CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
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PARAQUAT: A REVIEW OF THE ANIMAL AND HUMAN TOXICOLOGY

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PARAQUAT

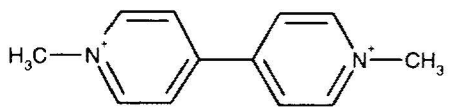
This report is a review of the animal and human toxicology which is available on this herbicide in the open literature. It will appear as a chapter in the 2nd edition of the Handbook of Pesticide Toxicology to be published by Academic Press sometime in 2000.

1. IDENTITY, PROPERTIES AND USE

1.1 Chemical name

Paraquat is 1,1'-dimethyl-4,4'-bipyridinium ion (IUPAC, CAS RN *[4685-14-7]*), also known as the 1,1'-dimethyl-4,4'-bipyridyldiylium ion.

1.2 Structure



Paraquat dichloride

1.3 Synonyms

The common name paraquat is in general use (BSI, E-ISO, ANSI, WSSA, JMAF), except in Germany. Paraquat is usually formulated as the dichloride salt (also known as methyl viologen) (CAS NR *[1910-42-5]*), the bis(methyl sulphate) salt (CAS NR *[2074-50-2]*) is no longer commercialised. Code designations for the material are PP148 (for the dichloride salt) and PP910 (for the bis(methyl sulphate) salt). Trade names for paraquat dichloride formulations include Crisquat®, Cyclone®, Dextrone X®, Esgram®, Efoxon®, Goldquat® 276, Gramoxone®, Herbaxon®, Katalon®, Osaquat Super®, Pilarxone®, R-Bix®, Speeder®, Starfire®, Sweep®, Total®, Weedless®. Mixtures of paraquat with diquat are sold under trade names including Actor®, Dukatalon®, Opal®, Pathclear® (also includes simazine and aminotriazole), Preeglox®, Preglone®, Seccatuto®, Spray Seed®, Weedol®.

Trade names of mixtures with urea herbicides include Dexuron®, Gramocil®, Gramonol®, Gramuron®, Tota-Col®.

1.4 Physical and chemical properties

The molecular formula of the cation is $C_{12}H_{14}N_2$ with a molecular weight of 186.3. The dichloride salt has the formula $C_{12}H_{14}Cl_2N_2$ and a molecular weight of 257.2. Paraquat dichloride forms colourless, hygroscopic crystals which decompose at 300 °C. It is practically non-volatile with a vapour pressure of <0.1 mPa. It is very soluble in water (700 g/l at 20 °C) and practically insoluble in most other organic solvents. It is stable in neutral and acidic media, but readily hydrolysed in alkaline media. Paraquat is photochemically decomposed by ultraviolet radiation in aqueous solution.

1.5 History, formulations and uses

Paraquat was first described in 1882 by Weidel and Russo. In 1933, Michaelis and Hill discovered its redox properties and called the compound methyl viologen. The herbicidal properties of paraquat were first described by Brian *et al.*, (1958) and it became commercially available in 1962. Paraquat is mainly formulated as an aqueous solution with surface-active agents. In some countries, a low-strength granular formulation (also containing diquat) is available. Paraquat is a fast-acting, non-selective contact herbicide, absorbed by the foliage with some translocation in the xylem. It is used for broadspectrum control of broad-leaved weeds and grasses in fruit orchards and plantations, and for inter-row weed control in many crops. It is also used for general weed control on non-crop land, as a defoliant on cotton and hops, for destruction of potato haulms, as a desiccant, and for control of aquatic weeds. Paraquat is rapidly deactivated upon contact with the soil and does not leach.

2. TOXICITY TO LABORATORY ANIMALS

2.1 Signs of toxicity

Following a lethal dose of paraquat to rats mortality is first seen on days 2-5 after dosing but deaths can also occur around days 10-12 (Clark *et al.*, 1966; Sharp *et al.*, 1972; Smith and

Rose, 1977), indicating there is considerable inter-individual animal response to the chemical. The major cause of death after a median lethal dose is due to lung damage, the animals develop acute pulmonary oedema with signs of laboured respiration and ultimately die of respiratory failure (Clark *et al.*, 1966; Kimbrough and Gaines, 1970; Sharp *et al.*, 1972; Murray and Gibson, 1972). Rabbits, however, do not show signs of respiratory distress, they stop eating and drinking and tend to die without overt toxicity, following oral dosing (Clark *et al.*, 1966; Butler and Kleinerman, 1971). Rats and mice given doses above the median lethal dose (MLD), by ip or sc administration show signs of hyperexcitability, ataxia and convulsions and usually die within a few hours of dosing, indicative of an effect on the central nervous system (Clark *et al.*, 1966; Bagetta *et al.*, 1992). Following chronic exposure signs of toxicity are few, but may include respiratory effects.

2.2 Acute toxicity

The acute oral toxicity of paraquat to the rat is shown in Table 1. The median lethal dose of pure paraquat dichloride expressed as the cation was about 150mg/kg to female rats and ranged from 100-143mg/kg in a number of different strains from a number of different laboratories. No sex difference in toxicity was seen and the toxicity was similar for the two different salts of paraquat (Table 1). Fasting rats prior to oral administration of paraquat made little difference to the toxicity, the 7 day MLD with 95% confidence limits were 143 (123-166), 130(106-159) and 126(102-156) mg paraquat ion/kg respectively for rats fasted for 0, 4 and 8hr (Murray and Gibson, 1971). Mice are less sensitive than the rats to orally administered paraquat, while guinea pigs, cats, monkeys and rabbits are more susceptible (Table 2).

Paraquat was more toxic when given by the intraperitoneal (ip) or intravenous (iv) routes with an MLD of approximately 20mg paraquat ion/kg (Table 1), indicating that following oral dosing the compound is poorly absorbed from the gastrointestinal tract (see later). The guinea pig and dog (Nagata *et al.*, 1992a) are also more sensitive to systemic administration with a MLD of 2-3mg/kg (Table 2), reflecting poor or incomplete absorption of paraquat from the gastrointestinal tract after oral administration. Rabbits given a single iv dose of paraquat at 40 or 80 mg/kg died within 24h, while they survived a single dose of 10mg/kg iv, no lung lesions were seen at these doses (Ilett *et al.*, 1974). The vehicle used to administer

paraquat can influence lethality in mice. For example, paraquat was more toxic when given by the ip or subcutaneous(sc) route in water than in isotonic saline, suggesting that the solvent may influence the absorption from the site of injection and hence the amount delivered to the lung (Drew and Gram, 1979).

The dermal toxicity of paraquat has been studied in rabbits (Table 3). The precise technique of application of paraquat to the skin, whether the site of application is open to the air or covered and whether the rabbits are prevented from grooming, affects the findings (Clark *et al.*, 1966; McElligott, 1972). Rabbits fitted with restraining collars to reduce grooming the site of application, followed by decontamination of the skin and removal of the collars, showed glossitis, anorexia, weakness and loss of weight with some skin erythema followed by hyperkeratosis and desquamation at the higher doses, indicating that some oral ingestion had still occurred. This technique resulted in a MLD following a single application of 236mg paraquat ion/kg. If however, the restraining collars were not removed then the erythema and desquamation was mild and the extent of glossitis and hence body weight loss was less. Under these conditions the MLD was found to be > 480mg ion/kg, the maximum dose possible to apply in a satisfactory manner (McElligott, 1972). Thus when compared to the systemic MLD of 18mg ion/kg (Table 2) indicates that little of the applied dose has been absorbed through intact skin. Dermal exposure of rats to paraquat gave an MLD of 80-90mg paraquat ion/kg (Table 3), however these authors (Kimbrough and Gaines, 1970) gave no information on the state of the skin after application, whether the site was occluded or free for the rats to groom. The absorption of paraquat across the skin has been reviewed by Smith, (1988) who concluded that paraquat is poorly absorbed by intact skin and raised technical concerns about the validity of the earlier dermal studies reported by Kimbrough and Gaines, (1970).

Paraquat is not volatile, but following inhalation exposure to an aerosol is irritant to the respiratory tract. At lethal concentrations under these conditions, death is usually delayed for several days and is due to respiratory failure. Following single exposures the MLD is a function of both the amount and duration of exposure, which in the rat is approximately 6µg/L. hr.(Table 3). Guinea pigs and male mice are of similar sensitivity to the rat, while female rats and rabbits are less sensitive. Dogs can tolerate a concentration-time product of 25µg/L. hr without ill effects (Gage, 1968a). The toxicity is also a function of particle size,

3μm was the most lethal to the rat. Large particles do not reach the alveolar region and are less toxic. Under normal conditions of manufacture, handling and use inhalation exposure is not considered to be a hazard.

Studies with rabbits have shown that the lung is susceptible to paraquat injury following intrabronchial deposition (Zavala and Rhodes, 1978) and inhalation exposure (Seidenfeld *et al.*, 1978), although as mentioned above it is refractory following oral or intraperitoneal administration. Local instillation of paraquat in the lungs of rats will also produce local injury and fibrosis (Kimbrough and Gaines,1970; Wyatt *et al.*, 1981).

2.3 Irritation and sensitisation

Paraquat is a skin and eye irritant but is not a skin sensitiser (Bainova, 1969). As discussed earlier skin irritation has been reported in rabbits when the area of application is occluded (Clark *et al.*, 1966; McElligott, 1972), resulting in local erythema followed by hyperkeratosis and desquamation.

Instillation of a 0.29% aqueous solution of paraquat into the rabbit eye produced no effect, however more concentrated solutions produced inflammation of the conjunctiva and nictitating membrane. This response developed gradually over 12h and lasted for 48-96h (Clark *et al.*, 1966). Instillation of higher concentrations of paraquat (3-48mg contained in 0.2ml of water into the rabbit eye produced a dose-related increase in ocular injury with doses of 48mg (about 16mg/kg) and above producing fatalities (Sinow and Wei, 1973). These findings indicate that absorption of paraquat from the eye is similar to that following systemic administration.

2.4 Subchronic toxicity

Daily administration of paraquat in the diet of rats at an inclusion rate of 100ppm (about 5mg ion/kg/day) was tolerated for several months. However, if increased to 250ppm (about 12.5mg ion/kg/day) the rats became ill and died within 27 to 57 days. Females appeared to be more susceptible than males, the primary target organ for toxicity being the lungs (Clark *et al.*, 1966). A number of other studies have shown that moderate daily doses of paraquat can be tolerated. Rats fed 125ppm (about 6.25mg/kg/day) for 2 years showed no toxic effects and dogs tolerated 50ppm (about 0.9mg/kg/day) for 2 years (Howe and Wright, 1965). Rats given

paraquat in their drinking water at 1.3mg/L or 2.6mg/L for 2 years showed some mortality and histological changes in the lung at the highest dose, while only minimal changes to the lung were seen at the lower dose (Bainova and Vulcheva, 1977). The MLD for paraquat fed in the diet for 90 days has been determined. Groups of female rats were fed 300, 400, 500, 600 and 700ppm paraquat and their food consumption recorded at intervals to enable the dose in mg/kg/day to be calculated. After 90 days the surviving rats were held for 2 weeks to allow time for any delayed deaths. The MLD was 21 mg/kg/day, giving a subchronic toxicity factor of 5.2 (ratio of acute to subchronic MLD's), indicating that paraquat has a moderate cumulative toxicity in this species (Kimbrough and Gaines, 1970).

Rabbits given paraquat ip at 10 or 20mg/kg at 48h intervals showed marked signs of toxicity with a high mortality following 3 to 5 doses, there was little evidence of lung damage and it is likely that the animals died from multi-organ failure (Butler and Kleinerman, 1971). Rabbits can however tolerate 3mg/kg day ip for up to 14 days, but when increased to 6mg/kg/day significant mortality was seen (Hassan *et al.*,1989). Daily oral dosing at 11mg/kg/day to male rabbits for 30 days produced few signs of toxicity, with only one animal showing lung damage (Dikshith *et al.*, 1979).

Subchronic exposure following dermal application has been examined in rabbits. The mortality observed with repeated daily applications beneath an occlusive dressing gave a MLD of 6.24 (4.6-8.5)mg ion/kg/day of paraquat over 20 days (McElligott, 1972). At the higher doses the skin was reddened and sloughing with local oedema was observed, while at the lowest dose some scab formation was seen after about 7 days of application. Systemic effects at post-mortem included renal tubular necrosis, focal hepatocellular necrosis and pulmonary congestion. Studies were also conducted where the skin was not occluded, rabbits were fitted with collars and these were either removed after decontamination of the skin or at the end of the observation period. The MLD for 20 days exposure was between 7.25 and 14.5mg paraquat ion/kg/day for the animals where the collars were removed after decontamination and at least 24mg ion/kg/day for those where the collars were left on all the time. The rabbits showed marked signs of salivation, which was associated with glossitis and ulceration of the tongue. The animals refused to eat and death occurred in a state of cachexia; this effect was less marked at the lower doses when the collars were kept in place all the time (McElligott, 1972). When the "Gramoxone" formulation of paraquat was diluted to spray

strength and applied to the skin of rabbits for 20 days (2.4mg ion/kg/day) no clinical signs of toxicity or pathological changes were seen (McElligott, 1972).

Daily subcutaneous dosing of paraquat to dogs for 4 weeks, resulted in some animals being terminated at the top dose of 0.495mg/kg/day, while at the other doses of 0.165 and 0.055mg/kg/day all animals appeared well (Nagata *et al.*, 1992b). Histopathology of the lungs showed proliferation of alveolar lining cells and some fibrosis at the top dose and pulmonary changes (thickening of the alveolar wall and pleura) at all doses. The 28 day MLD from this study was about 0.5mg/kg/day.

Repeated exposure of rats to paraquat by inhalation at 0.4µg/L for 6 hr per day for 15 days, over 3 weeks, led to intermittent respiratory problems after about 4 exposures. At post-mortem after 15 exposures the animals showed marginal paraquat-related pathology to the lungs. While, exposure to 0.1µg/L for 15 daily, 6 hr periods showed no signs of toxicity or pathology in the lungs (Gage, 1968a). Rats exposed to 0.003µg/L for 6 hr a day for 5 days per week for 2 months, put on body weight, remained in good condition and showed no histopathological evidence of lung damage. Bainova *et al.*,(1972) exposed rats to a paraquat aerosol at 1.1 or 0.05mg/L for 6hr /day for 4.5 months and found evidence of lung damage at the higher dose, with little effect at the lower dose. Seidenfeld *et al.*,(1978) exposed rabbits by inhalation to paraquat by an ultrasonic nebuliser (mean particle size 4µm) at a concentration of 0.1mg/ml for 2hr/day for 5 days per week for 3 months and found no lung damage, however rabbits exposed to 2mg/ml for 2 hr/day could only tolerate 3 exposures and developed a reduced arterial oxygen tension and specific compliance which was associated with marked lung injury.

Overall these studies show that following acute and chronic exposure the primary target organ for toxicity is the lung, with deaths from lung damage frequently taking many days to occur following a single dose. The rabbit is unusual in that it does not readily develop a lung lesion following oral or parenteral exposure, but if instilled into the lung or exposed via inhalation, lung injury ensues. Renal functional impairment with some renal tubular necrosis is the other major organ affected. Dose levels of paraquat that do not cause lung damage in laboratory animals following acute and chronic exposure have been clearly established.

2.5 Mutagenic and carcinogenic potential

Paraquat is not carcinogenic in either rats or mice. The activity seen in some short-term assays for mutagenesis is associated with cytotoxicity, and believed to arise as a consequence of the redox cycling ability of paraquat, leading to superoxide anion formation.

Paraquat has minimal to no genotoxic activity when evaluated in a wide range of *in vitro* and *in vivo* test systems. Many groups have reported the absence of an effect while others have reported weakly positive effects (IPCS, 1984; Ribas *et al.*, 1995; Dabney, 1995 and references therein). These later effects were usually associated with high cytotoxicity or mortality and are believed to arise as a consequence of the redox cycling ability of paraquat. It is known that DNA damage frequently occurs when cells are exposed to oxidative stress (Brawn and Fridovich, 1981; Repine *et al.*, 1981). Paraquat-mediated effects on DNA have been reported in bacteria (Moody and Hassan, 1982; Yonei *et al.*, 1986), Chinese hamster cells (Sofuni *et al.*, 1985; Nicotera *et al.*, 1985; Takana and Amano, 1989), isolated alveolar macrophages and epithelial type II cells (Dusinska *et al.*, 1998) and in a few cases in cells from treated mice (He and Yasumoto, 1994; Rois *et al.*, 1995) these responses are all considered to be secondary to superoxide anion generation.

Studies with cultured mammalian cells have shown that paraquat inhibits DNA synthesis leading to the arrest of the cells in S-phase (Yamagami *et al.*, 1994; Tomita, 1996). This effect occurs prior to the onset of cytotoxicity and is thought to be part of a cascade of events initiated by the production of oxygen free radicals by the redox cycling of paraquat. These findings have been extended to rat lung cells exposed to paraquat *in vivo* which also showed S-phase arrest at early times after dosing. Prior treatment of the rats with a diet enriched in sodium tungstate, an inhibitor of xanthine oxidase, to reduce the production of free radicals, prevented the S-phase arrest produced by paraquat (Matsubara *et al.* 1996) and reduced mortality (Kitazawa *et al.*, 1991). Once inside a cell, paraquat can redox cycle producing oxygen free radicals that can cause cell cycle arrest and inhibit DNA synthesis. These findings are consistent with early studies showing that paraquat reduces DNA synthesis at early times after dosing (Van Osten and Gibson, 1975; Smith and Rose, 1977).

Paraquat has been evaluated for its carcinogenic potential in both rats and mice and it was concluded that at all doses up to the maximum tolerated dose, paraquat did not result in a

compound related increase in tumour incidence (FAO/WHO, 1986; Bainova and Vulcheva, 1977).

2.6 Effects on reproduction, embryotoxicity and teratogenicity

Paraquat has no effect on fertility, is not teratogenic and only produces fetotoxicity at doses that are maternally toxic. The main finding in multigeneration studies was lung damage.

Paraquat does not readily cross the placenta and enter the embryo of mice when given either orally or by ip administration (Bus *et al.*, 1975). In contrast, paraquat appears to readily cross the placenta of rats, being detected in fetuses within 30 min of an iv injection to 20 day pregnant rats (Ingebrigtsen *et al.*, 1984). A three-generation reproduction study in rats maintained on dietary levels of paraquat of 30 or 100ppm showed no effect on food intake, fertility, fecundity, neonatal morbidity or mortality. No teratogenesis or other changes in gross or histological morphology were seen, except for a slight increase in the incidence of renal hydropic degeneration in the 3-4 week old young receiving 100ppm (about 10mg/kg/day). Pregnant and young animals did not appear to be more susceptible than adults (FAO/WHO, 1973). A two-generation reproduction study in mice maintained on dietary levels of 45, 90 or 125 ppm showed no effects on age to parturition, number born or abnormalities in the pups in the first generation following 45 or 90 ppm. However, at 125ppm an increase in mortality was seen in the dams and pups during the first few weeks of life (Dial and Dial, 1987). The second generation mice were more resistant to the effects of paraquat the only effect being an increase in the age of the mothers at second parturition on the highest dose of paraquat (Dial and Dial, 1987). Subsequent studies to explore the basis for the high mortality in the first generation dams and pups exposed to 125ppm paraquat in the diet, showed that they almost certainly died from lung damage. This only occurred in pups exposed pre-natally via the placenta, not in pups exposed post-natally (Dial and Dial, 1989). Bus and Gibson, (1975) also reported that paraquat given to mice in their drinking water at either 50 or 100ppm from day 8 of gestation and to the young until 42 days of age increased pup mortality at 100ppm but not 50ppm. The lungs of mice killed 42 days after 100ppm showed extensive alveolar consolidation and collapse, supporting the view that the deaths at this dose were probably due to lung damage. No dominant lethal effects were seen

in mice exposed to paraquat at oral doses up to 4mg/kg/day for 5 days (Anderson *et al.*, 1976).

High doses of paraquat injected ip into pregnant rats or mice on various days of gestation can produce significant maternal toxicity (Bus *et al.*, 1975; Khera and Whitta, 1970). Examination of the foetuses of mice exposed to 1.67 or 3.35mg/kg ip or 20mg/kg po daily on days 8-16 of gestation induced no teratogenic effects, although a slight increase in non-ossification of the sternbrae was seen (Bus *et al.*, 1975).

2.7 Pathology of the lung

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

The most extensive studies on the pathogenesis of lung damage produced by paraquat have been conducted in rats. The time course of development of the injury in rats given a single MLD ip was reported by Vijeyaratnam and Corrin (1971) and Smith and Heath, (1974a). Damage to the type I and II alveolar epithelial cells was seen within a day of dosing. This damage was more marked by days 2-4 with large areas of the alveolar epithelium being completely lost, alveolar oedema developed and in some areas haemorrhage into the air spaces occurred. At this time there was extensive infiltration of inflammatory cells into the alveolar interstitium, air spaces and perivascular areas, although the alveolar endothelial capillaries were mainly spared. The animals died as a consequence of severe anoxia usually within the first few days after dosing and this has been confirmed by others (Clark *et al.*, 1966; Sharp *et al.*, 1972; Smith and Rose, 1977). This phase has been called the destructive

phase (Smith and Heath, 1976). Similar early pathological changes have been reported by Kimbrough and Gaines, (1970); Brooks, (1971); Modee *et al.*, (1972); Wasan and McElligott, (1972); Smith *et al.*, (1974); Sykes *et al.*, (1977) and Smith and Heath, (1976). Some rats that survive for up to 10-12 days after dosing develop an extensive hypercellular lesion in the lung which is dominated by proliferation of fibroblasts. This phase of the lesion is called the proliferative phase and is characterised by attempts by the epithelium to regenerate and restore normal architecture of the alveolar epithelium (Kimbrough and Gaines, 1970; Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974a). The findings in these animals are typically extensive intra-alveolar and inter-alveolar fibrosis, which in association with residual oedema reduces gaseous exchange which results in death from anoxia. It appears that the initial damage to the alveolar epithelium, produced by paraquat, is the primary event in the development of the lung injury, with the proliferative fibrosis being a consequence of the extensive damage produced. For a more detailed review on pulmonary injury see the review by Smith and Heath, (1976).

2.8 Absorption

The first studies on the absorption and excretion of paraquat from the gastrointestinal tract were conducted by Daniel and Gage, (1966) in rats. Following a single oral dose of 4, 6 or 50 mg/kg [^{14}C-methyl]paraquat dichloride most of the radioactivity was excreted within 48h. Occasionally some appeared in the faeces 3 and 4 days after dosing at the higher doses, with small amounts also in the urine. Between 6 and 14% of the dose was excreted in the urine over 48h when given as the dichloride salt, and 16-23% when given as the dimethylthiosulphate salt, the remainder being in the faeces. In contrast, when paraquat as either salt was given sc then the bulk of the radioactivity appeared in the urine within 24h of dosing, showing that paraquat is poorly absorbed across the gastrointestinal tract of the rat. Subsequent studies have extended and essentially confirmed these findings (Molnar and Hayes, 1971; Murray and Gibson, 1974; Lock and Ishmael, 1979; Chiu *et al.*, 1988).

The concentration of paraquat in the plasma following an oral dose to the rat is determined largely by the amount of paraquat present in the small intestine (Smith *et al.*, 1974). Studies in the dog using tracer doses (129µg/kg) of [^{14}C-methyl]paraquat support this, as peak plasma concentrations following oral dosing were observed at 75-90min (Figure 1), with about 46-

66% of the dose absorbed, as judged by the amount excreted in the urine at 6h (Davies *et al.*, 1977). Thus, the dog absorbs a greater percentage of an orally administered dose of paraquat than the rat, which is consistent with the greater susceptibility of the dog to paraquat by this route of administration. Pre-treatment of dogs with a drug that will block gastric emptying, delayed the peak plasma concentration by 3 to 6h, indicating that the stomach is not the major site of absorption (Bennett *et al.*, 1976).

These data in both rats and dogs indicate that the absorption of paraquat from the gastrointestinal tract occurs somewhere beyond the stomach. It is assumed this is similar for humans but there is limited evidence to support this. Based on the cationic nature of paraquat, it would not be expected to readily cross cellular membranes, it seems unlikely that simple diffusion would explain the rapid but incomplete absorption seen in the rat and dog. Studies *in vitro* with isolated mucosa from a number of different regions of the rat gastrointestinal tract (Steffen and Konder, 1979) have confirmed that the jejunum and ileum have the greatest capacity to transport paraquat from the lumen into the bloodstream and also showed that a component of the transport is facilitated (Heylings, 1991).

Following oral administration of paraquat to rats, the peak plasma concentration is seen between 30-60 min (Figure 1 and 2) following either a tracer dose (Chui *et al.*, 1988) or a toxic dose (Murray and Gibson, 1974). This profile is similar to that seen in the dog (Figure 1) (Davies *et al.*, 1977). The peak plasma concentration in the monkey and guinea pig occurs within the first hr (Figure 2) and 30min respectively following a toxic oral dose (Murray and Gibson, 1974).

Overall, these studies indicate that paraquat is rapidly but incompletely absorbed from the gastrointestinal tract of laboratory animals and humans (see later), with peak plasma concentrations occurring within 30-90 minutes.

Paraquat is poorly absorbed across human skin *in vitro,* human skin being less permeable to paraquat than the skin of rats, rabbits or guinea pigs (Walker *et al.*, 1983). Application of a low dose of [^{14}C] paraquat (150nmol/kg) in acetone to rat skin resulted in a peak blood levels about 1 hr after dosing and a total of 3.5% of the dose absorbed (Chui *et al.*, 1988). It should be pointed out that a occlusive dressing was applied in these studies which has previously

been shown to greatly enhance the percutaneous absorption of paraquat in animals (McElligott, 1972).

Overall, these studies plus those of Hoffer *et al.*, (1989) in rabbits, indicate that paraquat is poorly absorbed across the intact skin of laboratory animals.

2.9 Distribution

In the rat, after a lethal oral dose, the plasma paraquat concentration remained relatively constant after the initial peak for up to 32 h (Murray and Gibson, 1974; Rose *et al.*, 1976a), during this time the concentration in the lung rose progressively to several times that found in the plasma. In no other organ, apart from the kidney, the major organ for the excretion of paraquat, was a time-dependent accumulation of paraquat detected (Murray and Gibson, 1974; Rose *et al.*, 1976a). These findings, plus the earlier observation of Sharp *et al.*, (1972) who administered paraquat iv and showed that paraquat was retained in the lung with a half-life of 50h, provided the key evidence showing that those organs that had the highest concentration of paraquat were those that were susceptible to injury, namely the lung and kidney. Many other groups have subsequently examined the pharmacokinetics and elimination of paraquat in the rat (Maling *et al.*, 1978; Chui *et al.*, 1988; Dey *et al.*, 1990), dog (Hawksworth *et al.*, 1981; Giri *et al.*, 1982; Pond *et al.*, 1993) rabbit (Ilett *et al.*,1974; Yonemitsu, 1986; Yu *et al.*, 1994) and mouse (Drew and Gram, 1979). The distribution of paraquat in the body is best described by a three-compartment model, with input to and removal from the central plasma compartment. Simulations of plasma concentrations in the peripheral compartments show there is a compartment with rapid uptake and removal of paraquat, which was assumed to be the highly vascular tissues such as the kidney, and a slow uptake compartment reaching a maximum about 4-5hr after iv dosing, which may be the lung (Hawksworth *et al.*, 1981).

Using lung slices, Rose *et al.*, (1974a) first described the time-dependent accumulation of paraquat into lung tissue. This process was shown to be energy-dependent in that it could be inhibited by the addition of the metabolic inhibitors cyanide plus iodoacetate to the incubation medium. The accumulation of paraquat into rat lung was shown to obey saturation kinetics with an apparent Km of 70μM and a V_{max} of 300nmol/h/g wet weight of lung slice (Table 4) (Rose *et al.*, 1974a). Other aspects of the accumulation of paraquat into the lung will be

discussed in more detail later. Hawksworth *et al.*, (1981) also showed that early onset of renal failure markedly affected the concentration of paraquat in the peripheral compartments, suggesting that any reduction in renal excretion of paraquat may allow more of the chemical to be transported into the lung. The distribution in the rabbit, which is refractory to lung damage following a single systemic dose, showed the organs with highest concentration of paraquat were the lung and kidney at 6 and 24 after dosing but the concentration in rabbit lung appeared to decline more rapidly than from rat lung (Ilett *et al.*, 1974).

Whole body autoradiography studies have provided valuable information on the tissue distribution of paraquat, early studies by Litchfield *et al.*, (1973) in mice given iv [^{14}C methyl]-paraquat, showed retention in the lung. A more detailed study using [^{3}H-methyl]-paraquat and thin tissue sections revealed localisation of radioactivity at all time intervals after dosing in the lung, choroid plexus, muscle and melanin in addition to excretory pathways such as the proximal tubules of the kidney, urine, liver, gall bladder and intestinal contents of the mouse (Waddell and Marlowe, 1980). Radioactivity in the lungs appeared to be higher in certain areas and higher cellular resolution autoradiography revealed that the radioactivity was confined to alveolar type II cells, which are one of the major target cells for paraquat toxicity. In these studies it was essential to keep the tissue frozen at all times to prevent diffusion of paraquat which is highly polar. An association of paraquat with melanin has been demonstrated and this is probably due to an ionic interaction (Larsson *et al.*, 1977, 1978; Lindquist *et al.*, 1988). Immunohistochemical approaches utilising specific antibodies to paraquat have shown immunoreactive material localised primarily in bronchiolar epithelial cells and walls of blood vessels in the lungs of rats, 3h to 10days after an iv dose. Other studies have localised immunoreactive material in the intestine, liver, kidneys and in the brain to capillary walls and glial cells but not neurones, after paraquat administration (Nagao *et al.*, 1990; 1991; 1993).

2.10 Metabolism

Paraquat is very poorly metabolised with the bulk of the administered dose being excreted unchanged in the urine and faeces. Daniel and Gage, (1966) compared the colourimetric assay for paraquat with that found by radiochemical detection on the urine and faeces of rats dosed with paraquat and demonstrated that there was very close agreement. Chromatography

of the urine and lung tissue from rats treated with paraquat also showed no evidence of biotransformation (Hughes *et al.*, 1973; Murray and Gibson, 1974; Rose *et al.*, 1974a). No radioactivity was excreted in expired air following paraquat administration to rats, indicating that it did not undergo metabolism to CO_2 (Murray and Gibson, 1974). Incubation of paraquat with rat caecal contents for up to 24 h showed up to a 50% loss, indicating microbial metabolism, the loss was not seen when the contents of the caecum were heat treated (Daniel and Gage, 1966). However, *in vivo* studies in rats, guinea pigs and dogs showed little evidence of biotransformation, indicating that the *in vitro* studies had over predicted the likely metabolism (Summers, 1980). The overriding weight of evidence is that metabolism does not contribute to the toxicity of paraquat.

