a doctor, who advised hospitalization. The patient rejected this advice, but was admitted into hospital 3 days later. He died of toxic shock and renal and respiratory insufficiency 12 h after admission. At autopsy, the findings were typical of paraquat poisoning with fibrosing interstitial pneumonitis and intra-alveolar haemorrhage in the lungs, renal tubular cell degeneration, cholestasis and necrosis of the skin of the back. A further case of a fatality from transdermal exposure to paraquat was reported from Papua New Guinea, the patient evidently thinking that Gramoxone (20% paraquat w/v) would kill lice, for which purpose he applied the material to his scalp and beard. This produced painful sores and his condition steadily deteriorated until death 6 days after applying the paraquat to his skin. At autopsy, there were skin lesions as well as solid and haemorrhagic lungs (Binns, 1976). In a further report, Garnier et al. (1994) reported two cases of percutaneous exposure. In the first case, a man aged 36 years applied 20% paraquat concentrate to his whole body to cure scabies. He developed extensive erythema followed by blistering and 2 days later he was admitted to hospital. He developed transient renal failure. Dyspnoea appeared 1 week after admission and he deteriorated, dying 26 days after exposure. The other case reported by Garnier et al. (1994) was much milder, with mainly skin effects, and the outcome was not fatal. Additional cases of fatal percutaneous paraquat intoxication were reported by Newhouse et al. (1978), Levin et al. (1979), Wohlfahrt (1982), Okonek et al. (1983) and Papiris et al. (1995). In general, systemic toxicity after percutaneous exposure of humans seems to be unusual (Hoffer & Taitelman, 1989).

There is evidence that as well as the route of exposure, the formulation may be important in determining the severity of effects. A case series of 14 instances of poisoning with granular paraquat and diquat at low concentrations was reported by Fitzgerald & Barniville in 1978. No deaths occurred, the illness was mild and necrotic lesions of the mouth and pharynx were not seen.

Initial management of cases of poisoning with paraquat comprises replacement of fluid loss, determination of the prognosis by measurement of the plasma concentration of paraquat, treatment of local damage to the oropharynx, and supportive care (Vale et al., 1987).

Numerous treatments have been tried in the management of cases of poisoning with paraquat, many concentrating on the prevention of absorption (Meredith & Vale, 1987). Gastric lavage, fuller's earth and activated charcoal have all been tested: other therapies that have been investigated include removal of paraquat from the blood by forced diuresis, peritoneal dialysis, haemodialysis or haemoperfusion using sorbent materials, including charcoal haemoperfusion (Tabei et al., 1982). Corticosteroids have also been tried (Bismuth et al., 1982; Chen et al., 2002), as have acetylcysteine and deferoxamine (Lheureux et al., 1995), and *S*-carboxymethylcysteine (Lugo-Vallin et al., 2003) and radiotherapy (Talbot & Barnes, 1988). Addo et al. (1984) reported that treatment with cyclophosphamide, dexam-

ethasone, forced diuresis with frusemide, triamterine and hydrochlorothiazide enabled the survival of 15 of 20 patients. This therapy was combined with routine measures, such as fuller's earth, activated charcoal and magnesium sulfate to eliminate paraquat from the gut. Time has, however, shown that none of the measures discussed above are consistently successful, therefore treatment is perforce symptomatic (Vale et al., 1987). The use of oxygen may increase the severity of pulmonary fibrosis (Bismuth et al., 1982) and should be delayed as long as possible. The therapy of paraquat poisoning has been reviewed (Flanagan & Jones, 2001).

3.2 Epidemiological studies

In an analysis of all cases of early onset Parkinson disease in persons born and raised in Saskatchewan, Canada, it was found that 20 out of 22 cases were exclusively exposed to a rural environment during the first 15 years of life. This distribution was significantly different from that of the general population ($p = 0.0141$). Further study included sampling and metal analysis of sources of drinking-water in childhood in 18 cases and in 36 age- and sex-matched controls. Drinking-water to which the individuals in the cases and controls had been exposed was collected and analysed for 23 metals. There was no difference in the metal composition of the water between the two groups. A review of pesticide usage from Saskatchewan agricultural records was undertaken to determine if there was an increased incidence of early onset Parkinson disease after use of any particular chemical. No increase was found in the incidence of the disease with the introduction of any pesticide, including paraquat, for agricultural use (Rajput et al., 1987).

