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THE DEVELOPMENT OF HERBICIDES, INCLUDING SAFETY ASPECTS

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SUMMARY

The development process for a new pesticide is a complex and time-consuming business. All aspects of safety to mankind and the environment require detailed consideration.

In the case of herbicides specifically, user safety and safety to the wider environment usually require greater attention than consumer safety since herbicide residues in crops at harvest are generally very small and frequently non-detectable.

For a new pesticide, safety to Man has to be assessed by extrapolation from studies in laboratory animals. On the other hand, where a pesticide has been in widespread use for many years, evidence from actual human exposure can often be used to assess safety. This is demonstrated in the case of paraquat where it can be predicted from a knowledge of the properties of the chemical that no harm will come to human health during normal occupational exposure. Health studies following long term occupation have confirmed this prediction. Problems of paraquat poisoning in Homo sapiens have arisen mainly as a result of suicides. There is an effective treatment of paraquat poisoning. ICI has also taken steps successfully to minimise drinking accidents with products containing paraquat.

The potential for environmental effects of a pesticide depend both upon its fate in the environment and its toxicity to environmental species. For example, paraquat reaching soil and hydrosoil is quickly inactivated through strong adsorption to clay minerals. As a result, it has no significant adverse effects in soil.

Paraquat is registered widely in the world and no country with a significant reliance upon agriculture has withdrawn its use.

OUTLINE OF THE PESTICIDE DEVELOPMENT PROCESS

The responsible and efficient use of pesticides is one important facet of modern agriculture. The development of a new herbicide, insecticide or fungicide is, however, a time consuming and expensive process. High quality expertise is needed in all parts of the process. The objective is to produce a product which will be of benefit in agriculture, without causing unacceptable risks to mankind and the environment.

The development process, from discovery to full marketing, usually takes ten years nowadays. During the process, three overall areas proceed in parallel. These are the manufacturing chemistry aspects (including finalising patent claims, the scale up from laboratory synthesis to manufacturing scale and the development of preferred formulations), the biological aspects - to define use recommendations and limitations - and the product safety aspects. Product safety includes the health of the employee involved in manufacture, formulation and packing, the health of the user of the product in the field, overall environmental safety and the safety of those who consume the treated crop (which can be either Man or livestock animals). The outcome of the development process is a specific product, bearing a given label, solid in a specific pack or packs.

SOME BASIC PRINCIPLES OF SAFETY EVALUATION

Evaluations of the safety of agrochemicals to Man and his environment depend upon two, interrelated aspects:

- Toxicity to Man and other life forms.

- The nature and level of the exposure which occur during use, in food and feed and in the wider environment.

In the case of pesticides used for weed control, residues in the crop at harvest are usually very small and frequently non-detectable (even with modern sensitive techniques). User exposure and potential for effects in the wider environment are usually the areas requiring particular attention in the case of herbicides. Typically, that is the situation with the herbicide, paraquat.

Toxicity to environmental species can be measured by direct tests. Potential toxicity to Man, however, has to be assessed in toxicological studies in laboratory animals. This is particularly the situation of new pesticides. On the other hand, when legitimately available, information on (lack of) effects in humans is particularly valuable since it relates directly to Homo sapiens and does not involve extrapolation from laboratory animal data. Directly-applicable data for Homo sapiens are more readily available for older compounds, ie those where we have experience of, and most especially data from, extended periods of use. This situation applies in the case of paraquat.

PARAQUAT : SAFETY TO USERS

In nearly 30 years of use, paraquat has been shown to be safe to human health when handled in a normal and reasonable manner. The reason for this is that the primary route of occupational exposure is dermal - to the hands and, in the case of people using knapsack sprayers, to the forelegs as well. However, paraquat is only poorly absorbed through normal skin, with the result that no discernible health effects arise.

Inhalational exposure to paraquat is negligible. To many people this seems surprising since it is well documented that the lung is the most sensitive target organ in the case of paraquat poisonings. It must be stressed, therefore, that those poisonings have arisen from systemic uptake (most usually from the stomach as a result of suicidal ingestion). The lack of inhalation exposure to paraquat, in practice, is consistent with both the ionic nature of the chemical (ie it has no significant vapour pressure) and the fact that the herbicide spray droplets, which are large (eg 100+ microns mass median diameter) and directed downwards for weed control, are of a size well above that which must be attained (below 7 microns) for inhalation to begin to occur.

The majority (99%+) of occupational exposure to paraquat is therefore dermal (Table 1). The highest exposures occur when spraying with hand-held equipment, as compared with aerial or vehicle-mounted applications [1-4].

The health consequences of dermal exposure will depend upon the rate at which a material is absorbed through the skin. Normal human skin routinely provides a barrier to the uptake of a wide range of chemicals encountered in daily life. In the specific case of paraquat, studies with human skin, using both *in vitro* laboratory techniques and *in vivo* human volunteers, have demonstrated that the compound is absorbed extremely poorly [5-7]. This poor absorption can be attributed to the protection provided by the *stratum corneum*, or horny layer on the surface of the skin.

The health consequences of the absorption of very small quantities of paraquat through human skin into the plasma have been studied using human lung slices. Human poisonings resulting from drinking of undiluted concentrate have shown that the lung is the most sensitive target organ to paraquat. Studies [8-10] of the dynamics of uptake into the lung, including human lung slices *in vitro*, have led to a conclusion that continuous plasma levels of 0.2-0.5 µg/ml of paraquat will not result in lung accumulation and damage in *Homo sapiens*.

In the use of paraquat under field conditions, both plasma and urinary concentrations of the herbicide have been measured [1, 12, 13]. The results lead to the conclusion that plasma paraquat concentrations in workers are likely to be very low, and indeed well below the normal limit of detection of an analytical method. Under field conditions, plasma paraquat levels have been consistently non-detectable (limit of determination 0.01 µg/ml). These findings and conclusions are consistent with the inherently poor absorption of paraquat through normal human skin. Thus, under all normal field circumstances, it can be predicted that lung accumulation and resultant lung damage due to paraquat will not occur. A safety margin of several orders of magnitude will normally exist.

The foregoing prediction has been verified through health studies which have been conducted under a range of field conditions [11-19]. These studies are reviewed in greater detail in Appendix I below. During the retrospective health studies of occupational exposure to paraquat, priority has been given to examining situations involving long term exposure and involving hand-held application since this type of use normally results in the greatest occupational exposure.

Of particular relevance is the work of Howard et al [17] who evaluated the health of agricultural workers with a long history of paraquat exposure in Malaysia. A spray gang had applied paraquat by knapsack sprayer for some 30 hours per week and ten months of the year for an average of more than five years. No adverse health effects were found. In particular, there was no evidence of lung, liver or kidney damage attributable to a long term exposure to paraquat. Similar findings were obtained in Sri Lanka [18].

The effects of paraquat seen during occupational exposure studies have been limited to minor and reversible local irritancy problems. Typically, they have included nail damage, nose bleeding and eye and skin irritation, notably of hands, feet, back and groin. Such findings are invariably indicative of inadequate standards of personal hygiene and equipment maintenance. They regress on termination of exposure. Urinary paraquat levels monitored among operators showing localised irritancy effects were very low [12] and certainly far lower that urinary paraquat levels found in patients who had swallowed paraquat and survived.

On rare occasions during the last 30 years there have been incidents, reported in the literature [20-25], in which **extensive** skin damage had led to increased dermal absorption and death from systemic paraquat poisoning. Without exception, these cases arose from gross misuse of the product. It must be stressed that contact with high concentrations of paraquat over a prolonged period, so as to cause extensive skin damage, is a prerequisite for the absorption of a lethal amount of paraquat.

In summary, therefore, the evidence produced from skin penetration studies, plus worker exposure studies, shows that before significant percutaneous absorption of paraquat can take place, extensive or severe skin damage must be present. In the absence of such damage, the skin provides a very effective barrier to paraquat absorption. This explains why paraquat, over nearly 30 years use worldwide, has had such a good record for safety in field use.

PARAQUAT POISONING BY INGESTION AND ITS TREATMENT

Almost all reported cases of paraquat poisoning have resulted from deliberate swallowing of a paraquat formulation. All the information available to ICI shows that accidental swallowing of paraquat has become a rare event. This has been well documented in the UK [26] and the evidence from other countries, while generally less well documented, fits in well with this pattern.

ICI recognises its responsibility to contribute to minimising accidental ingestion of the liquid concentrate to the extent which is practicable and reasonable, even though such incidents are few in number. The labels indicate clearly the toxic nature of the product and they carry a prominent warning against the dangers of decanting into unlabelled bottles, the major potential cause of accidents. A stenching agent and a blue dye have been added to act as deterrents to accidental swallowing, and an emetic has been included to induce vomiting and so reduce the amount of product retained following swallowing. In the UK, the combination of measures has reduced the rate of fatal drinking accidents from 6-8 per year in the early 1970s to (at most) two in the total period 1983-91.

Unfortunately, efforts to minimise instances of accidental poisonings have drawn paraquat to the attention of persons seeking to commit suicide. For example, in the Republic of Ireland, where we also have detailed information, the perception of the problem has shifted from one centred on accidents to one almost exclusively of suicides [27].

Instances of suicide involving paraquat are found in those countries where suicide itself is frequent. It is regrettable that people should try to commit suicide, but the fact is that they do, often using large quantities of various products. Where suicide is a problem, availability obviously will have some bearing on which means of suicide is chosen (although it may not be the sole deciding factor). However, removal of availability in the market of products which have previously been used in suicide attempts will not prevent suicide attempts. This has been shown in the UK. In 1977, the use of barbiturates, a common cause of suicidal death, was severely restricted, for reasons that were not solely related to their use in suicides. Since then, the number of suicide deaths from barbiturates has fallen dramatically, but the number from tricyclic antidepressants, paracetamol and aspirin has increased. The net effect has been no change in the total number of suicide fatalities.

It must also be recognised that a verdict of suicidal death often carries a social or religious, as well as legal, stigma, so that in the world overall the number of suicide fatalities will tend to be an understatement of the true position and accidental poisonings will tend to be overstated.

The approximate <u>minimum</u> lethal dose of paraquat in man is between 10 and 15 ml of product, equivalent to between 2 and 3 grammes paraquat ion. There are also many cases of paraquat poisoning where ingestion of volumes approaching 30-50 ml has been claimed and the patient has survived.

In practice, there is no risk of fatal poisoning by drinking paraquat solutions of spray strength, since it would be necessary to take a volume of the order of two litres. Fatalities have invariably involved either undiluted product or product receiving very limited dilution.

It would be misleading to state that even the smallest amount of paraquat absorbed can prove fatal. Many cases of proven paraquat poisoning have survived either as a result of treatment or because only a small quantity was ingested [28]. ICI knows of no cases in which a person surviving paraquat poisoning has subsequently suffered serious ill-effects as a result of that poisoning.

Paraquat is often described as a poison with no known antidote. It is not unusual in this respect; the majority of chemicals, including drugs and pesticides, have no known antidotes. Even for chemicals where an effective antidote is available, eg organophosphates, the poisoning problem may not necessarily be solved. Deaths, mainly suicides, from poisonings by some organophosphates still occur worldwide, despite the fact that an antidote exists.

Over the past 20 years, ICI has expended a great deal of resource, both in terms of money and manpower, in attempting to understand the nature of paraquat poisoning with a view to developing an antidote. Although the former objective has been well achieved, finding an acceptable antidote has so far eluded the researchers. Nevertheless efforts are still continuing.

Despite the lack of antidote, there is a treatment. Dr Howard[29] showed that treatment could be effective provided it was instituted early enough and provided massive amounts of product were not swallowed. In 1982 the US EPA acknowledged that there is an effective treatment for paraquat poisoning.

In summary, therefore, paraquat poisonings have arisen almost invariably as a result of oral intake, usually with suicide intent, of undiluted concentrate. Poisonings are not a feature of the normal use of the product.

PARAQUAT AND THE ENVIRONMENT

The potential for environmental effects of a pesticide depend both upon its fate in the environment and its toxicity to environmental species.

From an environmental perspective, one of the most important features of paraquat is its fate in soil. Paraquat reaching soil is quickly rendered inactive through strong adsorption to clay minerals. Many uses of paraquat depend upon this property. Most soils can deactivate many hundreds, if not thousands, of normal paraquat applications. Although the degradation rate of paraquat in soil is slow, it is nevertheless sufficient to ensure that, in the overwhelming majority of agricultural situations in which paraquat may be used routinely, residue levels in those soils will never exceed their capacities to deactivate the herbicide. Specific surveys of situations, in which paraquat has been used extensively (eg in Malaysia and Thailand) coupled with anecdotal experience of use over nearly 30 years worldwide, lend support to this conclusion.

Strongly adsorbed paraquat residues are not leached out of soil nor displaced by fertilisers or other agricultural chemicals. They are not taken up by crops, have no adverse effects on crop yields and no discernible effects upon soil microfauna and microflora.

The use of paraquat is consistent with systems of Integrated Pest Management (IPM). The major issue following the use of paraquat is one of toxicity to arthropods, caused by the change of environment caused by weed control.

Paraquat is of relatively low inherent toxicity to many environmental species, with the notable exception of green plant tissue and certain small mammals, such as hares. Extensive laboratory and field studies, coupled with experience of use, confirm that paraquat does not pose a risk in practice to domestic animals, most wild animals, birds and bees.

Paraquat reaching bodies of water, eg as a result of spray drift or other inadvertent contamination, rapidly becomes adsorbed to aquatic weeds and to hydrosoil. These processes quickly render it unavailable to aquatic species. Paraquat is, moreover, of low inherent toxicity to fish and invertebrates such as Daphnia. From practical experience, the greatest potential danger to fish and other aquatic species arises not from the direct toxicity of paraquat itself, but from deoxygenation of water during the decay of dense populations of aquatic weeds, where present.

PARAQUAT : SOIL PERSISTENCE AS A REGULATORY ISSUE

The soil persistence of paraquat has been a major regulatory issue in the Netherlands and in the Federal Republic of Germany.

In the Netherlands, an attempt was made to introduce a policy whereby pesticides which are chemically persistent in soil should not be registered or re-registered. This was regardless of whether or not that presence was associated with any adverse biological consequences. The case went to court. On the basis of advice from a panel of independent experts, the court found in favour of ICI and ordered the government to reinstate the paraquat registrations. While the case was being heard and the court's decision being implemented, sales continued unchanged, by court order.