2.11 Excretion

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

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

divalent cation quinine, cimetidine and to a lesser extent tetraethylammonium (Chan *et al.*, 1996b) suggesting that paraquat may be transported by both transport systems (Figure 3).

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

In the rat *in vivo,* the fractional excretion of paraquat decreased from 2.1 at a plasma concentration of about 0.4 nmol/ml to 1.2 at a plasma concentration of 21nmol/ml, demonstrating that the excretion of paraquat is greater than the glomerular filtration rate and that the process is saturable (Chan *et al.*, 1997). Thus, at low plasma concentrations paraquat will be readily cleared from the body, however at higher plasma concentrations the system will become saturated and less paraquat will be cleared. At toxic doses it is well established that paraquat can cause renal functional impairment. In rats, given 126mg ion/kg, po (Lock, 1979) and mice given 50mg ion/kg, iv (Ecker *et al.*, 1975b) renal impairment was observed 17-24h after dosing. In the cynomologus monkey given 85mg ion/kg, po the decline in renal clearance was seen 12h after dosing the first time examined (Purser and Rose, 1979). In dogs given 20mg ion/kg, iv (Hawksworth *et al.*, 1981) renal impairment was observed as early as 2.5 hr after dosing. An early report on the renal handling of paraquat by the dog, suggested that paraquat was reabsorbed by the proximal tubules, this study was conducted at high plasma concentrations (54-810nmol/ml) where the transport system will have been saturated and function impairment will almost certainly will have occurred (Ferguson, 1973). The weight of evidence strongly supports the view that paraquat is actively secreted by the kidney of laboratory animals and humans (see later). The implication of impairment of renal excretion is that more paraquat is available in the plasma, to accumulate into the lung.

Whole body autoradiography has shown that paraquat was present in the gall bladder of mice, indicating some biliary excretion (Waddell and Marlowe, 1980). The extent of biliary excretion of paraquat was < 5% when dosed to bile cannulated rats, rabbits or guinea pigs and measured over a three hour period (Hughes *et al.*, 1973). The bulk of the dose appeared

unchanged in the urine, these authors suggesting that the molecular weight of paraquat at 186 was below the minimal molecular weight of about 500 for chemicals that are excreted in bile. Radioactivity from paraquat was also detected in the bile of dogs given a single iv dose, indicating some biliary excretion in this species (Giri *et al.*, 1982).

2.12 Accumulation of paraquat into the lung

The original discovery of an energy-dependent accumulation of paraquat into rat lung tissue (Rose *et al.*, 1974a), lead to studies to look for this transport system in the lung of other species, including human. The accumulation of paraquat by slices of lung from a number of species was reported by Rose *et al.*, (1976a). The apparent kinetic constants for the uptake process were very similar for all species examined except the rabbit. Slices of rabbit had a very high affinity, but low capacity, to accumulate paraquat which is consistent with the *in vivo* findings that show that following oral or parenteral administration of paraquat the rabbit does not develop a lung lesion. For the rat the derived K_m was 70 μM with a V_{max} of 300nmol/h/g wet weight of lung (Table 4). The kinetic constants for rat and human lung were very similar, suggesting that the rat lung was a good surrogate for studying paraquat uptake into human lung (Rose *et al.*, 1976a). The kinetics of accumulation of paraquat into human lung slices has been confirmed by others, the V_{max} being similar at 370nmol/h/g wet weight while the K_m was lower at 244 μM (Hoet *et al.*, 1994). Considerable inter-individual variation is seen in paraquat accumulation into human lung slices (Brooke-Taylor *et al.*, 1983) which may either reflect individual variability or more likely the state of the tissue and delay between removal of the tissue and analysis of paraquat transport. Table 4 summarises the available data on the transport kinetics for paraquat in rat and human lung tissue. These observations, coupled with the finding that paraquat is not metabolised by the lung nor covalently bound to any degree (Ilett *et al.*, 1974; Forman *et al.*, 1982; Sullivan and Montgomery, 1983), suggests that this accumulation is mediated through binding to, and subsequent translocation into lung cells by a carrier-mediated system.

The finding that paraquat was actively transported into lung slices lead to a search for chemicals that might inhibit this process (Lock *et al.*, 1976; Maling *et al.*, 1978; Smith *et al.*, 1981; Ross and Krieger, 1981; Dunbar *et al.*, 1988) and hence provide protection against paraquat-induced lung toxicity. A number of chemicals were identified that could block

paraquat uptake into lung slices but none of these were effective in the whole animal (see later under treatment of poisoning).

Studies were also undertaken to try and identify the endogenous chemicals for this transport system. A wide range of chemicals were examined and a number of naturally occurring amines were identified as the most effect inhibitors of paraquat accumulation into slices of rat lung, and which themselves act as substrates. These amines include the diamine putrescine, the oligoamines spermidine and spermine (Smith and Wyatt, 1981; Smith *et al.*, 1982; Gordonsmith *et al.*, 1983) and the disulphide cystamine (Lewis *et al.*, 1989). The physiological role for this transport system is not know, but it has been suggested that polyamines, which are known to regulate cell growth, may play a role in the differentiation of alveolar type II cells to type I cells (Smith, 1982). It has also been proposed that cystamine represents a source of taurine, which may have an antioxidant role in the lung (Wright *et al.*, 1986; Lewis *et al.*, 1989). Cystamine has also been implicated in playing a role in regulating cellular NADPH levels in response to oxidative stress (Brigelius, 1985). The structural requirements of substrates for this system have been examined and at least two charged nitrogen atoms separated by a distance of at least four methylene groups (about 6.6°A) is essential for uptake (Ross and Krieger, 1981; Gordonsmith *et al.*, 1983; O'Sullivan *et al.*, 1991). It is probable that paraquat, which meets these criteria is recognised as a substrate and thereby accumulated (Smith, 1987).

Paraquat accumulation into rat lung slices is reduced in the presence of putrescine in a dose-related manner (Smith and Wyatt, 1981; Karl and Friedman, 1983). Subsequently studies showed that putrescine was accumulated into slices of rat lung by an saturable energy-dependant process with an apparent K_m of 7μM and a V_{max} of 330nmol/h/g wet weight of lung. The K_m is about 10-fold lower than that for paraquat, indicating that the endogenous substrate has a higher affinity for the uptake process than paraquat (Table 4). These studies stimulated work to try and identify the specific cell types into which both paraquat and putrescine are accumulated. Slices of rat lung from rats treated with paraquat, which had been shown to cause selective damage to alveolar type I and type II cells, had a decreased ability to accumulate both paraquat and putrescine, suggesting that the transport system resides at least in part in these cell types (Smith *et al.*, 1976; Smith and Wyatt, 1981). This finding is consistent with the autoradiographic studies reported by Waddell and Marlowe,

(1980), who showed the distribution of paraquat in mouse lung following iv administration to be consistent with localisation in alveolar type II cells. Studies with rat lung slices *in vitro* have shown localisation of [^{3}H]paraquat to alveolar type II cells (Wyatt *et al.*, 1988). Similar studies with rat lung slices using [^{3}H]-putrescine, [^{3}H]-spermidine or [^{3}H]-spermine have also shown localisation to alveolar type II cells and in addition provided evidence for accumulation of radiolabel in bronchiolar Clara cells and possibly alveolar type I cells (Wyatt *et al.*, 1988). Similar localisation of [^{3}H]-putrescine was reported by Nemery *et al.*, (1987) and the localisation confirmed by electron microscopy to the type I and type II alveolar epithelial cells and Clara cells (Dinsdale *et al.*, 1991). In contrast, in rabbit lung slices [^{3}H]-putrescine was localised to alveolar type II cells and macrophages but not in Clara cells (Saunders *et al.*, 1988). More recent studies with slices of human lung have established that [^{3}H]-putrescine also accumulates into type I and type II alveolar epithelial cells (Hoet *et al.*, 1993).

Paraquat accumulation has also been demonstrated in isolated alveolar type II cells from rat and rabbit lung (Forman *et al.*, 1982; Horton *et al.*, 1986, Chen *et al.*, 1992) and in isolated Clara cells from rabbit lung (Horton *et al.*, 1986) suggesting that paraquat transport resides in both cell types. Paraquat is toxic to isolate mouse Clara cells and the addition of putrescine affords some protection (Masek and Richards, 1990). No accumulation of paraquat was however detected in isolated rabbit lung macrophages, although Saunders *et al.*,(1988) have reported putrescine accumulation by rabbit lung macrophages, the basis for this difference is currently not clear but it is now well established that polyamine transport systems are present in a number of transformed and non-transformed blood cells (see Smith *et al.*, 1990).

The kinetics of transport of paraquat into isolated type II alveolar epithelial cells has been reported by Chen *et al.*, (1992) using freshly isolated cell suspensions, they found a K_m of 88 μM with a V_{max} of 20pmol/h/μM DNA. They also examined putrescine transport in these alveolar type II suspensions and found a K_m of 2.5 μM with a V_{max} of 33pmol/h/μM DNA. This finding is in broad agreement with that for rat lung slices where the V_{max} is very similar for both substrates while the K_m for putrescine is higher than that for paraquat (Table 4). The accumulation of both spermidine and putrescine have been characterised in rat alveolar type II cells in culture (Richards *et al.*, 1987; Kameji *et al.*, 1989; Oreffo *et al.*, 1991). The uptake of spermidine into isolated cells was inhibited by putrescine, spermine and paraquat as

described for slices of rat lung. The accumulation of putrescine has also been studied in human alveolar type II cells in culture, the uptake of putrescine and the competitive inhibition by paraquat was essentially the same as that seen in human lung slices (Hoet *et al.*, 1994). Some difficulties have been experienced by several groups in determining the kinetics of transport of paraquat into isolated alveolar type II cells in culture, this may reflect changes to the cell membrane during the isolation procedure, such that the findings in these cells may not accurately reflect that occurring *in vivo.*

A summary of the kinetic constants for the accumulation of both paraquat and putrescine by lung slices and isolate alveolar type II cells for rats and humans is shown in Table 4. This data shows that paraquat and putrescine are accumulated by lung slices and alveolar type II cells from both rats and humans and that putrescine has a higher affinity for this system than paraquat.

2.13 Efflux of paraquat from the lung

The amount of paraquat that accumulates into the lung is determined by both the rate of accumulation and the rate of efflux from the cells in which it concentrates. The loss of paraquat from rat lung following *in vivo* administration is slow. There appears to be a rapid phase of elimination over the first 20-30 min following iv administration of paraquat which is then followed by slower loss that obeys first-order kinetics with a half-life of about 50hr (Sharp *et al.*, 1972). Similar studies by Smith *et al.*, (1978) and Dey *et al.*,(1990) showed a rapid phase of elimination that was similar to that reported by Sharp *et al.*, (1972) while the second phase showed a half-life for paraquat loss from the lung of approximately 20 hr, which was independent of the plasma concentration. Studies *in vitro* using lung slices from rats dosed *in vivo* with paraquat also showed a biphasic elimination, with a rapid loss within 30 min presumably reflecting loss from the extracellular space followed by a slower phase with a half-life of 17 hr similar to that seen *in vivo* (Smith *et al.*, 1981).

Thus, the basis for the selective toxicity of paraquat to the lung resides in paraquat's ability to become concentrated in alveolar type I and II cells and Clara cells. The concentration of paraquat retained in the lung is a combination of that retained during the time of the peak plasma concentration, plus that accumulated via the carrier-mediated process. Paraquat, once accumulated into lung cells, is not then readily lost.

2.14 Biochemical mechanisms of paraquat toxicity

Paraquat can be reduced to form a free radical which is stable in aqueous solution in the absence of oxygen (Michaelis and Hill, 1933).

$$PQ^{2+} + e^{-} \rightarrow PQ^{+\cdot}$$

In the presence of oxygen, in biological systems, the radical will rapidly re-oxidise to the cation with the concomitant production of superoxide anion O_2^{-} (Farrington *et al.*, 1973).

$$PQ^{+} + O_2 \rightarrow PQ^{2+} + O_2^{-}$$

Thus, once paraquat enters a cell it will undergo alternate reduction followed by re-oxidation a process known as redox cycling. Gage (1968b) first reported that the paraquat cation could be reduced by rat liver NADPH-dependent microsomal flavoprotein reductase to form the radical, with the concomitant oxidation of NADPH. Redox cycling of paraquat has also been reported in microsomal preparations of lung, liver and kidney (Baldwin *et al.*, 1975) and in lung microsomal and slice systems (Adam *et al.*, 1990). Studies using antibodies against NADPH-cytochrome c reductase have shown that paraquat radical formation can be blocked, demonstrating a role for this enzyme in the reduction process (Bus *et al.*, 1974; Horton *et al.*, 1986). Further support for a key role for NADPH-cytochrome c reductase comes from the studies of Kelner and Bagnel (1989) using a lymphoblastoid cell line with a specific deficiency in this enzyme which they reported was very resistant to paraquat toxicity.

Thus, provided there is sufficient NADPH, as an electron donor, and O_2 as an electron acceptor, paraquat will redox cycle inside a cell, generating superoxide anion and consuming NADPH. This reaction is believed to be a key step in the mechanism of paraquat toxicity, however the biochemical consequence of this reaction which leads to lung cell death are complex and still not fully understood. Recent studies with endothelial cells in culture have indicated that xanthine oxidase can also mediate redox cycling of paraquat to produce superoxide anion (Sakai *et al.*, 1995) indicating that two intracellular enzyme systems are probably involved.

Mammalian cells have many enzyme systems which provide them with protection against free radical attack and it is assumed that once these defences have been overwhelmed that cell

death occurs. Superoxide dismutase (SOD), is a family of metalloenzymes that can dismutate superoxide anion to hydrogen peroxide and oxygen.

$$O_2^- + O_2^- \rightarrow H_2O_2 + O_2$$

The importance of this enzyme in cellular toxicity comes from studies where cellular SOD activity has been genetically modified either by spontaneous mutation or by the transfection of SOD genes. Bilinski and Litwinska (1987) isolated a mutant yeast deficient in SOD activity, which had a greater sensitivity to paraquat than its isogenic wild type. In contrast, Hela cells which possess a higher content of both manganese and copper/zinc SOD had an increased resistance to paraquat (Krall *et al.*, 1988). Transfection of human copper/zinc SOD into various cell lines also lead to resistance to paraquat toxicity (Elroy-Stein *et al.*, 1986; Krall *et al.*, 1988). Recent studies have shown that mice lacking copper/zinc SOD show a marked increase in sensitivity to paraquat (Figure 4). $Sod^{-/-}$ mice showed a median survival time of about 1.5 days after 10mg/kg ip, while the $Sod^{+/-}$ and $Sod^{+/+}$ mice appeared normal at the end of 7 days of observation (Ho *et al.*, 1998). These studies provide strong evidence for a role for superoxide anion radical in the mechanism of cellular toxicity and for the role of copper/Zn SOD in protecting the lungs against paraquat toxicity.

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

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

$$\downarrow$$

2e-

$$\downarrow \text{ catalase/GSH peroxidase}$$

$$H_2O$$

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

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

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$$

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

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

Many other studies too numerous to mention have been conducted both *in vitro* and *in vivo* to explore the effect of altered anti-oxidant status on the toxicology of paraquat. Examples include the role of GSH and GSH reductase (Bus *et al.*, 1976a; Keeling, *et al.*, 1982; Hardwick *et al.*, 1990), the role of selenium deficiency, vitamin E and glutathione peroxidase (Bus *et al.*, 1976b; Cagen and Gibson, 1977; Block, 1979; Omaye *et al.*, 1978; Kelner *et al.*, 1995) and the role of metallothionein (Satoh *et al.*, 1992; Lazo *et al.*, 1995). Metallothionein appears to have play a role as a free radical scavenger in addition to its well established role as a heavy metal chelator, metallothionein has been reported to quench both superoxide anion and hydroxyl radicals, with a significantly higher reactivity towards hydroxyl radicals (Thornalley and Vasak, 1985). Genetically engineered animals have been used as tools to try and elucidate the function of the various antioxidant defence mechanism

against paraquat-induced oxidant injury. In addition to the discussion above regarding mice deficient in copper/zinc SOD, Sato *et al.*, (1996) found mice deficient in metallothionein I and II genes to be more susceptible to paraquat toxicity. Glutathione peroxidase deficient mice show a increased susceptibility to paraquat toxicity with a mean survival time of 5hr compared to the wild type of 69hr following an ip dose of 50mg/kg (Cheng *et al.*, 1998). Mice over expressing glutathione peroxidase are more tolerant to paraquat toxicity, wild type mice given a large 125mg/kg ip dose of paraquat died within 5h while the mice over expressing the enzyme lived until about 54hr (Cheng *et al.*, 1998).

Figure 5 shows a schematic representation of the key requirements to enable paraquat to enter a cell and the subsequently redox cycling steps believed to lead to cytotoxicity. Three hypotheses have been proposed to account for the ensuing cytotoxicity, one involving lipid peroxidation, another the oxidation of NADPH and the third mitochondrial toxicity, none of these hypotheses are mutually exclusive.

2.15 Lipid peroxidation hypothesis

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

peroxidation in the alveolar type I and II cells and Clara cell that are only a small population of the total cells in the lung.

2.16 Oxidation of NADPH hypothesis

Intracellular redox cycling of paraquat results in the oxidation of NADPH leading to cellular depletion such that those cells that selectively accumulate paraquat can longer function normally. Fisher *et al.*, (1975) first suggested that the redox potential of lung cell may be altered by the redox cycling of paraquat. A marked stimulation of the activity of the pentose phosphate pathway in the lung has been observed following exposure to paraquat (Fisher *et al.*, 1975; Rose *et al.*, 1976b; Bassett and Fisher, 1978; Keeling *et al.*, 1982 and Fisher and Reicherter, 1984). Since this pathway represents the major cellular source of NADPH, it is inferred that this response represents an attempt by lung cells to maintain their levels of reducing equivalents under conditions of oxidative stress. In those cells in which paraquat is accumulated, the concentration may be very high and result in very fast rates of NADPH oxidation. If the rate of consumption exceeds the rate of formation via the pentose phosphate pathway, the concentration of NADPH will fall below that required to maintain cell viability. Witschi *et al.*, (1977) first demonstrated that the NADPH/$NADP^+$ ratio in the lungs of rats dosed iv with paraquat was decreased suggesting that oxidation of the reduced nucleotide had occurred. Later studies by Keeling and Smith, (1982) demonstrated that the shift in NADPH/NAD^+ ratio in the lung following sc administration of paraquat was the result of NADPH loss from the lung. A consequence of depletion of cellular NADPH is that the cell shuts down it synthetic pathways which are dependent on this nucleotide, such as the synthesis of fatty acids (Keeling *et al.*, 1982). A loss of NADPH may also have particular importance for alveolar type II cells which produce pulmonary surfactant (Brigelius *et al.*, 1986).

NADPH is also consumed in an attempt by the lung to detoxify hydrogen peroxide that is formed via the glutathione peroxidase/reductase enzyme system (Figure 5) to regenerate reduced glutathione (GSH) from its oxidised form (GSSG). In general large changes in lung GSH and GSSG are not seen after paraquat administration (Bus *et al.*, 1976a; Reddy *et al.*, 1977; Shu *et al.*, 1979; Keeling and Smith, 1982) this may explain why lipid peroxidation has not been conclusively demonstrated *in vivo* as this would not become apparent until both

NADPH and GSH were markedly reduced. However, formation of protein mixed disulphides are increased in the lung *in vivo* (Keeling *et al.*, 1982; Keeling and Smith, 1982) and in perfused liver (Brigelius *et al.*, 1982). These changes in protein mixed disulphides in the lung are presumably a response to oxidative stress and may not be critical to the cellular toxicity. This notion is supported by studies with the bipyridyl diquat which can undergo redox cycling in the lung (Rose *et al.*, 1976b; Witschi *et al.*, 1977). Diquat also produced increases in protein mixed disulphide content in the lung without affecting NADPH content at a dose that did not cause lung injury (Keeling and Smith, 1982). This indicates that NADPH depletion subsequent to redox cycling is a critical step in the mechanism of paraquat toxicity.

2.17 The role of mitochondria in the toxicity

Another hypothesis that has been proposed is that paraquat toxicity is due to mitochondrial damage, based on morphological findings of early mitochondrial changes in alveolar type II cells (Hirai *et al.*, 1985) Ultrastructural studies of the time course of development of paraquat-induced lung injury have also reported early changes to mitochondria such as swelling and altered staining density (Smith and Heath, 1974a; Sykes *et al.*, 1977; Keeling *et al.*, 1981). These mitochondrial changes were also observed in the lungs of rats exposed to paraquat and 85% oxygen, which enhances paraquat toxicity to the lung (Keeling *et al.*, 1981). However, as discussed with regard to the lipid peroxidation hypothesis the question is are the effects on mitochondria a cause, or a consequence, of paraquat toxicity. Early studies with isolated liver mitochondria reported only minor changes in mitochondria respiration by paraquat (Gage, 1968b). More recent studies have reported that paraquat cation can be reduced by NADH-ubiquinone oxidoreductase (Complex I) located on the inner mitochondrial membrane (Fukushima *et al.*,1993; Shimada *et al.*, 1998), these authors also showed that paraquat was able to stimulate lipid peroxidation in submitochondrial particles (Yamada and Fukushima, 1993). These findings show that mitochondria have the potential to generate superoxide anion from paraquat provided it can gain access. In general, studies with intact mitochondria support the orginal findings of Gage, (1968b) showing that little or no effects are seen (Lambert and Bondy 1989, Constantini *et al.*, 1995) unless very high concentrations of paraquat are present (Kopaczyk-Locke, 1977; Yamamoto *et al.*, 1987; Thakar and Hassan, 1988; Palmeira *et al*, 1995). Paraquat has been shown to induce a Ca^{2+}-dependent permeability transition of the inner mitochondrial membrane leading to membrane

depolarisation, uncoupling and matrix swelling in isolate rat liver mitochondria (Constantini *et al.*, 1995). This opening of the membrane permeability pore does not occur in the absence of added Ca^{2+} and requires the presence of rotenone, leading one to question the relevance of this observation to the *in vivo* situation. It seem likely that any intracellular increases in Ca^{2+} would be only occur once paraquat had entered the lung cell, undergone redox cycling and altered mixed disulphide status. In summary, mitochondrial damage has been observed in the lung prior to cell death, it seems likely that this response is secondary to changes taking place in the cytosol.

2.18 The involvement of oxygen

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

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

2.19 Effects on the kidney

The major route of elimination for paraquat once it has entered the blood stream is via the kidneys where it is actively secreted by organic cation transport systems (see review by Chan *et al.*, 1998). This process becomes saturated at fairly low plasma concentrations (3-4 nmol/ml; 0.5-0.75µg/ml) in the rat (Chan *et al.*, 1997). At higher plasma concentrations paraquat is nephrotoxic, large oral or systemic doses administered to rats or mice producing morphological changes to the proximal renal tubules, including hydropic degeneration with occasional evidence of necrotic epithelial cells and of renal tubular regeneration (Clark *et al.*, 1966; Lock and Ishmael, 1979). Chronic exposure to mice via their drinking water showed ultrastructural evidence for proliferation of smooth endoplasmic reticulum and the presence of lipid containing bodies in proximal tubule cells (Fowler and Brooks, 1971). Renal tubular necrosis is more marked in the dog and rabbit following large toxic doses with clear evidence of degeneration of proximal tubular cells with the presence of casts in the tubular lumen (Clark *et al.*, 1966; McElligott, 1972; Yonemitsu *et al.*,1986; Giri *et al.*, 1982; Nagata *et al.*, 1992a). Prior to the onset of renal tubular necrosis, paraquat-induced renal functional changes including diuresis, albuminuria, glucosuria and elevations in plasma urea and creatinine in the rat (Lock and Ishmael, 1979), dog (Giri *et al.*, 1982; Nagata *et al.*, 1992a) and cynomologus monkey (Purser and Rose, 1979). The precise mechanism of renal functional impairment is not known, it probably involves altered renal haemodynamics as well as accumulation of paraquat into proximal renal tubules leading to cellular necrosis. There is some evidence that paraquat may reduce renal blood flow based on the finding of elevated renal plasma renin activity in the dog after dosing (Giri *et al.*, 1982) and hypovolaemia in the rat (Lock, 1979). Paraquat is thought to enter renal tubular cells by an organic cation transport system, thereby enabling it to concentrate to many time that present in the plasma (Ecker *et al.*, 1975a; Lock and Ishmael, 1979; Hawksworth *et al.*, 1981; Wright and Wunz, 1995; Groves *et al.*, 1995; Chan *et al.*, 1996 a, 1996b, 1997). The

accumulation can be blocked by other organic cations such as tetraethylammonium and quinine but is not affected by the polyamines, putrescine or spermine (Groves *et al.*, 1995; Chan *et al.*, 1996a). Thus the accumulation of paraquat into renal tubular cells occurs via a different transport system to that which leads to its accumulation in the lung. Once inside a renal tubular cell paraquat can redox cycle (Baldwin *et al.*, 1975, Tomita, 1991) producing superoxide anion and hence trigger the cascade of biochemical events leading to cytotoxicity similar to that discussed for the lung (Lock and Ishmael, 1979; Molck and Friis, 1997).

Regardless of the mechanism, the consequence of a reduced renal excretion is that more paraquat is available in the plasma, to accumulate into the lung. Thus, maintenance of renal function to facilitate paraquat excretion from the body is critically important for cases of human poisoning (see later).

2.20 Effects on the central nervous system

No signs of neurotoxicity or neuropathological changes have been reported following oral gavage or dietary administration of paraquat to rodents or dogs (IPCS,1984). Paraquat as a di-cation does not readily cross the blood-brain barrier and enter the rat brain after either oral or systemic administration (Rose et al., 1976a; Dey *et al.*, 1990; Corasaniti *et al.*, 1991; Corasaniti and Nistico, 1993; Naylor *et al.*, 1995; Widdowson *et al.*, 1996a and b). The concentration associated with the rat brain is always lower than that in the plasma and decreases with time, the initial concentration detected in the brain may be largely associated with blood (Rose et al., 1976a; Dey *et al.*, 1990; Naylor *et al.*, 1995). Paraquat was however detected in brain regions such as the olfactory bulb, area postrema and hypothalamus, which do not possess an effective blood-brain barrier. Autoradiographic studies have detected paraquat in these regions and in the cerebrospinal fluid (ventricles and choroid plexus) but the concentrations were low and only represent a very small percentage of the administered dose, about 0.05% at the time of maximal blood concentration 1 hr after dosing (Waddell and Marlowe 1980; Naylor *et al.*, 1995). Immunohistochemical localisation of paraquat in rat brain has shown it is present in capillary walls and glial cells but was not detected in neurones (Nagao *et al.*, 1991).

Recent studies in the rat, using parenteral doses of paraquat at or above the MLD (20-100mg/kg, ip) produced signs of neurotoxicity with muscle fasciculation, some tremors and

"wet-dog" shakes and at the higher doses myoclonus, typically within 30 min of dosing (Bagetta *et al.*, 1992; Corasaniti *et al.* 1992; Hara *et al.*, 1993), which is the time of peak blood and brain concentrations. These authors also reported neuronal cell necrosis in the pyriform cortex of these animals 24h after dosing (Bagetta *et al.*, 1992; Corasaniti *et al.*, 1992). The neuronal cell necrosis could be reduced by administration of atropine but not methylatropine (Bagetta *et al.*, 1992), suggesting some involvement of central muscarinic receptors. No effects were seen after 5mg/kg ip paraquat. The basis for the selective injury to the pyriform cortex is currently not known, but it does not reflect the brain region with the highest concentration of paraquat (Corasaniti and Nistico, 1993; Naylor *et al.*, 1995). Others have reported that paraquat (20mg/kg, sc) does not produce neuronal cell necrosis in the pyriform cortex of perfused-fixed material from rats 24 and 48h after dosing (Naylor *et al.*, 1995; Widdowson *et al.*, 1996a) and have suggested the effect reported by the Italian group may be a fixation artefact. The precise basis for this variance is currently not understood. Similarly, daily oral dosing of paraquat at 5mg/kg/day for 14 days to rats produced no evidence of neuronal cell necrosis, despite particular emphasis on the pathology of the pyriform cortex, nigro-stratial region and hypothalamus, or behavioural changes indicative of neurotoxicity (Widdowson *et al.*, 1996b).

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

Comparisons have been drawn to the structural similarity between paraquat and 1-methyl-4-phenylpyridinium ion (MPP^+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which can induce a Parkinson-like syndrome in monkeys and humans. Administration of MPTP to susceptible animal species produces selective damage to dopaminergic neurons in the substantia nigra leading to a marked loss of dopamine and clear signs of neurotoxicity.

The mechanism for MPTP toxicity (see Markey *et al.*, 1986; Tipton and Singer, 1993) is due to its ability to cross the blood-brain barrier and enter glial cells where it can undergoes oxidative metabolism by the enzyme monoamine oxidase B to form MPP^+. This metabolite then accumulates selectively into dopaminergic neurons via the dopamine transport system, leading to inhibition of mitochondrial respiration which ultimately leads to the demise of the neurone. Structure-activity relationships suggest that, despite their apparent similarity, paraquat and MPTP are two very different chemicals (Koller, 1986): MPTP is uncharged and lipophilic and thereby able to cross the blood-brain barrier, whereas paraquat is charged and hydrophilic and does not readily enter the brain. Also, MPTP is a monoamine whose metabolite MPP+, the proximate toxin, is able to use a specific uptake system, particularly in the substantia nigra, whereas paraquat is a diamine. It is also very relevant that administration of MPP^+ to experimental animals did not produce neurotoxicity, due to its poor entry across the blood-brain barrier (Tipton and Singer, 1993). Thus like paraquat, MPP^+ does not readily enter the brain. Consistent with this systemic administration of paraquat to C57 black mice or rats did not lead to dopamine depletion or neuronal cell death in the striatum, like that seen with MPTP (Perry *et al.*, 1986; Widdowson *et al.*, 1996b). Others have reported changes in brain dopamine content following paraquat administration to mice (Endo *et al.*, 1988; Fredriksson *et al.*, 1993). In the latter case, paraquat was administered to pups on days 10 and 11 after birth at a time when the brain is undergoing rapid growth and hence might be a more vulnerable to chemical insult. The authors reported a small loss of dopamine and its metabolites and a decreased behavioural activity when measure at about 4 months of age (Fredriksson *et al.*, 1993). This suggests that the developing brain is potentially more sensitive to insult, however adverse effects have not been detected in developmental toxicity or multigeneration studies, where paraquat was given to pregnant rats and their offspring (see earlier section). Attempts to try and reproduce the findings of Fredriksson *et al.*, (1993) in C57 black mice, in another laboratory, have not proved possible (David Ray, personal communication).