In a case–control study, the personal histories of 57 cases and 122 age-matched controls were compared to identify possible determinants of Parkinson disease. Odds ratios (OR) adjusted for sex, age, and smoking were computed using stepwise logistic regression. A statistically significant increased risk for working in orchards was found (OR, 3.69; 95% CI, 1.34–10.27; $p = 0.012$). The relative risk of Parkinson disease decreased with smoking, an inverse relationship that was supported by the results of many studies (Hertzman et al., 1990).

A questionnaire-based case–control study to investigate possible risk factors for Parkinsonism involved 150 patients with Parkinson disease and 150 controls matched by age and sex. Use of well water and rural living were associated with Parkinsonism, but farming and pesticide/herbicide use was not (Koller et al., 1990).

In a case–control study of 130 cases of Parkinson disease and 260 age- and sex-matched controls from Calgary, Alberta, Canada, no significant association of Parkinson disease with rural or farm living or drinking well water in early childhood was found (Semchuk et al., 1991).

A retrospective case–control study, with 127 cases and 245 controls was carried out to identify possible risk factors for idiopathic Parkinsonism. Of the controls, 121 had cardiac disease and 124 were randomly selected from electoral lists. An occupational history was collected, and known contact with all pesticides associated with the tree-fruit sector of the agricultural industry was recorded. There was a significant association between Parkinsonism and having had an occupation in which exposure through handling or directly contacting pesticides was probable, but no specific chemicals were associated with the condition. The authors concluded that although occupations involving the use of agricultural chemi-

cals might predispose to the development of Parkinsonism, it was likely that the pathogenesis is multifactorial rather than related to a specific agent (Hertzman et al., 1994).

In a cross-sectional study undertaken in the Republic of Nicaragua to evaluate any relationship between respiratory health and paraquat exposure, the study population was selected from among workers at 15 banana plantations that used paraquat as a herbicide. All workers who reported never having applied paraquat and all who reported more than 2 years of cumulative exposure as knapsack sprayers of paraquat were invited for medical examination. There were 134 workers in the group that was exposed to paraquat and there were 152 workers that were not exposed. All took part in a questionnaire interview asking about exposure and respiratory symptoms, and underwent spirometric testing of forced expiratory volume in 1 s ($FEV_{1.0}$) and forced vital capacity (FVC). Of the persons in the exposed group, 53% reported having experienced a skin rash or burn resulting from exposure to paraquat, 25% reported epistaxis, 58% reported nail damage, and 42% reported paraquat splashes to the eyes. There was a consistent relationship between a history of skin rashes or burns and the prevalence of dyspnoea. This relationship was more marked for more severe dyspnoea. There was a three-fold increase in episodic wheezing accompanied by shortness of breath among the more intensely exposed workers. There was no relationship between exposure and $FEV_{1.0}$ or FVC. The authors considered that the high prevalence of respiratory symptoms associated with exposure, in the absence of spirometric abnormalities associated with exposure, could be a result of unmeasured gas exchange abnormalities among workers with long-term exposure to paraquat. They could also have been caused by recall bias (Castro-Gutiérrez et al., 1997).