In the Federal Republic of Germany, a dispute arose between ICI and the authorities over the precise interpretation of various of the data concerning soil persistence. As in the Netherlands, ICI's interpretation has the support of several independent experts. The matter was resolved in court. As a result, the authorities are now required to reinstate registrations that had not been renewed while the court case was pending, this reinstatement to be consistent with the proceedings of the case and consistent with the court's ruling.

OTHER CONSIDERATIONS RELATING TO PARAQUAT REGISTRATION

Paraquat is widely registered in the world, including the USA, UK, France, the Netherlands, Australia and Japan.

Paraquat is no longer registered in Sweden, Finland and Switzerland. These decisions need to be seen in the context of agriculture being minor in all cases and in the context of prevailing political attitudes in the northern half of Continental Western Europe. In considering these decisions in relation to the situation of Indonesia, it is salutary to note that no country with a significant reliance upon agriculture has withdrawn the use of paraquat. On the contrary, when weighing considerations of benefit and risk, there is every reason for a country such as Indonesia to continue to gain full advantage from the unique and extremely useful properties of paraquat in agriculture and horticulture.

TABLE 1

WORKER EXPOSURE TO PARAQUAT

APPLICATION METHOD	DERMAL EXPOSURE (mg/hour)	TOTAL INHALATIONAL* EXPOSURE (mg/hour)	REFERENCE
Hand-held knapsack	66	$(0.45\text{-}1.3) \times 10^{-3}$	1
Vehicle-mounted	0.4 (0.1-3.4)	$0\text{-}2 \times 10^{-3}$	2
Aerial:			
a) Flagger	0.1-2.4	$0\text{-}47 \times 10^{-3}$	3
b) Pilot	0.05-0.1	$0\text{-}06 \times 10^{-3}$	
c) Mixer/loader	0.18	$1.3\text{-}1.5 \times 10^{-3}$	

* Assumes ventilation of 1.8 m^3/hour involving exposure to particles in the non-respiratory range.

APPENDIX I

REVIEW OF HEALTH STUDIES WITH PARAQUAT

Howard [11] reviewed worker exposure to paraquat in normal usage. He concluded that the available evidence supports the contention that systemic poisoning from recommended agricultural use does not occur. A variety of local effects on skin, nails and mucous membranes may result from unwashed spillages, from splashes of commercial product or from prolonged exposure to dilute spray solutions. Howard noted that these local reactions are due to the delayed caustic effect of paraquat and that prompt first aid is frequently effective in preventing the development of the tissue response. Treatment is symptomatic and even though superficial damage may be severe, recovery is normally complete.

Swann [12] described one of the earliest studies demonstrating the safety in use of paraquat. The study was conducted in Malaysia, where exposure tends to be heavy and the use of paraquat is intense. It involved exposure to the herbicide over a twelve week period, and the workers were medically assessed before, during and after the spraying trial. None of the workers showed any serious adverse health effects which could be attributed to paraquat exposure. In particular no worker exhibited any lung abnormality detectable by chest radiography. Adverse effects that did occur were minor and reversible; they included nail damage, skin and eye irritation and one case of nose bleeding. It was concluded that most, if not all, of these side effects could be avoided with improved personal hygiene. Urine levels of paraquat were also monitored throughout this study and found to be very low, certainly far lower than urinary levels found in patients who had swallowed paraquat and survived.

In another study on the exposure of spray operators to paraquat dichloride in Ireland, Hogarty [14] points out that no fatalities have been reported from agricultural spraying in accordance with recommended practice. In this specific study, the results of the medical tests carried out indicated that paraquat was not inhaled or ingested during the trials, and that the spraying operation had no effect on the health of the spray operators. It was concluded that there is little or no risk attached to the use of paraquat dichloride as an agricultural herbicide, provided recommended methods of application are adhered to.

The results were largely confirmed by Hearn and Keir [15] who also demonstrated the development of local skin and nail effects in workers on Trinidad sugar estates, but found no evidence of effects from systemic absorption. The dilutions of 'GRAMOXONE' were from 1:100 to 1:200 (ie from 0.2 to 0.1% paraquat ion in the final spray solution).

In a survey of paraquat dichloride formulation workers in England and Malaysia, Howard [16] failed to show any system effects from the dermal absorption of paraquat, although the incidence of local reactions indicated that the workers had been exposed to significant quantities of the compound in the formulating process. It is worth noting that this group included persons who had been exposed to paraquat for long periods of time (up to 12 years of workplace exposure) and there was no evidence of chronic skin problems nor of any effects on lungs or other organs.

Howard et al [17] also evaluated the health of agricultural workers with a long history of paraquat exposure. A spray gang had applied paraquat by knapsack sprayer for some 30 hours per week and ten months of the year. The individuals had used paraquat for up to 12 years (average exposure 5.3 years). No adverse health effects were found. In particular there was no evidence of lung, liver or kidney damage which could have been attributed to a long term exposure to paraquat. Incidents of skin irritation or rashes associated with spraying were commonest on hands, legs or groin. Groin or buttock rashes were commonly associated with a leaking knapsack sprayer which had allowed liquid to run down the back and between the buttocks. All cases cleared rapidly in response to local treatment (usually a steroid cream).

Similar results were found by Gurunathan et al [18] in Sri Lanka. These authors studied the health of a group of tea plantation workers who had been occupationally exposed to paraquat for a minimum of five years and an average of twelve years. No adverse health effects due to paraquat were found, notably in tests of lung, liver and kidney function. The incidence of skin damage, nose bleeds and nail damage in this study group was slightly higher than in control groups but lower than that reported elsewhere (eg Malaysia and Trinidad).

Whitaker [19] reviewed the results of field surveys of smallholders using paraquat in seven Less Developed Countries (Brazil, Colombia, El Salvador, Guatemala, Honduras, Malaysia and Thailand). Under a range of circumstances, most smallholders had not experienced any ill-effects which they attributed to the use of the product. A low incidence of minor and reversible ill-effects among users included headaches and nausea (where the product included a stenching agent) plus reversible skin irritation, particularly of hands, feet, back and groin. Such symptoms are generally indicative of inadequate standards of personal hygiene and handling procedures.

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PARAQUAT

EXPLANATION

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

Data were submitted to the 1985 Meeting which met the 1982 request. These data were reviewed by the 1985 Meeting, but logistical difficulties precluded their full evaluation, especially in the light of the considerable amount of information previously evaluated by the Joint Meeting. The 1985 Joint Meeting was aware that the 2-year study in rats that was submitted had been considered by 1 national authority to indicate a possible oncogenic potential in the rat. The Meeting also noted differing interpretations of the observed lesions by different pathologists. The Meeting therefore recommended that a complete evaluation of all valid data available should be undertaken by the 1986 Joint Meeting. In addition, it requested submission of full discriptions of the lung lesions seen in the new long-term rat study and of historical control data on all lung lesions in the strain of rats utilized in the study in the laboratory in which it was conducted. The Joint Meeting extended the existing temporary ADI until 1986.

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

IDENTITY AND PROPERTIES

CHEMICAL NAMES

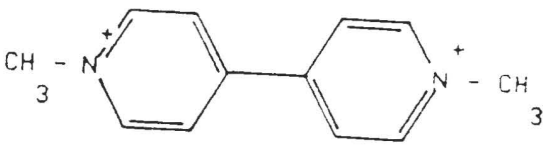
1,1'-dimethyl-4,4'-bipyridylium ion
1,1'-dimethyl-4,4'-bipyridinium ion
1,1'-dimethyl-4,4'-dipyridylium ion
N,N'-dimethyl-γ,γ'-dipyridylium ion

Present as the dichloride.

SYNONYMS

Methyl viologen, PP-148, Gramoxone, Gramoxone S, Gramoxone ZU, Dextrone X, Esgram, Dexuron, Tota-Col, Gramuron, Simpar, Toxer Total, PP-910, Para-Col, Pathclear, Gramonol, Cleansweep, Terraklene, Actar, Priglone, Preeglone, Mofisal, Sweep, Crisquat, Herboxone, Pillarquat, Pillarxone, Duanti, Dukatalon, Frankol Prompt, Gramazin, Gramixel, Katalon, Ortho Paraquat CL, Ortho Spot Weed & Grass Killer, Orvar, Paradi, Seythe, Spray Seed, Tryquat, Weedrite, Crisquat, Goldquat-276, Paraquat CL.

STRUCTURAL FORMULA



EMPIRICAL FORMULA

$[C_{12}H_{14}N_2]^{2+}$

MOLECULAR WEIGHTS

186.2 (ion)
257.2 (dichloride)

PHYSICAL STATE*	Colorless crystalline solid.
MELTING POINT	Decomposes at about 300 °C.
VAPOUR PRESSURE	Not measurable.
SOLUBILITY	Very soluble in water, slightly soluble in lower alcohols, insoluble in hydrocarbons.
STABILITY	Stable in acid or neutral solutions, unstable in alkaline solutions. Inactivated by inert clays, anionic surfactants, and ultraviolet light.
OTHER PROPERTIES	Solutions of paraquat become intensely purple on reduction, due to the formation of a water soluble, relatively stable free radical, which absorbs at 400 nm. The unreduced form absorbs at 258 nm. The extinction coefficients of the reduced and the oxidized paraquat at these absorption maxima are ε mM_{400} = 46.0 and ε mM_{258} = 53.6, respectively (Autor, 1977). Vigorous reduction gives tetrahydro derivatives and ultimately the fully saturated base. The redox potential (-446 mV) is independent of pH. Concentrated aqueous solutions of paraquat are corrosive to metal.
FORMULATIONS	These include aqueous concentrates (100 - 240 g/l) and water-soluble granules (24 g/kg) of paraquat dichloride.
COMBINATIONS	These include mixtures of paraquat with diquat (e.g., Weedol), diuron (e.g., Dexuron), monolinuron (e.g., Gramonol), and simazine (e.g., Terraklene).

* All chemical properties are for the dichloride.

ANALYTICAL METHODS These include spectrophotometric, gas chromatographic and radioimmunoassay methods. They have been extensively reviewed by WHO (1984).

EVALUATION FOR ACCEPTABLE INTAKE

BIOLOGICAL DATA

Biochemical aspects

Absorption, distribution and excretion

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

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

The low rate of paraquat absorption by the gut was confirmed in experiments in which rats, guinea pigs, and monkeys, orally administered with LD_{50} doses of ^{14}C-paraquat, had low peak serum concentrations (2.1 - 4.8 mg/litre). The radioactivity levels reached a maximum 30 - 60 minutes after administration and then remained relatively constant for 32 hours (Litchfield et al., 1973; Conning et al., 1969).

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

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

Dose-dependent data from dogs and whole-body autoradiography seem to suggest that absorption is facilitated in the small intestine (WHO, 1984).

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

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

Paraquat absorption through animal and human skin has been studied using an in vitro technique. Human skin was shown to be impermeable to paraquat, having a very low permeability constant of 0.73. Furthermore, human skin was found to be at least 40 times less permeable than that of the animals tested, including rats, rabbits, and guinea pigs (Walker et al., 1983).

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

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

An association between paraquat concentrations in the lung and degree of toxicity or lung injury has been reported (Sharp et al., 1972; Ilett et al., 1974; Waddell & Marlowe, 1980; Wyatt et al., 1981).

In 1 study toxic doses of ^{14}C-paraquat were administered orally and i.v. to rats. Paraquat concentrations in the whole blood were similar to those in the plasma. The distribution of the herbicide in various tissues was then followed for up to 10 days. The initial and secondary half-lives of paraquat in plasma following i.v. administration were 23 minutes and 56 hours, respectively. The concentration in the kidney, lung, and muscle declined at the same rate as in the plasma initially, but the rapid phase in the lung ended after 20 minutes (compared with 1 - 4 hours in other organs), after which it declined, with a half-life of 50 hours. The lung had the greatest retention and consequently contained the highest concentration 4 hours after dosing. Four to 10 days after dosing, the paraquat concentration in the lung was 30 - 80 times higher than in the plasma (Sharp et al., 1972).

Table 1. **Paraquat distribution in tissues***

Route of entry	Dose	Species	Time after treatment	Tissue	Concentration	Reference
Intra-bronchial	10 ng	rat	60 min	plasma	0.0092 g/l	Wyatt et al., 1981
				lung	5.2 ng	
				kidney	0.052 ng	
				liver	not measured	
				heart	not measured	
				brain	not measured	
I.v.	20 mg/kg	rat	24 h	plasma	0.07 mg/l	Sharp et al., 1972
				lung	6.00 mg/kg	
				kidney	1.45 mg/kg	
				liver	0.48 mg/kg	
				heart	1.20 mg/kg	
				brain	not measured	
I.v.	20 mg/kg	rat	24 h	plasma	not measured	Ilett et al., 1974
				lung	11.36 mol/kg	
				kidney	1.93 mol/kg	
				liver	0.90 mol/kg	
				heart	1.13 mol/kg	
				brain	0.87 mol/kg	
	20 mg/kg	rabbit	24 h	plasma	0.28 mol/l	
				lung	7.90 mol/kg	
				kidney	5.25 mol/kg	
				liver	1.59 mol/kg	
				heart	1.52 mol/kg	
				brain	0.49 mol/kg	
I.p.	15 mg/kg	rat	24 h	plasma	0.32 mol/kg	Maling et al., 1978
				lung	26.28 mol/kg	
				kidney	10.40 mol/kg	
				liver	5.04 mol/kg	
				heart	4.59 mol/kg	
				brain	1.22 mol/kg	
Oral	126 mg/kg	rat	16 h	plasma	0.9 mg/l	Murray & Gibson, 1974
				lung	5.0 mg/kg	
				kidney	7.0 mg/kg	
				liver	2.1 mg/kg	
				heart	2.7 mg/kg	
				brain	not measured	
	22 mg/kg	guinea pig	16 h	plasma	0.03 mg/l	
				lung	1.29 mg/kg	
				kidney	1.99 mg/kg	
				liver	0.08 mg/kg	
				heart	0.31 mg/kg	
				brain	not measured	

* From WHO, 1984

Table 2. **Paraquat distribution in tissues (in mg/kg (mean) tissue)***

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

* From WHO, 1984

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

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

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

No paraquat was detected in the kidney, brain, liver, or lungs when administered in the diet to rats at a concentration of 50 ppm for a period of 8 weeks. At 120 ppm it was found at low concentrations in the lung, kidney, gastrointestinal system, and brain. When administered at 250 ppm, it was detected in the tissues within 2 weeks. No sex differences or any clear pattern of accumulation were noted throughout the 8-week study. Within 1 week of return to a normal diet, no paraquat was detected in any tissue examined. Histological changes were observed in all lungs of animals fed paraquat at 250 ppm in the diet (Litchfield, et al., 1973).