Thus, paraquat as a charged di-cation, does not readily enter the brain. The behavioural effects observed in rats only occurs at lethal systemic doses.

2.21 Effects on other organs

Following oral ingestion of paraquat by humans, ulceration of the pharyngeal, oesophageal and gastric mucosa has been reported (see section on human toxicology). In animal studies there is often no direct contact with these tissues when gavage dosing is employed. However, focal necrosis of the gastrointestinal tract has been observed in primates, demonstrating the topical irritant nature of high oral doses of paraquat (Murray and Gibson, 1972).

Paraquat administration to the rat produced an increased synthesis of liver glycogen and an increase in blood glucose that appeared to be mediated by the adrenal, since adrenalectomy prevented these changes (Rose *et al.*, 1974b). These effects seen following paraquat and the related bipyridyl diquat are thought to be due to catecholamine release and high circulating concentrations of corticosteroids (Rose *et al.*, 1974b). This response is thought to be unrelated to the pulmonary damage produced by paraquat, but may account for some of the effects seen with paraquat on the adrenal and lymphoid tissues such as the spleen and thymus (Clark *et al.*, 1966; Butler and Kleinerman, 1971; Fisher *et al.*, 1973). The increase in circulating corticosterone seen with paraquat can also be prevented by lesioning the area postrema (Edmonds and Edwards, 1996). This area of the brain also controls the taste aversion to paraquat seen in rats (Dey *et al.*, 1987; Edmonds and Edwards, 1996).

Liver damage is not a major finding after paraquat administration, after large doses some central lobular necrosis has been reported in most species examined (Clark *et al.*, 1966; Murray and Gibson, 1972; Cagen *et al.*, 1976; Giri *et al.*, 1992; Nagata *et al.*, 1992a). Since paraquat is delivered to the liver following dosing and hepatocytes possess the relevant enzymes to facilitate redox cycling, presumably paraquat does not normally accumulate in hepatocytes to a sufficient concentration to overwhelm the protective antioxidant defence enzymes and produce necrosis. However, both mice and rats made selenium deficient show marked liver injury following paraquat administration (Cagen and Gibson, 1977; Burk *et al.*, 1980) supporting the view that selenium dependent enzymes such as glutathione peroxidase play an important protective role. These findings are consistent with recent studies using transgenic mice where glutathione peroxidase has either been deleted or over expressed showing that this selenium dependent enzyme plays a key role in paraquat-induced tissue injury (Cheng *et al.*, 1998; de Haan *et al.*, 1998).

2.22 Treatment of poisoning in animals

Over the past 30 years a variety of attempts to modify the toxicity of paraquat in experimental animals have been examined. To date the only approach that has been shown to clearly reduce mortality in rats is purgation of the gastrointestinal tract with a diatomaceous clay (bentonite or Fuller's earth) along with a cathartic (e.g. magnesium sulphate) (Clark, 1971; Smith *et al.*, 1974). Attempts to modify paraquat toxicity have been based on its known mechanism of toxicity and will be briefly discussed under the following headings: (a) prevention of absorption from the gastrointestinal tract; (b) removal from the blood stream; (c) prevention of accumulation into the lung; (d) attempts to scavenge oxygen free radicals and (e) attempts to prevent lung fibrosis. This aspect of paraquat toxicity has been reviewed by others see Bateman, (1987); Meredith and Vale, (1995); Jaeger *et al.*, (1995) and section 1.3 on human poisoning in this review.

2.22.1 Absorption from the gastrointestinal tract

As discussed earlier, paraquat is poorly absorbed from the gastrointestinal tract and therefore attempts to reduce its entry into the blood stream could be beneficial. Peak blood levels are detected within 60-90 min in rats, dogs and monkeys (Figure 1 and 2), therefore any interventions must be taken quickly after poisoning if they are to be effective. The bipyridilium herbicides have been shown to bind very strongly to soil and clay minerals (Knight and Tomlinson, 1967). Clark, (1971) demonstrated that bentonite and Fuller's earth where able to reduce mortality in rats given a lethal dose of paraquat when delayed for 2 or 3 hours after paraquat administration. Smith *et al.*, (1974) subsequently showed that repeated doses of a bentonite, castor oil and magnesium sulphate mixture protected rats against the lethal effects of paraquat when given 4hr after exposure and this regimen also reduced mortality when delayed for as long as 10hr after exposure. The basis for the protection was shown to be due to a reduction in the concentration of paraquat in the bloodstream and a concomitant reduction in the amount accumulated into the lung (Smith *et al.*, 1974). Several other absorbent or binding agents have been examined, activated charcoal was shown to be very effective in rats (Okonek *et al.*, 1982) and in mice in combination with magnesium citrate, the magnesium salt affording some protection on its own (Gaudreault *et al.*, 1985). Kayexalate (sodium polystyrene sulphate), Kalimate (calcium polystyrene sulphate), sodium dextrin sulphate, sodium glucose sulphate and a variety of alkylsulphates and

alkylsulphonates have been shown to afford some protection in rats and mice (Nokata *et al.*, 1984; Ukai *et al.*, 1987; Tsuchiya *et al.*, 1995). The use of this approach in clinical practice will be discussed in more detail later.

2.22.2 Removal from the bloodstream

Both peritoneal dialysis and haemodialysis have been suggested for removing paraquat from the blood stream and thereby reducing availability to the lungs. Charcoal haemoperfusion was initially demonstrated to remove paraquat from the blood of beagle dogs (Maini and Winchester, 1975). Haemoperfusion appeared to reduce mortality in dogs when given within 12 h of administration of paraquat (Widdop *et al.*, 1975), although more recent studies in the dog have indicated that unless started within 2 hours of exposure it is unlikely to reduce the paraquat content in the lungs (Pond *et al.*, 1993).

2.22.3 Prevention of accumulation into the lung

Paraquat is actively transported into alveolar type I and II cells where it accumulates. Studies *in vitro* using polyamines, diaminoalkanes and a number of other chemicals has identified chemicals that can reduce paraquat accumulation (Lock *et al.*, 1976; Maling *et al.*, 1978; Ross and Krieger, 1981 Smith and Wyatt, 1981; Gordonsmith *et al.*, 1983). However, attempts to reduce paraquat mortality in rats with these agents have failed to demonstrate significant protection (Maling *et al.*, 1978; Dunbar *et al.*, 1988).

Another approach has been to use antibodies to paraquat (polyclonal, monoclonal or specific Fab fragments) to try and reduce toxicity to the lung. This approach has been shown to reduce paraquat uptake and cytotoxicity in rat lung slices and isolated alveolar type II cells (Wright *et al.*, 1987a; Chen *et al.*, 1994). However, treatment of paraquat-intoxicated mice (Wright *et al.*, 1987b; Cadot *et al.*, 1985) or rats (Nagao *et al.*, 1989) by immunotherapy did not reduce the concentration of paraquat in the lung or affect the mortality.

2.22.4 Free radical scavenging

As discussed earlier, once inside a cell paraquat can redox cycle and produce superoxide anion, singlet oxygen and hydroxyl radicals. Many studies have been aimed at attempting to scavenge the radicals formed to reduce or protect the lung injury. In many of these cases significant protection can be demonstrated using isolated cell systems, but in whole animals the protection is limited or equivocal.

Superoxide dismutase has been reported to increase survival in rats exposed to paraquat (Autor, 1974; Wasserman and Block, 1978), while other studies have failed to confirm these observations (Frank, 1983; Patterson and Rhodes, 1982). The short plasma half -life of exogenous superoxide dismutase and the fact that it does not enter cells accounts for the lack of protection. A more recent report indicated that a low molecular weight metalloporphyrin superoxide dismutase mimetic afforded some protection against paraquat-induced injury to the lung, but its effect on mortality was not examined and its effect is likely to have been marginal (Day and Crapo, 1996).

Desferrioxamine (DF) is an iron chelating agent which has been used to scavenge free iron and thereby reduce hydroxyl radical production. Studies in mice suggested that DF given 24h before, and regularly after an acute dose of paraquat reduced mortality (Kohen and Chevion, 1985). In this same model these workers showed that iron increased paraquat toxicity. In rats however, DF appeared to afford no protection (Osheroff *et al.*, 1985; Hoffer *et al.*, 1992). Van Asbeck *et al.*, (1989) gave DF by continuous infusion to vitamin E deficient rats and showed it prevented the lung injury and hence reduced mortality. This group also examined the effect of DF and CP51 an hydroxypyridin-4-one iron chelator in rats with a normal vitamin E status and found no protection with DF while CP51 increased survival (Van der Wal *et al.*, 1992).

Xanthine oxidase inhibitors may also reduce superoxide anion formation and rats fed a diet rich in tungstenate showed a better survival following paraquat exposure than rats fed the diet alone (Kitazawa *et al.*, 1991).

Clofibrate induces hepatic peroxisomes in rodents and thereby increases hepatic catalase activity, and it was postulated that a similar effect in the lung might afford protection against paraquat toxicity. Prior administration of clofibrate to rats for 6 days followed by paraquat afforded significant protection, however when clofibrate was administered after paraquat it gave no protection (Frank *et al.*, 1982).

Vitamin E is a lipid soluble antioxidant and radical scavenger, some early studies showed that vitamin E deficient animals were more susceptible to paraquat than those with a normal vitamin E status (Bus *et al.*, 1975; Block, 1979). Acute administration of vitamin E to normal mice or rats did not however significantly protect against the toxicity (Bus *et al.*,

1976a; Redetzki *et al.*,1980) even when instilled into the trachea in a liposome either alone or in combination with reduced glutathione (Suntres and Shek, 1995, 1996).

The protective effect of selenium has been reported, animals fed selenium deficient diets being more sensitive to paraquat toxicity (Cagen and Gibson, 1977; Omaye *et al.*, 1978). This is probably related to the selenium-dependant enzyme glutathione peroxidase which plays an important role in protecting cells against oxidative stress. Evidence that glutathione peroxidase plays a key role in protecting animals against paraquat toxicity comes from recent studies in transgenic mice where deletion of this enzyme enhances toxicity while addition affords some protection (Cheng *et al.*, 1998; de Haan *et al.*, 1998).

Vitamin C a water soluble antioxidant has provided equivocal data with one study suggesting it might protect while others showed it either had no effect or enhanced paraquat toxicity (Matkovics *et al.*, 1980; McArn *et al.*, 1980; Montgomery *et al.*, 1982; Sullivan and Montgomery, 1984; Minakata *et al.*, 1996). A combination of vitamin C and riboflavin in rats produced a significant improvement in paraquat mortality (Schavartsman *et al.*, 1984), while vitamin C or riboflavin alone was not protective. These authors suggested that perhaps the combination of antioxidant plus an effect of riboflavin on glutathione reductase activity may have contributed to the protection.

Niacin has been reported to modestly reduce paraquat mortality in rats, this may be due to an effect of niacin on NAD synthesis which is reduced by paraquat (Brown *et al.*, 1981), however subsequent studies were unable to confirm any protection with niacin (Hooper *et al.*, 1983).

A number of sulphydryl compounds have been examined based on their antioxidant ability, and on an early observation by Bus *et al.*, (1976b) showing that diethylmaleate, which depletes glutathione, enhanced paraquat toxicity. In general precursors of glutathione synthesis which increase intracellular cysteine content have been shown by some workers to provide some increased survival in mice or rats, while others have found these reagents to produce equivocal effects. The protection may be due to alteration of the pharmacokinetics of paraquat or induction of some of the enzymes involved in providing protection against free radical damage. The following have been examined N-acetylcysteine (Shum *et al.*, 1982; Cramp, 1985; Wegener *et al.*, 1988; Hoffer *et al.*, 1993; Hybertson *et al.*, 1995);

glutathione (Matkovics *et al.*, 1980; Szabo *et al.*, 1986); cysteine and cystine (Szabo *et al.*, 1986; Kojima *et al.*, 1992); L-2-oxothiazolidine-4-carboxylate (Ali *et al.*, 1996); D-penicillamine (Szabo *et al.*, 1986) and sulphite or thiosulphate (Yamamoto, 1993).

The affect of the lung-surfactant stimulating drug, ambroxol, has been examined in rats and shown to increase the rate of survival after paraquat (Salmona *et al.*, 1992) while Nemery *et al.*, (1992) found no protective effect.

2.22.5 Prevention of lung fibrosis

Since delayed deaths with pulmonary fibrosis are a characteristic of paraquat poisoning in experimental animals and humans (see later) a number of agents have been examined to try and ameliorate the fibrotic response. Immunosuppressants such as methylprednisolone, dexamethasone and cyclophosphamide have been examined in experimental animals and in general they were either without effect (Seidenfeld, 1985) or only afforded some protection when give prior to paraquat, but not when given simultaneously (Reddy *et al.*, 1976; Smith and Watson, 1987; Kitazawa *et al.*, 1988). Lung irradiation (Saeghirunvattana *et al.*, 1992) and collagen synthesis inhibitors such as D,L-3,4-dehydroproline (Akahori and Oehme, 1983) were not effective in reducing paraquat lung damage.

A recent suggestion has been mechanical ventilation with additional inhalation of nitric oxide, based on nitric oxide's vasodilatory effect on the lungs (Berisha *et al.*, 1994). This approach has not been examined in experimental animals but some clinical experience in combination with other antidotes has been examined (see later).

In summary, removal of the ingested material by emesis and purgation of the gastrointestinal tract is currently the most effective method after paraquat exposure in experimental animals. As discussed later, a cocktail of many of these approaches is often used in cases of human poisonings.

3. TOXICITY TO HUMANS

3.1 Experimental exposure

The percutaneous absorption of radiolabelled paraquat has been determined in humans (Wester *et al.*, 1984). Following application of 9 $\mu g/cm^2$ the amount absorbed was 0.29% for the leg, 0.23% for the hand, and 0.29% for the forearm. This gave a calculated *in vivo* absorption rate of 0.03 $\mu g/cm^2$ for the 24 hr exposure period. Paraquat was thus only minimally absorbed, especially in comparison with other commonly available pesticides (Wester and Maibach, 1985).

3.2 Accidental and intentional poisoning

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

With the increasing use of paraquat throughout the world during the 1970's and 1980's it became apparent that the problem of accidental and intentional poisoning had shifted away from the British Isles and Europe (Onyon and Volans, 1987). A high incidence was reported in particular from Asian countries such as Japan (Naito and Yamashita, 1987), Malaysia (Amarasingham and Lee, unpublished report), Sri Lanka (Hettiarachi and Kodithuwakku,

1989) and Fiji (Goundar, 1984). Paraquat was also the most widely used chemical suicidal agent in Trinidad (Hutchinson *et al.*, 1991) and Surinam (Perriens *et al.*, 1989).

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

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

Data on mortality from paraquat poisoning are difficult to compare because of differences in circumstances, treatment and reporting systems. In a collection of data from 14 publications compiled by the International Programme on Chemical Safety (IPCS, 1984), mortality ranged from 36% to 100%, with an overall mortality of 48% (446 of 925 cases). A difference in mortality between ingestion of the liquid concentrate (20% paraquat ion) and a granular product (2.5% paraquat, 2.5% diquat) has been described by some authors. Park *et al.*, (1975) found that the fatality rate was 15 of 23 (65%) in patients who had ingested liquid concentrate and 3 of 8 (38%) in patients ingesting the granular product. Fitzgerald and Barniville (1978) reported no deaths in 14 patients ingesting the granular product compared to a mortality of 74% in 118 cases of ingestion of the liquid concentrate. In the series published by Howard (1979a) there were 36 deaths from 41 cases (88%) where liquid concentrate was ingested, and

5 deaths from 27 cases (19%) involving the granular product. These differences are largely a reflection of the size of dose ingested.

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

Although ingestion is the route of entry into the body for the overwhelming majority of poisoning cases, there are a few reports of systemic effects from inhalation and dermal exposure (localised skin, eye and upper respiratory effects will be discussed under 'Use Experience'). Inhalation exposure is not a prominent feature in paraquat poisoning cases because of the extremely low (not measurable) vapor pressure of paraquat. Respiratory exposure to paraquat during spray applications is very low because the large droplet size will prevent the material from going beyond the nasal cavity. Concerns about oral exposure to spray droplets as a result of drainage into the oral cavity and swallowing appear unwarranted because the typical spray concentration of paraquat for hand-held spray applications is 0.1 - 0.2%; and would thus require a dose of 1 - 2 litres of spray solution directly into the nose and into the oral cavity to achieve an lethal dose (Howard, 1980). It is therefore not surprising that there are no reports in the published literature of deaths arising from inhalation exposure. A review of 30 cases of presumed inhalation exposure found no evidence for systemic poisoning (Vlachos and Kontoes, 1987). Where paraquat was measured it was undetectable or at the limit of detection. Patients were either asymptomatic or had non-specific symptoms such as headache, nausea or feeling unwell. Two patients described nose bleeds. In two patients who presented with cough and fever, pneumonia was established as clinical diagnosis. A recent review (Garnier, 1995) concluded that there was only one convincing reported case of possible systemic poisoning following inhalational exposure to paraquat and

signs of toxicity were very mild and the patient made a full recovery (Fitzgerald *et al.*, 1978a). In this case, a 43 year old market gardener sprayed a 'stronger than usual' solution (no details of spray concentration available) in a greenhouse and complained of a burning sensation in throat and mouth and weakness. There was biochemical evidence of mild renal failure, but liver function tests and chest x-ray were normal. Paraquat tested positive in urine. Renal function parameters returned to normal within 10 days after exposure.

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

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

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

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

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

3.3 Use experience

Exposure to paraquat under actual field conditions has been assessed in studies with hand held (knapsack), vehicle mounted and aerial applications. Dermal exposure was measured either in patches placed on different body regions or, more recently, using whole body exposure assessments. Inhalation exposure (including oral exposure) was determined using personal air sampling and the air concentration of different particle sizes was measured. Internal dose was assessed using biological monitoring, for which paraquat is an ideal candidate: it is not metabolised, rapidly and completely excreted via the kidneys, stable in urine, and there are sensitive analytical techniques available. The data from these studies are summarised in Table 5.

There is an enormous variation in dermal exposure evident in the studies found in the literature. This is not surprising given the differences in spray strength, volume applied, application technique, environmental conditions, use of personal protective equipment, and differences in study design. Nevertheless, some patterns emerge across the variety of study conditions encountered. It is evident that skin exposure represents by far the most significant route of exposure for paraquat. For hand held applications, total dermal exposure was more than an order of magnitude higher than exposure to uncovered body parts (Chester and

Woollen, 1981, Van Wendel de Joode *et al.*, 1996). A similar difference was seen for vehicle-mounted spray applications (Staiff *et al.*, 1975, Wojeck *et al.*, 1983). The lowest dermal exposure was seen for pilots applying paraquat (Chester and Ward, 1984), whereas the total dermal exposure of flaggers is comparable to exposure of uncovered body parts in other spray applications.

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

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

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

splashes with paraquat concentrate (Cant and Lewis, 1968, Joyce, 1969, Peyresblanque, 1969, Watanabe *et al.*, 1979, Deveckova and Mydlik, 1980). Apart from eye irritation and blepharitis, more serious, delayed ocular damage may occur such as destruction of the bulbar and tarsal conjunctiva and erosion of the corneal epithelium. Anterior uveitis has also been noted. Progressive keratitis and decreased visual acuity may occur and persist for several weeks, however, complete restoration of vision is the norm.

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

The State of California has probably the most comprehensive surveillance system of pesticide-related illness in the world. Between 1971 and 1985 a total of 231 cases of ill health attributed to paraquat were notified to the Worker Health and Safety Branch, California Department of Food and Agriculture (Weinbaum *et al.*, 1995). Of these, 38.5% were listed as systemic effects (mainly dizziness, nausea, lightheadedness, headache, chest pain, vomiting and tiredness), 32% were eye effects (burning, itching, redness), 26% were skin effects (rash and irritation, itching) and 3.5% were local respiratory irritant effects (epistaxis, sore throat).

There were no cases of pulmonary fibrosis. Analysis of data from 1981 to 1985 showed that the overall incidence of illness was low at 0.6 per 1000 paraquat applications.

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

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

A recently published study was carried out in Nicaragua (Castro-Gutierrez *et al.*, 1997), although the investigation dates back to 1987/88. A population of 134 spray workers with at least 2 years spraying experience with paraquat from 15 banana plantations was interviewed, 63 out of which had not experienced skin irritation, and 71 who had a history of skin rash or

burn (used as a surrogate measure of intensity of exposure). A questionnaire was used to check for symptoms of respiratory illness and Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV_1) were measured. The results were compared with a control population of 152 unexposed workers. There was a difference in male:female ratio between the exposed and unexposed groups (100:34 and 88:64, respectively). Paraquat-exposed workers gave a significantly more frequent history of Grade 3 dyspnea, but not Grade 1 or 2 dyspnea. There was no difference in the occurrence of chronic bronchitis, and episodic dyspnea with wheezing was more frequent in the group with topical effects only. However, there were no differences between exposed and control workers with regard to restrictive (FVC <80% of predicted value) or obstructive (FEV1:FVC <70% of predicted value) spirometry parameters. In fact, the lowest incidence of restrictive changes was found in the 'intensive exposure' group.

3.4 Atypical cases of various origins

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

George and Hedworth-Whitty (1980) attributed a case of non-fatal lung disease to the inhalation of nebulised paraquat. A 64 year old woman noticed spray mist drifting into her garden from a spraying operation in an adjacent field. After some 10 minutes she noticed a chest tightening, and over the next week she became gradually more breathless. She was initially treated with a short course of steroids without much effect. Pulmonary function evaluation some two months later showed severe restriction, but there were no abnormalities

in the chest radiograph. She was kept on systemic steroids and her lung function had markedly improved some 7 months after the original incident. Hart (1980) commented that the diagnosis of paraquat-induced lung injury was doubtful. The woman had a history of allergic rhinitis and chronic sinusitis. No previous lung function recording was available and no transfer factor was measured at the time of assessment. The chest radiograph was clear and the description of exposure did not provide convincing arguments for a significant inhalation exposure.

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

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

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

clearance. However, it has also been suggested that alcoholism may have a protective effect against paraquat toxicity on the basis of increased synthesis of superoxide dismutase (Ragoucy-Sengler *et al.*, 1991).

Methaemoglobinaemia was described in a patient who ingested 'Gramonol', a formulation containing 100 g/l paraquat and 140 g/l monolinuron (Ng *et al.*, 1982). The authors speculated that the superoxide anion and hydrogen peroxide generated by paraquat could oxidise haemoglobin to methaemoglobin. However, in response, Proudfoot (1982) pointed out that monolinuron, along with other substituted urea herbicides, is metabolised to aniline derivatives which are well known methaemoglinaemia and haemolysis causing agents. Furthermore, administration of monolinuron alone had produced methaemoglobinaemia in experimental animals. Instead of a new feature of paraquat poisoning, it appeared therefore that Ng *et al.* had reported the first human case of monolinuron toxicity. Since then, a further case of paraquat-monolinuron poisoning has been described (Casey *et al.*, 1994) in which the severe methaemoglobinaemia (52%) was successfully treated with methylene blue. However, the patient died after 10 days from the consequences of paraquat poisoning.

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

A possible association between paraquat exposure and the development of Parkinson's disease has been the subject of much speculation. The reason for this is, as previously discussed, the apparent structural similarity between paraquat and the synthetic pyridine

MPTP which produced severe neuropathies in several dozen drug users in southern California (Langston *et al.*, 1983, Lewin, 1984). The first epidemiological work to draw attention to a possible role of pesticides in Parkinson's disease was published by Barbeau *et al.*, (1986) who showed that the regional incidence of the illness in Canada was non-uniform and correlated with a genetically determined enzyme deficiency. While there was certainly a strong correlation between disease incidence and pesticide use, such a correlation was also found for industrial areas and wood processing regions. Since then a number of case-control studies have been published, with varying methodologies and conflicting results. Some studies suggested that the use of herbicides was significantly associated with the development of Parkinson's disease (Ho *et al.*, 1989, Golbe *et al.*, 1990, Semchuk *et al.*, 1991); in two studies this was specifically linked to paraquat exposure (Hertzman *et al.*, 1990, Liou *et al.*, 1997). Others have found no such association (Ohlson and Hogstedt, 1981, Tanner *et al.*, 1989, Koller *et al.*, 1990, Zayed *et al.*, 1990, Tanner *et al.*, 1990).

Structure-activity relationships suggest that, despite their apparent similarity, paraquat and MPTP are two very different chemicals (Koller, 1986, see above). Barbeau's hypothesis that Parkinson patients may be more likely to have a specific hydroxylation defect in the P450 enzyme system which might inhibit their ability to metabolise toxins (Barbeau *et al.*, 1985) does not apply to paraquat because it is not metabolised in mammals. Furthermore, none of the health surveys of paraquat-exposed workers (see above) has revealed any neurological deficits, let alone Parkinson's disease. The strongest evidence against paraquat as a causative factor in Parkinson's disease, however, comes from the many published case reports of paraquat poisoning. There is no evidence of a specific effect of paraquat on the nervous system, nor have neurological sequelae been noticed in survivors of paraquat poisoning (Vieregge *et al.*, 1988). Zilker *et al.*, (1988) carried out detailed neurological follow-up examinations in 4 survivors of paraquat poisoning (latency period between ingestion and follow-up 5 - 10 years) and 3 patients who had had skin contact with paraquat. It was possible to exclude parkinsonism in all patients. One patient exhibited tardive dyskinesia most likely due to long term therapy with neuroleptic drugs. The authors concluded that acute paraquat exposure does not lead to Parkinson's disease.

3.5 Clinical findings and dosage response

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

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

3.5.1 Mild or subacute poisoning

The smallest fatal dose has been quoted as 16.7 mg/kg (Stevens and Sumner, 1991), however, the original reference (FAO/WHO, 1973) makes clear that this value is erroneously low, since the formulation ('Weedol') also contained an equal amount of diquat, so that the total bipyridyl ingestion was approximately 35 mg/kg. This is in line with clinical experience which shows that ingestion of less than 20 - 30 mg paraquat ion/kg has rarely serious consequences. Patients are either asymptomatic or develop nausea and vomiting. Renal and hepatic lesions are minimal or absent. An initial decrease of the diffusing capacity may be apparent in lung function measurements, but full recovery is the norm.

3.5.2 Moderate to severe acute poisoning

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

3.5.3 Fulminant or hyperacute poisoning

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

While this categorisation reflects experience with a large number of cases, it has to be emphasised that there are a significant number of cases reported in the literature where there was survival following the ingestion of alleged doses well above of what is usually considered to be fatal. Table 6 shows that there are 52 case reports where a dose apparently in excess of 55 mg/kg has been survived. While inaccuracies in estimating the dose may have led to exaggeration of the dose in some cases this appears unlikely in many others.

Talbot *et al.*, (1988b) reported a series of nine cases of suicidal paraquat poisoning in pregnant women. In the cases where the outcome was known, one foetus died probably unrelated to paraquat, three died in utero or after delivery but associated with respiratory distress in the mothers, two died in utero (one mother survived and subsequently had a normal pregnancy with no evidence of teratogenicity from the previous paraquat

intoxication). One foetus was aborted. Previously, Fennelly *et al.*, (1968) had reported the case of a woman who was 28 weeks pregnant and died 20 days after paraquat ingestion. Upon autopsy the foetus showed no abnormalities. A 20 week pregnant patient survived the ingestion of a small dose of paraquat and subsequently delivered a normal child (Musson and Porter, 1982).

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

The measurement of paraquat plasma concentration has proved to be a reliable indicator of the prognosis of the intoxication. Levitt (1979) was the first to demonstrate a relationship between plasma concentration of paraquat, the estimated time after ingestion and the eventual

outcome. Based on results from 79 patients with a reasonably well established time of ingestion, Proudfoot *et al.*, (1979) found that those patients whose plasma paraquat concentration did not exceed 2.0, 0.6, 0.3, 0.16, and 0.1 mg/l at 4, 6, 10, 16,and 24 hours after ingestion survived. This semi-logarithmic plot has become known as the predictive line, or 'Proudfoot's curve'. Because of the rapidly decreasing plasma concentration in the first few hours following ingestion no accurate prognosis could be given prior to 4 hours. The authors emphasised that the line to separate survivors and non-survivors was meant to be an approximate guide, and the main use should be to help clinicians in the decision which patients needed urgent aggressive treatment. Subsequently, several other methods have been described to establish the prognosis from plasma paraquat concentrations. None of those methods have been found to invalidate the original estimate by Proudfoot *et al.*, but they have added other dimensions which may be of help to clinicians. Scherrmann *et al.*, (1987) used data from 30 patients to extrapolate the predictive line beyond 24 hours up to 15 days after intoxication, this was later modified (Scherrmann, 1995) with data from a total of 52 patients (Table 7). The same authors evaluated the relationship between early urine concentrations and clinical prognosis. They also attempted to correlate urine results obtained by radioimmunoassay with those given by the simple colorimetric dithionite test. Data from 75 patients showed a wide variation in urine concentrations within 24 hours of ingestion. All 17 patients with concentrations of less than 1 µg/ml survived, whereas 51 out of 58 patients with urine paraquat concentrations of more than 1 µg/ml died. No colour was observed in the dithionite test at paraquat concentrations below 0.5 µg/ml (Scherrmann *et al.*, 1987, Scherrmann, 1995).

Using a sample size of 219 patients, Hart *et al.*, (1984) were able to calculate the probability of survival of the patient from the initial paraquat plasma concentration (Figure 6). It was noted that the line denoting a 50% probability of survival correlated well with Proudfoot's curve. Sawada *et al.*, (1988) categorised their patients into three groups: survivors (n=10), non-survivors who died from respiratory failure (n=9), and non-survivors who died from circulatory failure (n=11). They calculated a severity index of paraquat poisoning (SIPP) from time to treatment since ingestion of paraquat multiplied by the serum level at admission (µg/ml). A boundary SIPP of 10 separated survival from death by either cause, whereas a SIPP of 50 separated deaths from respiratory failure and deaths from circulatory failure.