3.3 Studies in human volunteers

A study of the percutaneous absorption of paraquat in vivo was undertaken in six human volunteers by Wester et al. (1984). ^{14}C-Labelled paraquat dichloride at a dose of 9 µg/cm^2 was applied to a 70 cm^2 area of the skin of the back of the leg, the back of the hand or the ventral surface of the forearm. The specific activity of the paraquat was 2.0 mCi per mmol per l and the concentrations of the solution were given as paraquat dichloride, not as paraquat ion. Urine samples were collected at 4, 8, 12, and 24 h, and then every 24 h for 5 days. The extent of percutaneous absorption was measured by comparing the excretion of ^{14}C after parenteral and topical administration; rather than administer the paraquat to humans, it was administered to rhesus monkeys. The percentage of the applied dose that was absorbed was 0.29 ± 0.2 (mean ± SD) for the leg, 0.23 ± 0.1 for the hand and 0.29 ± 0.1 the forearm. The absorption rate for the 24 h of exposure was 0.03 µg/cm^2. It was concluded that paraquat was poorly absorbed through human skin and that there was little difference between skin at different sites in ability to absorb paraquat.

Comments

The pharmacokinetics and metabolism of paraquat have been the subject of many studies. Paraquat is not well-absorbed when administered orally. After oral administration of radiolabelled paraquat to rats, more than half the administered dose (60–70%) appeared in the faeces and a small proportion (10–20%) in the urine. In studies involving single or repeated doses, excretion of the radiolabel was rapid; about 90% was excreted within 72 h. Residual radioactivity was primarily found in the lungs, liver and kidneys. Some studies have found small amounts in the brain, but only in structures outside the blood–brain barrier or in structures without a blood–brain barrier (the pineal gland and linings of the

cerebral ventricles, the anterior portion of the olfactory bulb, hypothalamus and area postrema). Paraquat is taken up into the lungs by an active process, whose normal substrate is endogenous diamines, e.g. putrescine and polyamines such as spermine and spermidine. In rats, dogs and monkeys, there are indications that paraquat is actively secreted in the kidneys.

Paraquat is largely eliminated unchanged; in rats, approximately 90–95% of radiolabelled paraquat in urine was excreted as the parent compound. Some studies have failed to show the presence of any metabolites after oral administration of paraquat, while others have shown a small degree of metabolism, which probably occurs in the gut as a result of microbial metabolism. Paraquat was not found in the bile.

The acute LD_{50} after oral administration was 290–360 mg/kg bw in mice and 112–350 mg/kg bw in rats, while the guinea-pig was more sensitive (LD_{50} of 22–30 mg/kg bw). The LD_{50} in cynomolgus monkeys was 50–70 mg/kg bw. Paraquat was considered to be a mild skin irritant and a moderate ocular irritant and was not a skin sensitizer in the Magnusson and Kligman test.

The predominant feature of exposure to repeated doses of paraquat was lung toxicity. Renal toxicity (proximal tubular damage) and toxicity to the liver (jaundice and elevations of enzyme activity) were also found. In some studies, lens opacities were seen. At higher doses, decreased body-weight gain, clinical signs (dyspnoea, increased respiratory sounds, swellings and sores in the genital area), haematological changes and effects on organ weight were reported, as well as increased mortality.

Lung abnormalities observed in mice, rats and dogs consisted of increased lung weight and gross pathological changes. Associated histopathological changes included cell necrosis, alveolar cell proliferation and hypertrophy, oedema, infiltration of macrophages and mononuclear cells and exudate. Dogs were most sensitive to paraquat-induced lung toxicity, followed by rats and mice; a NOAEL of 0.45 mg of paraquat ion/kg bw per day was found in a 1-year study in dogs, on the basis of signs of respiratory dysfunction and histopathological changes at higher doses. This finding was supported by the NOAEL of 0.55 mg of paraquat ion/kg bw per day from a 13-week study in dogs.