Rose et al. (1974) demonstrated an energy-dependent accumulation of paraquat in slices of rat lung that obeyed saturation kinetics. The same investigators later examined the ability of paraquat to accumulate in tissue slices from other organs in vitro. The uptake of the herbicide in brain, adrenal gland, and kidney slices was less than 10% of that observed in lung slices. The authors established the uptake of paraquat by the lung in various species (rat, rabbit, dog, monkey, and man). The human lung accumulated paraquat as readily as that of the rat. Indeed, the kinetics (V_{max} and K_m) of the process were found to be very similar in the 2 species. Moreover, there was a relationship between the concentration of paraquat in the different lung areas and the development of microscopic lung lesions (Rose et al., 1976a; Rose & Smith, 1977).

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

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

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

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

The dog was used as a model to evaluate the influence of paraquat-induced renal failure on the kinetics of paraquat elimination. After i.v. injection of a trace dose of ^{14}C-paraquat (30 - 50 g/kg b.w.), the kinetics of distribution was described by a 3-compartment model. To obtain a good fit of the curve, it was necessary to sample the central (plasma) compartment for at least 24 hours after dosing. Simulation of paraquat levels in the peripheral compartments suggested the existence of a compartment with rapid uptake and removal (kidney) and another with slow uptake (lung). The renal clearance of paraquat approximated total body clearance, indicating that paraquat elimination occurs through renal excretion. The urinary excretion rate of an

i.v. dose was rapid, approximately 80 - 90% of the dose being eliminated during the first 6 hours. Intravenous injection of a large toxic dose of paraquat (20 mg/kg b.w.), however, brought about a marked decrease in renal clearance, from 73 ml/minute to 18 ml/minute after 2.5 hours and 2 ml/minute after 6 hours. These data suggest that kidney damage could contribute to paraquat accumulation in the lung (Hawksworth et al., 1981).

Metabolism

Rats, dogs, and guinea pigs

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

These conclusions were in contrast with the results of other studies previously evaluated which indicated that when paraquat (50 mg/kg b.w. of ^{14}C-labelled dichloride salt) was given to rats, 25% of the radioactivity excreted in the faeces could be attributed to products of metabolism by gut microflora. Examination of extracts indicated the presence of only 1 metabolite in addition to paraquat. Thirty percent of the paraquat was broken down when incubated anaerobically with rat caecal contents; the metabolites were not identified. Urine from rats injected i.p. with ^{14}C-methyl-labelled paraquat contained 87% of the administered radioactivity in 24 hours, which was entirely unchanged paraquat (Plant Protection Ltd, 1972).

Hens

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

Continuous dosing of hens with radiolabelled paraquat for up to 22 days, at rates up to 30 ppm in the diet, resulted in total radioactive residues in the eggs of up to approximately 0.05 mg/kg paraquat ion equivalent. At least 80% of the radioactivity was due to unchanged paraquat. The residue was almost entirely in the yolk rather than in the albumin (Hemingway & Oliver, 1974; Hendley et al., 1976a).

Pigs

Pigs excreted an oral dose of paraquat principally in the faeces as unchanged paraquat. Two pigs were dosed with ^{14}C-labelled paraquat for 7 consecutive days at a rate equivalent to 50 ppm in the diet. One was dosed with ^{14}C-methyl- and the second with ^{14}C-ring-labelled paraquat. The pigs were sacrificed 2 hours after receiving the final dose. By this time 69 - 73% of the administered residue had been recovered in the faeces and approximately 3% had been recovered in the urine. More than 90% of the radioactivity in the faeces was present as unchanged paraquat. Total radioactive residues in the tissues were low. More than 90% of these residues were due to unchanged paraquat, except in liver, where approximately 70% was due to unchanged paraquat and 4 - 7% was due to monoquat ion (Leahey et al., 1976; Spinks et al., 1976).

Goats

^{14}C-ring-labelled paraquat was administered to a goat in mid-lactation twice daily for 7 days at a dose equivalent to 100 ppm in the diet. Total radioactive residues in the milk were less than 0.01 mg/kg paraquat ion equivalent; 76% was unchanged paraquat. Total radioactive residues were 0.74, 0.56, and 0.1 mg/kg in kidney, liver, and muscle, respectively. There was no significant metabolism of paraquat, except in the liver, where 50% of the residue was paraquat and about 5% was each of the metabolites monoquat ion and monopyridone ion (Hendley et al., 1976b).

Sheep

A dose of ^{14}C-methyl-labelled paraquat administered to a sheep via a rumen fistula was recovered quantitatively within 10 days. Approximately 4% of the dose was excreted in the urine and the remainder in the faeces. More than 95% of the radioactivity in urine and faeces was present as unchanged paraquat. Small amounts of monoquat ion (1%) and monopyridone ion (2.3%) were also detected (Hemingway et al., 1972).

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

Cows

When cows were given single oral doses of ^{14}C-methyl paraquat at 8 mg/kg, 96% of the radioactivity was recovered in the faeces during the following 9 days; 0.7% was recovered in the urine. Unchanged paraquat accounted for most of the radioactivity in the faeces (96%) and urine (62 - 90%), but traces of the monoquat ion and monopyridone ion were also detected in the urine. Only 0.003 - 0.004% of the radioactivity was recovered in milk; the maximum radioactive residue (0.005 mg/kg, paraquat ion equivalent) was observed on the day after dosing. About 15% of this radioactivity was present as unchanged paraquat. Monoquat ion and monopyridone ion (3 - 25%) were also found in the milk. The radioactivity not identified as paraquat, monoquat, or monopyridone was incorporated into natural constituents of milk resulting from the anabolism of the radioactive methyl group cleaved from paraquat (Hemingway et al., 1974).

Cows were fed for 3 months diets containing 24, 80, or 170 ppm paraquat ion (equivalent to 0.8, 2.5, or 5.5 mg/kg b.w./day). The paraquat was present as a residue in dried grass obtained from a pasture that had been sprayed with Gramoxone and subsequently weathered. The diet was accepted satisfactorily and no toxicological effects were observed during the trial. Pathological examination of tissues from animals slaughtered within 24 hours of the end of the feeding trial showed no toxic effects attributable to paraquat. The tissue residues, including muscle and liver, determined in cows at the 2 higher dose rates, varied between 0.01 and 0.09 mg/kg except in the kidney, where 0.21 - 0.31 mg/kg was found. These fell to low (0.04 mg/kg in the kidney) or non-detectable levels in an animal fed the high-paraquat diet for 30 days and then maintained on an untreated diet for 12 days before slaughter. Very low residues of paraquat were present in milk samples taken weekly during the trial (121 samples ranging from 0.0001 - 0.0006 mg/kg; 1 sample = 0.001 mg/kg) (Edwards et al., 1974).

Effects on enzymes and other biochemical parameters

Several reviews or monographs have summarised the biochemical mechanism of paraquat toxicity in plants (Calderbank, 1968), bacteria (Fridovich & Hassan, 1979), and animals (Bus et al., 1976; Autor, 1977; Smith et al., 1979; Smith, 1985). The mechanism of the toxic action of paraquat has also been extensively reveiwed by WHO (1984).

Paraquat has long been known to participate in cyclic reduction-oxidation reactions in biological systems. The compound readily undergoes a single electron reduction in tissues, forming a free radical. In an aerobic environment, the free radical is immediately oxidised by molecular oxygen, generating the superoxide anion radical. The reoxidized paraquat is capable of accepting another electron and continuing the electron transfer reactions in a catalytic manner (Figure 1).

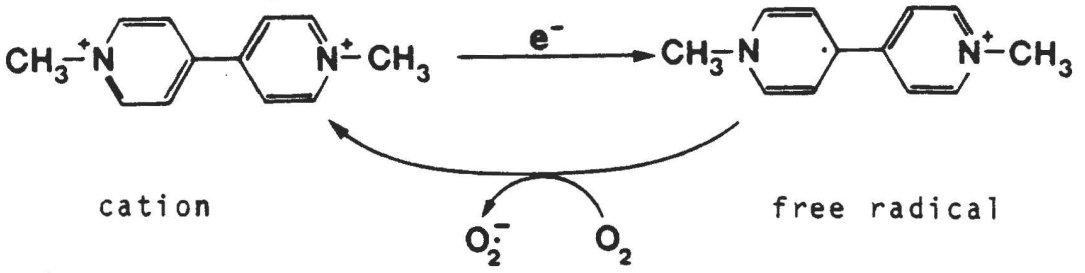


FIGURE 1. Paraquat reduction-oxidation (modified from Bus and Gibson, 1984).

Research into the mechanism of paraquat toxicity has identified at least 2 partially toxic consequences of the redox cycling reaction: a) generation of the superoxide anion radical, and b) oxidation of cellular NADPH, which is the major source of reducing equivalents for the intracellular reduction of paraquat. Generation of the superoxide anion radical can lead to the formation of more toxic forms of reduced oxygen, hydrogen peroxide (H_2O_2), and hydroxyl radicals. Hydroxyl radicals have been implicated in the

initiation of membrane damage by lipid peroxidation, depolymerization of hyaluronic acid, inactivation of proteins, and damage to DNA. Depletion of NADPH, on the other hand, may disrupt important NADPH-requiring biochemical processes such as fatty acid synthesis (Hassan & Fridovich, 1980; Smith et al., 1979).

The importance of molecular oxygen and the potential role of superoxide anion radical generation in mediating paraquat toxicity have been implicated in studies on plants, bacteria, and in vitro and in vivo mammalian systems. In cultures of E. coli, Hassan & Fridovich (1977, 1978, & 1979) demonstrated that paraquat stimulated cyanide-resistant respiration, which could be almost entirely accounted for by the NADPH-dependent formation of the superoxide anion radical.

The possibility that formation of the superoxide anion radical might be responsible for the toxicity of paraquat in bacteria is supported by observations that bacteria containing elevated activities of superoxide dismutase, an enzyme that detoxifies the superoxide anion radical, were resistant to paraquat toxicity (Hassan & Fridovich, 1977, 1978; Moody & Hassan, 1982).

In vitro studies on lung and liver preparations from various animal species have supported the hypothesis that paraquat redox cycling and associated superoxide anion radical and H_2O_2 generation also occur in mammalian systems (Gage, 1968; Ilett et al., 1974; Montgomery, 1976, 1977; Steffen & Netter, 1979; Talcott et al., 1979).

Bus et al. (1974) reported that the single electron reduction of paraquat in mammalian systems was catalysed by microsmal cytochrome P-450 reductase and NADPH. The observation that the in vivo toxicity of paraquat in animals is markedly potentiated by exposure to elevated oxygen tensions further supports the potential role for molecular oxygen in mediating toxicity (Fisher et al., 1973; Autor, 1974; Bus & Gibson, 1975; Witschi et al., 1977; Kehrer et al., 1979; Keeling et al., 1981; Selman et al., 1985).

The results of in vivo studies conducted by Bus et al. (1974) suggest that stimulation of lipid peroxidation, which is dependent on paraquat redox cycling and associated superoxide anion radical generation, might be an important toxic mechanism in mammalian systems. Consistent with this hypothesis, animals fed diets deficient in selenium or vitamin E in order to diminish cellular antioxidant defenses were significantly more sensitive to paraquat toxicity than control animals (Bus et al., 1975a; Omaye et al., 1978). Moreover, selenium deficiency potentiated paraquat-induced lipid peroxidation in isolated perfused rat lung (Glass et al., 1985). In contrast to these studies, a number of studies have shown that paraquat inhibited in vitro microsomal lipid peroxidation (Ilett et al., 1974; Montgomery & Niewoehner, 1979; Steffen & Netter, 1979; Kornburst & Mavis, 1980). Subsequent studies have indicated, however, that paraquat would stimulate microsomal lipid peroxidation when an adequate supply of electrons (NADPH) and in vitro oxygen tension were maintained (Trush et al., 1981, 1982).

Despite the evidence described above, the hypothesis that lipid peroxidation is the underlying toxic mechanism functioning in vivo has not been conclusively demonstrated. Direct quantification of paraquat-induced lipid peroxidation damage in vivo by analysis of tissue malonadialdehyde levels or ethane exhalation, both markers of peroxidation injury, has been

largely unsuccessful (Reddy et al., 1977; Shu et al., 1979; Steffen et al., 1980), although significant increases of serum malondialdehyde levels have been recently reported in patients with paraquat poisoning (Yasaka et al., 1986). Furthermore, attempts to counteract paraquat toxicity by administration of various antioxidants have also been unsuccessful (Fairshter, 1981).

Superoxide radicals generated in paraquat redox cycling may induce biochemical changes other than the initiation of the peroxidation reaction. Ross et al. (1979) demonstrated that paraquat increased DNA strand breaks in cultured mouse lymphoblasts. Paraquat was also reported to induce a superoxide-dependent stimulation of guanylate cyclase activity in rat liver (Vesely et al., 1979) and guinea pig lung (Giri & Krishna, 1980). These investigators postulated that increased cyclic-GMP might stimulate the pulmonary fibroproliferative changes characteristic of paraquat toxicity. In other studies, paraquat has also been found to increase collagen synthesis in the rat lung (Greenberg et al., 1978; Thompson & Patrick, 1978; Hussain & Bhatnagar, 1979).