Using data from 128 patients, Ikebuchi *et al.*, (1993) separated survivors and fatal cases by multivariate analysis and established a discriminate function D. Their toxicological index of paraquat (TIP) could then be divided into three types: TIP 1 is characterised by $D > 0.1$ (100% survival probability); TIP 2 has the characteristic $-0.1 < D < 0.1$ and here urgent treatment may influence the outcome; in TIP 3 the discriminate function $D < -0.1$, and the probability of a fatal outcome is 100%.

All these methods depend on the availability of paraquat analysis, and this is often not the case, or at least not in a timely fashion. Investigators have therefore attempted to predict the outcome of the intoxication using biological indices rather than plasma paraquat concentrations. Suzuki *et al.*, (1989) measured the respiratory index (RI) from blood gas analysis and used it as an index of lung oxygenation in 51 patients. Progressive deterioration of the RI above 1.5 was found in 43 non-survivors, whereas the RI remained below 1.5 in the 8 survivors. Furthermore, the time taken from ingestion for the RI to exceed 1.5 was found to be a good indicator for predicting the survival period in fatal cases. The major weakness of this method is that it cannot predict the outcome at the point of first contact with the patient, unlike the methods relying on plasma paraquat analysis. Also, conditions which may influence the RI such as pneumothorax, cardio-pulmonary rescuscitation, septic shock, pulmonary oedema and pneumonia limit the usefulness of this method. On the other hand, it can be used at any time after the intoxication, and it is independent from an estimate of time of ingestion. Yamaguchi *et al.*, (1990) reviewed the medical records of 160 patients who had ingested paraquat and calculated an equation derived from serum creatinine and potassium concentrations and arterial blood bicarbonate level. When plotted against time of ingestion they were able to estimate the probability of survival in three categories (90%, 38% and 3%). Most recently, a different biological index using creatinine measurement from 18 patients has been proposed by Ragoucy-Sengler and Pileire (1996a). They found that the time evolution of blood creatinine in intoxicated patients was linear during the first 24 hours after admission. The rate of increase of creatinine in the patients with fatal outcome was equal to a constant (zero order kinetics). A rate of creatinine increase over 5 hours (dCreat/dt) of > 3 µmol/l/h was found in the 12 fatal cases whereas this value remained < 1.26 for the survivors. As with the method of Suzuki *et al.*, (1989) this biological index is independent from an estimate of time elapsed since ingestion. It has the advantage that a prognosis can be established within a

few hours after admission of the patient using a standard biochemical analysis. However, it is currently based on data from a relatively small number of patients and will thus require further confirmation from a larger dataset.

3.6 Laboratory findings

If performed early and serially, pulmonary function tests may be of diagnostic value. However, it has been pointed out that any changes seen are not specific for paraquat poisoning, since they may also occur in other clinical conditions such as pneumonia, pulmonary oedema, pulmonary thromboembolism and advanced degrees of the alveolar capillary block syndrome (Cooke *et al.*, 1973). The abnormalities must be interpreted in conjunction with the clinical picture. As mentioned above, pulmonary function tests in patients with moderate to severe paraquat poisoning are likely to be abnormal much earlier than clinical or radiological findings. A decrease in the carbon monoxide diffusing capacity or transfer factor (DL_{CO} or TL_{CO}) can be noted as early as the first day after intoxication (Baguley *et al.*, 1983). Beginning between the fifth and sixth day there may be a restriction of the FEV_1 and the FVC. These changes are followed by a drop in the arterial oxygen tension and an increase in the gradient of alveolar to arterial tension. Finally, there is the development of a functional shunt by which a decreasing fraction of the blood passing through the lungs is oxygenated (Cooke *et al.*, 1973).

In patients who died within 11 - 14 days, the extent of lipid peroxidation, expressed as malondialdehyde, was higher than in controls or in patients who survived. Massive doses (death in 1 - 3 days) did not result in increased levels of malondialdehyde (Yasaka *et al.*, 1981, 1986). Serum superoxide dismutase (SOD) levels were significantly decreased in cases of lethal paraquat poisoning (Nemeth *et al.*, 1985). Better clinical courses were detected if SOD levels were normal or slightly elevated. Extremely increased levels were measured several times in the terminal state and were interpreted as the consequence of liver cell necrosis and intravascular haemolysis.

Other laboratory findings, including those reflecting renal and hepatic failure, are non-specific. Detailed renal function studies were performed in three cases of paraquat poisoning who developed acute renal failure (Vaziri *et al.*, 1979). The glomerular filtration rate (estimated by using creatinine clearance) improved for two patients who survived two weeks,

illustrating the reversible nature of the renal failure. A mild to moderate transient proteinuria but little albuminuria was observed during the first two weeks after intoxication. Other findings consistent with proximal tubular dysfunction included glucosuria, amino aciduria and increased fractional excretion of phosphorus, sodium and uric acid.

Many case reports have shown a transient rise in liver enzymes such as ALT and AST, reflecting the centrilobular necrosis and cholestasis often seen at autopsy (Vale *et al.*, 1987). Serum protein was decreased in one case (Bullivant, 1966), but increased in another with a large increase in the globulin fractions (Matthew *et al.*, 1968). Peak total serum bilirubin concentration correlated significantly with the alveolar-arterial oxygen difference in a series of 21 patients (Lin *et al.*, 1995).

Normochromic anaemia developed rapidly in five cases reported by Lautenschläger *et al.*, (1974). This was accompanied by suppression of erythropoietin in the bone marrow, but little effect on other aspects of haematopoesis. The bone marrow had returned to normal in one patient who survived and was re-examined 6 months after the intoxication. In the above mentioned study by Lin *et al.*, (1995), the alveolar-arterial oxygen difference also showed a negative correlation with the initial platelet count.

Paraquat analysis in plasma and urine has already been mentioned as the key to diagnosis and prognosis of paraquat poisoning. A simple spot test can be performed with urine or gastric aspirate and is based on the reduction of paraquat cation to a blue radical in the presence of alkali and sodium dithionite (Berry and Grove, 1971, Widdop, 1976). These methods can detect concentrations of paraquat in urine down to 1-2 µg/ml and may be made semi-quantitative if a range of standards are prepared in control samples. Quantitative methods based on the dithionite reaction with a spectrophotometric end-point have also been described to determine paraquat in plasma (Knepil, 1977, Jarvie and Stewart, 1979). An improved spot test using extraction with a silica cartridge has allowed lower detection limits between 0.1-0.5 µg/ml (Woollen and Mahler, 1987). The lower limit of detection for paraquat using spectrophotometry following solid phase extraction was 45 ng/ml (Smith *et al.*, 1993).

Other methods which have been described include a radioimmunoassay with a sensitivity of 6 ng/ml (Levitt, 1979). Gas chromatography and mass spectroscopy has been used (Draffan *et al.*, 1977) giving a sensitivity of 25 ng/ml. A fluoroimmunoassay achieved a sensitivity of

20 ng/ml (Coxon *et al.*, 1988). Gill *et al.*, (1983) described a high performance liquid chromatography method involving ion-pair extraction on disposable cartridges of octadecyl silica. Most of these methods can be applied to the analysis of plasma, urine and tissue samples.

3.7 Absorption

No adequate data exist on absorption of paraquat in humans. However, Davies (1987) has pointed out that early estimates of an absorption of less than 5% of an ingested dose (Conning *et al.*, 1969) may be an underestimate. He suggested that absorption kinetics in man may be more similar to those seen in the dog, where a rapid, but incomplete paraquat absorption occurs, with peak plasma levels occurring at 75 - 90 minutes, and almost 40% of the dose absorbed in 6 hours, as judged by the amount excreted in urine (Bennett *et al.*, 1976, Davies *et al.*, 1977). Limited clinical data suggest that having a full stomach may effectively decrease the bioavailability of paraquat (Bismuth *et al.*, 1982, 1995).

In humans, the precise time at which the plasma paraquat concentration peaks is unknown. However, paraquat may be detected in urine as early as one hour after ingestion (Meredith and Vale, 1987). To judge by the plasma concentration data published by Proudfoot *et al.*, (1979), peak plasma concentrations in humans are certainly attained within 4 hours. This is in line with the toxicokinetic analysis of data from 18 patients by Houze *et al.*, (1990), who estimated peak plasma concentrations to occur between 2 and 4 hours. However, most patients were admitted to hospital comparatively late, and they could measure peak plasma concentrations in only 2 cases, in both they were seen around 3.5 hours after ingestion.

3.8 Distribution

The distribution of paraquat appears to be similar in humans and dogs (Davies *et al.*, 1977, Van den Bogaerde *et al.*, 1984), suggesting that the three compartment model described by Hawksworth *et al.*, (1981) (see above) in the dog is also applicable to humans. Smith (1987) pointed out that the concentration of paraquat in plasma in human poisoning cases falls rapidly to much lower levels than described in the rat. In their series, Houze *et al.*, (1990) found that the concentration-time curve in 15 adult patients (not haemodialysed) was best

described by a bi-exponential curve, with the elimination half lives of the early and late phase being 5 and 84 hours, respectively. These patients could be divided into three groups:

1. Patients admitted early and having a rapidly fatal course from cardiovascular collapse showed only mono-exponential decreases with a mean half life of 7 hours. However, because of the early death of the patients, evaluation of the late phase was precluded.

2. The second group included patients who were admitted early and survived long enough for an evaluation of the late phase. They showed a bi-exponential decrease with mean half lives of 7 and 103 hours, respectively.

3. In the third group hospital admission was delayed and only late paraquat plasma concentrations could be measured. Accordingly, a mono-exponential decrease in plasma paraquat concentrations was observed with a mean half life of 101 hours.

Acute renal failure occurred in all but one of the patients. The terminal half-life, however, was very long even in the patient with normal renal function, suggesting that the prolonged elimination phase depends not only on renal function but also on the gradual release of paraquat by extravascular tissue into the blood circulation.

In six of their cases with fatal outcome, Houze *et al.,* (1990) also determined tissue paraquat concentrations. High concentrations were found in the lungs, kidneys, heart and liver and much lower concentrations in lipophilic organs such as brain and adipose tissue. The apparent volume of distribution ranged from 1.2 to 1.5 l/kg, compared to 2.75 l/kg in the study by Davies *et al.,* (1977). The mean value of the distribution half-life in humans is greater than that reported from animal studies (see above). Assuming a first-order distribution rate constant and an early half-life of 5 hours, paraquat distribution would be achieved within approximately 30 to 40 hours (Houze *et al.,* 1990).

The active transport of paraquat into lung tissue in different species, including humans, has been described in detail above. Paraquat accumulation in tissue could be considered as a slow process from a pharmacological point of view, but it is rapid in clinical terms (Bismuth *et al.,* 1987). In a study of the kinetics of paraquat through the heart-lung block, Baud *et al.,* (1988) showed that concentrations in the radial artery were usually higher than or equal to the corresponding value in the pulmonary artery. Only one patient who was examined

approximately 4 hours after ingestion showed a pulmonary artery concentration clearly higher than that in the radial artery, providing evidence of pulmonary uptake of paraquat. The arteriovenous difference disappeared approximately eight hours after ingestion followed by inversion of this ratio. This suggests that lethal concentrations of paraquat in the lung may be reached less than 10 hours after ingestion.

Paraquat crosses the placenta and a case reported by Talbot *et al.*, (1988b) suggests that it is concentrated in the foetus. Following suicidal ingestion of paraquat a premature infant (32 weeks) was delivered by Caesarean section. Both mother and infant died shortly thereafter. Paraquat was measured in maternal blood at 5.6 µg/ml and in the infant's blood at 20.6 µg/ml.

3.9 Metabolism

As in experimental animals, paraquat is not metabolised in humans, but is reduced to an unstable free radical which is then re-oxidised to produce a superoxide radical (see above). Paraquat is excreted unchanged in urine.

3.10 Excretion

As in experimental animals, paraquat elimination is essentially renal via glomerular filtration with an element of tubular secretion (Bismuth *et al.*, 1988). With normal renal function, clearance of paraquat is greater than creatinine clearance, which enables excretion of high concentrations and large amounts of paraquat within the first hours after ingestion (Davies *et al.*, 1977, Scherrmann *et al.*, 1983). However, ingestion of large doses of paraquat causes tubular necrosis with a rapid decrease of glomerular filtration and tubular secretion.

In four cases described by Houze *et al.*, (1990), renal paraquat clearance was lower than creatinine clearance, even in a patient with apparently normal creatinine clearance. Urinary and plasma elimination half lives correlated well. Paraquat may be detectable in urine for a long period of time. Beebeejaun *et al.*, (1971) found paraquat excreted in urine until 26 days after ingestion. In the case of a 14 month old boy, Houze *et al.*, (1990) could detect paraquat in urine for up to three months after ingestion, suggesting on-going release of paraquat from a deep body compartment.

Small amounts of paraquat have been recovered in bile samples at post-mortem examination, suggesting that a minor enterohepatic cycle may exist in humans (Van Dijck *et al.*, 1975). As in experimental animals, the amount of paraquat excreted in faeces corresponds to 60-70% of the ingested dose in humans. This excretion may be prolonged (Van Dijck *et al.*, 1975).

3.11 Pathology

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

3.11.1 The destructive phase

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

3.11.2 The proliferative phase

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

Renal pathology is common, but rarely responsible for the death of the patient. Macroscopically, the kidneys are swollen and soft. There is degeneration or necrosis of proximal tubular cells (Bullivant, 1966, Campbell 1968) with nuclear loss and cast formation

(Parkinson, 1980). Depending on the time after poisoning, there may be signs of regeneration.

While early studies made little mention of liver damage, Mullick *et al.*, (1981) found evidence of cholestasis, usually localised to the centrilobular region in the majority of their 13 autopsy cases. There was cholangiocellular injury involving the small and medium-sized bile ducts in portal areas. The authors hypothesised that paraquat injury to the liver is biphasic with an initial hepatocellular injury followed after 2 days by a cholangiocellular phase.

Toxic myocarditis is frequently seen in cases with ingestion of larger amounts of paraquat. Parkinson (1980) described a patchy but widespread polymorphonuclear leucocyte infiltration in the presence of normal myocardial fibres.

In some cases adrenal cortical necrosis has been described (Nagi, 1969, Reif and Lewinsohn 1983) in patients who died early after ingestion of paraquat. This lesion was diffuse and involved mainly the zona fasciculata and zona reticularis. Fitzgerald *et al.*, (1977) found adrenal cortical necrosis upon autopsy in 12 of 23 patients. The severity of the lesion appeared dose-related with patients showing complete cortical necrosis after ingestion of higher doses.

Brain pathology has been studied in a series of 8 patients (Grant *et al.*, 1980). Changes included generalised oedema, haemorrhages (these two findings being the most consistent changes), glial reactions and meningeal inflammation. The authors suggested that paraquat may damage the cerebral blood vessels. These changes were also seen in a case reported by Hughes (1988) who suggested that, apart from a direct toxicity of paraquat on cerebral blood vessels, the neuronal depletion, myelin breakdown and astrocytic fibrous gliosis seen were a secondary effect due to prolonged anoxia.

3.12 Treatment of poisoning

The therapy of paraquat intoxication has focussed on three main areas: prevention of absorption from the gastrointestinal tract, enhancement of elimination of paraquat from the body, and therapy directed against the mechanisms of toxicity. In addition, there have been attempts to use lung transplantation as a means to overcome the consequences of paraquat lung toxicity.

3.12.1 Prevention of absorption

Following the first reports of paraquat poisoning it was suggested that the immediate therapy of paraquat poisoning should be directed towards prevention of absorption from the gastrointestinal tract (Malone *et al.*, 1971). There is little information available on the use of gastric lavage in paraquat poisoned patients. Bismuth *et al.*, (1982) were not able to establish a beneficial effect from gastric lavage in their series of 28 patients. Bramley and Hart (1983) did not find an improved prognosis resulting from the use of gastric lavage in a series of 262 patients. McDonagh and Martin (1970) proposed urgent gastric lavage with a 1% bentonite solution to inactivate paraquat. Following the studies by Clark (1971) who found that bentonite (sodium montmorillonite) and Fuller's Earth (calcium montmorillonite) had a high adsorption capacity for paraquat, Douglas *et al.*, (1973) reported three cases of survival after paraquat poisoning, two of which had been treated with 7% bentonite as adsorbent. Smith *et al.*, (1974) suggested a treatment regime of repeated administration of cathartics together with large volumes of Fuller's Earth or bentonite which had been shown to effectively protect rats against an otherwise lethal dose of paraquat. Vale *et al.*, (1977) used this approach together with charcoal haemoperfusion in 10 patients with paraquat poisoning. Only one patient who had the initially lowest plasma paraquat concentration survived, prompting the authors to conclude that the treatment was likely to be of benefit only in less severely poisoned patients. This was also the conclusion of Fitzgerald *et al.*, (1979a) who analysed 62 cases of paraquat poisoning with respect to treatment with Fuller's Earth and survival. They found that the majority of patients who survived had not taken what was regarded as a lethal dose. Also, death occurred in all patients who had ingested more than 30 ml of the concentrate, irrespective of therapy. In the group of patients who ingested between 5 and 30 ml and who received therapy within 6 hours after ingestion 4 out of 7 survivors and 2 out of 5 non-survivors had received Fuller's Earth. The authors suggested that Fuller's Earth may have been of benefit in a few cases who had taken slightly in excess of the lethal dosage, but was unlikely to affect the outcome in the majority of patients with paraquat poisoning.

While Fuller's Earth is still widely used in the first-line treatment of paraquat poisoning, the original claim by Clark (1971) that activated charcoal did not bind paraquat has been disputed. On the basis of *in vitro* binding studies and *in vivo* experiments, Okonek *et al.*, (1982) suggested that the use of activated charcoal instead of Fuller's Earth was equally

effective. This has prompted a revision of the advice given to medical practitioners in the United Kingdom (Department of Health, 1996) since activated charcoal is more likely to be immediately available in most hospitals and treatment centres. Other adsorbents such as the cation exchange resin kayexalate have been used (Yamashita *et al.*, 1987) but it is doubtful whether these have any benefit over the use of Fuller's Earth and activated charcoal.

From 1979 onwards a potent emetic, the phosphodiesterase inhibitor PP796, was gradually introduced in all paraquat formulations made by the major manufacturer (Denduyts-Whitehead *et al.*, 1985). It has been shown that following ingestion of emeticised formulations vomiting occurs earlier, is more profuse and prolonged than following ingestion of non-emeticised product (Meredith and Vale, 1987). However, a comparison of data from patients who had ingested paraquat concentrate with or without added emetic failed to show an overall benefit of the emetic on survival rate (Bismuth *et al.*, 1982, Bramley and Hart, 1983, Onyon and Volans, 1987). Nevertheless, the emetic has been retained with the rationale that in particular accidental paraquat ingestions usually involve small quantities of the product, where early gastric emptying could have an effect on the outcome.

It can be concluded that there is no clear evidence that gastric emptying and the use of adsorbents have improved the survival of patients with paraquat poisoning. The main reasons for this are the high dose of paraquat ingested by the majority of patients with deliberate ingestion, and the frequent delay in hospital admission. Most authors concede that, on theoretical grounds, therapy designed to prevent absorption of paraquat should be able to help those patients who have a realistic chance of survival. However, clear evidence for this from clinical studies has so far not been obtained,

3.12.2 Elimination of paraquat from the body

Since the kidney is the primary excretory organ for absorbed paraquat, enhancement of urinary elimination was one of the first therapeutic options considered. Kerr *et al.*, (1968) published the first case report where forced diuresis had been used to treat paraquat poisoning. The exact fluid volume was not given, but their patient's urine excretion was more than 11 litres over 24 hours. The total urine excretion of paraquat was 46 mg and the patient survived. Another patient was treated with a total of 27 litres of fluid over 48 hours (Fennelly *et al.*, 1971). During the course of the forced diuresis he developed seizures, a

metabolic alkalosis and electrolyte disturbances, but the therapy was successfully completed. He developed transient mild hepatic and renal failure, but the only sign of pulmonary involvement was a slight temporary reduction in transfer factor. The authors suggested that this was a case of severe poisoning but were unable to attribute his survival to the forced diuresis therapy because the patient had also received immunosuppressive therapy with azathioprin and prednisolone. Bismuth *et al.*, (1982) suggested that forced diuresis per se does not enhance the urinary elimination of paraquat. Nevertheless, they believed the therapy might be of value in the prevention of paraquat-induced renal damage because of a reduction in the tubular concentration of paraquat. However, of the 18 patients with developing renal failure who were treated with frusemide, only one survived despite the fact that diuresis was maintained in nine patients.

Removal of paraquat by means of peritoneal dialysis, haemodialysis and haemoperfusion has been advocated to reduce paraquat plasma concentrations and enhance elimination. Of these, dialysis procedures were found to be ineffective (Vale *et al.*, 1977, Bismuth *et al.*, 1982) and the value of charcoal haemoperfusion remains controversial. Experimental haemoperfusion in dogs was able to improve survival (Widdop *et al.*, 1977), but early results in paraquat poisoned patients were disappointing (Vale *et al.*, 1977). In 1979, Okonek and co-workers published a report on the successful treatment of two patients with what they described as 'continuous haemoperfusion'. Plasma paraquat analysis prior to haemoperfusion indicated a very poor prognosis, but under an aggressive haemoperfusion therapy over several weeks both survived. Subsequently, a further 6 patients were treated with this regime and had a positive outcome (Okonek *et al.*, 1982/83). However, these apparent successes proved to be rare. Hampson and Pond (1988) carried out a meta-analysis of data from 35 cases published in the literature and 7 cases from their own hospital which had sufficient comparative data, as well as details of the haemoperfusion procedure. They showed that none of the patients whose initial plasma paraquat concentration was higher than 3 mg/l survived, regardless of time after ingestion and treatment. Overall, the outcome was in line with predictions and did not appear to be affected by haemoperfusion, single or repeated. The authors concluded that haemoperfusion should only be considered for patients whose initial plasma concentration was below 3 g/l, those, in whom the probability of survival was between 20 and 70%, and those who present within a few hours of ingestion. Subsequently, Böhler *et al.*, (1992)

reported a case where the use of continuous arterio-venous haemoperfusion was effective in lowering the plasma paraquat concentration below the limit of detection. However, the patient died on the second day after ingestion from gastrointestinal complications. Suzuki *et al.*, (1993) compared the effect of 'aggressive' (> 10 hours in the first 24 after ingestion) vs. 'conventional' (< 10 hours) haemoperfusion on the outcome of the intoxication in 40 patients. Aggressive haemoperfusion did not improve the overall outcome, but significantly increased survival time. Finally, Lee and Lee (1995) found that 8 out of 18 patients treated with haemoperfusion survived, whereas none of 20 who did not receive haemoperfusion died. No plasma paraquat concentrations were measured, but the authors stated that the estimated volume ingested was not significantly different between the two groups.

In conclusion, no clear benefit has been demonstrated from therapies aimed at enhancing elimination of paraquat from the body. The best chances appear to lie in the maintenance of renal function through adequate diuresis. As for extracorporeal elimination, haemoperfusion appears the only technique which may be of benefit in some patients, and the early and aggressive use of this technique may have contributed to survival in a few cases.

3.12.3 Pathophysiological treatment

A wide range of therapeutic substances have been studied experimentally to try and prevent the specific lung toxicity of paraquat from occurring. Some have been used in humans, but most of the published work is based on single or a small number of cases. Usually, more than one therapy was employed, and information on the severity of poisoning and the initial probability of survival is often limited. For these reasons a critical evaluation of the benefit of any one therapy is difficult and, in many cases, impossible.

Since oxygen is required to set off the biochemical cascade of paraquat toxicity the use of supplementary oxygen should be avoided as long as possible. Bismuth *et al.*, (1982) used a hypoxic breathing mixture and hypothermia in six patients. The arterial oxygen tension was maintained below 6.6 kPa. Only one patient survived who had clinical evidence of only mild poisoning. In the other patients, the FiO_2 had to be increased on a daily basis, all of them requiring > 0.5 (50%) prior to their death.

Since redox cycling and the generation of free radicals are considered to be the principle steps in the development of alveolar epithelial cell damage, a number of agents which, at least

theoretically, interfere with this process have been tried therapeutically. One of the first steps in the biochemical cascade of injury is the generation of the superoxide anion which is detoxified by the enzyme superoxide dismutase. This has been given either intravenously (Davies and Conolly, 1975), intramuscularly (Harley *et al.*, 1977), intrapulmonary during fibreoptic bronchoscopy (Bateman, 1987) or as a nebulized aerosol (Davies and Conolly, 1975, Hong *et al.*, 1996). In some cases there was co-administration with the antioxidants vitamin C (Hong *et al.*, 1996) or vitamin E (Harley *et al.*, 1977) which has also been given on its own (Shahar *et al.*, 1980). The doses given appeared to have been determined empirically, and no conclusive evidence of a beneficial effect has so far been shown. N-acetyl cysteine (NAC) is a glutathione precursor which readily crosses the cell membrane, and glutathione depletion is one of the features of paraquat-induced cellular damage. Lheureux *et al.*, (1995) treated a patient with high doses of NAC (300 mg/kg/day) over three weeks. However, the patient who survived also received early haemodialysis and desferrioxamine. The latter, an iron chelating agent, has been proposed because iron has a catalytic effect in the production of hydroxyl radicals. However, no other data exist on its clinical use.

3.12.4 Prevention of lung fibrosis

The development of the paraquat lung lesion is characterised by early infiltration of inflammatory cells, followed by fibroblast proliferation. Attempts have therefore been made to halt this process by giving immunosuppressive therapy. A few case reports involved the use of azathioprine, in one case with successful outcome (Laithwaite, 1975), in two other cases the patients died (Malcolmson and Beesley, 1975). In one patient who survived, bleomycin was used over three days (Mahieu *et al.*, 1977). Most experience exists with a combination treatment of cyclophosphamide and corticosteroids which was first advocated by Malone *et al.*, (1971). Addo *et al.*, (1984) claimed a 75% survival rate in 20 patients treated with cyclophosphamide (5 mg/kg/day to a maximum total of 4 g) and dexamethasone (8 mg eight-hourly over two weeks). Two years later they published a case series using the same regime with 72 patients, 52 (72%) of which survived (Addo and Poon-King, 1986). However, the plasma paraquat data of 25 patients showed that 7 survivors had no measurable paraquat levels, and of the other 18 only the six patients with the lowest plasma concentration survived. Following a preliminary report (Lin *et al.*, 1996) on the use of pulse therapy with cyclophosphamide (1g/day over 2 days) and methylprednisolone (1g/day over 3 days), Lin*et*

al., (1999) reported results of a prospective study in 142 patients. Seventy-one patients died from fulminant poisoning within one week, and cyclophosphamide did not make any difference. In the group of moderately to severely poisoned patients, only 4/22 patients treated with cyclophosphamide died, compared to 16/28 in the control group. Plasma paraquat concentrations were not available, but the authors stated that there was no difference in severity of poisoning between the two groups based on the urine dithionite test. However, the beneficial effects of the cyclophosphamide-dexamethasone regime have been disputed (Nogue *et al.*, 1989), and in a prospective study Perriens *et al.*, (1992) did not find any difference in mortality between 14 patients who had received standard treatment and the 33 patients who had received high-dose cyclophosphamide and dexamethasone. A final answer regarding the usefulness of this therapy can therefore not been given at this stage.

Because of the radiosensitivity of fibroblasts *in vitro*, Webb *et al.*, (1984) treated a patient who had developed diffuse alveolar damage following paraquat ingestion initially with cyclophosphamide and, after further deterioration with fractionated radiotherapy over 11 days. The patient survived. It was noted that the severity of poisoning in this patient was mild (Proudfoot *et al.*, 1984) and the majority of patients in subsequent reports died (Bloodworth *et al.*, 1986, Williams and Webb, 1987). This may have been due to differences in the severity of intoxication, as well as the therapy employed. Following the successful treatment of a patient with poor prognosis (Talbot *et al.*, 1988a), Talbot and Barnes (1988) treated a further 8 patients with radiotherapy. Only 2 survived and the authors suggested that a definite benefit of radiotherapy could not be demonstrated in their study.

3.12.5 Other treatments

Beta-blocking agents such as propranolol have been shown to block the uptake of paraquat into the lung (Maling *et al.*, 1978). However, their limited therapeutic use has not been successful (Davies and Conolly, 1975, Fairshter *et al.*, 1976, 1979).

Recently, there have been two case reports on the use of nitrogen oxide inhalation (NO) in paraquat poisoning. On the basis that NO is a potent endogenous vasodilator and that NO inhalation exerts a beneficial effect on pulmonary gas exchange, Köppel *et al.*, (1994) treated a 52 year old patient with severe paraquat poisoning (plasma concentration 4 days after ingestion 1 mg/l). She received 25 ppm in the inhalation mixture, her respiratory parameters

improved immediately and she was stabilised for three days. However, the patient died with massive pleural effusions and ventilatory failure on day 11 after ingestion. In the second case, Eisenman *et al.*, (1998) treated a 52 year old male whose plasma paraquat concentration predicted only a 30% chance of survival, with NO because of developing respiratory distress. In addition, the patient had received Fuller's Earth, forced diuresis, haemofiltration, N-acetyl cysteine, methyl prednisolone, cyclophosphamide, vitamin E, and colchicine. Because of the multiple therapy it was impossible to be sure which of the therapeutic measures had contributed to this patient's survival. Nevertheless, it was felt that the use of NO deserved further evaluation (Hall, 1998).

There are five reports in the literature where lung transplantation has been performed after paraquat poisoning. Matthew *et al.*, (1968) described a single lung transplantation 6 days after accidental paraquat ingestion in a 15 year old boy whose plasma paraquat levels at the time of the operation were still at toxic levels (0.4 µg/ml). The patient died 13 days after the operation in respiratory failure and the autopsy showed changes typical for paraquat poisoning, although no paraquat was measurable in the transplanted lung. A contribution of rejection to the disease process could not be excluded. The same group subsequently reported a further unsuccessful lung transplantation in an 18 year old farm worker (Cooke *et al.*, 1973). A further single lung transplantation with fatal outcome in a 25 year old man was reported in 1984 by Kamholz *et al.* This patient died after 45 days from the consequences of a bronchopleural fistula. A sequential bilateral lung transplantation was described by the Toronto Lung Transplant Group (Saunders *et al.*, 1985). This 31 year old patient had received a lung transplant at a time when his plasma paraquat levels were below what was considered to be a toxic level (0.03 µg/ml). In the postoperative period there was a seven-fold increase in paraquat plasma levels, possibly due to the release of paraquat from muscle stores. The transplanted lung failed and following several days of intensive therapy including haemoperfusion a second transplant was undertaken. The transplant worked well over two months, however, the patient developed a progressive toxic myopathy and ultimately died from a cerebrovascular accident. Nevertheless, this case showed the feasibility of lung transplantation in paraquat poisoning. The most recent case (Licker *et al.*, 1998) is the only one with a successful outcome. A 17 year old farmer developed respiratory failure of unknown origin. Repeated plasma paraquat measurements were negative. Following

mechanical ventilation for five weeks a single lung transplantation was carried out. Recovery was complicated by myopathy, and paraquat was confirmed in the excised lung and a muscle biopsy. The patient subsequently admitted to having taken paraquat. The patient was discharged after 88 days and was able to lead an independent life at the last follow-up 13 months after transplantation.