Ophthalmoscopy in-life and histopathological examination of eyes at necropsy revealed corneal opacity and cataracts in animals receiving doses of 3.75 mg and 7.5 mg of paraquat ion/kg bw per day in a lifetime study in Fischer rats. Other ocular effects included lenticular degeneration, lens capsular fibrosis and/or lens ruptures, peripheral retinal degeneration, and proteinaceous vitreous humour. At time-points after 2 years (i.e. after the study would have ended according to current guidelines), rats receiving the lowest dose exhibited age-related peripheral morgagnian corpuscles and slight peripheral and moderate mid-zonal lenticular degeneration. Histopathological evidence of cataracts was also found at the highest dose (7.67 mg of paraquat ion/kg bw per day) in a 2-year study in Fischer rats, but not at lower doses. In another 2-year study in Wistar rats, no intergroup differences in the prevalence of cataracts were seen. These differences between effects on the lens in the three long-term studies in rats may be indicative of a difference between Wistar and Fischer rats.

Paraquat elicited renal toxicity, which comprised changes in the proximal tubules of the kidneys (hydropic degeneration, eosinophilia and dilatation) in mice fed with 15.0 mg of paraquat ion/kg bw per day in a lifetime study. Some very mild changes were also

observed in males at 5.62 mg of paraquat ion/kg bw per day, however, there was a clear NOAEL at 1.88 mg of paraquat ion/kg bw per day. There were some histopathological effects on renal distal tubular cells at 1.75 mg and 3.52 mg of paraquat ion/kg bw per day in a 13-week study in dogs, the NOAEL being 0.55 mg of paraquat ion/kg bw per day.

The frequency of pulmonary adenoma was increased in females in a 2-year study in rats receiving a dose of 8.47 mg of paraquat ion/kg bw per day; however, there was a clear NOAEL at 3.13 mg of paraquat ion/kg bw per day. In males, adenocarcinoma was found in three animals (out of 80) receiving a dose of 10.6 mg of paraquat ion/kg bw per day, one animal (out of 80) receiving 3.52 mg of paraquat ion/kg bw per day and two animals (out of 80) receiving 1.34 mg of paraquat ion/kg bw per day. The NOAEL for males in this study was 0.77 mg of paraquat ion/kg bw per day on the basis of histopathology of the lungs. In a second 2-year study in rats, no intergroup differences in tumour incidence were seen at any site. After review of the histopathological findings in the lifetime study in rats, it was concluded that the incidence of lung neoplasms in the test groups was comparable to that in the control groups. Thus tumours were seen in only one out of three long-term studies in rats. The Meeting concluded that the weight of evidence suggested that paraquat was not carcinogenic in the rat. Paraquat was not considered to be tumorigenic in two studies in mice.

Paraquat has been tested extensively in a broad range of assays for genotoxicity in vitro and in vivo, with mixed results. Studies more commonly gave positive results when DNA damage or clastogenicity were the end-points. Paraquat is known to produce active oxygen species and the available evidence indicates that it is probably this property that is responsible for its genotoxicity. Consequently, there is a threshold below which genotoxic activity will not be evident, provided that normally functioning antioxidant defence mechanisms have not been overwhelmed. The Meeting concluded that paraquat is unlikely to pose a genotoxic risk to humans.

Because of the nature of the genotoxicity observed and the lack of carcinogenicity in rats and mice, the Meeting concluded that paraquat was unlikely to pose a carcinogenic risk to humans.

Three studies of reproductive toxicity in rats were reported. The overall NOAEL for parental toxicity was 1.67 mg of paraquat ion/kg bw per day, and the NOAEL for pup toxicity was 5.0 mg of paraquat ion/kg bw per day. Impaired fertility was not seen in these studies. Two studies of developmental toxicity in rats and two in mice were available for evaluation. The lowest NOAELs observed for both maternal and developmental toxicity in rats were 1 mg of paraquat ion/kg bw per day on the basis of clinical signs, and reduced body-weight gain in the dams and reduced mean fetal weights and retarded ossification in the fetuses. Higher NOAELs for maternal and developmental toxicity were seen in mice. Teratogenicity was not seen at any dose in any study in either rats or mice.

