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THE EVALUATION OF THE TOXIC EFFECT OF PARAQUAT AND ITS MECHANISM OF ACTION ON REPRODUCTIVE SYSTEM OF MALE RATS

By

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Thesis submitted in fulfillment of the requirements
For the degree of
Master of Science

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been acknowledged.

27th May 2007

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P-UM 1107

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THE EVALUATION OF THE TOXIC EFFECT OF PARAQUAT AND ITS MECHANISM OF ACTION ON REPRODUCTIVE SYSTEM OF MALE RATS

ABSTRACT

Paraquat® - PQ (1,1' – dimethyl 4,4' – bipyridillium dichloride) is a non-selective contact herbicide. Its herbicidal and toxicological properties are dependent on the ability of the parent cation to undergo a single electron addition to form a free radical. The present study was conducted to evaluate the toxic effect of low dose of PQ and its mechanism of action on the reproductive system of adult male rats. Two routes of administration were selected; oral and dermal which are the most common exposure routes in human being. Groups of six male Sprague-Dawley rats (n=6) were orally gavaged in selected dose of 2mg/kg, 5mg/kg and 20mg/kg (1/80, 1/32 and 1/8 of oral LD_{50}) and dermally applied in selected dose of 6mg/kg, 15mg/kg and 30mg/kg (1/15, 1/6, and 1/3 of dermal LD_{50}) for five consecutive days separately and scheduled for 7, 28, 42, 84 and 105 days for oral, and 7, 14, 28 and 42 days for dermal applications in separate groups after the last dose. Body weight did not show any change in the treated groups compared to the control. Reproductive organ weight (testis, epididymis, seminal vesicles and prostate), seminiferous tubular diameter (STD), seminiferous epithelial height (SEH) and epididymal epithelial height (EEH) decreased significantly ($p<0.05$) in all treated groups except in 2mg/kg dose. STD was significantly decreased only in 20mg/kg dose following 7, 28 and 42 days oral treatment and in 15 and 30mg/kg dermal dose following 7 and 14 days. A significant decline ($p<0.05$) in SEH and EEH were observed in most of the groups. Epithelial

sloughing, number of degenerated cells as well as loss of cell integrity in the seminiferous tubules of the testis was observed, except in 2mg/kg treatment group. Number of spermatogonia, spermatocytes, spermatids, ‘dividing cells’ and Leydig cells were decreased on exposure to PQ ($p<0.05$). Increase in sperm mortality and abnormal sperm morphology was significant ($p<0.05$). The hormones testosterone, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and prolactin were decreased significantly in most of the doses as well as testicular marker enzyme lactate dehydrogenase (LDH) were increased significantly ($p<0.05$). But exposure to paraquat did not change the level of Acid Phosphatase (ACP) in oral treated rats. In conclusions, PQ is a toxic substance both by dermal and oral route affecting the male reproductive function on exposure to a short duration of five consecutive treatment. Most of the parameters exhibited a transient change with an initial response such as decline in hormone level, increase in LDH level and decreased STD, SEH and member of different cell population of spermatogenic cycle. Based on our findings we propose that PQ is toxic to male reproductive functions both by oral and dermal route of exposures. Our hypothesis, for the possible mechanism of PQ toxicity in male reproductive system is by inducing a derangement in pituitary-gonadal-hormone synthesis/secretion, and elevation of testicular marker enzyme-LDH indicative of cell damage/destruction/death and also due to the direct toxicity of free radicals generated as reported earlier by other researchers.