These cases demonstrate that, over the years, lung transplantation has become feasible in cases of paraquat poisoning. While the early attempts were hampered by problems with immune suppression as well as a lack of understanding of the pathophysiological events following paraquat poisoning, these problems appear now to have been satisfactorily resolved. However, the authors of the latest paper make the point that the use of such a scarce and expensive resource is questionable in cases of deliberate self-harm.

4. DISCLAIMER

The positions on certain aspects of the toxicology of paraquat in this chapter may not be aligned with the Zeneca positions; the latter are derived mainly from internal Zeneca reports many of which have not been published in the open literature.

5. REFERENCES

Ackrill P, Hasleton P S, and Ralston A J (1978). Oesphagal perforation due to paraquat. *Br. Med. J* **1**, 1252-1253.

Adam A, Smith L L and Cohen G M (1990). An evaluation of the redox cycling potencies of paraquat and nitrofurantoin in microsomal and lung slice systems. *Biochem. Pharmacol.* **40**, 1533-1539.

Addo E and Poon-King T (1986). Leucocyte suppression in treatment of 72 patients with paraquat poisoning. *The Lancet* **I**, 1117-1120.

Addo E, Ramdial S and Poon-King T (1984). High dosage cyclophosphamide and dexamethasone treatment of paraquat poisoning with 75% survival. *West. Indian Med. J* **33**, 220.

Akahori F and Oehme F W (1983). Inhibition of collagen synthesis as a treatment for paraquat poisoning. *Vet. Human Toxicol.* **25**, 321-327.

Aldrich T K, Fisher A B, Cadenas E and Chance B (1983). Evidence for lipid peroxidation by paraquat in the perfused rat lung. *J. Lab. Clin. Med.* **101**, 66-73.

Ali S, Abdulla M and Athar M (1996). L-2-oxothiazolidine-4-carboxylate, an in situ inducer of glutathione, protects against paraquat-mediated pulmonary damage in rat. *Med. Sci. Res.* **24**, 699-701.

Amarasingham R D and Lee S E. A review of human poisoning cases examined by the Toxicology Division of the Department of Chemistry, Petaling Jaya, Malaysia, from 1977 to 1981 (unpublished report).

Anderson D, McGregor D B and Purchase I F H (1976). Dominant lethal studies with paraquat and diquat in male CD-1 mice. *Mutat. Res.* **40**, 349-58.

Athanaselis S, Qammaz S, Alevisopoulos G and Koutselinis A (1983). Percutaneous paraquat intoxication. *J. Toxicol. Cut. & Ocular Toxicol.* **2**, 3-5.

Autor A P (1974). Reduction of paraquat toxicity by superoxide dismutase. *Life Sci.* **14**, 1309-1319.

Bagetta G, Corasaniti M T, Iannone M, Nistico G and Stephenson J D (1992). Production of limbic motor seizures and brain damage by systemic and intracerebral injection of paraquat in rats. *Pharmacol. Toxicol.* **71**, 43-448.

Bagetta G, Iannone M, Vecchio I., Rispoli V, Rotiroti D, Nistico G (1994). Neurodegeneration produced by intrahippocampal injection of paraquat is reduced by systemic administration of the 21-aminoacid U74389F in rats. *Free Rad. Res.* **21**, 85-89.

Baguley E, Iles P B and Wright N (1983). Serial lung function tests in paraquat poisoning. *Human Toxicol.* **2**, 418.

Bainova A (1969). Experimental appraisal of the effect of dipyridylium herbicides on skin. *Letopisi. HEI.* **9**, 25-30.

Bainova A and Vulcheva V (1977). Lung changes after chronic paraquat intoxication. *Dokl. Bolg. Akad. Nauk.* **30**, 1788-90.

Bainova A, Zlateva M and Vulcheva V I (1972). Chronic inhalation toxicity of dipyridylium herbicides. *Khig. Zdraveopazvane* **15**, 25-31.

Baldwin R C, Pasi A, MacGregor J T and Hine C H (1975). The rates of radical formation from the dipyridylium herbicides, paraquat, diquat and morfamquat in homogenates of rat lung, kidney and liver: an inhibitory effect of carbon monoxide. *Toxic. Appl. Pharmac.* **32**, 298-304.

Barbeau A, Cloutier T, Roy M, Paris S, Plasse L and Poirier J (1985). Ecogenetics of Parkinson's disease: 1 - The 4-hydroxylation of debrisoquine. *Lancet* **2**, 1213-1216.

Barbeau A, Roy M, Clourier L, Plasse L and Paris S (1986). Environmental and Genetic factors in the etiology of Parkinson's disease. In: Yahr, M. D., and Bergmann, K. J. (eds.). Parkinson's disease. *Adv. Neurol.* **45**, 299-306.

Bassett D J P and Fisher A B (1978). Alterations of glucose metabolism during perfusion of rat lung with paraquat. *Am. J Physiol.* **234**, E653-E659.

Bateman D N (1987). Pharmacological treatments of paraquat poisoning. *Human Toxicol.* **6**, 57-62.

Baud F J, Houze P, Bismuth C, Scherrmann J M, Jaeger A and Keyes C (1988). Toxicokinetics of paraquat through the heart lung block six cases of acute human poisoning. *Clinical Toxicology* **26**, 35-50.

Beebeejaun A R., Beevers G and Rogers W N (1971). Paraquat poisoning-prolonged excretion. *J Toxicol. Clin. Toxicol.* **4**, 397-407.

Bennett PN, Davies D S and Hawksworth G M (1976). In vivo absorption studies with paraquat and diquat in the dog. *Br. J Pharmacol.* **58**, 284P.

Berisha H I, Pakbaz H, Absood A and Said S I (1994). Nitric oxide as a mediator of oxidant lung injury due to paraquat. *Proc. Natl. Acad. Sci. USA* **91**, 7445-9.

Berry D J and Grove J (1971). The determination of paraquat 1,1-dimethyl-4,4'- bipyridylium cation in urine. *Clin. Chim. Acta* **34**, 5-11.

Bilinski T and Litwinska J (1987). On the ideas alternative to the theory of superoxide mediated oxygen toxicity. *Bull. Pol. Acad. Sci. Biol. Sci.* **35**, 25-31.

Binns C W (1976). A deadly cure for lice: a case of paraquat poisoning. *Papua New Guinea Med. J.*, **19**, 105-107.

Bismuth C, Garnier R, Dally S, Fournier P E and Scherrman J M (1982). Prognosis and treatment of paraquat poisoning: a review of 28 cases. *Clin. Tox.* **19**, 461-474.

Bismuth C, Scherrmann J M, Garnier R, Baud F J and Pontal P G (1987). Elimination of paraquat. *Human Toxicol.* **6**, 63-7.

Bismuth C, Baud F J, Garnier R, Muszinsk J and Houze P (1988). Paraquat poisoning biological presentation. *J Toxicol. Clin. Exp.* **8**, 211.

Bismuth C, Hall A H and Wong A (1995). Paraquat ingestion exposure symptomatology and risk. In: Bismuth C and Hall A H (eds) Paraquat poisoning. pp195-210, Marcel Dekker Inc, New York.

Bloch CA and Ausubel F M (1986). Paraquat-mediated selection for mutations in the manganese-superoxide dismutase gene soda. *J Bacteriol.* **168**, 795-798.

Block E R (1979). Potentiation of acute paraquat toxicity by Vitamin E deficiency. *Lung* **156**, 195-203.

Bloodworth L L, Kershaw J B, Stevens P E, Alcock C J and Rainford D J (1986). Failure of radiotherapy to reverse progressive pulmonary fibrosis caused by paraquat. *Br. J Radiol.* **59**, 1037-1038.

Böhler J, Riegel W, Keller E, Logemann E, Just H and Schollmeyer P J (1992). Continuous arteriovenous haemoperfusion for treatment of paraquat poisoning. *Nephrology Dialysis Transplantation* **7**, 875-878.

Bramley A and Hart T B (1983). Paraquat poisoning in the United Kingdom. *Human Toxicol.* **2**, 417.

Brawn K and Fridovich I (1981). DNA strand scission by enzymatically generated oxygen radicals. *Arch. Biochem. Biophys.* **206**, 414-419.

Brian R C, Homer R F, Stubbs J and Jones R L (1958). A new herbicide, 1:1-ethylene-2,2'-dipyridylium dibromide. *Nature (Lond)* **181**, 446.

Brigelius R (1985). Mixed disulphides: biological functions and increase in oxidative stress. In *Oxidative Stress.* (H. Sies, Ed), Academic Press Inc, London, pp 243-247.

Brigelius R, Dostal L A, Horton J K and Bond J R (1986). Alteration of the redox state of NADPH and glutathione in perfused rabbit lung by paraquat. *Toxicol. Ind. Hlth.* **2**, 417-428.

Brigelius R, Lenzen R and Sies H (1982). Increase in hepatic mixed disulphide and glutathione disulphide levels elicited by paraquat. *Biochem. Pharmacol.* **31**, 1637-1641.

Brine D R, David K H and Wall M E (1981). Combustion of paraquat contaminated marijuana - analysis of paraquat and its degradation products. Final Report, Contract No. 273-79-C-0003, RTI/1755/OF, Research Triangle Park, NC, Research Triangle Institute.

Brooks R E (1971). Ultrastructure of lung lesions produced by ingested chemicals. 1. Effect of the herbicide paraquat on mouse lung. *Lab. Invest.* **25**, 536-45.

Brooke-Taylor S, Smith L L and Cohen G M (1983). The accumulation of polyamines and paraquat by human peripheral lung. *Biochem. Pharmacol.* **32**, 717-720.

Brown O R, Heitkamp M and Song C (1981). Niacin reduces paraquat toxicity in rats. *Science* **212**, 1510-1512.

Bullivant C M (1966). Accidental poisoning by Paraquat : Report of two cases in man. *Br. Med. J* **1**, 1272-1273.

Burk R F, Lawrence R A and Lane J M (1980). Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration - Effect of selenium deficiency. *J Clin. Invest.* **65**, 1024-1031.

Bus J S, Aust S D and Gibson J E (1974). Superoxide and singlet oxygen catalysed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity. *Biochem. Biophys. Res. Commun.* **58**, 749-755.

Bus J S and Gibson J E (1975). Postnatal toxicity of chronically administered paraquat in mice and interactions with oxygen and bromobenzene. *Toxicol. Appl. Pharmacol.* **33**, 461-70.

Bus J S, Preache M M, Cagen S Z, Posner H S, Eliason B C, Sharp C W and Gibson J E (1975). Fetal toxicity and distribution of paraquat and diquat in mice and rats. *Toxicol. Appl. Pharmacol.* **33**, 450-60.

Bus J S, Aust S D and Gibson J E (1976a). Paraquat toxicity: Proposed mechanism of action involving lipid peroxidation. *Environ. Hlth. Perspect.* **16**, 139-46.

Bus J S, Cagen S Z, Olgaard M and Gibson J E (1976b). A mechanism of paraquat toxicity in mice and rats. *Toxicol. Appl. Pharmacol.* **35**, 501-513.

Butler C (1975). Pulmonary interstitial fibrosis from paraquat in the hamster. *Arch. Pathol.* **99**, 503-7.

Butler C and Kleinerman J (1971). Paraquat in the Rabbit. *Br. J Ind. Med.* **28**, 67-71.

Cadot R, Descotes J, Grenot C, Cuilleron C Y, Evreux J C (1985). Increased plasma araquat levels in intoxicated mice following anti-paraquat F(ab')2 treatment. *J Immunopharmacol.* **7**, 467-477.

Cagen S Z, Janoff A S, Bus J S and Gibson J E (1976). Effect of paraquat (methyl viologen) on the liver function in mice. *J Pharm. Exp. Therap.* **198**, 222-228.

Cagen S Z and Gibson J E (1977). Liver damage following paraquat in selenium deficient and diethyl maleate pretreated mice. *Toxicol. Appl. Pharmacol.* **40**, 193-200.

Calo M, Iannone M, Passafaro M and Nistico G (1990). Selective vulnerability of hippocampal CA3 neurones after microinfusion of paraquat into the rat substantia nigra or into the ventral tegmental area. *J Comp. Path.* **103**, 73-78.

Campbell S (1968). Paraquat poisoning. *Clin. Toxicol.* **1**, 245-249.

Cant J S and Lewis D R H (1968). Ocular damage due to paraquat and diquat. *Br. Med. J* **3**, 59.

Carson D J L and Carson E D (1976). The increasing use of paraquat as a suicide agent. *Forensic Sci.* **7**, 151-160.

Casey P and Vale J A (1994). Deaths from pesticide poisoning in England and Wales: 1945-1989. *Hum. Exp. Toxicol.***13**, 95-101.

Casey P, Buckley B M and Vale J A (1994). Methemoglobinemia following ingestion of a monolinuron/paraquat herbicide. *J Toxicol. Clin. Toxicol.* **32**, 185-9.

Castro-Gutierrez N, McConnell R, Andersson K, Pacheco-Anton F and Hogstedt C(1997). Respiratory symptoms, spirometry and chronic occupational paraquat exposure. *Scand. J Work Environ. Health* **23**, 421-427.

Cavalli R D and Fletcher K (1977). An effective treatment for paraquat poisoning In 'Biochemical Mechanisms of Paraquat Toxicity : Proc. 1st Iowa Symp. on Toxic Mechanisms, June 28-29 (1976)'. Chapter 5, pp 213-230. Ed : Autor AP, Publ: Academic Press.

Chan B S H, Lazzaro V A, Seale J P and Duggin G G (1996a). Characterisation and uptake of paraquat by rat renal proximal tubular cells in primary culture. *Human Exp. Toxicol.* **15**, 949-956.

Chan B S H, Lazzaro V A, Kirwan P D, Seale J P and Duggin G G (1996b). The effect of paraquat on two renal cell lines-LLC-PK_1 and MDCK. *Res. Commun. Pharmacol. Toxicol.* **1**, 99-112.

Chan B S H, Seale J P and Duggin G G (1997). The mechanism of excretion of paraquat rats. *Toxicol. Lett.* **90**, 1-9.

Chan B S H, Lazzaro V A, Seale J P and Duggin G G (1998). The renal excretory mechanisms and the role of organic cations in modulating the renal handling of paraquat. *Pharmacol. Therap.* **79**, 193-203.

Chen N, Pond S M and Bowles M R (1992). Competition between paraquat and putrescine for uptake by suspensions of rat alveolar type II cells. *Biochem. Pharmacol.* **44**, 1029-1036.

Chen N, Bowles M R and Pond S M (1994). Prevention of paraquat toxicity in suspensions of alveolar type II cells by paraquat-specific antibodies. *Human Exp. Toxicol.* **13**, 551-557.

Cheng W H, Ho Y S, Valentine B A, Ross D A, Combs G F Jr and Lei X G (1998). Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. *J Nutr.* **128**, 1070-1076.

Chester G and Ward R J (1984). Occupational exposure and drift hazard during aerial application of paraquat to cotton. *Arch. Environ. Contam. Toxicol.* **13**, 551-63.

Chester G and Woollen B H (1981). Studies of the occupational exposure of Malaysian plantation workers to paraquat. *Br. J Ind. Med.* **38**, 23-33.

Chester G, Gurunathan G, Jones N and Woollen B H (1993). Occupational exposure of Sri Lankan tea plantation workers to paraquat. *Bulletin of World Health Org.* **71**, 625-633.

Chui Y-C, Poon G and Law F (1988). Toxicokinetics and bioavailability of paraquat in rats following different routes of administration. *Toxicol. Ind. Health* **4**, 203-219.

Clark D G (1971). Inhibition of the absorption of paraquat from the gastrointestinal tract by absorbents. *Br. J Ind. Med.* **28**, 186-188.

Clark D G, McElligott T F and Weston Hurst E (1966). The toxicity of paraquat. *Br. J. Ind. Med.* **23**, 126-132.

Conning D A, Fletcher K and Swan A A B (1969). Paraquat and related bipyridyls. *Br. Med. Bull.* **25**, 245-249.

Cooke N J, Flenley D C and Matthew H (1973). Paraquat poisoning: Serial studies on lung function. *Quart. J Med.* **62**, 683-692.

Corasaniti M T, Defilippo R, Rodino P, Nappi G and Nistico G (1991). Evidence that paraquat is able to cross the blood-brain barrier to a different extent in rats at various ages. *Functional Neurology* **6**, 385-391.

Corasaniti M T, Bagetta G, Rodino P, Gratteri S and Nistico G (1992). Neurotoxic effects induced by intracerebral and systemic injection of paraquat in rats. *Human Exp. Toxicol.* 11, 535-9.

Corasaniti M T and Nistico G (1993). Determination of paraquat in rat brain by high-performance liquid chromatography. *J Chromatography* **643,** 419-425.

Costantini P, Petronilli V, Colonna R and Bernardi P (1995). On the effects of paraquat on isolated mitochondria. Evidence that paraquat causes opening of the cyclosporin A-sensitive permeability transition pore synergistically with nitric oxide. *Toxicology* **99**, 77-88.

Coxon R E, Rae C, Gallacher G and Landon J (1988). Development of a simple fluoroimmunoassay for paraquat. *Clin. Chim. Acta.* **175**, 297-306.

Cramp T P (1985). Failure of N-acetylcysteine to reduce renal damage due to paraquat in rats. *Human Toxicol.* **4**, 107.

Dabney B J (1995). Genetic, carcinogenic and reproductive effects of paraquat *In Paraquat poisoning, mechanisms, prevention and treatment*, Ed. C. Bismuth and A. H. Hall Marcel Dekker, New York, pp 235-248.

Daniel J W and Gage J C (1966). Absorption and excretion of diquat and paraquat in rats. *Br. J Ind. Med.* **23**, 133-136.

Davies D S, Hawksworth G M and Bennett PN (1977). Paraquat Poisoning. *Proc. Europ. Soc. Toxicol.* **18**, 21-6.

Davies D S and Connolly M E (1975). Paraquat poisoning - Possible therapeutic approach. *Proc. Royal Soc. Med.* **68**, 442.

Davies D S (1987). Paraquat poisoning : the rationale for current treatment regimes. *Human Toxicol.* **6**, 37-40.

Day B J and Crapo J D (1996). A metalloporphyrin superoxide dismutase mimetic protects against paraquat induced lung injury *in vivo*. *Toxicol. Appl. Pharmacol.* **140**, 94-100.

De Gori N, Froio F, Strongoli M C, De Francesco A, Calo M and Nistico G (1988). Behavioural and electrocortical changes induced by paraquat after injection in specific areas of the brain of the rat. *Neuropharmacology* **27**, 201-207.

De Haan J B, Bladier C, Griffiths P, Kelner M, O'Shea R D, Cheung N S, Bronson R T, Silverstro M J, Wild S, Zheng S S, Beart P M, Hertzog P J and Kola I (1998). Mice with a homozygous null mutation for the abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. *J Biol. Chem.* **273**, 22528-22536.

Denduyts-Whitehead A, Hart T B and Volans G N (1985). Effects of the addition of an emetic to paraquat formulations on acute poisoning in man. *J Toxicol. Clin. Toxicol.* **23**, 422-3.

Department of Health (1996). *Pesticide Poisoning* (2nd edition). Notes for the guidance of medical practitioners. Ed. A. Proudfoot. The Stationary Office, London.

Deveckova D and Mydlik M (1980). Gramoxone ocular burns. *Cesk. Oftalmol.* **36**, 7-10.

Dey M S, Krieger R I and Ritter RC (1987). Paraquat-induced, dose-dependent conditioned task aversions and weight loss mediated by the area postrema. *Toxicol. Appl. Pharmacol.* **87**, 212-221.

Dey M S, Breeze R G, Hayton W L, Karara A H and Kreiger R I (1990). Paraquat pharmacokinetics using a subcutaneous toxic low dose in the rat. *Fundam. Appl. Toxicol.* **14**, 208-216.

Dial C A B and Dial N A (1987). Effects of paraquat on reproduction and mortality in two generations of mice. *Arch. Environ. Contam. Toxicol.* **16**, 759-764.

Dial C A B and Dial N A (1989). Effects of paraquat on parent generation female and F1 suckling mice using different treatment regimes. *Bull. Environ. Contam. Toxicol.* **43**, 66-73.

Dikshith T S S, Datta K K, Raizada R B and Kushwah H S (1979). Effect of paraquat dichloride in male rabbits. *Ind. J Exp. Biol.* **17**, 926-8.

Dinsdale D, Preston S G and Nemery B (1991). Effects of injury on ^{3}H-putrescine uptake by type I and II cells in rat lung slices. *Exp. Mol. Pathol.* **54**, 218-229.

Douglas J F, McGeown M G and McEnvoy J (1973). The treatment of paraquat poisoning: three cases of recovery. *Ulster Med. J* **42**, 209-212.

Douze J M C, van Dijk A, Gimbrere J S F, van Heijst A N P, Maes R and Rauws A G (1974). Intensive therapy after Paraquat intoxication. *Intensivmedizin* **11**, 241-250.

Douze J M C and van Heijst A N P (1977a). The paraquat intoxication - oxygen a real problem. *Acta. Pharmac. Tox.* **41** Suppl II, 241-5.

Douze J M C, van Dijk A, Gimbrere J S F, van Heijst A N P and Maes R (1977b). Intensive therapy after paraquat intoxication: clinical aspects of paraquat poisoning. *RIV. Tossicol. Sper. Clin.* **5**, 333-335.

Draffan, G. H., Clare, R. A., Davies, D. L., Hawksworth, G., Murray, S., and Davies, D. S. (1977) Quantitative determination of the herbicide paraquat in human plasma by gas chromatographic and mass spectrometric methods. *J Chromat.* **139,** 311-320.

Drew R and Gram T E (1979). Vehicle alteration of paraquat lethality in mice. *Toxicol. Appl. Pharmacol.* **48**, 479-487.

Dunbar J R, Delucia A, Acuff R V and Ferslew K E (1988). Prolonged intravenous paraquat infusion in the rat. I. Failure of co-infused putrescine to alternate pulmonary paraquat uptake,

paraquat-induced biochemical changes or lung injury. *Toxicol. Appl. Pharmacol.* **94**, 207-220.

Dusinska M, Kovacikova Z, Vallova B and Collins A (1998). Responses of alveolar macrophages and epithelial type II cells to oxidative DNA damage caused by paraquat. *Carcinogenesis* **19**, 809-812.

Dutt A, Priebe T S, Teeter L D, Kuo M T and Nelson J A (1992). Postnatal development of organic cation transport and MDR gene expression in mouse kidney. *J Pharmacol Exp Therap.* **261**, 1222-1230.

Ecker J L, Gibson J E and Hook J B (1975a). In vitro analysis of the renal handling of paraquat. *Toxicol. Appl. Pharmacol.* **34,** 170-177.

Ecker J L, Hook J B and Gibson J E (1975b). Nephrotoxicity of paraquat in mice. *Toxicol. Appl. Pharmacacol.* **34**, 178-186.

Edmonds B K and Edwards G L (1996). The area postrema is involved in paraquat-induced condition aversion behaviour and neuroendocrine activation of the hypothalamic-pituitary-adrenal axis. *Brain Res.* **712**, 127-133.

Eisenman A, Armali Z, Raikhlin-Eisenkraft B, Bentur L, Bentur Y, Guralnik L and Enat R (1998). Nitric oxide inhalation for paraquat-induced lung injury. *J Toxicol. Clin. Toxicol.* 36, 575-584.

Elroy-Stein O, Bernstei Y and Groner Y (1986). Overproduction of human Cu/Zn-superoxide dismutase in transfected cells extenuation of paraquat mediated cytotoxicity and enhancement of lipid-peroxidation. *EMBO J* **5**, 615.

Endo T, Hara S, Kano S and Kuriiwa F (1988). Effects of a paraquat-containing herbicide, Gramoxone, on the central monoamines and acetylcholine in mice. *Research Comm. Psychology Psychiatry Behaviour* **13**, 261-270.

Ernouf D, Boussa N, Legras,A and Dutertre-Catella H (1998). Acute paraquat poisoning: increased toxicity in one case with high alcohol intake. *Human Exp. Toxicol.* **17**, 182-184.

Fairshter R D, Rosen S M, Smith W R, Glasser F L, McRae D M and Wilson A F (1976). Paraquat poisoning: New aspects of therapy. *Quart. J Med.* **45**, 551-565.

Fairshter R D, Dubir-Vaziri N, Smith W P, Glauser F L and Wilson A F (1979). Paraquat poisoning: an analytical toxicological study of three cases. *Toxicology* **12**, 259-266.

FAO/WHO (1973). *Paraquat.* In: 1972 Evaluations of some pesticide residues in food, Rome, Food and Agricultural Organisation of the United Nations.

FAO/WHO (1986). *Paraquat.* In 1986 Evaluations of some pesticide residues in food, Rome, Food and Agricultural Organisation of the United Nations.

Farrington J A, Ebert M, Land E J and Fletcher K (1973). Bipyridilium quaternary salts and related compounds. V. Pulse radiolysis studies on the reaction of paraquat radical with oxygen, implications for the mode of action of bipyridilium herbicides. *Biochim. Biophys. Acta.* **314**, 372-381.

Fennelly J J, Gallagher J T and Carroll R J (1968). Paraquat poisoning in a pregnant woman. *Br. Med. J* **3**, 722-723.

Fennelly J J, Fitzgerald M X and Fitzgerald O (1971). Recovery from severe Paraquat poisoning following forced diuresis and immunosuppressive therapy. *J Ir. Med. Ass.* **64**, 69-71.

Ferguson D M (1973). Factors influencing renal excretion of paraquat. Toxicol. Appl. Pharmacol. **25**, 486.

Fisher H K, Clements J A and Wright R R (1973). Enhancement of oxygen toxicity by the herbicide paraquat. *Am. Rev. Resp. Dis.* **107**, 246-252.

Fisher H K, Clements J A, Tierney D F and Wright R R (1975). Pulmonary effects of paraquat in the first day after injection. *Am. J Physiol.* **228**, 1217-1223.

Fisher A B and Reicherter J (1984). Pentose pathway of glucose metabolism in isolated granular pneumocytes metabolic regulation and stimulation by paraquat. *Biochem. Pharmacol.* **33**, 1349-1353.

Fitzgerald G R and Barniville G (1978). Poisoning by granular paraquat. *J Irish Phys. Surg.* **7**, 133-136.

Fitzgerald G R, Barniville G, Black J, Silke B, Carmody M and O'Dwyer W F (1978a). Paraquat poisoning in agricultural workers. *J Irish Med. Ass.* **71**, 336-342.

Fitzgerald, G. R., Barniville, G., Fitzpatrick, P., Edwards, A., and Silke, B. (1977) Adrenal abnormalities in paraquat poisoning: An indication for corticosteroid therapy? *Irish J Med. Sci.* **146,** 421-423.

Fitzgerald, G. R., Carmody, M., Barniville, G., O'Dwyer, W.F., Flanagan, M., and Silke, B. (1978b). The changing pattern of paraquat poisoning : An epidemiologic study. *J Irish Med. Ass.* **71,** 103-108.

Fitzgerald, G. R., Barniville, G., Dickstein, K., Carmody, M., and O'Dwyer, W.F. (1979a)Experience with Fuller's Earth in paraquat poisoning. *J Irish Med. Ass.* **72**, 149-152.

Fitzgerald, G.R., Barniville, G., Gibney, R. T. N., and Fitzgerald, M. X. (1979b) Clinical, radiological and pulmonary function assessment of 13 long-term survivors of paraquat poisoning. *Thorax* **34,** 414-429.

Florkowski, C. M., Bradberry, S. M., Ching, G. W. K., and Jones, A. F. (1992)Acute renal failure in a case of paraquat poisoning with relative absence of pulmonary toxicity. *Postgraduate Med. J* **68,** 660-662.

Forman, H. J., Aldrich, T. K., Posner, M. A., and Fisher, A. B. (1982) Differential paraquat uptake and redox kinetics of rat granular pneumocytes and alveolar macrophages *J Pharmacol. Exp. Therap.* **221,** 428-433.

Fowler, B. A., and Brooks, R. E. (1971) Effects of the herbicide paraquat on the ultrastructure of the mouse kidney. *Am. J Path.* **63,** 505-520.

Frank, L. (1983) Superoxide dismutase and lung toxicity. *TIPS* **4,** 124-128.

Frank, L., Neriishi, K., Sio, R., and Pascal, D. (1982) Protection from paraquat-induced lung damage and lethality in adult rats pretreated with clofibrate. *Toxicol. Appl. Pharmacol.* **66,** 269-277.

Frank, L. and Massaro, D. (1979) The lung and oxygen toxicity. *Arch. Intern.Med.* **139,** 347-350.

Fredriksson A, Fredriksson M and Eriksson P (1993). Neonatal exposure to paraquat or MPTP induces permanent changes in striatum dopamine and behaviour in adult mice. *Toxicol. Appl. Pharmacol.* **122**, 258-264.

Fukushima T, Yamada K, Isobe A, Shimwaku K and Yamane Y (1993). Mechanism of cytotoxicity of paraquat I NADH oxidation and paraquat radical formation via complex I. *Exp. Toxic. Pathol.* **45**, 345-349.

Gage J C (1968a). Toxicity of paraquat and diquat aerosols generated by a size selective cyclone: effect of particle size distribution. *Br. J Ind. Med.* **25**, 304-314.

Gage J C (1968b). The action of paraquat and diquat on the respiration of liver cell fractions. *Biochem. J* **109**, 757-761.

Garnier R (1995). Paraquat poisoning by inhalation and skin absorption. In: Bismuth C, Hall A H (eds) *Paraquat poisoning*. pp 211-34. Marcel Dekker Inc, New York.

Garnier R, Chataigner D, Efthymiou M L, Morallion I and Bramary F (1994). Paraquat poisoning by skin absorption: report of two cases. *Vet. Human Toxicol.*, **36**, 313-315.

Gaudreault P, Friedman P A and Lovejoy F H (1985). Efficacy of activated charcoal and magnesium citrate in the treatment of oral paraquat intoxication. *Ann. Emerg. Med.* **14**, 123-125.