Paraquat is structurally similar to the known dopaminergic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). As a result, paraquat has been considered as a possible etiologic factor in Parkinson disease. However, paraquat is a quaternary nitrogen compound and therefore crosses biological membranes poorly, unlike MPTP, the precursor of the neurotoxicant methylphenylpyridinium ion. Data made available to the Meeting suggested that paraquat is not taken up by the dopamine transporter. Studies on the effects of paraquat on the central nervous system have used a variety of routes, including subcuta-

neous or intraperitoneal injection and direct injection into the central nervous system, and end-points observed have been behavioural, morphological and neurochemical. Behavioural effects and loss of neurones in the substantia nigra were observed and, neurochemically, depletion of dopamine was reported in many, but not all of these studies. The design of these studies, however, renders the relevance of these data questionable for the risk assessment of dietary exposure to paraquat residues.

Persistent hypoactivity was observed in mice given paraquat by mouth on postnatal days 10 and 11. Reduced striatal content of dopamine and its metabolites was seen, but concentrations of serotonin were not affected. In a similar study of which the Meeting was aware, these findings had not been reproduced.

The Meeting concluded that the available mechanistic and other animal studies did not support the hypothesis that paraquat residues in food are a risk factor for Parkinson disease in humans.

Two studies carried out to assess the potential involvement of combined exposure to paraquat and maneb, a manganese-containing ethylene*bis*dithiocarbamate fungicide, in the etiology of idiopathic Parkinson disease were evaluated by the Meeting. Paraquat or maneb, or a combination of the two, was given intraperitoneally to mice. The study was not designed appropriately to investigate potentiation and the results could have reflected dose-additivity.

Intentional and accidental poisonings with paraquat have been a major cause of death in many countries. Most incidents are caused by ingestion of the concentrate intended for agricultural use. Local effects include damage to the skin, nails, mouth, eyes and nose. Sore throat, dysphagia and epigastric pain may occur. Systemic effects, which produce the fatal outcome seen in those who have ingested a sufficient quantity of paraquat, mainly involve the respiratory system. The changes in the lungs that underly the symptoms and clinical signs comprise a proliferative alveolitis similar to that seen in most experimental animals treated with paraquat. In most, but not all, patients who develop the characteristic lung changes, the condition progresses inevitably towards a fatal outcome, death being due to respiratory failure. Numerous therapies have been tested, but none has been consistently successful.

A number of epidemiological (case–control) studies have been carried out in humans with Parkinson disease. In some of these, associations with exposure to chemicals including pesticides (in some cases specifically paraquat) were sought. Some but not all studies have shown a relationship between working in situations that might involve contact with or use of pesticides and Parkinson disease, but associations with exposure to specific pesticides have not been shown consistently.

The Meeting established an ADI of 0–0.005 mg of paraquat ion/kg bw based on a NOAEL of 0.45 mg of paraquat ion/kg bw per day in the 1-year study in dogs and using a safety factor of 100. Although a 1-year study in dogs is not considered to be a long-term study, the nature and time-course of the pathogenesis of the lung lesions were such that the application of an additional safety factor was not considered to be necessary.

The Meeting established an acute RfD of 0.006 mg of paraquat ion/kg bw based on the NOAEL of 0.55 mg of paraquat ion/kg bw per day in the 13-week study in dogs, with

a safety factor of 100. Histopathological changes in the lungs were present at higher doses in both studies in dogs.