PENILAIAN KESAN TOKSIK PARAQUAT DAN MEKANISMA TINDAKANNYA KE ATAS SISTEM PEMBIAKAN TIKUS JANTAN

ABSTRAK

Paraquat® (1,1' – dimetil 4,4' – bipiridilium diklorida) merupakan sejenis racun herbisid bukan selektif. Kesan herbisid dan toksikologinya bergantung kepada keupayaan kompoun utamanya untuk mengubah kepada elekron tunggal dan membentuk radikal bebas. Kajian ini dijalankan untuk menilai kesan toksik paraquat dan mekanisma tindakannya ke atas sistem reproduktif tikus jantan dewasa. Sebagai sebahagian daripada penilaian kesan herbisid terhadap sistem reproduktif dan juga melalui beberapa kes pendedahannya terhadap manusia, dua pendedahan dipilih, iaitu pendedahan secara oral dan kulit. Kumpulan enam ekor tikus Sprague-Dawley bagi satu kumpulan (n=6) telah diberi oral dalam dos dedahan sebanyak 2mg/kg, 5mg/kg dan 20mg/kg (1/80, 1/32 dan 1/8 dari LD_{50}) dan secara dedahan kulit dalam dos dedahan sebanyak 6mg/kg, 15mg/kg dan 30mg/kg (1/15, 1/6 dan 1/3 dari LD_{50}) selama 5 hari berturutan bagi jangkamasa 7, 28, 42, 84 dan 105 hari bagi dedahan oral dan 7, 14, 28 dan 42 dari bagi dedahan kulit. Berat badan tikus tidak menunjukkan sebarang perubahan berbanding kumpulan tikus kawalan. Berat organ reproduktif (testis, epididimis, vesikel semen dan prostat), STD, SEH dan EEH menunjukkan penurunan yang signifikan ($p<0.05$) didalam semua kumpulan rawatan kecuali kumpulan 2mg/kg. Didapati STD menurun secara signifikan didalam kumpulan 20mg/kg pada 7, 28 dan 42 hari dedahan oral dan juga didalam kumpulan 15mg/kg dan 30mg/kg pada hari 7

dan 14 hari dedahan dermal. Penurunan EEH & SEH yang signifikan ($p<0.05$) didapati dalam kesemua kumpulan rawatan. Kerosakan epitelium, peningkatan bilangan sel degenerasi selain daripada kehilangan integriti tubul seminiferous didapati pada semua kumpulan rawatan kecuali pada kumpulan dedahan 2mg/kg. Bilangan spermatogonia, spermatosit, spermatid, sel membahagi, dan sel Leydig didapati menurun didalam semua kumpulan rawatan PQ ($p<0.05$). Hormon testosteron, FSH, LH dan prolaktin didapati menurun secara signifikan pada kesemua kumpulan rawatan selain daripada peningkatan aras enzim Laktat dehidrogenase ($p<0.05$). Kesimpulannya, PQ merupakan bahan toksik bagi kedua-dua dedahan dermal dan oral dan juga kesannya terhadap fungsi sistem reproduktif pada jangkamasa pendek selama 5 hari dedahan. Kebanyakkan parameter menunjukkan perubahan yang mendadak seperti penurunan aras hormon, peningkatan aras LDH, penurunan STD, SEH dan juga bilangan populasi sel didalam kitaran spermatogenesis. Berdasarkan penemuan ini, mencadangkan bahawa PQ merupakan bahan toksik bagi dedahan oral dan juga dermal. Hipotesis kesan toksik ialah mekanisma toksik PQ terhadap fungsi sistem reproduktif ialah melalui gangguan pada sistem pituitari-gonadal-hormon, peningkatan aras penanda LDH terhadap kerosakan sel dan juga kesan langsung radikal bebas sepertimana dilaporkan pada kajian terawal.

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ABBREVIATIONS

ABP	-	Androgen binding protein
ACP	-	Acid Phosphatase
ALP	-	Alkaline Phosphatase
ALT	-	Alanine Aminotransferase
AST	-	Aspartate Aminotranferase
EEH	-	Epididymes Epithelial Height
EPA	-	Environmental Protection Agency
FP	-	Fluorescent pigmentation
FSH	-	Follicle Stimulating Hormone
G6PDH	-	Glucose-6-phosphate dehydrogenase
GSH	-	Glutathione
i.p.	-	intraperitoneal
IPCS	-	International Programme on Chemical Safety
IRMA	-	Immunoradiometricassay
LDH	-	Lactate dehydrogenase
LH	-	Luteinizing Hormone
MDA	-	malonialdehyde
Mg/kg	-	milligram per kilogram
NIOSH	-	National Institute of Occupational Safety and Health
OSHA	-	Occupational Safety and Health Administration
PQ	-	Paraquat

RIA	-	Radioimmunoassay
ROS	-	Reactive Oxygen Species
SDH	-	Sorbitol dehydrogenase
SEH	-	Seminiferous Epithelial Height
STD	-	Seminiferous Tubule Diameter
TDS	-	Testicular Dysgenesis Syndrome

CHAPTER 1

INTRODUCTION

General

Modern technology, including the use of pesticides to control insects, weeds, and disease-inducing agents, enables food production to support the world population of nearly 6.5 billion. Without the use of pesticides, food production would be further reduced and the number of individuals suffering from malnutrition would increase.