George M and Hedworth-Whitty R B (1980). Non-fatal lung-disease due to inhalation of nebulised paraquat. *Br. Med. J* **280**, 902.

Gill R, Qua S C and Moffat A C (1983). High-performance liquid chromatography of paraquat and diquat in urine with rapid sample preparation involving ion-pair extraction on disposable cartridges of octadecyl-silica. *J Chromat.* **255**, 483-490.

Giri S N, Parker H R, Spangler W L, Misra H P, Ishizaki G, Schiedt M J and Chandler D B (1982). Pharmacokinetics of ^{14}C-paraquat and associated biochemical and pathologic

changes in beagle dogs following intravenous administration. *Fund. Appl. Toxicol.* **2**, 261-269.

Golbe L J, Farrell T M and Davis P H (1990). Follow-up study of early-life protective and risk factors in Parkinson's disease. *Mov. Dis.* **5**, 66-70.

Gordonsmith RH, Brooke-Taylor S, Smith L L and Cohen G M (1983). Structural requirements of compounds to inhibit pulmonary diamine accumulation. *Biochem. Pharmacol.* **32**, 3701-3709.

Goundar R P (1984). Paraquat poisoning in Fiji. *J Forensic. Sci. Soc.* **24**, 376.

Grant H C, Lantos P L and Parkinson C (1980). Cerebral damage in paraquat poisoning. *Histopathology* **4**, 185-195.

Groves C E, Morales M N, Gandolfi A J, Dantzler W H and Wright S H (1995). Peritubular paraquat transport in isolated renal proximal tubules. *J Pharmacol. Exp. Therap.* **275**, 926-932.

Grundemann D, Gorboulev V, Gamabaryan S, Veyhl M and Koepsell H (1994). Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* **372**, 549-552.

Grundies H, Kohmar D and Bennhold I (1971). Paraquat intoxication. Case report with special reference to hemodialysis. *Dtsch. Med. Wochenschr.* **96**, 588-589.

Hall A H (1995). Paraquat and diquat exposures reported to US Poison Centers 1983-1992. In: Bismuth C, Hall A H (eds) *Paraquat poisoning*. pp53-63, Marcel Dekker Inc, New York.

Hall A H (1998). Nitric oxide inhalation for paraquat - surviving both poisoning and therapy? J Toxicol. Clin. Toxicol. **36**, 585-586.

Halliwell B and Gutteridge J M C (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J* **219**, 1-14.

Hampson E C and Pond S M (1988). Failure of hemoperfusion and hemodialysis to prevent death in paraquat poisoning - a retrospective review of 42 patients. *Med. Toxicol. Adverse Drug Experience* **3**, 64-71.

Hara S, Iwata N, Kuriiwa F, Kano S, Kawaguchi N and Endo T (1993). Involvement of opioid receptors in shaking behaviour induced by paraquat in rats. *Pharmacol. Toxicol.* **73**, 146-149.

Hardwick S J, Adam A, Smith L L and Cohen G M (1990). Potentiation of the cell specific toxicity of paraquat by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU): Implications for the heterogeneous distribution of glutathione (GSH) in rat lung. *Biochem. Pharmacol.* **39**, 581-589.

Harley J B, Grinspan S and Root R K (1977). Paraquat suicide in a young women: Results of therapy directed against the superoxide radical. *Yale J Biol. Med.* **50**, 481-488.

Hart T B (1980). Non-fatal lung disease due to inhalation of nebulised paraquat. *Br. Med. J* **281**, 63-64.

Hart T B (1984). Letter to the editor: on percutaneous paraquat intoxication. *J. Toxicol. - Cut & Ocular Toxicol.*, **3**, 239-240.

Hart T B, Nevitt A and Whitehead A (1984). A new statistical approach to the prognostic significance of plasma paraquat concentrations. *The Lancet* **II**, 1222-3.

Hassan R A, Afzal M, Ali M and Gubler C J (1989). Effect of paraquat administered intraperitoneally on the nonpolar lipids of rabbits. *Ecotox. Environ. Saf.* **17**, 47-58.

Hawksworth G M, Bennett P N and Davies D S (1981). Kinetics of paraquat elimination in the dog. *Toxicol. Appl. Pharmacol.* **57**, 139-145.

He P and Yasumoto K (1994). Dietary butylated hydroxytoluene counteracts with paraquat to reduce the rate of hepatic DNA single strand breaks in senescence-accelerated mice. *Mech. Ageing Development* **76**, 43-48.

Hearn C E D and Keir W (1971). Nail damage in spray operators exposed to paraquat. *Br. Med. J* **28**, 399-403.

Hertzman C, Wiens M, Bowering D, Snow B and Caine D (1990). Parkinson's disease: a case-control study of oocupational and environmental risk factors. *Am. J Ind. Med.* **17**, 349-355.

Hettiarachchi J and Kodithuwakku G C (1989). Pattern of poisoning in Sri Lanka. *Int. J Epid.* **18**, 418-422.

Heylings J R (1991). Gastrointestinal absorption of paraquat in the isolated mucosa of the rat. *Toxicol. Appl. Pharmacol.* **107**, 482-493.

Hirai K, Witschi H and Cote M G (1985). Mitochondrial injury of pulmonary alveolar epithelial cells in acute paraquat intoxication. *Exp. Mol. Path.* **43**, 242-253.

Ho S C, Woo J and Lee C M (1989). Epidemiologic studies of Parkinson's disease in Hong Kong. *Neurology* **39**, 1314-1318.

Ho Y S, Magnenat J L, Gargano M and Cao J (1998). The nature of antioxidant defense mechanisms: a lesson from transgenic mice. *Environ. Health Perspec.* **106**, 1219-1228.

Hoet P H M, Dinsdale D, Lewis C P L, Verbeken J M, Lauweryns J M and Nemery B (1993). Kinetics and cellular localisation of putrescine uptake in human lung tissue. *Thorax,* **48**, 1235-1241.

Hoet P H M, Lewis C P L, Demedts M and Nemery B (1994). Putrescine and paraquat uptake in human lung slices and isolated type II pneumocytes. *Biochem. Pharmacol.* **48**, 517-24.

Hoet P.H.M., Demedts, M., and Nemery, B. (1997) Effects of oxygen pressure and medium volume on the toxicity of paraquat in rat and human type II pneumocytes. *Human Exp. Toxicol.* **16,** 305-310.

Hoffer E, Tzipiniuk A, Gal M and Taitelman U (1989). Skin versus blood distribution of paraquat in experimental animals during percutaneous absorption. *J Tox. Cut. Ocular Tox.* **8**, 179-182.

Hoffer E and Taitelmann N (1989). Exposure to paraquat through skin absorption: Clinical and laboratory observations of accidental splashing on healthy skin of agricultural workers. *Human Tox.* **8**, 483-485.

Hoffer E, Zonis Z, Tabak A and Taitelman U (1992). The administration of desferrioxamine to paraquat intoxicated rats. *Vet. Human Toxicol.* **34**, 300-303.

Hoffer E, Avidor I, Benjaminar O, Shenker L, Tabak A, Tamir A, Merzbach D and Taitelman U (1993). N-Acetylcysteine delays the infiltration of inflammatory cells into the lungs of paraquat intoxicated rats. *Toxicol. Appl. Pharmacol.* **120**, 8-12.

Hogarty C (1976). Exposure of spray operators to paraquat. Internal report Institute for Industrial Research and Chemical Engineering Department, University College, Dublin.

Hong SY, Yang D H, Kim Y D (1996). Successful management of severe paraquat poisoning with free radical scavenger. *Korean. J Med.* **51**, 99-107.

Hooper R G, Bates P D, Thomas A B, Beechler C R and Kunkle D B (1983). The effect of therapeutic vitamin E and niacin on paraquat toxicity. *Clin. Res.* **31**, A417.

Horton J K, Brigelius R, Mason R P and Bend J R (1986). Paraquat uptake in freshly isolated rabbit lung epithelial cells and its reduction to the paraquat radical under anaerobic conditions. *Mol. Pharmacol.* **29**, 484-8.

Houze P, Baud F J, Mouy R, Bismuth C, Bourdon R and Scherrmann J M (1990). Toxicokinetics of Paraquat in Humans. *Human Exp.Toxicol.* **9**, 5-12.

Howard J K (1979a). Recent experience with paraquat poisoning in Great Britain - A review of 68 cases. *Vet. Hum. Toxicol.* **21** Suppl, 213-216.

Howard J K(1979b). A clinical survey of paraquat formulation workers. *Br. J Ind. Med.* **36**, 220-223.

Howard J K (1980). Paraquat: A review of worker exposure in normal usage. *J Soc. Occup. Med.* **30**, 6-11.

Howard J K, Sabapathy N N and Whitehead P A (1981). A study of the health of Malayan plantation workers with particular reference to Paraquat spraymen. *Br. J Ind. Med.* **38**, 110-116.

Howe D J T and Wright N (1965). The toxicity of paraquat and diquat. *Proc 18th NZ Weed and Pest Control Conference* 105-14.

Hudson M, Smith C C, Patel S B, Friend J A R and Ewen S W B (1991). Paraquat induced pulmonary fibrosis in three survivors. *Thorax* **46**, 201-204.

Hughes J T (1988). Brain damage due to paraquat poisoning. *Neurotoxicology* **9**, 140.

Hughes R D, Millburn P, Williams R T (1973). Biliary excretion of some diquaternary ammonium cations in the rat, guinea-pig and rabbit. *Biochem. J* **136**, 979-84.

Hutchinson G, Daisley H, Simmons V and Gordon A N (1991). Suicide by poisoning. *West Ind. Med. J* **40**, 69-73.

Hybertson B M, Lampey A S, Clarke J H, Koh Y and Repine J E (1995). N-acetylcysteine pretreatment attenuates paraquat-induced lung leak in rats. *Redox Report* **1**, 337-342.

Iff H W, Brewis R A L, Mallick N P, Mawer G E, Orr W McN and Stern M A (1971). Paraquat poisoning. *Schweiz Med Wschr.* **101**, 84-88.

Ikebuchi J, Proudfoot A T, Matsubara K, Hampson E O G M, Tomita M, Suzuki K, Fuke C, Ijiri I., Tsunerari T, Yuasa I and Okada K (1993). Toxicological index of paraquat: A new strategy for assessment of severity of paraquat poisoning in 128 patients. *Forensic Science International* **59**, 85-87.

Ilett K F, Stripp B, Menard R H, Reid N D and Gillette J R (1974). Studies on the Mechanism of the Lung Toxicity of Paraquat: Comparison of Tissue Distribution and some Biochemical Parameters in Rats and Rabbits. *Toxicol. Appl. Pharmacol.* **28**, 216-226.

Ingebrigtsen K, Nafstad I and Andersen R A (1984). Distribution and transplacental transfer of paraquat in rats and guinea-pigs. *Gen. Pharmacol.* **15**, 201-204.

IPCS (1984). Environmental Health Criteria No 39, *Paraquat and Diquat*, World Health Organisation, Geneva.

Jaeger A, Saunder P and Scherrmann J M (1995). Mechanisms of paraquat toxicity and therapeutic implications. *In Paraquat poisoning: mechanisms, prevention, treatment*, Ed Bismuth C and Hall A H, Marcel Dekker, New York, pp141- 160.

Jaros F, Zuffa L M, Kritinova R, Skakala I and Domsova J (1978). Acute percutaneous intoxication by Gramoxone. *Pracov. Lek.* **30**, 260-263.

Jarvie DR and Stewart M J (1979). The rapid extraction of paraquat from plasma using an ion pair technique. *Clin. Chim. Acta.* **94**, 241-251.

Jenkinson SG (1982). Pulmonary oxygen toxicity. *Clin. Chest Med.* **3**, 109-119.

Joyce M (1969). Ocular damage caused by paraquat. *Br. J Ophthal.* **53**, 688-690.

Kameji R, Rannels S R, Pegg A E and Rannels DE (1989). Spermine uptake by type II pulmonary epithelial cells in primary culture. *Am. J. Physiol.* **256**, C160-167.

Kamholz S, Veith F J, Mollenkopf F, Montefusco C, Nehlsen-Cannarella S, Kaleya R, Pinsker K, Tellis V, Soberman R and Sablay L (1984). Single lung transplant in paraquat intoxication. *NY State J Med.* **84**, 82-84.

Karl P I and Friedman P A (1983). Competition between paraquat and putrescine for accumulation by rat lung slices. Toxicology **26**, 317-323.

Katopodis K, Logothetis E, Noussias C and Hadjiconstantinou V (1993). Survival of a paraquat poisoned patient despite late (4 days) referral and initiation of conventional haemoperfusion treatment. *Nephrology Dialysis Transplantation* **8**, 570-571.

Keeling PL, Pratt I S, Aldridge W N and Smith L L (1981). The enhancement of paraquat toxicity in rats by 85% oxygen-lethality and cell-specific lung damage. *Br. J Exp. Path.* **62**, 643-654.

Keeling P L and Smith L L (1982). Relevance of NADPH depletion and mixed disulphide formation in rat lung to the mechanism of cell damage following paraquat administration. *Biochem. Pharmacol.* **31**, 3243-3249.

Keeling P L, Smith L L and Aldridge W N (1982). The formation of mixed disulphides in rat lung following paraquat administration. Correlation with changes in intermediary metabolism. *Biochem. Biophys. Acta.* **716**, 249-257.

Kerr F, Patel A R, Scott P D R and Tompsett S L (1968). Paraquat poisoning treated by forced diuresis. *Br. Med. J* **3**, 290-291.

Kehrer J P, Haschek W and Witschi H P (1979). The influence of hyperoxia on the acute toxicity of paraquat and diquat. *Drug Chem. Tox.* **2**, 397-408.

Kelner M J and Bagnell R D (1989). Paraquat resistance associated with reduced NADPH reductase in an energy- dependent paraquat-accumulating cell line. *Arch. Biochem. Biophys.* **274**, 366-374.

Kelner M J, Bagnell R D, Uglik S F, Montoya M A and Mullenbach G T (1995). Heterologous expression of selenium dependent glutathione peroxidase affords cellular resistance to paraquat. *Arch. Biochem. Biophys.* **323**, 40-46.

Khera K S, Whitta L L and Clegg D J (1970). Embryopathic effects of diquat and paraquat in rats. *Ind. Med. Surg.* **37**, 257-261.

Kimbrough R D and Gaines T B (1970). Toxicity of paraquat to rats and its effect on rat lung. *Toxicol. Appl. Pharmacol.* **17**, 679-90.

Kitazawa K, Kobayashi T, Shibamoto T and Hirai K (1988). Effects of methyl prednisolone on acute lung paraquat toxicity in sheep. *Am. Rev. Resp. Dis.* **137**, 173-180.

Kitazawa Y, Matsubara M, Takeyama N and Tanaka T (1991). The role of xanthine oxidase in paraquat intoxication. *Arch. Biochem. Biophys.* **288**, 220-224.

Knepil J (1977). A short simple method for the determination of paraquat in plasma. *Clin. Chim. Acta.* **79**, 387-390.

Knight B A G and Tomlinson T E (1967). The interaction of paraquat (1:1'-dimethyl-4,4'dipyridilium dichloride) with mineral soils. *J Soil Sci.* **18**, 233.

Kohen R and Chevion M (1985). Paraquat toxicity is enhanced by iron and reduced by desferrioxamine in laboratory mice. *Biochemical. Pharmacol.* **34**, 1841-1843.

Kojima S, Miyazaki Y, Honda T, Kiyozumi M, Shimada H and Funakoshi T (1992). Effect of L-cystine on toxicity of paraquat in mice. *Toxicol. Lett.* **60**, 75-82.

Koller W C (1986). Paraquat and Parkinsons disease. *Neurology* **36**, 1147.

Koller W, Vetere-Overfield B, Gray C, Alexander C, Chin T, Dolezal J, Hassanein R and Tanner C (1990). Environmental risk factors in Parkinson's disease. *Neurology* **40**, 1218-1221.

Kopazyk-Locke K (1977). In vitro and in vivo effects of paraquat on rat liver mitochondria. In *Biochemical mechanisms of paraquat toxicity*. Ed. Autor, A.P, Academic Press, pp 93-115, Academic Press.

Köppel C, Wissmann CV, Barckow D, Rossaint R, Falke K, Stoltenburg-Didinger G and Schnoy N (1994). Inhaled nitric oxide in advanced paraquat intoxication. *J Toxicol. Clin. Toxicol.* **32**, 205-214.

Kornbrust D J and Mavis R D (1980). The effect of paraquat on microsomal lipid peroxidation in vitro and in vivo. *Toxicol. Appl. Pharmacol.* **53**, 323-332.

Krall J, Bagley A C, Mullenbach G T, Hallewell R A and Lynch R E (1988). Superoxide mediates the toxicity of paraquat for cultured mammalian cells. *J Biol. Chem.* **263**, 1910-1914.

Kuo T L and Nanikawa R (1990). Effect of ethanol on acute paraquat toxicity in rabbits. *Jpn. J Legal Med.* **44**, 12-17.

Laithwaitte J A (1975). Paraquat poisoning treated with immunosuppressants and potassium aminobenzoate. *Br. Med. J* **1**, 266-267.

Lambert C E and Bondy S C (1989). Effects of MPTP, MPP^+ and paraquat on mitochondrial potential and oxidative stress. *Life Sci.* **44**, 1277-1284.

Landrigan P J, Powell K E, James L E and Taylor P R (1983). Paraquat and marijuana - epidemiologic risk assessment. *Am. J Public Health* **73**, 784-788.

Langston J W, Ballard P, Tetrud J W and Irwin I (1983). Chronic Parkinsonism in humans due to a product of mereperidine analog. *Science* **219**, 979-980.

Larsson B, Oskarsson J A and Tjalve H (1977). Binding of paraquat and diquat in melanin. *Exp. Eye Res.* **25**, 353-59.

Larsson B, Oskarsson A and Tjalve H (1978). On the binding of the bisquaternary ammonium compound paraquat to melanin and cartilage in vivo. *Biochem. Pharmacol.* **27**, 1721-4.

Lautenschläger J, Grabensee B and Poettgen W (1974). Paraquat poisoning and isolated aplastic anaemia. *Dtsch. Med. Wschr.* **99**, 2348-2351.

Lazo J S, Kondo Y, Dellapiazza D, Michalska A E, Choo K H A and Pitt B R (1995). Enhanced sensitivity to oxidative stress in cultured embryonic cells from transgenic mice deficient in metallothionein I and II genes. *J Biol. Chem.* **270**, 5506-5510.

Lee K Y and Lee T H (1995). Effect of hemoperfusion on treatment for paraquat intoxication. *Blood Purification* **13,** 60-61.

Lee S H, Lee K S, Ahn J M, Kim S H and Hong S Y (1995). Paraquat poisoning of the lung: thin-section CT findings. *Radiology* **195**, 271-274.

Levin P J, Klaff L J, Rose A G and Ferguson A D (1979). Pulmonary effects of contact exposure to paraquat: a clinical and experimental study. *Thorax.*, **34**, 150-160.

Levitt T (1979). Determinations of paraquat in clinical practice using radioimmunoassay. *Proc. Analyt. Div. Chem. Soc.* 72-76.

Lewin R (1984). Trails of ironies to Parkinson's disease. *Science* **224**, 1083-1085.

Lewis C P L (1989). The pulmonary uptake and metabolism of cystamine. PhD thesis, University of London.

Lewis C P L, Haschek W M, Wyatt I, Cohen G M and Smith L L (1989). The accumulation of cystamine and its metabolism to taurine in rat lung slices. *Biochem. Pharmacol.* **38**, 481-488.

Lheureux P, Leduc D, Vanbinst R and Askenasi R (1995). Survival in a case of massive paraquat ingestion. *Chest* **107**, 285-289.

Licker M, Schweizer A, Hohn L, Morel D R and Spiliopoulos A (1998). Single lung transplantation from adult respiratory distress syndrome after paraquat poisoning. *Thorax* **53**, 620-6211.

Liddle J A, Needham L L, Rollen Z J, Roark B R and Bayse D D (1980). Characterization of the contamination of marijuana with paraquat. *Bull. Environ. Contam. Toxicol.* **24**, 49-53.

Lin J L, Liu L and Leu M L (1995). Recovery of respiratory function in survivors with paraquat intoxication. *Arch. Environ. Health* **50**, 432-439.

Lin, J. L., Wei, M.C., and Liu, Y. C. (1996) Pulse therapy with cyclophosphamide and methylprednisolone in patients with moderate to severe paraquat poisoning: A preliminary report. *Thorax* **51,** 661-663.

Lin, J.L., Leu, M.L., Liu, Y.C., and Chen, G.H. (1999) A prospective clinical trial of pulse therapy with glucocorticoid and cyclophosphamide in moderate to severe paraquat-poisoned patients. *Am. J Resp. Crit. Care Med.* **159,** 357-360.

Lindquist N G, Larsson B S and Lydensokolowski A (1988). Autoradiography of C-14 paraquat or C-14 Diquat in frogs and mice - accumulation in neuromelanin. *Neurosci. Letters* **93**, 1-6.

Liou H-H, Chen R-C, Tsai Y-F, Chen W-P, Chang Y-C and Tsai M-C (1996). Effects of paraquat on the substantia nigra of the Wistar rats: neurochemical, histological and behavioural studies. *Toxicol. Appl. Pharmacol.* **137**, 34-41.

Liou H H, Tsai M C, Chen C J, Jeng J S, Chang Y C, Chen S Y and Chen R C (1997). Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. *Neurology* **48**, 1583-1588.

Litchfield M H, Daniel J W, Longshaw S (1973). The tissue distribution of the bipyridylium herbicides, diquat and paraquat in rats and mice. *Toxicology* **1**, 155-165.

Liu D, Yang J, Li L and McAdoo D J (1995). Paraquat - a superoxide generator - kills neurons in the rat spinal cord. *Free Rad. Biol. Med.* **18**, 861-867.

Lock E A(1979). The effect of paraquat and diquat on renal function in the rat. *Toxicol. Appl. Pharmacol.* **48**, 327-336.

Lock E A, Smith L L and Rose M S (1976). Inhibition of paraquat accumulation in rat lung slices by a component of rat plasma and a variety of drugs and endogenous amines. *Biochem. Pharmacol.* **25**, 1769-1772.

Lock E A and Ishmael J (1979). The acute effects of paraquat and diquat on the rat kidney. *Toxicol. Appl. Pharmacol.* **50**, 67-76.

Mahieu P, Hassoun A, Fautsch G, Lauwerijs R and Tremouroux J (1977). Paraquat poisoning: Survival without pulmonary insufficiency after early bleomycin treatment. *Acta. Pharmac. Tox.* **41**, Supp **11**, 246-248.

Maini R and Winchester J F (1975). Removal of paraquat from blood by haemoperfusion over sorbent materials. *Br. Med. J* **3**, 281-282.

Malcolmson E and Beesley J (1975). Unsuccessful immunosuppressant treatment of paraquat poisoning. *Br. Med. J* **3**, 650-651.

Maling H M, Saul W, Williams M A, Brown E A D and Gillette J R (1978). Reduced body clearance as the major mechanism of the potentiation by B_2- adrenergic agonist of paraquat lethality in rats. *Toxicol. Appl. Pharmacol.* **43**, 57-72.

Malone J D G, Carmody M, Keogh B and O'Dwyer W F (1971). Paraquat poisoning- A review of nineteen cases. *J Irish Med. Ass.* **64**, 59-68.

Markey S P, Castagnoli N, Trevor A J and Kopin I J (1986). (Eds) MPTP: a neurotoxin producing a Parkinson syndrome. Academic Press, New York.

Masek I and Richard R J (1990). Interactions between paraquat, endogenous lung amine antioxidants and isolated mouse Clara cells. *Toxicology* **63**, 315-326.

Matkovics B, Barabas K, Szabo L and Berencsi G (1980). In vivo study of the mechanism of protective effects of ascorbic acid and reduced glutathione in paraquat poisoning. *Gen. Pharmac.* **11**, 455-461.

Matsubara M, Yamagami K, Kitazawa Y, Kawamoto K and Takana T (1996). Paraquat causes S-phase arrest of rat liver and lung cells in vivo. *Arch. Toxicol.* **70**, 514-518.

Matthew H, Logan A, Woodruff M F A, Heard B (1968). Paraquat poisoning-lung transplantation. *Br. Med. J* **3**, 759-763.

McArn G E, Gee S J and Krieger R I (1980). Ascorbic acid potentiated pathologic and toxicologic effects of paraquat (MV) and n-Propylviologen (PV) in rats. *19th Society of Toxicology* **Abstr 14**, p A101.

McCord J M and Da E D (1987). Superoxide dependant production of hydroxyl radical catalysed by iron-EDTA complex. *FEBS Lett,* **86**, 139-142.

McDonah B J and Martin J (1970). Paraquat poisoning in children. *Archs. Dis.Child* **45**, 425-427.

McElligott T F (1972). The dermal toxicity of paraquat: differences due to techniques of application. *Toxicol. Appl. Pharmacol.* **21**, 361-8.

McKean W I (1968). Recovery from paraquat poisoning. *Br. Med. J* **3**, 292.

Mehani S (1972). The toxic effect of paraquat in rabbits and rats. *Ain. Shams. Med. J* **23**, 599-601.

Meredith T J and Vale J A (1987). Treatment of paraquat poisoning in man: methods to prevent absorption. *Human Toxicol* **6**, 49-55.

Meredith T and Vale J A (1995). Treatment of paraquat poisoning: gastrointestinal decontamination. In *Paraquat poisoning: mechanisms, prevention, treatment*, Ed Bismuth C and Hall A H, Marcel Dekker, New York, pp297-314.

Michaelis L and Hill E S (1933). Potentiometric studies on semiquinones. *J. Am. Chem. Soc.* **55**, 1481-1494.

Minakata K, Suzuki O, Saito S and Harada N (1996). Effect of dietary paraquat on a rat mutant unable to synthesis ascorbic acid. *Arch. Toxicol.* **70**, 256-258.

Ming F K, Chun C H and Khoo T K (1980). Paraquat poisoning is not always fatal. *Singapore Medical J* **21**, 703-707.

Ministerio de Salud, Division de Saneamiento Ambiental, Depto. Registro y Control de Sustancias Toxicas (1997). Reporte Oficial Intoxicaciones con Plaguicidas, Costa Rica.

Mirchev N (1977). Acute poisoning with Gramoxone (Paraquat). *Vetr. Bol.* **16**, 99-101.

Modee J, Ivemark B I and Robertson B (1972). Ultrastructure of the alveolar wall in experimental paraquat poisoning. *Acta. Path. Microbiol. Scand.* **80**, 54-60.

Molck A-M and Friis C (1997). The cytotoxic effect of paraquat to isolated renal proximal tubular segments from rabbits. *Toxicology* **122**, 123-132.

Molnar I G and Hayes W J (1971). Distribution and metabolism of paraquat in the rat. *Toxicol. Appl. Pharmacol.* **19**, 405.

Montgomery M R, Furry J, Gee S J and Krieger R I (1982). Ascorbic acid and paraquat: oxygen depletion with concurrent oxygen activation. *Toxicol. Appl. Pharmacol.* **63**, 321-329.

Moody C S and Hassan H M (1982). Mutagenicity of oxygen free radicals. *Proc. Nat. Acad. Sci.* **79**, 2855-9.

Mullick F G, Ishak K G, Mahabir R and Stomeyer W F (1981). Hepatic injury associated with paraquat toxicity in humans. *Liver* **1**, 209-221.

Murray R E and Gibson J E (1971). Lethality and pharmacokinetics of paraquat in rats. *Toxicol. Appl. Pharmacol.* **19**, Abstr 115.

Murray R E and Gibson J E (1972). A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. *Exp. Mol Pathol.* **17,** 317-25.

Murray R E and Gibson J E (1974). Paraquat disposition in rats, guinea-pigs and monkeys. *Toxicol. Appl. Pharmacol.* **27**, 283-91.

Musson F A and Porter C A (1982). Effect of ingestion of paraquat on a 20-week gestation fetus. *Postgrad. Med. J* **58**, 731-732.

Nagao M, Takatori T, Wu B, Terazawa K, Gotouda H and Akabane H (1989). Immunotherapy for the treatment of paraquat poisoning. *Human Toxicol.* **8**, 121-123.

Nagao M, Takatori T, Inoue K, Shimizu M, Terazawa K and Akabane H (1990). Immunohistochemical localisation and dynamics of paraquat in small intestine, liver and kidney. *Toxicology* **63**, 167-182.

Nagao M, Takatori T, Wu B, Terazawa K, Getouda H and Akabane H (1991). Immunohistochemical localisation of paraquat in lung and brain. *Med. Sci .Law* **31**, 61-65.

Nagao, M., Zhang, W.D., Itakura, Y., Kobayashi, M., Yamada, Y., Yagi, K., Oono, T., and Takatori T (1993). Immunohistochemical localisation and dynamics of paraquat in the stomach and esophagus of rats. *J Legal Med.* **106**, 142-144.

Nagata T, Kono I, Masaoka T and Akahori F (1992a). Acute toxicological studies on paraquat pathological findings in beagle dogs following single subcutaneous injections. *Vet. Human Toxicol.* **34**, 105-112.

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

Nagi A H (1969). Paraquat and adrenal cortical necrosis. *Br. Med. J* **2**, 669.

Naito H and Yamashita M (1987). Epidemiology of paraquat in Japan and a new safe formulation of paraquat. *Human Toxicol.* **6**, 87-8.

Naylor J, Widdowson P S, Simpson M G, Ellis M K and Lock E A (1995). Studies of systemically administered paraquat on brain penetration and neurotoxicity. *Human Exp. Toxicol.* **14**, 370.

Nemery B, Smith L L and Aldridge W N (1987). Putrescine and 5-hydroxytryptamine accumulation in rat lung slices: cellular localisation and responses to cell-specific lung injury. *Toxicol. Appl. Pharmacol.* **91**, 107-120.

Nemery B, Van Lommel S, Verbeken E K, Lauweryns J .M and Demedts M (1992). Lung injury induced by paraquat, hyperoxia and cobalt chloride: Effects of ambroxol. *Pulmonary Pharmacol.* **5**, 53-60.

Nemeth P, Racz L, Varga J, Lang A, Nemeth A and Nemeth A (1985). Changes of the serum superoxide dismutase content in gramoxone poisoned patients, measured by anti-SOD monoclonal antibody. *Arch. Toxicol.* Suppl **8**, 288.

Newhouse M, McEvoy D and Rosenthal D (1978). Percutaneous paraquat absorption: An association with cultaneous lesions and respiratory failure. *Arch. Dermatol.* **114**, 1516-1519.