Toxicological evaluation

Levels relevant to risk assessment

Species	Study	Effect	NOAEL[a]	LOAEL[a]
Mouse	13-week study	Toxicity	100 mg/kg, equal to 8.33 mg of ion/kg bw per day	300 mg/kg, equal to 25.9 mg of ion/kg bw per day
	97–99-week study	Toxicity	12.5 mg/kg, equivalent to 1.88 mg of ion/kg bw per day	37.5 mg/kg, equivalent to 5.62 mg of ion/kg bw per day
		Carcinogenicity	100 mg/kg equivalent to 15.0 mg of ion/kg bw per day[b]	—
	Study of developmental toxicity	Maternal toxicity	10 mg/kg bw per day[b]	—
		Embryo- and fetotoxicity	10 mg/kg bw per day[b]	—
Rat	13-week study	Toxicity	100 mg/kg, equal to 4.74 mg/kg bw per day	300 mg/kg, equal to 14.2 mg/kg bw per day
	104-week study	Toxicity	30 mg/kg, equal to 0.77 mg/kg bw per day	100 mg/kg, equal to 2.55 mg/kg bw per day
		Carcinogenicity	300 mg/kg, equal to 7.67 mg of ion/kg bw per day[b]	—
	Multigeneration study of reproductive toxicity	Parental toxicity	25 mg/kg, equivalent to 1.67 mg/kg bw per day	75 mg/kg, equivalent to 5.0 mg/kg bw per day
		Pup toxicity	75 mg/kg, equivalent to 5.0 mg/kg bw per day	150 mg/kg, equivalent to 10.0 mg/kg bw per day
	Study of developmental toxicity	Maternal toxicity	1 mg/kg bw per day	5 mg/kg bw per day
		Embryo- and fetotoxicity	1 mg/kg bw per day	5 mg/kg bw per day
Dog	13-week study	Toxicity	20 mg/kg, equal to 0.55 mg/kg bw per day	60 mg/kg, equal to 1.75 mg/kg bw per day
	1-year	Toxicity	15 mg/kg, equal to 0.45 mg/kg bw per day	30 mg/kg, equal to 0.93 mg/kg bw per day

[a] Dietary concentrations are expressed as dichloride or paraquat ion as in the study report; intakes and doses are expressed as paraquat ion

[b] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.005 mg of paraquat ion/kg bw

Estimate of acute reference dose

0.006 mg of paraquat ion/kg bw

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for paraquat

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Poor
Dermal absorption	Poor; 0.25–0.29% absorbed (humans)
Distribution	Highest concentrations found in the lungs, liver and kidneys
Potential for accumulation	No potential for passive accumulation; active uptake into type II pneumocytes
Rate and extent of excretion	Rapid, about 64% in 24 h; 10% in urine, the remainder in the faeces; none is found in bile
Metabolism	Some metabolism (<5%) in gut (probably microbial); paraquat is largely excreted unchanged
Toxicologically significant compounds (animals, plants and environment)	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	100–300 mg paraquat ion/kg bw
Rat, LD_{50}, dermal	80→660 mg of paraquat ion/kg bw
Rat, LC_{50}, inhalation	0.0006–0.0014 mg of paraquat ion/l (4 h exposure)
Rabbit, skin irritation	Mild
Rabbit, eye irritation	Moderate
Skin sensitization	Not sensitizing (Magnusson and Kligman test)
Short term toxicity	
Target organ/critical effect	Lung toxicity
Lowest relevant oral NOAEL	0.55 mg of paraquat ion/kg bw per day (13-week study in dogs); 0.45 mg of paraquat ion/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1.15 mg of paraquat ion/kg bw per day (21-day study in rabbits)
Lowest relevant inhalation NOAEC	0.00001 mg/l (21-day study in rats)
Genotoxicity	Paraquat was clastogenic at high concentrations Unlikely to pose a genotoxic risk to humans at dietary concentrations
Long term studies of toxicity and carcinogenicity	
Target organ/critical effect	Lung toxicity
Lowest relevant NOAEL	0.77 mg of paraquat ion/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Lung toxicity in pups
Lowest relevant reproductive NOAEL	5 mg of paraquat ion/kg bw per day (three-generation study in rats)
Developmental target/critical effect	Not teratogenic; reduced fetus weight and ossification at maternally toxic dose
Lowest relevant developmental NOAEL	1 mg of paraquat ion/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
	Not neurotoxic by oral route
Other toxicological studies	Mechanistic studies on lung, liver and kidney toxicity
Medical data	Causes acute poisoning

Summary	Value	Study	Safety factor
ADI	0–0.005 mg/kg bw	Dog, 1-year study	100
Acute RfD	0.006 mg/kg bw	Dog, 13-week study	100

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