Redox cycling of paraquat has also been proposed to lead to increased oxidation of cellular NADPH (Brigelius et al., 1981; Keeling et al., 1982). The activity of pentose shunt enzymes in the lung rapidly increased in rats treated with paraquat, which suggested an increased demand for NADPH (Fisher et al., 1975; Rose et al., 1976b). The observation that paraquat decreased fatty acid synthesis in lung slices (Smith et al., 1979) further supported this hypothesis, since fatty acid synthesis requires NADPH. Direct analysis of NADPH in the lung has long confirmed that paraquat treatment decreases the NADPH content in rat lung (Witschi et al., 1977; Smith et al., 1979). More recently, both oxygen consumption and NADPH oxidation in lung microsomes were found to be significantly and specifically stimulated by the addition of paraquat (Rossouw et al., 1984). The above observations led Smith et al. (1979) to propose that oxidation of NADPH might interrupt not only vital physiological processes, such as fatty acid synthesis, but also may render tissues more susceptible to lipid peroxidation by decreasing the equivalents (NADPH) necessary for functioning of the antioxidant enzyme glutathione peroxidase (Figure 2). Indeed, a significant increase (589%) in lung-oxidized glultathione (GSSG) content was found over control levels after perfusion of isolated rabbit lung with a 0.4 mM paraquat solution. This effect was significantly increased (225%) by hyperoxia (Dunbar et al., 1984).

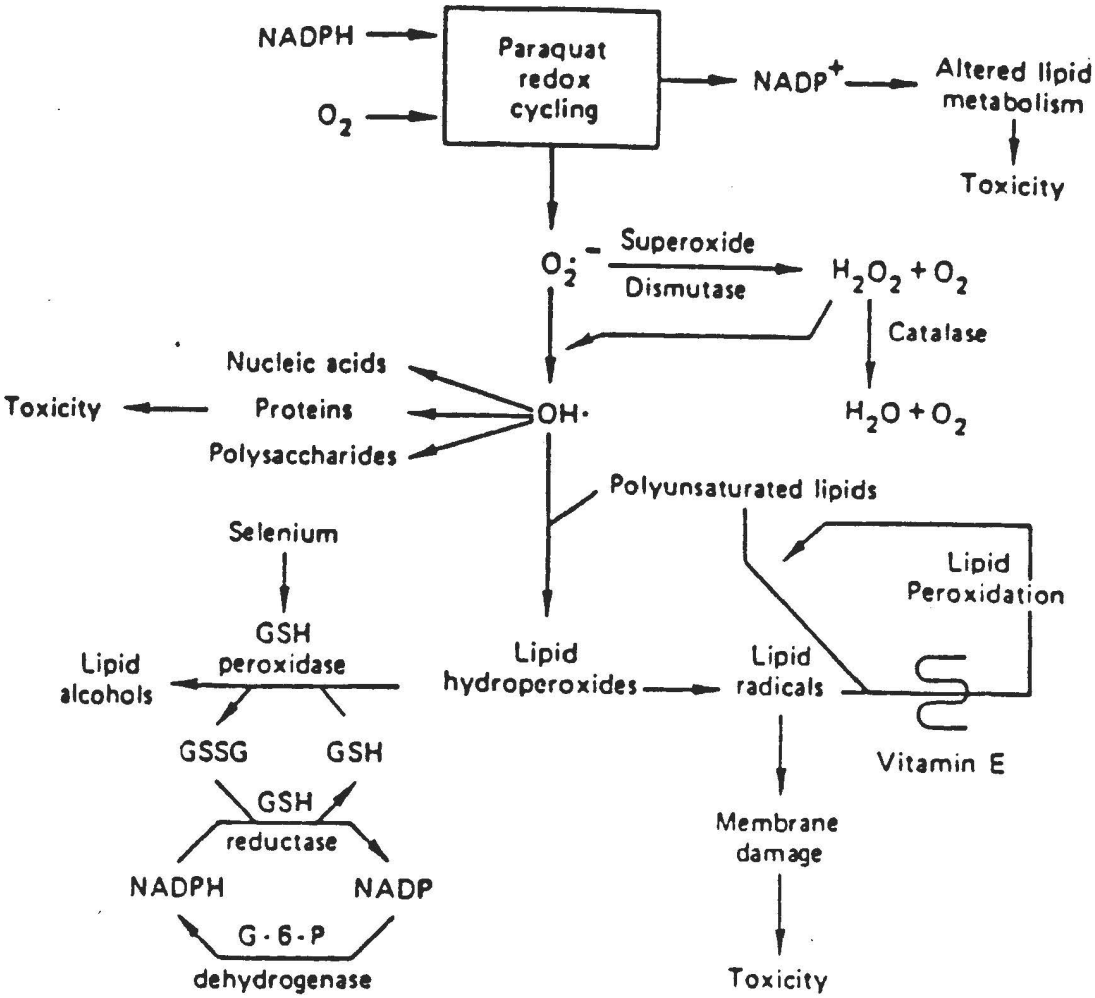


FIGURE 2. Proposed biochemical mechanism of paraquat toxicity (Bus and Gibson, 1984)

Toxicological studies

Special studies on carcinogenicity

Mice

Groups of 60 male and 60 female Alderly Park SPF mice were fed diets containing 0 (2 groups), 12.5, 37.5, or 100/125 ppm paraquat cation for 97 - 99 weeks. The initial top dose of 100 ppm was increased to 125 ppm at week 36 in order to evoke a toxic effect. The study was terminated at weeks 97 - 99 when 80% mortality was reached in a female control group and was approaching 80% overall. Clinical observations, body-weight gain, food consumption, and

urinary paraquat were measured throughout the study. Histopathological examination of approximately 40 tissues was performed on animals killed or dying during the study and at termination. Further groups of 10 males and 10 females were fed the same dose levels as above for 52 weeks for measurement of paraquat concentrations in the kidney, lung, and plasma at termination.

Mortality ranged from 32 - 55% at 80 weeks and from 58 - 87% at termination and was higher in the 37.5 ppm and 125 ppm groups than in the combined controls. Effects due to treatment were renal lesions in both sexes at 100/125 ppm and, in males, at 37.5 ppm; lung lesions in both sexes at 100/125 ppm; and decreased food consumption and body-weight gain and increased mortality in females at 100/125 ppm. Histopathologically, the treatment-related renal lesions were manifest as mild dilatation and degenerative changes in the tubules. The incidences of tubular degeneration (with and without dilatation) in male mice dying during the study were 31/48 at 100/125 ppm, 15/47 at 37.5 ppm, 9/45 at 12.5 ppm, and 8/45 and 3/35 in controls. The paraquat-induced lung lesions noted at 100/125 ppm included focal pneumonitis/alveolitis and hypercellularity of the alveolar walls. Statistically-significant increases in the incidence of fatty changes of the liver were reported at 37.5 and 100/125 ppm in males, when compared to controls. Other hepatic changes were noted, with a significantly-higher incidence in treated compared to control mice. These changes, however, were not considered by the authors of the study to be treatment-related. There were no effects observed at 12.5 ppm. Histopathological examination showed no clear evidence of treatment-related neoplastic changes in these mice. The incidence of pulmonary tumours in both males (7/24) and females (8/20) in the 100/125 ppm dose group dying from 79 - 98 weeks was somewhat higher than in controls (5/37 in males and 6/39 in females). However, the incidences of pulmonary tumours in the animals of the same groups surviving to termination were lower than in controls.

The authors of the study concluded that paraquat was not oncogenic to the mouse. Based on the renal lesions, the no-effect level of paraquat cation for Alderley Park SPF mice in this study was 12.5 ppm, equal to 1.4 mg/kg b.w./day in males and 37.5 ppm, equal to 4.3 mg/kg b.w./day in females.

Rats

Groups of 80 male and 80 female Fisher SPF rats were maintained on diets containing 0, 7.2, 22, 72, or 217 ppm paraquat cation for 104 weeks. Eight rats/sex/group were sacrificed after urinalysis at 26, 52, and 78 weeks and were subjected to haematological examination. All surviving animals were sacrificed at 104 weeks and, among these, 10 rats/sex/group were subjected to haematological and biochemical examination. All animals, including those killed on schedule and those found moribund and killed during the study, were autopsied and subjected to gross necropsy and histopathological examination of approximately 30 tissues.

Mortality was increased in female rats of the 217 ppm group from week 66 to week 74 when compared with that of other groups, including controls. Both male and female rats at the 217 ppm dietary level showed a marked statistically-significant reduction in body-weight gain when compared to control groups. Food consumption, efficiency of food utilisation, and water consumption were also staistically-significantly lower in these rats when compared to control animals.

Haematological examination showed a statistically-significant reduction in total white cell count in male rats of the 217 ppm group, when compared to controls, at 26, 52, and 78 weeks, but not at 104 weeks. This change was not considered by the authors of the study to be attributable to the administration of paraquat. Biochemical examination indicated a statistically-significant reduction in globulin in male rats of the 217 ppm group at 26, 78, and 104 weeks when compared to controls. Clinical observations, RBC counts, haemoglobin, mean red-cell volume (MCV), mean cell hamoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet counts, differential WBC counts, plasma alkaline phosphatase, lactic acid dehydrogenase, blood urea nitrogen, glucose, total cholesterol, GOT, GPT, total and direct bilirubin, GGPT, calcium, total protein, albumin, and urinalysis indicated no significant effects attributable to the administration of paraquat at any dose levels.

Throughout the entire administration period, a statistically-significant reduction was found in the absolute weights of various organs of male and female rats of the 217 ppm group at interim sacrifices. This change was considered by the authors of the study to be related to the reduction in body weight observed in these animals. Histological examination of the lung at termination showed a marked, treatment-related, statistically-significant increase in the incidence of proliferation of interalveolar septum cells and of hyperplasia of alveolar epithelium in both male and female rats at 217 ppm and in male rats at 72 ppm, when compared to controls. There was a marked, statistically-significant increase in the incidence of cataract in male and female rats of the 217 ppm group killed or found dead after week 79. This treatment-related change was reported to be the same microscopically as that observed in the tissues collected from those control rats which had spontaneous, age-related cataracts. Male rats of the 217 ppm group also showed a statistically-significant increase in the incidence of local atrophy of renal tubules when compared to controls. Females of the same dietary group had a statistically-significant increase in the overall incidence of diffusive fatty changes of the liver and pulmonary fibrosis when compared to controls. Kidney and liver lesions were not considered by the authors of the study to be attributable to the administration of paraquat. A significant increase (details of statistical analysis were not available) in the incidence of pulmonary adenoma (7/80) was found in female rats of the 217 ppm group when compared to controls (1/80). There was no significant increase in the incidence of lung adenoma in male rats, but a few of them had lung adenocarcinoma (1 in each of the 22 and 72 ppm groups, 3 in the 217 ppm group, and none in the controls). The authors noted that, although the historical incidence of pulmonary adenoma in rats of this strain is reportedly rather low (about 2%), 6/80 (7.5%) of the control rats developed pulmonary adenoma in a 24-month chronic toxicity study carried out separately in their laboratory. Based on these considerations, the authors of the study concluded that the incidence of pulmonary adenoma found in the present paraquat study in female rats in the 217 ppm group did not exceed the background incidence of pulmonary adenoma in rats of this strain. On the basis of the lung and eye lesions the no-effect level of paraquat cation determined in this study for Fisher SPF rats after 104-week treatment was 22 ppm, equal to 0.77 mg/kg b.w./day in male rats and 72 ppm, equal to 3.12 mg/kg b.w./day in female rats (Yoshida et al., 1982).

Historical control incidence data of neoplasia in F-344 rats in the laboratory in which the preceding study was conducted were made available and are summarized in Table 3.

Table 3. **Spontaneous lung tumours observed in F-344 rats at the Institute of Environmental Toxicology from 1980 to 1983**[1]

	No. of tumour-bearing animals (%)[2]	
	Males	Females
Adenoma	40 (4.2%)	21 (2.2%)
Adenocarcinoma	5 (0.5%)	1 (0.1%)
Bronchial gland adenoma	1 (0.1%)	-
Total	46 (4.8%)	22 (2.3%)

1 From Maita, 1986
2 These data were taken from 12 studies that included 960 male and 959 female F-344/DuCrj rats.

Groups of 60 male and 60 female Fischer 344 rats were maintained on diets containing paraquat cation at 0 (2 groups), 25, 75, or 150 ppm for at least 113 weeks (males) or 122 weeks (females). Further groups of 10 rats/sex/group received the same diets for 1 year. All animals were studied for mortality, food and water consumption, and body weight, and were subjected to periodical ophthalmoscopic and haematological examinations throughout the study.

The distribution of mortality was unaffected by treatment. There was approximately 50% mortality in all groups at the end of the study. At 150 ppm, statistically-significant reductions in body-weight gain, food consumption, and efficiency of food utilisation in both sexes were observed. There was a statistically-significant depression of body-weight gain in the first year of the study in males receiving 75 ppm paraquat . Water consumption was not significantly affected at any dietary level tested. Paraquat accelerated, in a dosage-dependent manner, the onset and progression of cataract changes, ranging from minor opacity to total cataract in both males and females. Treatment-related ocular lesions were first seen at 52 weeks. Thereafter, ophthalmoscopy revealed a statistically-significant dosage-related increase in the incidence, progression, and severity of lenticular cataract in the 150 ppm group and, toward the end of the study (103 weeks), in the 75 ppm group. There was evidence of paraquat-dependent ocular effects in all treatment groups of both sexes at termination. A statistically-significant higher incidence of secondary eye lesions was found at termination in females receiving 75 or 150 ppm paraquat when compared to controls. Haematological investigation (RBC counts, total and differential leucocyte counts, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin concentration, platelet and reticulocyte counts, and prothrombin and partial thromboplastin times) and blood biochemistry (urea, glucose, ALT, and AST) indicated no significant effects attributable to paraquat administration. Urinalysis did not reveal any treatment-related changes. Reductions in liver and testicular weights were noted at termination in the 150 ppm dietary group.