Nicotera T M, Block A W, Gibas Z and Sandberg A A (1985). Induction of superoxide dismutase,chromosomal aberrations and sister-chromatid exchanges by paraquat in Chinese hamster fibroblasts. *Mutat. Res.* **151**, 263-8.

Nogue S, Munne P, Campana E, Bertran A, Reig R and Rodamilana M (1989). Failure of the combination cyclophosphamide-dexamethasone in paraquat poisoning. *Medicina Clinica-Spa.* **93**, 61-63.

Nokata M, Tanaka T, Tsuchiya K and Yamashita M (1984). Alleviation of paraquat toxicity by kayexalate and kalimate in rats. *Acta. Pharmacol. Toxicol.* **55**, 158-160.

Ng L L, Naik R B and Polak A (1982). Paraquat ingestion with methaemoglobinemia treated with methylene blue. *Br. Med. J* **284**, 1445-1446.

Ohlson C G and Hogstedt C (1981). Parkinson's disease and occupational exposure to organic solvents, agricultural chemicals and mercury-a case-referent study. *Scand. J Work Environ. Health* **7**, 252-6.

Ogata T and Manabe S (1990). Correlation between lipid peroxidation and morphological manifestation of paraquat-induced lung injury in rats. *Arch. Tox.* **64**, 7-13.

Okonek S, Setyadharma H, Borchent A and Krienke E G (1982). Activated charcoal is as effective as Fullers Earth or bentonite in paraquat poisoning. *Klin. Wochenschr.* **60**, 207-210.

Okonek S, Baldamus C A., Holmann A, Schister C J, Bechstein P B and Zoller B (1979). Two survivors of severe paraquat intoxication by 'continuous haemoperfusion'. *Klin. Wschr.* **57**, 957-959.

Okonek S, Baldamus C A and Hofmann A (1980). Survival despite potentially fatal plasma paraquat concentrations. *The Lancet* **II**, 589.

Okonek S, Weilemann L S, Majdanzic J, Sethyadarma H and Reinecke H J (1982/83). Successful treatment of paraquat poisoning. Activated charcoal per os and 'continuous hemoperfusion'. *J Toxicol. Clin. Toxicol.* **19**, 807-819.

Okonek S, Wronski R, Niedermeyer W, Okonek M and Lamer A (1983). Near fatal percutaneous paraquat poisoning. *Klin. Wochenschr.* **61**, 655.

Okuda M, Saito H, Urakami Y, Takano M and Inui K (1996). cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem.Biophys. Res. Commun.* **224**, 500-507.

Omaye S T, Reddy K A and Cross C E (1978). Enhanced lung toxicity in selenium deficient rats. *Toxicol. Appl. Pharmacol.* **43**, 237-247.

Ongom V I, Owor R and Tomusangi E T (1974). Paraquat (Gramoxone) used as a pediculocide. Uses and Abuses of Drugs and Chemicals in Tropical Africa - East Africa Lit. Bureau, Nairobi. 229-233.

Onyon L J and Volans G N (1987). The epidemiology and prevention of paraquat poisoning. *Hum. Toxicol.* **6**, 19-29.

Oreffo V I C, John R A and Richards R J (1991). Diamine uptake by rat lung type II cells in vitro. *Biochem. Pharmacol.* **41**, 1209-1215.

Oreopoulos D G, Soyannwo M A, Sinniah R, Fenton S S and Bruce J H (1968). Acute renal failure in case of paraquat poisoning. *Br. Med. J* **1**, 749-750.

Osheroff M R, Schaich M K, Drew R T and Borg D C (1985). Failure of desferrioxamine to modify the toxicity of paraquat in rats. *J Free Rad. Biol. Med.* **1**, 71-82.

O'Sullivan M C, Golding B T, Smith L L and Wyatt I (1991). Molecular features necessary for the uptake of diamines and related compounds by the polyamine receptor of rat lung slices. *Biochem. Pharmacol.* **41**, 1839-1848.

Palmeira C M, Moreno A J and Madeira V M C (1995). Mitochondrial bioenergetics is affected by the herbicide paraquat. *Biochim. Biophys. Acta.* **1229**, 187-192.

Papiris S A, Maniati M A, Kyriakidis V and Constantopoulos S H (1995). Pulmonary damage due to paraquat poisoning through skin absorption. *Respiration.* **62**, 101-103.

Park J, Proudfoot A T and Prescott L F (1975). Paraquat poisoning - a clinical review of 31 cases. In: *Clinical aspects of paraquat poisoning*, Proceedings of an International Meeting held on 7th October 1975, published by Imperial Chemical Industries Limited, London.

Parkinson C (1980). The changing pattern of Paraquat poisoning in man.. *Histopathology* **4**, 171-183.

Patterson C E and Rhodes M L (1982). The effect of superoxide dismutase on paraquat mortality in mice and rats. *Toxicol. Appl. Pharmacol.* **62**, 65-72.

Perriens T, Van der Stuyft P, Chee H and Benimadhos S (1989). The epidemiology of paraquat intoxications in Surinam. *Tropical and Geographical Medicine* **41**, 266-69.

Perriens J H, Benimadho S, Kiauw I L, Wisse J and Chee H (1992). High-dose cyclophosphamide and dexamethasone in paraquat poisoning: A prospective study. *Human Exp. Toxicol.* **11**, 129-134.

Perry T L, Yong V W, Wall R A and Jones K (1986). Paraquat and two endogenous analogues of the neurotoxic substance N-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine do not damage dopaminergic nigrostriatal neurons in the mouse. *Neurosci. Lett.* **69**, 285-9.

Peyresblanques M J (1969). Ocular burns caused by Gramoxone. *Bull. Soc. Ophthalmol.* **69**, 928.

Pond S M, Rivory L P, Hampson E C G M and Roberts M S (1993). Kinetics of toxic doses of paraquat and the effects of haemoperfusion in the dog. *J Toxicol. Clin. Toxicol.* **31**, 229-246.

Proudfoot A T (1982). Methaemoglobinaemia due to monolinuron - not paraquat. *Brit. Med. J* **285**, 812.

Proudfoot A T, Stewart M S, Levitt T and Widdop B (1979). Paraquat poisoning: significance of plasma paraquat concentrations. *The Lancet* **II,** 330-332.

Proudfoot A T, Prescott L F, Simpson D, Buckley B M and Vale J A (1984). Radiotherapy for paraquat lung toxicity. *Br. Med. J* **289**, 112.

Purser D A and Rose M S (1979). The toxicity and renal handling of paraquat in cynomologus monkeys. *Toxicology* **15**, 31-41.

Ragoucy-Sengler C and Pileire B (1996a). A biological index to predict patient outcome in paraquat poisoning. *Human Exp. Toxicol.* **15**, 265-268.

Ragoucy-Sengler C and Pileire B (1996b). Survival after paraquat poisoning in a HIV positive patient. *Human Exp. Toxicol.* **15**, 286-288.

Ragoucy-Sengler C, Pileire B and Daijardin J B (1991). Survival from severe paraquat intoxication in heavy drinkers. *The Lancet* **2**, 1461.

Ramasamy S and Nursiah M T A (1988). A survey of pesticide use and associated incidences of poisoning in Peninsular Malaysia. *J Pl. Prot. Tropics* **5**, 1-9.

Reddy K A, Litov R E and Omaye S T (1977). Effect of pretreatment with anti-inflammatory agents on paraquat toxicity in the rat. *Res. Commun. Chem. Path. Pharm.* **17**, 87-100.

Reddy K, Omaye S, Chiu M, Litov R, Hasehawa G and Cross C (1976). Effect of aspirin, indomethacin and hydrocortisone pretreatments on selected aspects of rat lung metabolism before and after paraquat administration. *Am. Rev. Resp. Dis.* **113**, 102.

Redetzki H M, Wood C D and Grafton W D (1980). Vitamin E and paraquat poisoning. *Vet. Human Tox.* **22**, 395-397.

Repine J E, Pfinninger O S, Talmage D W, Berger E M and Pettijohn D E (1981). Dimethyl sulphoxide prevents DNA nicking mediated by ionising radiation or iron/hydrogen peroxide-generated hydroxyl radical. *Proc. Natl. Acad. Sci. USA* **78**, 1001-1003.

Restuccia A, Foglini A, De Alentis-Nannini D (1974). Paraquat toxicity for rabbits. *Vent. Ital.* **25**, 555-565.

Rhodes M L, Zavala D C and Brown D (1976). Hypoxic protection in paraquat poisoning. *Lab. Invest.* **35**, 496-500.

Ribas G, Frenzilli G, Barale R and Marcos R (1995). Herbicide induced DNA damage in human lymphocytes evaluated by the single cell gel electrophoresis SCGE assay. *Mutat. Res.* **344**, 41-54.

Richards R J, Davies N, Atkins J and Oreffo V I C (1987). Isolation, biochemical characterisation and culture of lung type II cells of the rat. *Lung* **165**, 143-158.

Reif R M and Lewinsohn G (1983). Paraquat myocarditis and adrenal cortical necrosis. *J Forensic Sci.* **28**, 505.

Rios A C C, Salvadori D M F, Oliveira S V and Ribeiro L R (1995). The action of the herbicide paraquat on somatic and germ cells of mice. *Mutat. Res.* **328**, 113-118.

Rose M S, Smith L L and Wyatt I (1974a). Evidence for the energy-dependant accumulation of paraquat into rat lung. *Nature* **252**, 314-5.

Rose M S, Crabtree H C, Fletcher K and Wyatt I (1974b). Biochemical effects of diquat and paraquat: disturbance of the control of corticosteroid synthesis in rat adrenal and subsequent effects in the control of liver glycogen utilisation. *Biochem. J* **138**, 437-443.

Rose M S, Lock E A, Smith L L and Wyatt I (1976a). Paraquat accumulation: tissue and species specificity. *Biochem. Pharmacol.* **25**, 419-423.

Rose M S, Smith L L and Wyatt I (1976b). The relevance of pentose phosphate pathway stimulation in rat lung to the mechanism of paraquat toxicity. *Biochem. Pharmacol.* **25,** 1763-1767.

Ross J H and Krieger R I (1981). Structure activity correlation's of amines inhibiting active uptake of paraquat (Methyl Viologen) into rat lung slices. *Toxicol. Appl. Pharmacol.* **59**, 238-249.

Sabapathy N N (1995). Paraquat formulation and safety management. In: Bismuth C, Hall A H (eds). *Paraquat poisoning*. pp335-47, Marcel Dekker Inc, New York.

Saenghirunvattana S, Sermswan A, Piratchvej V, Rochanawutanon M, Kaojaren S and Rattananenya A T (1992). Effect of lung irradiation on mice following paraquat intoxication. *Chest* **101**, 833-835.

Saito M, Thomas C E and Aust S D (1985). Paraquat and ferritin-dependant lipid peroxidation. *J Free Rad. Biol. Med.* **1**, 179-185.

Sakai M, Yamagami K, Kitazawa Y, Takeyama N and Tanaka T (1995). Xanthine oxidase mediates paraquat-induced toxicity on cultured endothelial cell. *Pharmacol. Toxicol.* **77**, 36-40.

Salmona M, Donnini M, Perin L, Diomede L, Romano M, Marini M G and Luisetti M (1992). A novel pharmacological approach for paraquat poisoning in rat and A549 cell line using ambroxol, a lung surfactant synthesis inducer. *Food Chem. Toxicol.* **30**, 789-794.

Samman P D and Johnston E N M (1969). Nail damage associated with handling of paraquat and diquat. *Br. Med. J* **1**, 818-819.

Sandy M S, Moldeus P, Ross D and Smith M T (1986). Role of redox cycling and lipid-peroxidation in bipyridyl herbicide cytotoxicity - studies with a compromised isolated hepatocyte model system. *Biochem. Pharmacol.* **35**, 3095-3101.

Sata T, Takeshige K, Takayanagi R and Minakami S (1983). Lipid peroxidation by bovine heart submitochondrial particles stimulated by 1,1'-dimethyl-4,4-bipyridylium chloride (paraquat). *Biochem. Pharmacol.* **32**, 13-19.

Sato M, Apostolova M D, Hamaya M, Yamki J, Choo K H A, Michalaska A E, Kodama N and Tohyama C (1996). Susceptibility of metallothionein-null mice to paraquat. *Env. Tox. Pharm.* **1**, 221-225.

Satoh M, Naganuma A and Imura N (1992). Effect of pre-induction of metallothionein on paraquat toxicity of mice. *Arch. Toxicol.* **66**, 145-148.

Saunders N A, Rigby P J, Ilett K F and Minchin R F (1988). Autoradiographic localisation of putrescine accumulation by type II pneumocytes of rabbit lung slices. *Lab. Invest.* **59**, 380-386.

Saunders N R, Alpert H M and Cooper J D (1985). Sequential bilateral lung transplantation for paraquat poisoning - A case report. *J Thorac. Cardiovasc. Surg.* **89**, 734-742.

Sawada Y, Yamamoto I, Hirokane T, Nagai Y, Satoh Y and Ueyama M (1988). Severity index of paraquat poisoning. *The Lancet* **1**, 1333.

Scherrmann J M (1995). Analytical procedures and predictive value of late plasma and urine paraquat concentrations. In: Bismuth C, Hall A H (eds). *Paraquat poisoning*. pp285-296 Marcel Dekker Inc, New York.

Scherrmann J M, Galliot M, Garnie R and Bismuth C (1983). Intoxication aigue par le paraquat: Interet prognostique et therapeutique du dosage sanguin. *Toxicol. Eur. Res.* **3**, 141-146.

Scherrmann J M, Houze P, Bismuth C and Bourdon R (1987). Prognostic value of plasma and urine paraquat concentration. *Human Toxicol.* **6**, 91-3.

Schvartsman S, Zyngier S and Schvartsman C (1984). Ascorbic acid and riboflavin in the treatment of acute intoxication by paraquat. *Vet .Human Toxicol.* **26**, 473-475.

Scott M D, Meshnik S R and Eaton J W (1987). Superoxide dismutase-rich bacteria: paradoxical increase in oxidant toxicity. *J. Biol. Chem.* **262**, 3640-3645.

Scott M D and Eaton J W (1996). Superoxide is not the proximate cause of paraquat toxicity. *Redox. Report* **2**, 113-119.

Seidenfeld J J, Wycoff D, Zavala D C and Richerson H B (1978). Paraquat lung injury in rabbits. *Br. J Ind. Med.* **35**, 245-57.

Seidenfeld J J (1985). Steroid pretreatment does not prevent paraquat pneumonitis in rabbits. *Am. J Med. Sci.* **289**, 51-54.

Semchuk K M, Love E J and Lee R G (1991). Parkinson's disease and exposure to rural environmental factors: a population-based case-control study. *Can. J Neurol. Sci.* **18**, 279-286.

Senawayake N, Gurunathan G, Hart T B, Amerasingne P, Babquille M, Ellapola S B, Udupitille M and Basanayake V (1993). An epidemiological study of the health of Sri Lankan tea plantation workers associated with long term exposure to paraquat.*Br. J Ind. Med.* **50**, 257-263.

Shahar E, Barzilay Z and Aladjem M (1980). Paraquat poisoning in a child: Vitamin E in amelioration of lung injury. *Arch. Dis. Child* **55**, 830.

Sharp C W M, Ottolenghi A and Posner H S (1972). Correlation of paraquat toxicity with tissue concentration and weight loss of the rat. *Toxicol. Appl. Pharmacol.* **22**, 241-251.

Shimada H, Hirai K, Simamura E and Pan J (1998). Mitochondrial NADH-quinone oxidoreductase of the outer membrane is responsible for paraquat cytotoxicity in rat livers. *Arch. Biochem. Biophys.* **351**, 75-81.

Singmaster J A and Liu L C (1998). Low paraquat inhalation exposure for applicators spraying properly with knapsacks. *J Agric. Univ. PR.* **82**, 97-107.

Shu H, Talcott R E, Rice S A and Wei E T (1979). Lipid peroxidation and paraquat toxicity. *Biochem. Pharmacol.* **28**, 327-331.

Shum S, Hale T W and Habersang R (1982). Reduction of paraquat toxicity by N-acetyl-1-cysteine. *Vet. Human Tox.* Suppl **24**, 158-160.

Sinow J and Wei E (1973). Ocular toxicity of paraquat. *Bull. Environ. Contam. Tox.* **9**, 163-168.

Sion A, Samuni A and Chevion M (1989). Mechanistic aspects of paraquat toxicity in E. coli. A spin trapping study. *Biochem. Pharmacol.* **38**, 3903-3907.

Smith J G (1988). Paraquat poisoning by skin absorption : a review. *Human. Toxicol.* **7**, 15-19.

Smith L L (1982). The identification of an accumulation system for diamines and polyamines into the lung and its relevance to paraquat toxicity. *Arch. Toxicol.* Suppl **5**, 1-14.

Smith L L (1987). Mechanism of paraquat toxicity in the lung and its relevance to treatment. *Human Toxicol.* **6**, 31-36.

Smith L L and Rose M S (1977). A comparison of the effects of paraquat and diquat on the water content of rat lung and the incorporation of thymidine into lung DNA. *Toxicology* **8**, 223-30.

Smith L L, Wright A, Wyatt I and Rose M S (1974). Effective treatment for paraquat poisoning in rats and its relevance to the treatment of paraquat poisoning in man. *Br. Med. J* **4**, 569-71.

Smith L L, Lock E A and Rose M S (1976). The relationship between 5-hydroxytryptamine and paraquat accumulation into rat lung. *Biochem. Pharmacol.* **25**, 2485-7.

Smith L L, Wyatt I and Rose M S (1978). A comparison of the uptake and elimination of paraquat in rat lung slices with that *in vivo*. In *'Industrial and Environmental Xenobiotics*: In vitro versus *in vivo* biotransformation'. p135-140, Publ Excerpta Medica.

Smith L L and Wyatt I (1981). The accumulation of putrescine into slices of rat lung and brain and its relationship to the accumulation of paraquat. *Biochem. Pharmacol.* **30**, 1053-1058.

Smith L L, Wyatt M S and Rose M S (1981). Factors affecting the efflux of paraquat from rat lung slices. *Toxicology* **19**, 197-207.

Smith L L, Wyatt I and Cohen G M (1982). The accumulation of diamines and polyamines into rat lung slices. *Biochem. Pharmacol.* **31**, 3029-3033.

Smith L L and Watson S C (1987). An assessment of the protective effect of cyclophosphamide and dexamethasone in rats. *Human Toxicol.* **6**, 99.

Smith L L, Lewis C P L, Wyatt I and Cohen G M (1990). The importance of epithelial uptake systems in lung toxicity. *Environ. Health Perspec.* **85**, 25-30.

Smith N B, Mathialagen S and Brooks K E (1993). Simple and sensitive solid-phase extraction of paraquat using cyanopropyl columns. *J Anal. Toxicol.* **17**, 143-145.

Smith P and Heath D (1974a). The ultrastructure and time sequence of the early stages of paraquat lung in rats. *J Path.* **114**, 177-84.

Smith P and Heath D (1974b). Paraquat lung: A reappraisal. *Thorax* **29**, 643-653.

Smith P and Heath D (1975). The pathology of the lung in paraquat poisoning. *J Clin. Path.* **28**, Suppl **9** 81-93.

Smith P and Heath D (1976). Paraquat. *CRC Crit. Rev. Toxicol.* **4**, 411-45.

Smith P, Heath D and Kay J M (1974). The pathogenesis and structure of paraquat-induced pulmonary fibrosis in rats. *J Path.* **114**, 57-67.

Sofuni T, Hatanaka M and Ishidate M (1985). Chromosomal aberrations and superoxide generating systems. II. Effects of paraquat on Chinese hamster cells in culture. *Mutat. Res.* **147**, 273-4.

Sokol P P, Holohan P D, Grass S M and Ross C R (1988). Proton-coupled organic cation transport in renal brush-border membrane vesicles. *Biochem. Biophys. Acta* **940**, 209-218.

Staiff D C, Comer S W, Armstrong J F and Wolfe H R (1975). Exposure to the herbicide paraquat. *Bull. Environ. Contam. Toxicol.* **14**, 334-340.

Steffen C and Konder H (1979). Absorption of paraquat by rat gut in vitro regional differences. *Arch. Tox.* **43**, 99-103.

Steffen C, Muliawan H and Kappus H (1980). Lack of in vivo lipid peroxidation in experimental paraquat poisoning. *Arch. Pharmacol.* **310**, 241-3.

Stephens D S, Walker D H, Schaffner W, Kaplovitz L G, Brashear R, Roberts R and Spickard W A (1981). Pseudodiptheria: prominent pharygeal membrane associated with fatal paraquat ingestion. *Ann. Intern. Med.* **94**, 202-4.

Stevens J T and Sumner D D (1991). Herbicides. In: Hayes W J, Laws E R (eds.), *Handbook of Pesticide Toxicology*, pp 1317-1408, Academic Press, San Diego.

Sullivan T M and Montgomery M R (1983). The relationship between paraquat accumulation and covalent binding in rat lung slices. *Drug Metab. Dispos.* **11**, 526-530.

Sullivan T M and Montgomery M R (1984). Ascorbic acid nutritional status does not affect the biochemical response to paraquat. *Fundam. Appl. Toxicol.* **4**, 754-9.

Summers L A (1980). The bipyridinium herbicides. Academic Press, London.

Suntres Z E and Shek P N (1995). Liposomal alpha-tocopherol alleviates the progression of paraquat-induced lung damage. *Drug Targeting* **2**, 493-500.

Suntres Z E and Shek P N (1996). Alleviation of paraquat induced lung injury by pretreatment with bifunctional liposomes containing alpha tocopherol and glutathione. *Biochem. Pharmacol.* **52**, 1515-1520.

Sutton H C, Vile G F and Winterbourn C C (1987). Radical driven Fenton reactions evidence from paraquat radical studies for production of tetravalent iron in the presence and absence of ethylenediaminetetraacetic acid. *Arch. Biochem. Biophys.*, 462-471.

Suzuki K, Takasu N, Arita S, Maenosono A, Ishimatsu S, Nishina N, Tanaka S and Kohama A (1989). A new method for predicting the outcome and survival period in paraquat poisoning. *Human Toxicol.* **8**, 33-38.

Suzuki K, Takasu N, Okabe T, Ishimatsu S, Ueda A, Tanaka S, Fukuda A, Arita S and Kohama A (1993). Effect of aggressive haemoperfusion on the clinical course of patients with paraquat posioning. *Human Exp. Toxicol.* **12**, 323-7.

Swan A A B (1967). Paraquat poisoning. *Br. Med. J* **4**, 551.

Swan A A B (1969). Exposure of spray operators to paraquat. *Br. J Ind. Med.* **26**, 322-329.

Sykes B I, Purchase I F H and Smith L L (1977). Pulmonary ultrastructure after oral and intravenous dosage of paraquat to rats. *J Path.* **121**, 233-41.

Szabo L, Matkovics B, Barabas K and Oroszlan G (1986). Effects of various thiols on paraquat toxicity. *Comp. Biochem. Physiol.* **Pt C 83**, 149-154.

Tabei K, Asano Y and Hosoda S (1982). Efficacy of charcoal hemoperfusion in paraquat poisoning. *Artif. Organs* **6**, 37-43.

Taki K, Hirahara K, Tomita S and Totoki S (1996). Case report: Case of recovery from paraquat poisoning without pulmonary fibrosis. *Therap. Res.* **16**, 521-529.

Talbot A R and Barnes M R (1988). Radiotherapy for the treatment of pulmonary complications of paraquat poisoning. *Human Toxicol.* **7**, 325-332.

Talbot A R, Barnes M R and Ting R S (1988a). Early radiotherapy in the treatment of paraquat poisoning. *Br. J Radiol.* **61**, 405.

Talbot A R, Fu C C and Hsieh M F (1988b). Paraquat intoxication during pregnancy: a report of 9 cases. *Vet. Human Toxicol.* **30**, 12-17.

Tanaka R and Amano Y (1989). Genotoxic effects of paraquat and diquat evaluated by sister chromatid exchange, chromosomal aberration and cell-cycle rate. *Toxicol. In Vitro* **3**, 53-57.

Tanner C M, Chen B, Wang W, Peng M, Liu Z, Liang X, Kao L C, Gilley D W, Goetz C G and Schoenberg B S (1989). Environmental factors and Parkinson's disease: a case-control study in China. *Neurology* **39**, 660-604.

Tanner C M, Grabler P and Goetz C G (1990). Occupation and the risk of Parkinson's disease (PD): a case-control study in young-onset patients. *Neurology* 40 Suppl **1**, 422.

Thakar J H and Hassan M N (1988). Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), cyperquat (MPP+) and paraquat on isolated mitochondria from rat striatum, cortex and liver. *Life Sci.* **43**, 143-150.

Thomas P D, Thomas D, Chan Y-L and Clarkson A R (1977). Paraquat poisoning is not necessarily fatal. *Med. J Aust.* **2**, 564-565.

Thornalley P J and Vasak M (1985). Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim. Biophys. Acta.* **827**, 36-44.

Tipton K F and Singer T P (1993). Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds. *J Neurochem.* **61**, 1191-1205.

Tomita M (1991). Comparison of one-electron reduction activity against the bipyridylium herbicides, paraquat and diquat, in microsomal and mitochondrial fractions of liver, lung and kidney (in vitro). *Biochem. Pharmacol.* **42**, 303-309.

Tomita M (1996). Studies on paraquat toxicity on deoxyribonucleic acid of cultured mammalian cells using flow cytometry. *Redox Report* **2**, 19-24.

Toner P G, Vetters J M, Spilg W G S and Harland W A (1970). Fine structure of the lung lesion in a case of paraquat poisoning. *J Path.* **102**, 182-185.

Trush M A, Mimnaugh E G, Ginsburg E and Gram T E (1981). *In vitro* stimulation by paraquat of reactive oxygen-mediated lipid peroxidation in rat lung microsomes. *Toxicol. Appl. Pharmacol.* **60**, 279-286.

Tsatsakis A M, Perakis K and Koumantakis E (1996). Experience with acute paraquat poisoning in Crete. *Vet. Hum. Toxicol.* **38**, 113-117.

Tsuchiya T, Yoshida T, Imaeda Y A, Khio T and Ukai S (1995). Detoxification of paraquat poisoning: Effects of alkylsulfates and alkylsulfonates on paraquat poisoning in mice and rats. *Biol. Pharm. Bulletin* **18**, 523-528.

Tungsanga K, Israsena S, Chusilp S and Sitprija V (1983). Paraquat poisoning: evidence of systemic toxicity after dermal exposure. *Postgrad. Med. J.*, **59**: 338.

Turner C E, Elsohly M A, Cheng F P and Torres L M (1978). Marijuana and paraquat *JAMA* **240**, 1857.

Ukai S, Nagai K, Kiho T, Tsuchiya T and Nochida Y (1987). Effectiveness of dextran sulfate on acute toxicity of paraquat in mice and rats. *J Pharmacobio. Dyn.* **10**, 682-4.

Vale J A, Crome P, Volans G N, Widdop B and Goulding R (1977). The treatment of paraquat poisoning using oral sorbents and charcoal haemoperfusion. *Acta. Pharmac. Tox.* **41** Suppl 11, 109-117.

Vale J A, Meredith T J and Buckley B M (1987). Paraquat poisoning: clinical features and immediate general management. *Human Toxicol.* **6**, 41-7.

Van Asbeck B S, Hillen F C, Boonen H C M, De Jong Y, Dormans J A M A, Marx J J M and Sangster B (1989). Continuous intravenous infusion of deferoxamine reduces mortality by paraquat in vitamin E-deficient rats. *Am. Rev. Respir. Dis.* **139**, 769-773.

Van der Wal N A, Van Oirschot J F L M, Van Dijk A, Verhoef J and van Asbeck B S (1990). Mechanism of protection of alveolar type II cells against paraquat-induced cytotoxicity by deferoxamine. *Biochem. Pharmacol.* **39**, 1665-1671.

Van der Wal N A, Smith L L, van Oirschot J F and van Asbeck B S (1992). Effect of iron chelators on paraquat toxicity in rats and alveolar type II cells. *Am. Rev. Resp. Dis.* **145**, 180-186.

Van den Bogaerde J, Schelstraete J, Colardyn F and Heyndrickx H (1984). Paraquat poisoning. *Forensic Sci. Int.* **26**, 103-114.

Van Dijck A, Maes R A A, Drost R H, Douze J M C and Van Heyst A N P (1975). Paraquat poisoning in man. *Arch. Toxicol.* **34**, 129-136.

Van Osten G K and Gibson J E (1975). Effect of paraquat on the biosynthesis of deoxyribonucleic acid, ribonucleic acid and protein in the rat. *Fd. Cosmet. Toxicol.* **13**, 47-54.

Van Wendel de Joode B N, De Graaf I A M, Wesseling C and Kromhout H (1996). Paraquat exposure of knapsack spray operators on banana plantations in Costa Rica. *Int. J Occup. Environ. Health* **2**, 294-304.

Vargas E and Sabapathy N N (1995). An epidemiology study on fatalities from ingestion and occupationally-related injuries of agricultural workers in Costa Rica. Report Series TMF4620B, Zeneca Agrochemicals, Fernhurst, Haslemere, UK.

Vaziri N D, Ness R L, Fairshter R D, Smith W R and Rosen S M (1979). Nephrotoxicity of paraquat in man. *Arch. Intern. Med.* **139**, 172-174.

Vieregge P, Kömpf D and Fassl H (1988). Environmental toxins in Parkinson's Disease.

The Lancet **1**, 362-3.

Vijeyaratnam G S and Corrin B (1971). Experimental paraquat poisoning: a histological and electron-optical study of the changes in the lung. *J Path.* **103**, 123-9.

Vlachos P and Kontoes P (1987). A study of 30 cases of paraquat inhalation. *Vet. Hum. Toxicol.* **29** (Suppl 2), 147.

Waddell W J and Marlowe C (1980). Tissue and cellular disposition of paraquat in mice. *Toxicol. Appl. Pharmacol.* **56**, 127-140.

Waight J J (1979). Fatal percutaneous paraquat poisoning. *JAMA.*, **242**, 472.

Walker M, Dugard P H and Scott R C (1983). Absorption through human and laboratory animal skins: in vitro comparison. *Acta. Pharm. Sci.* **20**, 52-53.

Wasan S M and McElligott T F (1972). An electron microscopic study of experimentally induced interstitial pulmonary fibrosis. *Am. Rev. Resp. Dis.* **105**, 276-82.

Wasserman B and Block E R (1978). Prevention of acute paraquat toxicity in rats by superoxide dismutase. *Aviat. Space Environ. Med.* **49**, 805-9.