Macroscopic examination at necropsy revealed a treatment-related increase in the incidence of focal subpleural changes in animals killed at termination in all dietary groups. This effect was most marked in females receiving 75 ppm and in both sexes receiving 150 ppm paraquat. Microscopic examination of lung tissues indicated that treatment with paraquat at 150 ppm, in both sexes, and possibly at 75 ppm in males, was associated with proliferative lesions of the alveolar epithelium. These lesions were not easily classified into non-neoplastic or neoplastic, nor into adenoma or carcinoma. This study provided strong evidence for the induction by paraquat of a proliferative lesion of the alveolar epithelium and some controversial evidence for the induction of lung adenomas in female Fischer 344 rats. There was no treatment-related increase in the incidence of lung adenocarcinoma at any dose level in either sex. At 25 ppm, significant increases in the incidence of proliferative lung lesions, compared to the controls, were not observed. Microscopic examination of the eyes confirmed a dose-related effect of paraquat on the onset and progression of cataract lesions normally present in both male and female F-344 rats. Slight dilation of the fourth ventricle of the brain was evident in females receiving 150 or 75 ppm paraquat, but not in males at these dosages or in either sex at 25 ppm. A statistically-significant increase in the incidence of apparent degeneration of occasional/several sciatic nerve fibers was noted in decedent males receiving 75 or 150 ppm paraquat. Both hydrocephalus and nervous tissue changes were considered by the authors of the study possibly to be associated with paraquat treatment. Pathology summaries indicate that atrophy of the testes was recorded in the high-dietary group (5/33) but not in controls at termination, and moderate lymphoid hyperplasia was observed in the respiratory epithelium of males receiving 75 and 150 ppm paraquat and dying between 52 weeks and termination.

The authors of the study concluded that "a wide range of tumour types was observed in treated and control animals, and there was no evidence that treatment with paraquat resulted in a marked change in the group distribution of any of these tumours". A no-effect level for paraquat was not found in this study due to the higher incidence of cataract observed in animals of the 25 ppm group when compared to controls. Paraquat accelerated, in a dosage-dependent manner, the onset and progression of cataract change in F-334 rats. The authors considered 25 ppm to be near the no-effect level for this change at the end of the study (Ashby et al., 1983; Busey, 1986; Ishmael & Godley, 1983).

Data on the historical incidence of lung neoplastic lesions in F-344 rats from 7 studies performed in the laboratory in which the preceding study was conducted were made available and are summarised in Table 4.

Table 4. **Spontaneous lung tumours observed in F-344 rats at Life Science Research**[1]

	No. of tumour-bearing animals (%)[2]	
	Males	Females
Adenoma	6/357 (1.7%)	4/363 (1.1%)
Carcinoma	3/357 (0.8%)	0/363 (0%)
Total pulmonary tumours	9/357 (2.5%)	4/363 (1.1%)

[1] From Ashby et al., 1983

[2] The range (% incidence) of adenoma was 0 - 4.4% in males and 0 - 4.0% in females; the range of carcinoma was 0 - 4.0% in males.

Special studies on embryotoxicity and teratogenicity

Mice

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

The same authors examined the effects of paraquat dichloride on the development of Swiss-Webster mice when administered in the drinking water at concentrations of 0, 50, or 100 ppm. Paraquat was given to pregnant mice from day 8 of gestation and administration was continued to the newborns until 42 days after birth, when both the control and paraquat-treated mice were sacrificed and subjected to histopathological examination of the lungs, liver, and kidneys. A significant increase in postnatal mortality in mice receiving 100 ppm paraquat was observed. Histopathological examination of the lungs of these mice showed extensive alveolar consolidation and collapse, and areas of thickening of intra-alveolar septa. No significant pathological changes were seen in the lungs of the 50 ppm or control mice, nor in the liver or kidneys of mice of any treatment group. There were no treatment-related effects on the number of live fetuses nor on postnatal growth rate at either treatment level (Bus & Gibson, 1975).

Four groups of at least 20 pregnant SPF Alderley Park mice were given orally 0, 1, 5, or 10 mg/kg b.w./day of paraquat cation during days 6 to 15 of pregnancy, inclusive. On day 18 the animals were killed, their uteri were examined, and the fetuses were removed, weighed, sexed, and observed for gross abnormalities. There was some evidence of maternal toxicity in the form of slight reductions in body-weight gain at 5 and 10 mg/kg b.w./day, although only that of the middle-dose group was statistically significant. There were no clinical signs nor pathological changes in maternal animals attributable to paraquat administration. Water and food consumption were not quantified in this study. Numbers of implantations, viable fetuses and resorptions, sex ratios, and fetal and litter weights showed no significant differences between treated and control groups. There were no increases in fetal external or soft-tissue abnormalities which could be associated with paraquat treatment. There were occasional statistically-significant differences in ossification of individual bones between treated and control groups, but no dose-related trend indicating either retardation of ossification or increased abnormalities was observed. The authors of the study concluded that paraquat was not teratogenic and had no significant influence on embryonic or fetal development of the mouse at levels up to and including 10 mg/kg b.w./day (Hodge et al., 1978a).

Rats

The teratogenic effects of paraquat were studied in 4 groups of at least 20 pregnant SPF Alderley Park rats after oral administration of 0, 1, 5, or 10 mg/kg b.w./day of paraquat cation during days 6 to 15 of pregnancy, inclusive. On day 21 the animals were killed. There were clear clinical signs of maternal toxicity at 5 and 10 mg/kg b.w./day. Apparently, 6 rats at the highest-dose level and 2 at the middle-dose level died or became moribund during the experiment. Histological changes found in the lungs and kidneys of the animals receiving 10 mg/kg b.w./day paraquat which died or became moribund were those known to be associated with oral paraquat poisoning. Slight fetotoxicity was seen at 5 and 10 mg/kg b.w./day, as shown by a statistically-significant reduction in fetal weight and retardation in ossification, and by a decrease in the number of viable fetuses per number of implants. According to the authors of the study, these effecs were probably associated with maternal toxicity. There were no effects on embryonic or fetal survival and increases in fetal abnormalities were not observed. The authors of the study concluded that paraquat was not teratogenic when administered orally to rats, even when there was clear evidence of maternal toxicity. However, it did cause slight fetotoxicity at the 2 highest-dose levels (Hodge et al., 1978b).

Special studies on eye irritation

The effects of paraquat on the eye have been reviewed by WHO (1984). The instillation of diluted paraquat (up to 500 g/litre) in rabbits' eyes induced inflammation within 24 hours, and this continued for 96 hours (Clark et al., 1966). In another experiment, 62.5, 125, 250, 500, or 1000 g/litre of paraquat was introduced into the eyes of rabbits. Concentrations of 62.5 and 125 g/litre caused severe conjunctival reactions; higher levels (250 - 500 g/litre) provoked ititis and pannus, while at the 500 g/litre concentration corneal opacification, iritis, and conjunctivitis occurred. All rabbits receiving 0.2 ml of paraquat at 1000 g/litre in 1 eye or 0.2 ml of 500 g/litre paraquat in both eyes died within 6 days of application (Sinow & Wei, 1973).

Special studies on mutagenicity

In a review of published mutagenicity data (WHO, 1984) it was noted that paraquat had been found to have minimal to no genotoxic activity when evaluated in a variety of in vitro and in vivo test systems. In in vitro studies producing weakly positive results, paraquat genotoxicity was accompanied by high cytotoxicity (Moody & Hassan, 1982; Parry, 1973 & 1977; Tweats, 1975; Benigni et al., 1979; Bignami & Grebelli, 1979). Moody and Hassan (1982) have shown that the mutagenicity of paraquat in bacterial test systems (Salmonella typhimurium TA98 and TA100) was mediated by the formation of superoxide. More recently, paraquat was found to induce superoxide dismutase, chromosomal aberrations, and sister-chromatid exchange in Chinese hamster fibroblasts, suggesting that superoxide production is responsible for the chromosomal damage (Nicotera et al., 1985). Other investigators (Anderson et al., 1972; Levin et al., 1982) have not found mutagenic activity in bacterial test systems. Furthermore, paraquat was not mutagenic when evaluated in human leukocytes nor in in vivo cytogenic tests on mouse bone marrow (Selypes & Paldy, 1978) or in dominant lethal tests on mice (Pasi et al., 1974; Anderson et al., 1976).

A set of recently completed studies indicate that in most tests paraquat was not mutagenic (see Table 5). Clastogenic potential has been shown in vitro at very high concentration levels which were themselves cytotoxic. Paraquat was not found to be mutagenic in vivo.

Table 5. **Mutagenicity assays on paraquat**

Test system	Test object	Concentration used	Purity (%)	Results	Reference
Ames test[1]	S. typhimurium TA98 TA100 TA1535 TA1538	0.12, 0.6, 2.9, 14, 72, 361, & 1807 µg/plate (dissolved in H_2O)	99	Negative	Anderson, 1977
Ames test[1]	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	0.4, 0.7, 3.6, 7, 36, 72, & 360 µg/plate (dissolved in H_2O)	100	Negative, growth inhibition at 72 & 360 µg/plate[2]	Shirasu et al., 1978
Host-mediated assay	S. typhimurium G 46 (host: male ICR mice)	3.6 & 14 mg/kg (2 equal doses orally)	100	negative[2]	Shirasu et al., 1978
Rec-assay	B. subtilis	14 - 361 µg/disk	100	negative[2]	Shirasu et al., 1978
Mouse lymphoma test[1]	L 5178 Y mouse lymphoma cells	23, 45, 90, 180, & 361 µg/ml	99	?	Clay & Thomas, 1985
Clastogenic potential test[1]	Human lymphocytes in vitro	90, 903, & 1807 µg/ml	99.6	positive at 2 highest levels (also cytotoxic)	Sheldon et al., 1985a
Mouse micronucleus test	Male & female C 57/BL/6J/Alpk mice	51.7 & 82.8 mg/kg (single dose orally)	99.4	negative	Sheldon et al., 1985b
In vitro sister chromatid exchange[1]	Chinese hamster lung fibroblasts	0.9, 1.8, 9, 18, 90 & 177 µg/ml	99.4	positive (reduced with metabolic activation)	Howard et al., 1985
Unscheduled DNA synthesis	Hepatocyte cultures from male Alderley Park albino rats	1 nM - 10 mM (0.19 ng/ml - 1.86 mg/ml)	99.6	negative	Trueman et al., 1985

1 Both with and without metabolic activation
2 Positive control compounds gave positive responses

Special studies on reproduction

Rats

In a 3-generation study, groups of 15 male and 30 female (F_0 parents) weanling Alderley Park SPF rats were fed diets containing 0, 25, 75, or 150 ppm paraquat cation. After 12 weeks, animals were mated to produce the first (F_{1a}) litter and subsequently re-mated to produce a second (F_{1b}) litter. The breeding programme was repeated twice with F_1 parents selected from the F_{1b} offspring and F_2 parents selected from the F_{2b} offspring. Test diets were fed continuously throughout the study.

There were no adverse effects on parental body weights or food consumption, and no treatment-related changes were found in the reproductive performance (male and female fertility, live-born and survival indexes, and litter size) or in the reproductive tract of parents or offspring. Development of the reproductive tract in all treated offspring was substantially comparable to that in controls. The mild atrophy of seminal tubules found in a few of the treated males of the F_{2b} offspring at termination was considered by the authors of the study to have no toxicological significance. Lung changes due to paraquat administration occurred mainly in females receiving 150 ppm paraquat. One pulmonary adenoma was found in 1 female receiving 150 ppm paraquat. Death resulting from severe, acute, or sub-acute lung damage was confined to females with litters of weaning age, and to 3 F_0 females which died during the first 2 weeks of the study. There were dose-related increases in the incidence and severity of focal alveolar histiocytosis in the lungs of male and female parents receiving 75 and 150 ppm paraquat. A mild perivascular inflammation was observed in the lungs of F_{1b} pups receiving 150 ppm paraquat. No changes due to paraquat were seen in animals receiving 25 ppm paraquat. The authors concluded that paraquat had no effect on reproductive performance or development of the reproductive organs of Alderley Park rats when administered at dietary levels up to 150 ppm over 3 generations (Lindsay et al., 1982).

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

In a 3-generation study with 2 litters per generation, groups of 30 male and 30 female Sprague-Dawley rats (F_0 parental generation) were given diets containing 0, 72, 145, or 290 ppm paraquat cation from 5 weeks of age (13 weeks prior to mating to obtain the first litter, F_{1a}) until the end of the second lactation (lactation of F_{1b} litters). In the second generation (F_{1b}), 30 males and 30 females per group were treated from immediately after weaning until the end of the second lactation (lactation of F_{2b} litters). In the third generation (F_{2b}), the same number of rats were treated immediately after weaning for at least 13 weeks. In a teratology study sub-group 5 pregnant females of the parental generation (F_0) and 10 pregnant females of the second generation (F_{1b}) were killed on day 20 of pregnancy and examined macroscopically. Fetuses were examined for number, sex, weight,

external and internal abnormalities, and progress of ossification. Another subgroup was used as a postnatal investigation group, where natural parturition of pregnant females was permitted to occur. In this group the duration of gestation, parturition conditions, the number of live and still-born pups, sex, and external abnormalities were recorded. Live pups were investigated until weaning. Five male and 5 female rats per group of the first (F_0) and second (F_{1b}) generations and 10 male and 10 female rats per group of the F_{2b} litters were subjected to histopathological examination of approximately 25 tissues.

In the parental generation there were significant increases in mortality and clinical signs attributable to paraquat (asthmatoid wheezing) in several rats of the 290 ppm group of each generation from the early stage of the dosing period. There were 29 treatment-related deaths/moribund animals, all from the 290 ppm dietary level (5 among F_{1b} animals and 24 among F_{2b} animals), but only 4 deaths among control rats (1 among F_0 female rats and 2 among F_{1b} female rats at parturition and 1 among F_{2b} rats during the dosing period). Histopathological examination of these dead or moribund F_{2b} rats showed, in some cases, hyperplasia of the alveolar epithelium and, in most cases, diffuse thickening and fibrosis of the alveolar walls. There was a decrease in body-weight gain in both male and female rats of the F_0 and F_{2b} generations at 290 ppm during the early stage of the dosing period. Body-weight gain was also reduced in F_{1b} females at 290 ppm during the gestation and lactation periods. Reductions in food consumption and efficiency of food utilisation were seen in F_0 and F_{2b} females. There was a significant decrease in water consumption in F_0 and F_{1b} females during lactation. No effect of the compound was observed on the reproductive performance of parental rats. Macroscopic examination revealed an apparently higher incidence of white spots in the lungs of both male and female rats of the 290 ppm group in all 3 generations. Treatment-related changes of the lung were confirmed by histopathology in rats of each generation. These lesions were dose-dependent and included zonal thickening and fibrosis of the alveolar walls, zonal atelectasis, and accumulation of foam cells. There were no treatment-related changes in organ weights. A treatment-related statistically-significant reduction in the lactation index was found in F_{1a} and F_{1b} litters of the 290 ppm group. A statistically-significant reduction in the lactation index was also observed in F_{2a} litters of the 290 ppm group, but it was not clear to the authors of the report whether this change was attributable to treatment. There were no statistically-significant differences in lactation index between other treatment groups and controls nor in the number of still-births and live births, sex ratios, or viability indexes in any of the treated groups of both generations, when compared to controls. The prolonged duration of gestation in F_0 rats of the parental group at 145 and 290 ppm was considered by the authors of the report to be accidental. The teratology phase showed a statistically-significant delay in ossification in F_{1b} fetuses from F_0 parents treated with 290 ppm paraquat and in F_{2b} fetuses from F_{1b} parents of all treated groups. It was not clear to the authors of the report whether the retarded growth was due to the treatment. There was a treatment-related statistically-significant higher incidence of female pups with retarded opening of the vagina in both F_{1b} and F_{2b} litters at 290 ppm. There was a statistically-significant decrease in body weight in male, but not in female, fetuses at 72 and 290 ppm. There were no statistically-significant differences between fetuses from treated rats and control fetuses in the number of corpora lutea or implantations, percentage of implantations, number of dead or live fetuses, sex ratios, or placental weights. No external or internal malformations were detected in fetuses of any treatment group.

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

Rabbits

Forty rabbits received paraquat i.p. at total dosages of 2 to 100 mg/kg b.w. in 1 to 5 separate administrations. Multinuclear giant cells were found in testicular tubules of 7/20 rabbits receiving 50 mg/kg b.w. or more paraquat (Butler & Kleinerman, 1971). However, it has been reported that, when paraquat was orally administered at 4 mg/kg b.w. to male rats for 60 days and testes were examined, there were no significant deviations in the spermatozoa count or motility, nor were there any biochemical changes in the several enzymes of testes homogenates. The histoenzyme activity of lactate dehydrogenase, succinate dehydrogenase, DPN-diaphorase, alkaline phosphatase, and acid phosphatase in the treated animals did not differ from those of the controls, nor did quantitative and qualitative histological examination of the testicular tubule cells reveal any abnormalities (WHO, 1984).

Special studies on skin irritation

The effects of paraquat on the skin have been reviewed by WHO (1984). Paraquat can provoke local irritation of the skin and eyes. Clark et al. (1966) found skin irritation in rabbits only when paraquat was applied beneath occlusive dressings in aqueous solutions (total doses 1.56, 5.0, and 6.25 mg ion/kg b.w.). In mice and rats, the application of solutions of 5 - 20 g paraquat/litre in single and 21-day repeated dermal toxicity tests provoked dose-related toxic dermatitis with erythema, oedema, desquamation, and necrosis (Bainova, 1969). Doses from 1.56 to 50 mg/kg, in repeated 20-day studies using the occlusive technique (McElligott, 1972), resulted in local erythema and scab formation. The histological changes consisted of parakeratosis and occasional intra-epidermal pustules. A delayed skin-irritant action of the herbicide was reported by Fodri et al. (1977) in guinea pig studies.

Acute toxicity

The LD_{50} and LC_{50} values for paraquat in various species are given in Table 6.

Table 6. **Acute toxicity of paraquat in various species**

Species	Route	Sex	LD_{50} (mg/kg b.w.)	LC_{50} (mg/l)	Reference
Mouse	oral	M	260	--	Shirasu &
		F	210	--	Takahashi, 1977
	i.p.	M & F	29-30	--	Shirasu & Takahashi, 1977 Bus et al., 1975a
	i.v.	--	50	--	Ecker et al., 1975
	s.c.	M	30	--	Shirasu &
		F	27	--	Takahashi, 1977
	dermal	--	62	--	Bainova, 1971
Rat	oral	M	161	--	Shirasu &
		F	187	--	Takahashi, 1977
		M	110	--	Kimbrough &
		F	100	--	Gaines, 1970
		--	126	--	Murray & Gibson, 1972
		--	200	--	Howe & Wright, 1965
	i.p.	M	18	--	Shirasu &
		F	19	--	Takahashi, 1977
		F	19	--	Clark et al., 1966
	s.c.	M	19	--	Shirasu &
		F	23	--	Takahashi, 1977
		--	22	--	Makovskii, 1972
	dermal	M	90	--	Kimbrough &
		F	80	--	Gaines, 1970
		--	350	--	Makovskii, 1972
	inhal-ation	M & F	--	10	Bainova & Vulcheva, 1972
		--	--	1	Gage, 1968
		--	--	6	Makovskii, 1972

Species	Route	Sex	LD_{50} (mg/kg b.w.)	LC_{50} (mg/l)	Reference
Guinea pig	oral	M	30	--	Clark et al., 1966
		--	40-80	--	Howe & Wright, 1965
		--	22	--	Murray & Gibson, 1972
		--	42	--	Makovskii, 1972
	i.p.	F	3	--	Clark et al., 1966
	s.c.	--	5	--	Makovskii, 1972
	dermal	--	319	--	Makovskii, 1972
	inhal-ation	--	--	4	Makovskii, 1972
Cat	oral	F	35	--	Clark et al., 1966
		--	40-50	--	Howe & Wright, 1965
Hen	oral	--	300-380	--	Howe & Wright, 1965
		--	262	--	Clark et al., 1966
Turkey	oral	--	250-280	--	Smalley, 1973
	i.p.	--	100	--	Smalley, 1973
	i.v.	--	20	--	Smalley, 1973
	dermal	--	375	--	Smalley, 1973
Monkey	oral	--	50	--	Murray & Gibson, 1972
Sheep	oral	--	50-75	--	Howe & Wright, 1965
Cow	oral	--	50-75	--	Howe & Wright, 1965

Following a single high dose of paraquat to animals, the earliest ultrastructural changes were observed in the Type I alveolar epithelial cells, approximately 4 - 6 hours after treatment, and were usually characterised by cellular and mitochondrial swelling, increased numbers of mitochondria, and the appearance of dark granules in the cytoplasm. When a high dose was given (equal to approximately the LD_{50} or greater), the lesions in the Type I cells often progressed to the point of complete cellular disintegration, leaving areas of exposed basement membrane (Kimbrough & Gaines, 1970; Smith et al., 1973; Smith & Heath, 1974; Vijeyaratnam & Corrin, 1971; Klika et al., 1980). In contrast to the effects on Type I pneumocytes, however, the capillary endothelial cells were remarkably resistant to the toxic effects of paraquat (Sykes et al., 1977).

Ultrastructural lesions in the alveolar Type II pneumocytes were also observed shortly after single-dose paraquat exposure, although, generally, these lesions were not apparent until after the first lesions were seen in the Type I cells (Kimbrough & Gaines, 1970). Swollen mitochondria and damage to the lamellar bodies usually occurred between 8 and 24 hours after a high dose of paraquat (Robertson, 1973; Robertson et al., 1976). Progressive deterioration of the Type II cells continued, resulting in completely denuded alveolar basement membranes and debris-filled alveolar spaces (Vijeyaratnam & Corrin, 1971). Infiltration and proliferation of fibroblasts may produce fibrosis that obliterated the alveolar structure (Smith & Heath, 1974).

Vijeyaratnam & Corrin (1971) observed that less-severely affected parts of the lung appeared to undergo epithelial regeneration 7 - 14 days after a single dose of paraquat. Electron microscopic examination revealed the alveoli to be lined with cuboidal epithelial cells that closely resembled Type II pneumocytes, except for a general lack of lamellar bodies. Similar phenomena have also been noted by other investigators who administered paraquat in the diet (Kimbrough & Linder, 1973) or as repetitive i.p. administrations (Smith et al., 1974). Thus, in animals where the paraquat dose was sufficient to kill only the Type I pneumocytes, the surviving Type II cells repaired the damaged epithelium by proliferating and subsequently differentiating into Type I epithelial cells. Inhaled paraquat in aerosol produced initial necrosis of the epithelia and Type II pbeumocyte hyperplasia, fibroblast proliferation, and increased synthesis of collagen in mice (Popenoe, 1979).

The pathogenesis of the paraquat lung lesion has been well characterised, and has been reviewed by Smith & Heath (1976). The acute pulmonary toxicity of paraquat in animals has been described as occuring in 2 phases. In the initial "destructive" phase, alveolar epithelial cells were extensively damaged and their subsequent disintegration often resulted in a completely denuded alveolar basement membrane. Pulmonary oedema was also a characteristic of the destructive phase, and was frequently of sufficient severity to result in the death of the animals. Animals surviving the initial destructive phase, which occurred in the first 1 - 4 days after acute paraquat overexposure, progressed to what has been termed the "proliferative" phase. In this phase, the lung was infiltrated with prolifroblastic cells that rapidly differentiated into fibroblasts which, in some cases, progressed to fibrosis. The histopathological outcome of the second phase may be influenced by the treatment regimen, however. Administration of repeated low doses of paraquat, which less-severly damaged the alveolar epithelial cells, was also able to induce a hyperplasia of the Type II cells. This response may represent an attempt by the lung to repair the damaged epithelium (WHO, 1984).

When rabbits were injected i.p. with total doses of paraquat from 2 - 100 mg/kg b.w., thymic atrophy was observed, but most lungs showed only occasional and small histological deviations that were poorly correlated with the clinical signs of paraquat intoxication. These results confirmed the resistance of the rabbit to paraquat-induced lung lesions (Butler & Kleinerman, 1971).

According to Murray & Gibson (1972) and Hundsdorfer & Rose (1980), guinea pigs treated with paraquat either orally or s.c. did not develop the same type of progressive pulmonary fibrosis as paraquat-intoxicated rats. In hamsters, a single administration of paraquat did not induce lung damage, but prolonged exposure resulted in lung fibrosis (Butler, 1975).

In conclusion, from lung toxicity studies, a characteristic dose-related pulmonary fibrosis can be induced in rats, mice, dogs, and monkeys, but not in rabbits, guinea pigs, or hamsters.

In paraquat toxicity, kidney damage often precedes signs of respiratory distress (Clark et al., 1966; Butler & Kleinerman, 1971; Murray & Gibson, 1972). Paraquat is excreted primarily via the urine and the concentrations of the herbicide in the kidneys are relatively high (see Table 1). Gross pathological and histological examination of paraquat-poisoned rats, guinea pigs, rabbits, and dogs revealed vacuolation of the convoluted renal tubules and proximal tubular necrosis (Murray & Gibson, 1972). The nephrotoxicity caused by paraquat is pronounced and appears to be restricted to the proximal nephron (Ecker et al., 1975; Gibson & Cagen, 1977; Lock & Ishmael, 1979; Purser & Rose, 1979). The degeneration of the proximal tubular cells has also been confirmed by electron-optical studies (Fowler & Brooks, 1971; Marek et al., 1981).

In contrast with lung and kidney damage, liver damage in experimental animals has not been severe and serum enzyme activities (SGOT, SGPT, LAP) only increased when large amounts of paraquat were given (Giri et al., 1979). Recently, electron microscopic examination of the liver of paraquat-treated rats showed early, localised changes (degranualtion of the RER, proliferation of the SER, and mitochondrial swelling) in hepatocytes within 2 layers around the central vein (Matsumori et al., 1984).

Short-term studies

Mice

Groups of 20 male and 20 female ICR-CRJ SPF mice were maintained on diets containing 0, 7.2, 22, 72, or 217 ppm paraquat cation for 13 weeks. At the 217 ppm dietary level 2 female mice died from pulmonary damage. Both males and females in this group showed significantly reduced body-weight gain and a slight reduction in efficiency of food utilisation. Food intake and water intake were not affected. No abnormalities considered related to paraquat treatment were seen during haematological, blood biochemistry, or urine analysis. A few statistically-significant changes in absolute and relative organ weights were seen at termination, mainly in males and females of the 217 ppm group. However, only an increase in lung weight of females in the 217 ppm group was reported by the authors of the study to coincide with histopathological changes of the same organ, namely eosinophilic swelling of the alveolar epithelium walls which was observed in both sexes at this dietary level. The no-effect level in this study with respect to pulmonary damage and other parameters was 72 ppm, equal to 12 (males) and 14 (females) mg/kg b.w./day (Maita et al., 1980a).

Rats

Groups of 20 male and 20 female Fischer 344 rats were maintained on diets containing 0, 7.2, 22, 72, or 217 ppm paraquat cation for 13 weeks. During the study there were no deaths. Body-weight gain was reduced markedly in both sexes at the 217 ppm dietary level, at which level food consumption, efficiency of food utilization, and water consumption were also reduced. Histopathological examination revealed swelling of alveolar epithelium cells in males and increased deposits of brown pigment in the spleen of females at the 217 ppm dietary inclusing level. No abnormalities considered attributable to paraquat administration were observed during heamatological, blood biochemistry, urine, organ weight, or gross necropsy investigations. The no-effect level in this study with respect to lung lesions and other parameters was 72 ppm, equal to 6.5 (males) and 7.1 (females) mg/kg b.w./day (Maita et al., 1980b).

Dogs

Groups of beagle dogs, 3 males and 3 females per group, received diets containing 0, 7, 20, 60, or 120 ppm paraquat cation for 13 weeks. Two males and 2 females in the 120 ppm group showed marked paraquat toxicity and were killed in extremis between days 16 and 23, having shown marked dyspnoea and body-weight loss. Both surviving dogs at 120 ppm also showed body-weight loss. A slight overall reduction in body-weight gain among the females of the other treatment groups was not considered by the authors of the study to be treatment-related. Lung weights were increased in all animals in the 120 ppm group and in 2 animals from the 60 ppm group. All other organ weights were in the normal range. Distinct gross and histological treatment-related lung lesions were seen in all dogs in the 60 and 120 ppm groups. Minor renal lesions (swelling of the cortical tubules) were also found histologically in a few of these animals. There were no discernible gross or histological treatment-related pulmonary lesions in the dogs of the 7 or 20 ppm groups. The focal pulmonary lesions in these animals were of a type and incidence similar to those found in the controls. Microscopic examination of 34 other tissues from each animal showed no treatment-related changes. There were no treatment-related effects on food intake except for 1 surviving high-dose female which showed a loss of appetite from week 8 onward. There were no distinct treatment-related changes in any of the haematological (RBC counts, mean cell volume, haemoglobin, total and differential white cell counts, platelets, prothrombin, and partial thromboplastin time), biochemical (glucose, cholesterol, blood urea nitrogen, bilirubin, total and partial protein, GOT, GPT, ALP, and CPK), or urinary parameters examined. Slight haemoconcentration was seen in 1 high-dose dog at termination. The no-effect level in this study after administration of paraquat for 13 weeks to beagle dogs, on the basis of lung and kidney lesions, was considered to be 20 ppm, equal to 0.57 mg/kg b.w./day (Sheppard, 1981).