Watanabe T, Sakai K, Toyama K, Ueno M and Watanabe M (1979). On three cases of ocular disturbance due to Gramoxone, a herbicide containing 24% paraquat dichloride. *Ganka. Rinsho. Iho.* **73**, 1244-1246.

Webb D B (1983). Nephrotoxicity of paraquat in the sheep and the associated reduction in paraquat secretion. *Toxicol. Appl. Pharmacol.* **68**, 282-289.

Webb D B, Williams M V, Davies B H and James K W (1984). Resolution after radiotherapy of severe pulmonary damage due to paraquat poisoning. *Br. Med. J* **288**, 1259-1260.

Wegener T, Sandhage B, Chan K W and Saldeen T (1988). N-acetylcysteine in paraquat toxicity - toxicological and histological evaluation in rats. *Upsala J Med. Sci.* **93**, 81-89.

Weidel H and Russo M (1882). Studien uber das pyridin. *Monatsh. Chem.* **3**, 850-885.

Weinbaum Z, Samuels S J and Schenker M B (1995). Risk factors for occupational illnesses associated with the use of paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride) in California. *Arch. Environ. Health* **50**, 341-348.

Wesseling C, Castillo L and Elinder C G (1993). Pesticide poisonings in Costa Rica. *Scand. J Work Environ. Health* **19**, 227-35.

Wesseling C, Hogstedt C, Picado A and Johansson L (1997). Unintentional fatal paraquat poisonings among agricultural workers in Costa Rica: Report of 15 cases. *Am. J Ind. Med.* **32**, 433-41.

Wester R C and Maibach H I (1985). In vivo percutaneous absorption and decontamination of pesticides in humans. *J Toxicol. Environ. Hlth.* **16**, 25-37.

Wester R C, Maibach H I, Bucks D A and Aufrere M B (1984). In vivo percutaneous absorption of paraquat from hand leg and forearm of humans. *J Toxicol. Environ. Hlth.* **14**, 759-762.

Whitaker M (1989a). The handling and use of paraquat by Malaysian rubber and oil palm smallholders. *J Pl. Prot. Tropics* **6**, 231-249.

Whitaker M (1989b). Normas de manipulacion y uso del paraquat por los pequenos productores de maiz en Centroamerica. *Turrialba* **39**, 260-274.

Whitaker M, Pitakpaivan C and Daorai A (1993). The use of paraquat by smallholder maize, cassava, fruit and rubber farmers in Thailand. *Thai. J Agric. Sci.* **26**, 43-81.

Widdop B (1976). Detection of paraquat in urine. *Br. Med. J* **2**, 1135.

Widdop B, Medd R K, Braithwaite R A and Vale J A (1975). Haemoperfusion in the treatment of paraquat poisoning. *Proc. Europ. Soc. Artific. Organs* **2**, 244-247.

Widdop B, Medd R K and Braithwaite R A (1977). Charcoal haemoperfusion in the treatment of paraquat poisoning. *Proc. Europ. Soc .Toxicol.* **18**, 156-159.

Widdowson P S, Farnworth M J, Simpson M G and Lock E A (1996a). Influence of age on the passage of paraquat through the blood-brain barrier in rats: a distribution and pathological examination. *Human Exp Toxicol* **15**, 231-336.

Widdowson P S, Farnworth M J, Upton R and Simpson M G (1996b). No changes in behaviour, nigro-stratial system neurochemistry or neuronal cell death following toxic multiple oral paraquat administration to rats. *Human Exp Toxicol* **15**, 583-591.

Williams M V and Webb D B (1987). Paraquat lung : is there a role for radiotherapy. *Human Toxicol.* **6**, 75-81.

Winterbourn C C and Sutton H C (1984). Hydroxyl radical production from hydrogen peroxide and enzymatically generated paraquat radicals catalytic requirements and oxygen dependence. *Arch. Biochem. Biophys.* **235**, 116-126.

Witschi H, Kacew S, Hirai K I and Cote M G (1977). In vivo oxidation of reduced nicotinamide adenine dinucleotide phosphate by paraquat and diquat in rat lung. *Chem. Biol. Interact.* **19**, 143-160.

Wohlfahrt D J (1982). Fatal paraquat poisonings after skin absorption. *Med. J. Aust.* **1**, 512-513.

Wojeck G A, Price J F, Nigg H N and Stamper J H (1983). Worker exposure to paraquat and diquat. *Arch. Environ. Contam. Toxicol.* **12**, 65-70.

Woollen B H and Mahler J D (1987). An improved spot-test for the detection of paraquat and diquat in biological samples. *Clin. Chim. Acta.* **167**, 225-229.

Wright C E, Tallan H H, Lin Y Y and Gaull G E (1986). Taurine: biological update. *Ann. Rev. Biochem.* **55**, 427-453.

Wright A F, Green T P, Robson R T, Niewola Z, Wyatt I and Smith L L (1987a). Specific polyclonal and monoclonal antibody prevents paraquat accumulation into rat lung slices. *Biochem. Pharmacol.* **36**, 1325-1331.

Wright A F, Green T P, Daley-Yates P and Smith L L (1987b). Monoclonal-antibody does not protect mice from paraquat toxicity. Vet. Human. Toxicol. **29(Suppl 2)**, 102.

Wright S H and Wunz T M (1995). Paraquat 2+/H+ exchange in isolated renal brush-border membrane vesicles. *Biochem. Biophys. Acta.* **1240**, 18-24.

Wyatt I, Doss A W, Zavala D C and Smith L L (1981). Intrabronchial instillation of paraquat in rats: lung morphology and retention study. *Br. J Ind. Med.* **38**, 42-48.

Wyatt I, Soames A R, Clay M F and Smith L L (1988). The accumulation and localisation of putrescine, spermidine, spermine and paraquat in the rat lung. *Biochem. Pharmacol.* **37**, 1909-1918.

Yamada K and Fukushima T (1993). Mechanism of cytotoxicity of paraquat. II. Organ specificity of paraquat-stimulated lipid peroxidation in the inner membrane of mitochondria. *Exp. Toxic. Pathol.* **45**, 375-380.

Yamagami K, Matsubara M, Kitazawa Y, Takeyama N, Tanaka T and Kawamoto K (1994). Flow cytometric analysis of the direct toxic effects of paraquat on cultured MDCK cells. *J Appl. Toxicol.* **14**, 155-159.

Yamaguchi H, Sato S, Watanabe S and Naito H (1990). Pre-embarkment prognostication for acute paraquat poisoning. *Human Exp. Toxicol.* **9**, 381-384.

Yamamoto H (1993). Protection against paraquat induced toxicity with sulfite or thiosulfate in mice. *Toxicology* **79**, 37-43.

Yamamoto T, Anno M and Sato T (1987). Effects of paraquat on mitochondria of rat skeletal muscle. *Comp. Biochem. Physiol.* **86**, 375-8.

Yamashita M, Naito H and Takagi S (1987). The effectiveness of a cation resin (kayexalate) as an adsorbent of paraquat: experimental and clinical studies. *Human Toxicol* **6**, 89-90.

Yasaka T, Ohya I, Matsumoto J, Shiramizu T and Sasaguri J (1981). Acceleration of lipid peroxidation in human paraquat poisoning. *Arch. Intern. Med.* **141**, 1169-1171.

Yasaka T, Okudaira K, Fujito H and Shiramitsu T (1986). Further studies of lipid-peroxidation in human paraquat poisoning. *Arch. Intern. Med.* **146**, 681-685.

Yonemitsu K (1986). Pharmacokinetic profile of paraquat following intravenous administration to the rabbit. *Forensic Sci. Int.* **32**, 33-42.

Yonei S, Noda A, Tachibana A and Akasaka S (1986). Mutagenic and cytotoxic effects of oxygen free radicals generated by methyl viologen (paraquat) on Escherichia Coli with different DNA-repair capacities. *Mutat. Res.* **163**, 15-22.

Yoshimura Y, Watanabe Y and Shibuya Y (1993). Inhibitory effects of calcium channel antagonists on motor dysfunction induced by intracerebroventricular administration of paraquat. *Pharmacol. Toxicol.* **72**, 229-235.

Younes M, Cornelius S and Seigers C P (1985). Iron-supported in vivo lipid peroxidation induced by compounds undergoing redox cycling. *Chem-Biol. Interact.* **54**, 97-103.

Yu H Y, Lai Y R, Kuo T L and Shen Y Z (1994). Effects of ethanol on pharmacokinetics and intestinal absorption of paraquat in animals. *J Toxicol. Sci.* **19**, 67-75.

Zavala D C and Rhodes M L (1978). An effect of paraquat on the lungs of rabbits: Its implications in smoking contaminated marijuana. *Chest* **74**, 418-420.

Zayed J, Ducic S, Campanella G, Panisset J C, Andre P, Masson H and Roy M (1990). Facteurs environnementeaux dans l'etiologie de la maladie de Parkinson. *Can. J Neurol. Sci.* **17**, 286-291.

Zilker T, Fogt F and von Clarmann M (1988). Kein Parkinsonsyndrom nach akuter paraquat intoxikation. *Klin. Wochenschr.* **66**, 1138-1141.

TABLE 1 - ACUTE TOXICITY OF PARAQUAT TO THE RAT

Data expressed as mg paraquat ion/kg.

Paraquat dichloride	Sex	Strain	Route of administration	Median lethal dose (time studied)	Reference
Pure salts	F	NS[a]	po	112 (104-122), 150 (139-162), 141[b] (140-142) (14 days)	Clark *et al.*, 1966
Pure salt	F	NS	po	150 (110-173) (21 days)	Mehani, 1972
Formulation	M	Sprague Dawley	po	143 (123-166) (7 days)	Murray & Gibson, 1971
Formulation	M	Sherman	po	100 (87-117) (15 days)	Kimbrough & Gaines, 1970
Formulation	F	Sherman	po	110 (90-134) (15 days)	Kimbrough & Gaines, 1970
Formulation	M	Sprague Dawley	po	115 (90-150) (30 days)	Sharp *et al.*, 1972
Formulation	M	Wistar	po	95 (79-114) (30 days)	Sharp *et al.*, 1972
Pure salt	F	NS	ip	19 (16-21) 16[b] (14-19)	Clark *et al.*, 1966
Pure salt	F	NS	ip	16 (10-26) (21 days)	Mehani, 1972
Formulation	M	Sprague Dawley	iv	21 (19-25)	Sharp *et al.*, 1972

a , Not stated
b, Dimethosulphate salt

Acute toTABLE 2 - ACUTE TOXICITY OF PARAQUAT TO LABORATORY ANIMALS

Data expressed as mg paraquat ion/kg

Species	Sex	Route of administration	Median lethal dose	Reference
Mouse	F[a]	ip	30[c],	Ecker *et al.*, 1976b;
	F		30[c](26.5-35.1)	Bus *et al.*, 1976a
Mouse	F	po	196[c]	Bus *et al.*, 1975b
Guinea pig	F	ip	3	Clark *et al.*, 1966
Guinea pig	M[b]	po	30 (22-41)	Clark *et al.*, 1966
Guinea pig	M & F	po	22[c] (15-33)	Murray and Gibson, 1972
Cat	F	po	35 (27-46)	Clark *et al.*, 1966
Monkey (*Macaca fascicularis*)	M & F	po	50[c]	Murray and Gibson, 1972
Rabbit	M	po	100[c]	Kuo & Nanikawa, 1990
Rabbit	M	po	50 (45-58)	Mehani, 1972
Rabbit	M	ip	25 (15-30)	Mehani, 1972
Rabbit	M	ip	18 (11-31)	McElligott, 1972
Dog	M	sc	1.8[c] (1-6.1)	Nagata *et al.*, 1992a
Dog	F	sc	3.5[c] (2.4- 10.1)	Nagata *et al.*, 1992a

a Female
b Male
c Paper refers to paraquat, not clear if salt or ion.

TABLE 3 - ACUTE TOXICITY OF PARAQUAT TO LABORATORY ANIMALS FOLLOWING DERMAL APPLICATION OR INHALATION EXPORURE

Data expressed as mg paraquat ion/kg

Species	Sex	Route of administration	Median lethal dose	Reference
Rat	M	Dermal	80 (60-96)	Kimbrough and Gaines, 1970
Rat	F	Dermal	90 (74-110)	Kimbrough and Gaines, 1970
Rabbit	M	Dermal	236 (collars removed) >480 (with collars)	Clark *et al.*, 1966; McElligott, 1972
Rat	M & F	Inhalation	6µg/L. hr.	Gage, 1968a

TABLE 4 - KINETIC CONSTANTS FOR THE ACCUMULATION OF PARAQUAT AND PUTRESCINE BY RAT AND HUMAN LUNG SLICES OR ISOLATED ALVEOLAR TYPE II CELLS

Species/ Tissue	Paraquat accumulation		Putrescine accumulation		Reference
	Km (μM)	Vmax[a]	Km (μM)	Vmax[b]	
Rat-lung slice	70	300			Rose *et al.*, 1974a
	210	710			Ross & Krieger, 1981
	119	636	8	480	Karl & Friedman, 1983
			7	330	Smith & Wyatt, 1981
			31	870	Nemery *et al.*, 1987
			12-18		O'Sullivan *et al.*, 1991
			13.5	720	Hardwick *et al.*, 1990
			13.1	723	Smith *et al.*, 1982
Human-lung slice	40	300			Rose *et al.*, 1974a
	244	370	7	376	Hoet *et al.*, 1994
			2-11	99-249	Brooke Taylor *et al.*, 1983
			7	414	Hoet *et al.*, 1993
Cultured rat-type II cells			5	18	Lewis, 1989
			8-14	58	Richards *et al.*, 1987
	29				Van der Wal *et al.*, 1990
	64		15	128	Oreffo *et al.*, 1991
Suspensions of rat-type II cells	88	29	2.5	34	Chen *et al.*, 1992
Cultured human-type II cells			6-8	12-14	Hoet *et al.*, 1994

a Vmax in lung slices expressed as nmol/h/g wet weight of slice
b Vmax Alveolar type II cells expressed as pmol/h/μM DNA

TABLE 5 - WORKER EXPOSURE AND ABSORPTION OF PARAQUAT

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

ND Not detected
a Exposure to uncovered skin
b Total Dermal exposure
+ Aerial - flagger
# Aerial - pilot
$ mg/g paraquat sprayed
& extrapolation from indirect measurement using copper as marker

TABLE 6 - SUMMARY OF CASE REPORTS WITH DOSES ABOVE 55 mg/kg TAKEN BY SURVIVORS OF PARAQUAT POISONING BY INGESTION

Calculated ingested dose (mg/kg)[1]	Dose stated[2]	Number of cases	Body weight (kg)[3]	Age Range (years)	References
55 - 75	15 - 20 ml (9 cases) 1 - 2 mouthful (2 cases) 1 sachet (2 cases)	13	25 - 70	3 - 75	Addo *et al.*, 1984 Iff *et al.*, 1971 Lloyd 1969 (cited in Cavalli, 1977) Mahieu *et al.*, 1977 Ming *et al.*, 1980 Mirchev, 1977 Taki *et al.*, 1996 Talbot *et al.*, 1988
76 - 100	10 - 40 ml (14 cases) 1 - 2 mouthful (4 cases)	18	25 - 70	3 - 65	Addo *et al.*, 1984 Douze *et al.*, 1977 Malone *et al.*, 1971 McKean, 1968 Ragoucy-Sengler *et al.*, 1991 Shahar *et al.*, 1980 Tabei, 1982 Taki *et al.*, 1996 Thomas *et al.*, 1977 Tsatsakis *et al.*, 1996
101 - 200	40 - >50 ml (7 cases) 3 - 4 mouthful (2 cases)	9	70	17 - 59	Addo *et al.*, 1984 Douze *et al.*, 1974, 1977 Florkowski *et al.*, 1992 Grundies *et al.*, 1971 Lheureux *et al.*, 1995 Okonek *et al.*, 1980
> 201	50 - 150 ml (8 cases) 3 - 4 mouthful (2 cases) 1 glass or cup (2 cases)	12	40 - 70	10 - 50	Addo *et al.*, 1984 Douze *et al.*, 1974, 1977 Malone *et al.*, 1971 Okonek *et al.*, 1979, 1980 Tabei *et al.*, 1982 Tsatsakis *et al.*, 1996

[1]All doses expressed as paraquat ion

[2]Volumes (ml) refer to the 20% liquid concentrate. A volume of 17.5 ml has been used for 'a mouthful'. 'Sachet' refers to a granular formulation containing 2.5% paraquat and 2.5 g diquat

[3]Where the body weight was not explicitly stated, the following assumptions were used:

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

TABLE 7 - PREDICTIVE PLASMA PARAQUAT CONCENTRATIONS BEYOND 24 HOURS SEPARATING SURVIVING AND NON-SURVIVING PATIENTS (FROM SCHERRMANN, 1995)

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

Legends to Figures

Figure 1
Plasma levels of paraquat in the rat and dog following a single non-toxic oral dose.

The dog was given a total dose of 1.03mg of paraquat, while the rats were dosed at 0.038mg/kg. Data adapted from Davies *et al.*, (1987) and Chui *et al.*, (1988).

Figure 2
Plasma levels of paraquat in the rat and monkey following a single toxic oral dose.

The rats were given 126mg/kg paraquat while the monkeys received 50mg/kg. Data adapted from Murray and Gibson, (1974).

Figure 3
Mechanism of paraquat transport across renal tubular cells.

A schematic representation of the proposed transport systems for paraquat across renal tubular cells. The transporters are OCT 1 at the basolateral membrane and P-glycoprotein and the cation/H^+ exchange system at the brush border membrane. Adapted from Chan *et al.*, (1998). Reproduced with permission from *Pharmacol. Ther.*

Figure 4
Increased susceptibility of mice lacking Cu/Zn superoxide dismutase to paraquat

The survival times of age-matched, male *Sod1*$^{+/+}$, *Sod1*$^{+/-}$ *and* *Sod1*$^{-/-}$ mice was determined following ip administration of paraquat at 10mg/kg . From Ho *et al.*, (1998). Reproduced with permission from *Environmental Health Perspectives.*

Figure 5
Mechanism of toxicity of paraquat

A schematic representation of the mechanism of toxicity of paraquat. 1 = structure of paraquat and putrescine showing the geometric standards of the distance between the n atoms; 2 = transport system which recognises paraquat, minimum separation of charge of approximately 0.5nm; 3 = redox cycling of paraquat utilising NADPH; 4 = formation of hydroxyl radical leading to lipid peroxidation; 5 = detoxification of H_2O_2 via glutathione reductase/peroxidase couple, utilising NADPH. From Smith, (1987). Reproduced with permission from *Human Toxicology.*

Figure 6
Relationship between the concentration of paraquat in the plasma and the survival of the patient
From Hart *et al.*, 1984, reproduced with permission from *The Lancet.*

FIGURE 1 - PLASMA LEVELS OF PARAQUAT IN THE RAT AND DOG FOLLOWING A SINGLE NON-TOXIC ORAL DOSE

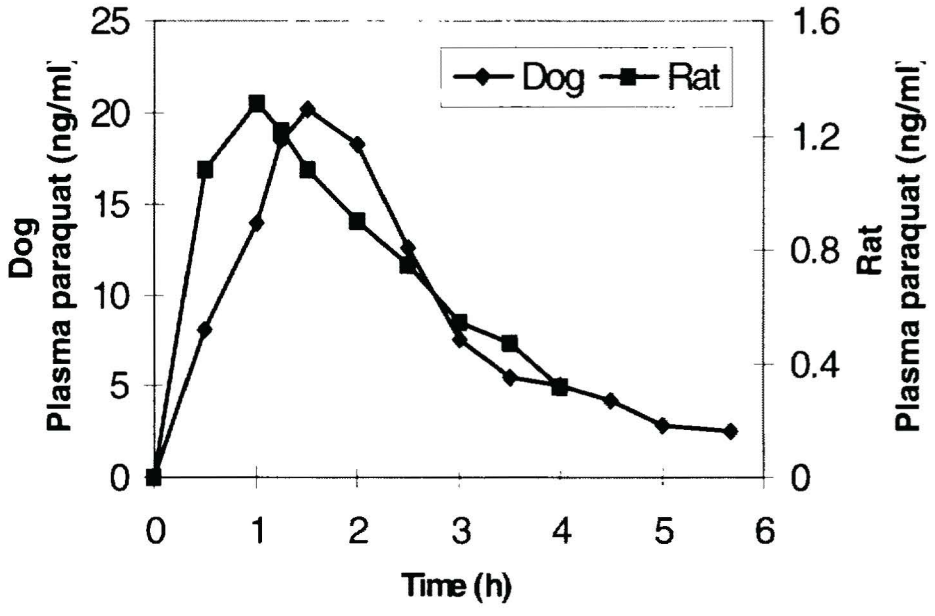


FIGURE 2 - PLASMA LEVELS OF PARAQUAT IN THE RAT AND MONKEY FOLLOWING A SINGLE TOXIC ORAL DOSE

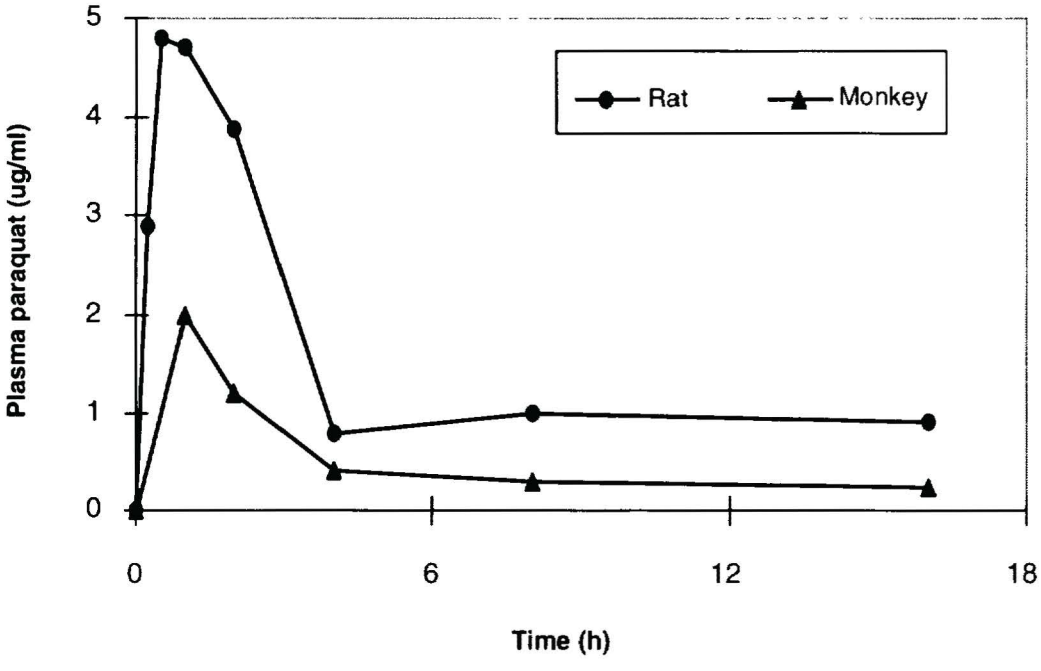


FIGURE 3 - MECHANISM OF PARAQUAT TRANSPORT ACROSS RENAL TUBULAR CELLS

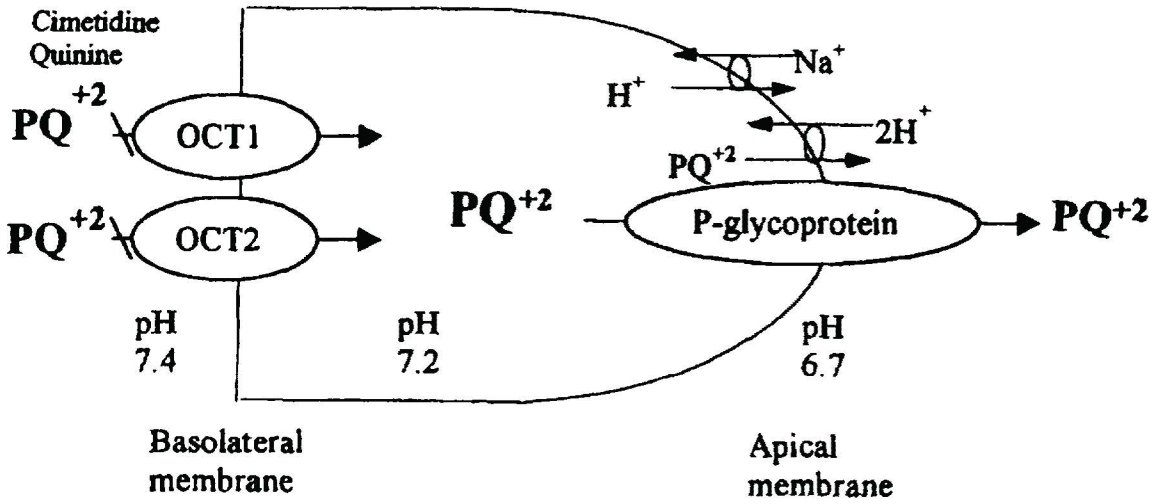


FIGURE 4 - INCREASED SUSCEPTIBILITY OF MICE LACKING Cu/Zn SUPEROXIDE DISMUTASE TO PARAQUAT

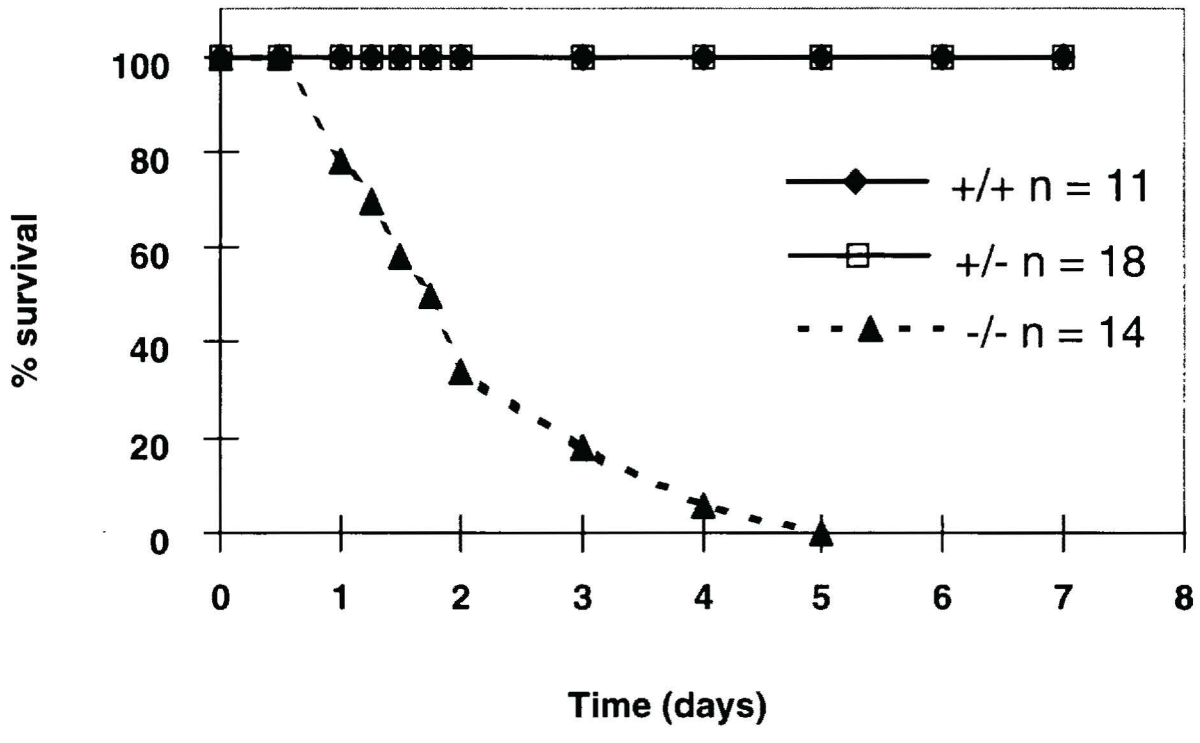


FIGURE 5 - MECHANISM OF TOXICITY OF PARAQUAT

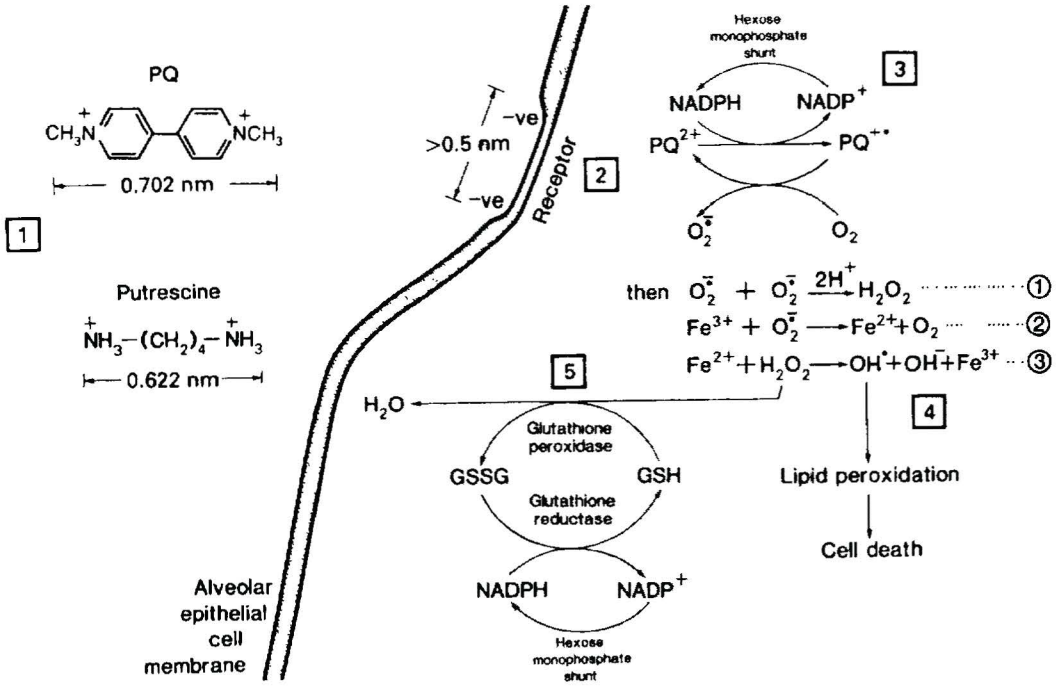


FIGURE 6 - RELATIONSHIP BETWEEN THE CONCENTRATION OF PARAQUAT IN THE PLASMA AND THE SURVIVAL OF THE PATIENT