Long-term studies

See also under "Special studies on carcinogenicity".

Mice

Groups of 60 male and 60 female JCL:ICR mice were maintained on diets containing 0, 1.4, 7.2, 22, or 72 ppm paraquat cation for 104 weeks and then killed and examined. Further groups of 10 males and 10 females receiving the same diets were sacrificed after 26 weeks or 52 weeks of treatment. Animals

in each group, including the control groups, showed approximately 60 - 70% mortality at the end of the study. Haematological changes, attributed by the authors of the study to the administration of paraquat, included reduced erythrocytes, hematocrit, and haemoglobin at the 72 ppm level in both sexes. Total serum protein was decreased and blood glucose increased at the 72 ppm dietary level in both sexes. Various tumours, mostly malignant, were obseved in all groups in this study, the main types being lung adenocarcinoma in males and leukaemia in females. However, no tumour type showed a higher incidence in the treated groups than in controls and no correlations between tumour incidence and concentration of the test substance were observed. No effects attributable to paraquat were observed in absolute or relative organ weights, urinalysis, body-weight gain, food consumption, efficiency of food utilization, or water intake. Based on the haematological and blood biochemistry changes observed, the no-effect level for paraquat after 104 weeks of administration to JCL:ICR mice in this study was 22 ppm (as paraquat cation), equal to 2.8 mg/kg b.w./day (Toyoshima et al., 1982a).

Rats

Groups of 50 male and 50 female JCL:Wistar rats were maintained on diets containing 0, 4.3, 22, 72, or 217 ppm paraquat cation for 104 weeks and then killed and examined. Further groups of 6 males and 6 females receiving the same diets were sacrificed after 26 or 52 weeks of treatment. There was 38 - 66% mortality in all groups at the end of the study. The distribution of mortality and of abnormalities were not signficantly affected by treatment. Females in the 217 ppm group showed a transient tendency to lower body-weight gain, compared to controls, at weeks 34, 42 - 48, and 54. Food consumption, efficiency of food utilization, and water consumption were not affected by paraquat administration. Haematological changes attributed by the authors of the study to paraquat administration at the 217 ppm level included reduced erythrocytes and haemoglobin in both sexes and decreased haematocrits and increased reticulocytes in males after 26 weeks. Total serum protein was slightly but constantly decreased at the 217 ppm level in both sexes.

During histopathological examination of approximately 20 tissues, various tumours were observed in this study in all groups, the main types being benign pituitary tumours in males and benign mammary tumours in females. However, none of the tumours were present at a significantly-higher incidence in the treated groups than in controls. Body weight, food consumption, food efficiency, water intake, leucocyte counts, platelet counts, prothrombin time, GOT, GPT, alkaline phosphatase activity, blood glucose, blood urea nitrogen, cholesterol, Na^+, K^+, Cl^-, creatine, and brain, serum, and corpuscular cholinesterase activities, as well as ophthamological examination indicated no significant effects attributable to paraquat at any dose level. Based on the haematological and blood biochemistry changes the no-effect level of paraquat after 104 weeks of administration to JCL:Wister rats in this study was 72 ppm (as paraquat cation) equal to 3.0 (males) and 3.7 (females) mg/kg b.w./day (Toyoshima et al., 1982b).

Dogs

Groups of 6 male and 6 female beagle dogs received diets containing 0, 15, 30, or 50 ppm paraquat cation for 1 year. During the study there were no deaths. No effects due to paraquat were observed on body weight. The reduced food consumption of 1 male and 1 female dog, both in the 50 ppm group, was considered by the authors of the study to be treatment-related. There was clinical evidence of respiratory dysfunction (hyperpnoea) in some dogs fed

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

Observations in humans

Information on the effects of paraquat in humans has been obtained from occupational exposure studies (epidemiological data and case reports), descriptions of accidental or suicidal poisonings, and volunteer studies. These data have been extensively reviewed by WHO (1984).

In 1965, a study was carried out on a team of 6 sprayers, and in 1967 on 4 teams in Malaysian rubber plantations, to estimate the efficacy of individual protective measures. The operators used a spray dilution containing paraquat at 0.5 g/litre for 12 weeks. Attention was paid to personal hygiene. Each man was given a thorough physical examination, and urine samples were taken before spraying began and at weekly intervals throughout the study. Chest X-rays were taken before the study started and at the end of the 6th and 12th weeks. In the 2 studies, a total of 528 urine samples were examined. Paraquat was found on 131 occasions (78/134 and 53/394 in the 2 studies, respectively), the maximum concentration detected being 0.32 mg/litre in the first study and 0.15 mg/litre in the second. Average urine levels of paraquat of 0.04 mg/litre were found in the 1965 study and of 0.006 mg/litre in the 1967 study. After spraying ceased, these levels declined steadily to become undetectable within a week, with 1 exception. Both trials showed that about half of the men had suffered mild irritation of the skin and eyes, but had recovered rapidly with treatment. Two cases of scrotal dermatitis occurred in workers wearing trousers that were continuously soaked by the spray solution. There were also 2 cases of epistaxis. All chest radiographs were normal (Swan, 1969).

Studies performed over a period of several years on 296 Trinidad sugar estate workers drew attention to nail damage that ranged in severity from localized discoloration to nail loss following gross contamination with paraquat at 1 - 2 g/litre. The typical distribution of the lesions, affecting the index, middle, and ring fingers of the working hand, suggested that they had occurred through leakage from the knapsack sprayer and inadequate personal hygiene. Apart from 2 cases of contact dermatitis of the hands, no skin, eye, or nose irritation was reported, nor were there any systemic effects (Hearn & Keir, 1971).

Similar data were obtained on several groups of workers spraying paraquat as an herbicide and dessicant in cotton fields during the hot season. These workers were exposed to paraquat aerosol concentrations of 0.13 - 0.55 mg/m^3 air. Dermal exposure was low, not more than 0.05 - 0.08 mg paraquat on the hands and face. There were no complaints, nor did the clinical and laboratory examinations of the workers demonstrate any significant deviations from the matched control groups (Makovskii, 1972).

In the USA, the exposure of field workers operating tractor-mounted spray equipment in orchards was determined. About 4.6 litres of paraquat liquid concentrate (291 g/litre) was used in 935 litres of water per hour. In addition, exposure from yard and garden applications were studied in volunteers using pressurized hand dispensers containing paraquat solution (4.4 g/litre). Dermal contamination was measured by adsorbent cellulose pads attached to the worker's body or clothing, and by hand-rinsing in water in a polyethylene bag. Special filter pads were used in the filter cartridges of the respirators worn by the subjects under study. In all, 230 dermal and respiratory exposure pads, 95 samples of hand-rinse water, and 130 urine samples, collected during and following spraying, were analysed, which involved 35 different paraquat application situations. The exposure of field workers was found to range from about 0.40 mg/hour (dermal) to less than 0.001 mg/hour (inhalation). As for individuals spraying yards or gardens, exposure ranged from 0.29 mg/hour (dermal) to less than 0.001 mg/hour (inhalation). In almost all cases dermal exposure affected the hands. The respiratory paraquat values were generally below the sensitivity levels of the analytical method. No detectable paraquat concentrations were found in the urine samples (lower limit 0.02 mg/litre). This study confirmed the general safety of paraquat under correct conditions of use (Staiff et al., 1975).

The potential long-term hazard associated with the use of paraquat has been studied by comparing the health conditions of 27 sprayers who had been exposed to paraquat for many months per year for an average of 5.3 years with those of 2 unexposed control groups consisting of 24 general workers and 23 factory workers. The workers were given full clinical examinations; lung, liver and kidney function tests were also carried out. There were a few skin lesions resulting from poor spraying techniques and 1 case of eye injury. There were no significant differences between exposed and control groups in any health parameters measured, which led the authors to suggest that the long-term use of paraquat is not associated with harmful effects on health (Howard et al., 1981).

To evaluate the effects of protective equipment on occupational human exposure to paraquat, a paraquat formulation (240 g/litre) diluted 300 times by volume with water was sprayed for 2 hours on weedy ground. During the spraying operations, the concentrations of paraquat aerosol were 11 - 33 $\mu g/m^3$ air. The total dermal exposure was about 0.22 mg. No irritation of the eyes or the skin was reported. The urine of the workers who wore gauze masks contained significant amounts of paraquat 24 hours after spraying. The urine of the workers who had worn a high-performance mask did not contain detectable levels of paraquat. The authors discussed the need for protective equipment to decrease skin contact with paraquat and to avoid aerosol inhalation (Kawai & Yoshida, 1981).

Quantitative estimates of dermal and respiratory exposure of 26 plantation workers in Malaysia have shown a mean dermal dose of 1.1 mg/kg b.w./hour. The highest individual total exposure was equivalent to 2.8 mg/kg b.w./hour; the mean respiratory exposure was 0.24 - 0.97 g/paraquat/m^3 air, which is 1% or less of a TLV of 0.1 mg/m^3 for respirable paraquat. Urine levels of paraquat were generally below 0.05 mg/litre (Chester & Woollen, 1982).

A study was carried out on a group of 14 sprayers in Thailand using conventional high-volume knapsack sprayers and low-volume spinning-disc applicators with paraquat ion concentrations of 1.5 g/litre and 20 g/litre, respectively. Irritation of unprotected skin was found, and this was severe (caustic burns on the feet) in workers using high spray conentrations and

spinning-disc applicators. Urinary paraquat levels ranging from 0.73 - 10.2 mg/litre after 14 days of spraying were detected in unprotected men using both concentrations, and there was evidence that urinary levels of paraquat increased as the trial progressed. No evidence of systemic toxicity was discovered among the sprayers undergoing clinical and radiographic examination 1 week after spraying ended. The author concluded that spray concentrations in hand-held equipment should not exceed 5 g paraquat ion/litre (Howard, 1982).

After tomato spraying in the USA, the total body exposure to paraquat was determined to be 169 mg/hour. The use of enclosed tractor cabs or a high-clearance tractor reduced total body exposures to 27 mg/hour or 18 mg/hour, respectively. The authors reported that the total body exposure of tractor sprayers working in 2 citrus locations was proportional to the tank concentrations (paraquat dilutions of 0.7 g/litre and 1.1 g/litre were applied). Exposure levels of 13 and 28 mg/hour were found for workers using the lower and high concentrations, respectively. In all situations studied, the respiratory exposure was consistently a small fraction ($< 0.1\%$) of the total body exposure, which was primarily through the skin (Wojeck et al., 1983).

Two groups of workers exposed to paraquat formulations were examined. The first group of 18 workers, in England, consisted of subjects exposed to dust and liquid paraquat formulations during a 37.5-hour working week, the mean length of exposure being 5 years. The second group also consisted of 18 males, from Malaysia, exposed to liquid concentrate formulations during a 42-hour working week, the mean length of exposure being 2.3 years. Partly protective clothing was worn. However, in Malaysia, no gloves, rubber aprons, or goggles were used. The medical records and the dermatological examinations revealed acute skin rashes, nail damage, epistaxis, blepharitis, and delayed wound healing in 12 - 66% of these workers. Delayed caustic effects were often found among the Malaysian formulation workers, where low levels of safety and hygiene were apparent. Clincal examination did not reveal any evidence of chronic contact dermatitis, hyperkeratosis, or eczematous lesions (Howard, 1979).

Some studies designed to estimate dermal and inhalation exposure to paraquat are summarized in Table 7. From the data reported it can be seen that:

(a) the main route of exposure of agricultural workers to paraquat is via the skin; respiratory exposure is negligible.

(b) The worst case of exposure (of those examined) was via knapsack spraying).

From 1956 - 1973, no deaths attributable to paraquat were registered among agricultural workers in the USA, but in 1974 4 fatal cases were associated with this herbicide. However, it is not clear whether they were accidental, suicidal, or occupational (Hayes & Vaughan, 1977).

Fitzgerald et al. (1978a) summarized the clinical findings and pathological details concerning 13 accidents involving paraquat among agricultural workers, 6 of which were fatal. In 5 of these cases, swallowing was involved. Of the 6 fatalities studied, 3 swallowed Gramoxone (a 20% solution of paraquat dichloride in water) after sucking the outlet of a sprayer. In 1 non-fatal case, the man had sucked out a nozzle containing diluted paraquat, while in another case the man who had blown into the jet, to clear it, escaped with only minor signs of poisoning. The use of a leaking sprayer by another worker with severe extensive dermatitis probably resulted in fatal absorption of paraquat through the damaged skin.

Table 7. **Comparison of dermal and inhalation exposure to paraquat resulting from various methods of application**[1].

Method of application	Dermal exposure (mg/hour)	Respiratory exposure (mg/hour)	Reference
Hand-held knapsack	66 (12.1 - 169.8)	(0.45 - 1.3) x 10^{-3}	Chester & Woollen, 1982
Vehicle mounted	0.4 (0.1 - 3.4)	(0 - 2) x 10^{-3}	Staiff et al., 1975
Aerial			Chester & Ward, 1981
Flagman	0.1 - 2.4	(0 - 47) x 10^{-3}	
Pilot	0.5 - 0.1	(0 - 0.6) x 10^{-3}	
Mixer/loader	0.18	(1.3 - 1.5) x 10^{-3}	

1 From WHO, 1984

Several other cases of fatal poisoning resulting from dermal absorption of paraquat have been reported. Jaros (1978) has described how the use of concentrated solutions of paraquat (50 g/litre instead of 5 g/litre), with an old leaking knapsack sprayer, resulted in paraquat contamination of the neck, back, and legs of a worker. After 4 hours of work, the worker complained of a burning sensation on the neck and scrotum. On admission to a hospital 6 days later, cough and respiratory difficulties were recorded. Three days later the patient died of renal and respiratory failure.

Severe skin damage, followed by death due to respiratory insufficiency, occurred in a woman 8 weeks after initial contact with paraquat. The toxic dermatitis started with scratches on the arms and legs from the branches of fruit trees. The patient had often failed to wear protective clothing or to shower after spraying. During the 4 weeks preceding her first admission to the hospital, she developed ulcers and respiratory complaints combined with anorexia. Damaged and broken skin was thus exposed to paraquat. A chest X-ray and needle biopsy of the lung revealed pulmonary lesions. Seventeen days after discharge from hospital, without a specific diagnosis, she was re-admitted, and died 2 weeks later with progressive lung, hepatic, and renal dysfunction (Newhouse et al., 1978).

The clinical and pathomorphological investigation of a patient who died of hypoxia after repeated dermal exposure to paraquat (28 g/litre) and diquat (29 g/litre) in a water-oil dilution has been described recently. The worker had used a leaking sprayer. A characteristic ulcer developed at the site of paraquat contact. There was also lung damage (Levin et al., 1979).

Another reported fatal case of dermal poisoning with paraquat occurred after prolonged contact with a concentrated formulation following spillage from a bottle in the back trouser pocket (Waight & Wheather, 1979).

Wohlfahrt (1982) discussed the factors related to severe paraquat poisoning due to dermal absorption in tropical agriculture. Three fatal incidents followed skin contamination; 1 victim used paraquat to treat scabies infestation, and 1 used it to treat lice. In all cases, the skin was blistered and ulcerated. The patients died of progressive respiratory failure 4 - 7 days after the accidents. In 1 of these cases, the presence of mouth and throat ulceration strongly suggested that ingestion might also have occurred (Davies, 1982).

Local skin and nail effects of paraquat have been reviewed by WHO (1984). Brief contact with liquid formulations, as well as repeated exposure to dilute solutions, produced skin irritation, desquamation, and, finally, necrosis at the site of contact (Ongom et al., 1974; Binns, 1976; Newhouse et al., 1978; Waight & Wheather, 1979; Levin et al., 1979; Horiuchi et al., 1980). Harmful dermal effects have been reported among sprayers who worked without protective clothes and with naked feet (Howard, 1982). The blistering and ulceration of the skin were due to excessive contact and inadequate hygiene. Horiuchi and Ando (1980) carried out patch testing on 60 patients with contact dermatitis due to Gramoxone. In 8 patients (13.3%) positive allergic reactions were established. In another survey with 52 persons, a positive photo-patch response was reported in 11 patients. Nail damage may also occur after frequent exposure to paraquat concentrations during the formulation of the herbicide or the preparation of the working dilution (Howard, 1979).

There have been some reports (Malone et al., 1971; Mircev, 1976; Bismuth et al., 1982) of adverse effects as a result of inhalation exposure to paraquat. However, inhalation of droplets in normal paraquat spraying does not appear to represent a significant health hazard (Howard, 1980), and the effects of occupational inhalation have usually been limited to nose bleeds and nasal and throat irritation (Swan, 1969; Howard, 1979).

Ocular damage may result from splashes of concentrated paraquat that come into contact with the eye. Apart from irritation of the eye and blepharitis, a week later more serious ocular damage may occur, such as destruction of the bulbar and tarsal conjunctiva and of the corneal epithelium. Anterior uveitis, conjunctival necrosis, progressive keratitis with gross corneal opacity, and decreased visual acuity may also occur (WHO, 1984).

It has been noted that when the recommended dilution rates were correctly used, systemic effects of oral, inhalation, or dermal exposure to paraquat have not been observed. Skin and eye irritation have occurred only when protective measures were disregarded (WHO, 1984).

In a volunteer study the percutaneous absorption of ^{14}C-paraquat through the legs, hands, and forearms of 6 human subjects was studied. The total dose absorbed in 5 days after a single application of 0.64 mg paraquat dichloride was very low (0.3%) at all sites of application (Wester et al., 1984).

A large number of cases of accidental or suicidal poisoning have been reported since 1966, the earlier cases being mostly accidental which apparently resulted from the habit of decanting the liquid formulations into small unmarked or incorrectly labelled containers such as beer, wine, or soft-drink bottles. An increasing ratio of suicidal to accidental poisonings has been noted in recent years (Fitzgerald et al., 1978b; Bramley & Hart, 1983). This change from accidental to suicidal poisoning was considered to be reflected by enhanced percentages of fatal cases, shorter survival times, and higher tissue and body fluid levels (WHO, 1984). The vast majority of cases

of paraquat poisoning have been due to ingestion. A few cases of fatal or non-fatal poisonings have been reported following either skin contamination (McDonagh & Martin, 1970; Kimura et al., 1980) or skin application in order to kill body lice (Ongom et al., 1974; Binns, 1976). Symptoms of poisoning depend on the dose absorbed.

It is difficult to estimate the dose absorbed from case histories since in many cases the patients spat out part of the paraquat concentrate or vomited profusely after swallowing the herbicide. Some patients have survived after apparently ingesting 10 - 20 g paraquat, whereas some died after taking as little as 2.5 g paraquat. The probability of survival after paraquat poisoning can be predicted from plasma paraquat concentration at a given time after ingestion (Proudfoot et al., 1979; Hart et al., 1984). The minimum lethal dose of paraquat for human beings has been estimated to be about 35 mg/kg b.w. (Pederson et al., 1981; Bismuth et al., 1982). The common factor of most, if not all, cases of paraquat poisoning is damage to the lung. Cases of fatal paraquat poisoning have been divided into 2 broad categories:

a) Cases of acute fulminant poisoning due to massive amounts of paraquat absorbed, resulting in death within 1 to 5 days from ingestion. Death is due to multi-organ failure associated with damage to the lungs, kidneys, liver, brain, and adrenals.

b) Cases of poisoning due to ingestion or absorption of smaller doses of paraquat resulting in death 6 to several days later. In these cases death is due primarily to lung and kidney damage.

COMMENTS

A small amount of paraquat is rapidly absorbed by the gut of rats, guinea pigs, dogs, monkeys, and man, most of the oral dose being excreted as unchanged paraquat. Following administration by different routes to animals, paraquat is rapidly distributed in most tissues, the highest concentrations being found in the lungs and kidneys. The compound accumulates slowly in the lung owing to uptake by an energy-dependent process which is also responsible for the uptake of putrescine. Saturation kinetics for the uptake of paraquat by the lung have been shown to be similar in rats and man. Excretion of absorbed paraquat is biphasic, owing to lung accumulation, and occurs largely in the urine as unchanged paraquat, but also to a limited extent in the bile. Biotransformation of absorbed paraquat is, in general, remarkably poor in all species studied (rats, guinea pigs, dogs, hens, pigs, goats, and sheep) although there is some controversy as to the possibility and extent of its metabolism by the gut microflora. Metabolism occurs via demethylation (monomethyl dipyridone ion) or oxidation (paraquat pyridone ion and paraquat dipyridone ion).

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

Two teratogenicity studies, 1 in mice and 1 in rats, showed paraquat to be non-teratogenic at doses up to 10 mg/kg b.w./day. Slight maternal toxicity was observed in mice and high maternal toxicity and some fetal toxicity were observed in rats at 5 and 10 mg/kg b.w./day paraquat ion. In a teratology sub-group in a 3-generation reproduction study in rats, paraquat was not teratogenic, but delays in ossification were found at all 3 dose levels tested (72, 145, and 290 ppm paraquat cation).

In previous in vitro studies both positive and negative results were obtained, with mutagenicity usually associated with high cytotoxicity. Recent studies have shown paraquat to be non-mutagenic, both in the presence and absence of metabolic activation, when tested by the Ames test, mouse micronucleus test, unscheduled DNA synthesis assay, rec-assay, and host-mediated assay. A mouse lymphoma cell test was inconclusive. The herbicide was clastogenic to human lymphocytes in vitro at cytotoxic doses and induced sister chromatid exchange in Chinese hamster lung fibroblasts.

In 2 rat 3-generation reproduction studies paraquat had no effects on the reproductive performance or development of the reproductive organs of rats. In 1 of these studies a reduction of lactation index was observed at 290 ppm paraquat cation.

The acute oral toxicity of paraquat is higher in guinea pigs, monkeys, cattle, and man than in rats and birds. Confidence limits of LD_{50} values are small. There are no significant differences between the 2 sexes in the oral, s.c., or i.p. LD_{50} values of paraquat in rats or mice. Paraquat induces characteristic dose-related fibrotic changes in the lungs of mice, rats, dogs, and monkeys, but not in rabbits, guinea pigs, or hamsters. The acute pulmonary toxicity of paraquat in rats and man is biphasic. In the early, destructive phase, the alveolar epithelial cells are extensively damaged. Death may occur within a few days due to pulmonary oedema. In the later, proliferative phase, fibroblasts and collagen accumulate in the lungs of surviving animals and humans, possibly resulting in fibrosis.

In a short-term study in mice, treatment-related lung, body weight, and organ-weight changes were found. Another short-term study in rats indicated that paraquat was responsible for lung lesions and other minor changes. In a short-term study in dogs using 3 animals/sex/group, paraquat-dependent lung and kidney lesions were observed.

In a 1-year feeding study in dogs, paraquat caused lung changes at the 2 highest dietary levels; the no-effect level in this study was 15 ppm (as paraquat cation), equal to 0.45 mg/kg b.w./day in males and 0.48 mg/kg b.w./day in females.

In 2 long-term feeding studies, 1 in mice and 1 in rats, haematological and blood biochemical changes were observed in both species. These changes have not been reported previously and were considered to be of little toxicological significance.

A lifetime feeding study in mice showed no oncogenic potential for paraquat, although a slightly higher incidence of lung tumours was noted in animals of the high-dose group dying between 79 and 98 weeks of treatment when compared with control mice. Based on renal lesions observed in males, the no-effect level in this study was 12.5 ppm (as paraquat cation), equal to 1.4 mg/kg b.w./day.

A long-term feeding study in F344 rats indicated that paraquat administration for 104 weeks at 217 ppm was responsible for a significant increase in the incidence of pulmonary adenomas in females. This incidence (8.7%) was significantly higher than that observed in historical control animals of the same laboratory (2.2%). Paraquat was also cataractogenic in both male and female rats. Based on the lung and eye changes the no-effect level in this study was 22 ppm (as paraquat cation), equal to 0.77 mg/kg b.w./day.

In another long-term study in F344 rats paraquat induced proliferative benign lung lesions in females in the high-dose group, which were considered neoplastic by 1 pathologist and non-neoplastic by 2 others (the total incidence of pulmonary adenoma in females ranged from 0/70 to 8/70). The reasons for the discrepancy were unclear, owing to the lack in most cases of adequately detailed histopathological descriptions of the lung lesions. The historical incidence of lung adenoma in control female F344 rats in the laboratory in which the study was conducted was reported to be 1.1%, and the incidences reported in the literature are 1.4% for females and 1.9% or 0% for males. The results of these studies indicate a weak, organ-specific, sex-related potential of paraquat to produce benign proliferative changes in the lungs of female F344 rats.

The following observations were considered by the Joint Meeting:

a) paraquat was shown to be non-mutagenic in most in vitro and, apparently, all in vivo tests;

b) the lung is the target organ for both acute and chronic paraquat toxicity in rats;

c) only female F344 rats, but not male F344 rats, developed treatment-related benign neoplastic lesions;

d) proliferative changes were observed in rats and not in mice; and

e) paraquat selectively interferes with the uptake of polyamines by pneumocytes.

Polyamines are endogenous substrates playing an important role in cell division and growth. These observations suggest that the potential of paraquat to induce lung cell proliferation may be modulated by species-, sex-, and organ-dependent factors, such as hormonal activity, cell growth, defense and repair mechanisms, etc. No lung changes were observed at 24 ppm in any of the dose groups, and significant increases in the incidence of lung adenocarcinomas were not observed in any of the treated groups when compared to controls in these studies.

Observations in man, including reports on accidental or suicidal poisonings, confirm the type of acute and subacute toxicity observed in some experimental animals. Death occurred after oral and, in some cases, dermal absorption of high doses of paraquat. The organs primarily involved are the lung and kidney and, to a lower extent, the intestinal tract, liver, pancreas, adrenals, and CNS. The minimal lethal dose of paraquat in man is estimated to be about 35 mg/kg b.w. Skin, nail, and eye lesions were found in subjects with prolonged occupational exposure to paraquat. Significant paraquat concentrations (up to 10 mg/l) were detected in the urine of workers using high spray concentrations, but not in protected workers. The use of protective measures has proved to be effective in preventing lesions due to skin contact.

TOXICOLOGICAL EVALUATION

LEVEL CAUSING NO TOXICOLOGICAL EFFECT

Mice: 13 ppm (as paraquat cation), corresponding to 17 ppm (as paraquat dichloride) in the diet, equal to 1.9 mg/kg b.w./day for males and 38 ppm (as paraquat cation, corresponding to 52 ppm (as paraquat dichloride), equal to 5.9 mg/kg b.w./day for females.

Rats: a) 22 ppm (as paraquat cation), corresponding to 30 ppm (as paraquat dichloride) in the diet, equal to 1.1 mg/kg b.w./day for males and 1.2 mg/kg b.w./day for females (cataract).

b) 25 ppm (as paraquat cation), corresponding to 35 ppm (as paraquat dichloride) in the diet, equal to 1.4 mg/kg b.w./day for males and 1.7 mg/kg b.w./day for females (proliferative lung changes).

Dogs: 15 ppm (as paraquat cation), corresponding to 20 ppm (as paraquat dichloride) in the diet, equal to 0.62 mg/kg b.w./day for males and 0.66 mg/kg b.w./day for females.

ESTIMATE OF ACCEPTABLE DAILY INTAKE FOR MAN

0 - 0.004 mg/kg b.w. as paraquat cation (0 - 0.006 mg/kg b.w. expressed as paraquat dichloride).

STUDIES WHICH WILL PROVIDE INFORMATION VALUABLE FOR THE CONTINUED EVALUATION OF THE COMPOUND

1. Further observations in humans.
2. Studies on the mechanism of paraquat-induced proliferative lung changes.

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