Since the early development of agricultural practices, people have always sought different ways to increase their crop yield. The early use of pesticides included a variety of substances, such as urine, lime, soap, vinegar, tobacco, and similar simple compounds. Agrochemical production began as a relatively simple process, based primarily on combinations of a few chemical substances such as copper, mercury salts, elemental sulphur, arsenic, and cyanide (BMA 1992).

The development of highly complex, chemical methods of pest control started around World War II, with the introduction of the first synthetic organochlorine (OC) insecticides, which included DDT, lindane (HCH), aldrin and dieldrin. Thousands of different pesticides manufactured today fall roughly into the following chemical categories; organochlorines, halogenated hydrocarbons, carbamates, heterocyclic compounds, organophosphates, chlorinated phenoxy substances, amines and ureas, benzonitrics, phenolic compounds and pyrethroids. They consist of a mixture of active ingredients; designed to destroy the pests, together with many other chemical additives, such as solvents, combined into usable products.

The growth of the agrochemical industry since World War II has been enormous, and now covers the globe. It is estimated that the industry worldwide produces about 45-50,000 different pesticides based on about 600 active ingredients. In one year alone 23,504 tonnes of active ingredients were sold by UK pesticide manufacturers, which amounts to nearly 420g for a person in the UK population (Robbins 1991). The actual sales of pesticides by UK manufacturers increased from £30 million a year from the late 1940s to £150 million in the mid-1970s. In 1985, total sales of pesticides amounted to almost £900 million, of which approximately 60% was accounted for export purposes (BAA 1989; Robbins 1991; BMA 1992). Pesticide manufacture is dominated by a few large chemical companies worldwide, including Ciba Geigy, Bayer and ICI. Many of these companies also have interests in other chemical productions, including the manufacture of pharmaceuticals (Brian 1988; BMA 1992).

The number of people who produce food has changed dramatically during the past 200 years. In 1787, 90 percent of the U.S. population lived on the farm and produced enough food for themselves and one other individual. By 1950, the percentage of individuals who lived on the farm had decreased to 16 percent, but those individuals produced enough food for themselves and 27 others. In 1990, only 2 percent of the U.S. population remained on the farm, but produced food for 120 people, in addition to themselves. Ninety-five of these 120 people live in the United States and the other 25 live overseas (Anonymous 1991).

Mechanization and technological advances of the early 1900's created a need for employees in factories and at manufacturing sites. High wages, combined with less physical labor, attracted laborers from the farm to the factory. Fewer and fewer laborers remained on the farm to plant, cultivate, and harvest crops. Fortunately, along with this decrease in labor came farm mechanization, which replaced horses with tractors and cultivators, and herbicides replaced hoes for weed control. These advancements enabled farmers to grow and manage more acres of crops with the reduced labor force.

Since pesticides play an important role in every day of life, their unwanted toxicities are of much concern as there are a number of diseases affecting humankind. Since fertility is one of the important aspects to carry forward the progeny, it is important to study any toxic effects affecting the fertility adversely.

In spite of gaps in our knowledge of reproductive vulnerability to xenobiotics across species, data and theoretical approaches are not available that would allow more rational prediction of human reproductive and developmental risk. Conducting physiologically based toxicological studies are essential to understand the exact mechanism of action of chemical compounds especially pesticides. A more rational approach for risk assessment would be to determine the physiologic, pharmacologic, toxicologic, cellular, and molecular characteristics that control reproductive and developmental toxicity in experimental models and translate that information into predicted human risk based on human characteristics that are similar.

Besides that, most of the farming population is directly exposed to pesticides, and as the fertility index by means of sperm count declining throughout the world, a probable cause may be the usage of these chemicals. This study helps to explore the possibilities of any toxic effects of PQ exposed either by oral or dermal route in very low concentration on male reproductive system and further, extrapolation of the same on human model may yield a supportive evidence. Usage of this chemical eventhough known to be fatal, low doses of PQ in environment may have adverse effect on reproductive system and may become a major threat to humankind affecting their reproductive ability. Eventhough there are few reports of PQ on male reproductive system, a thorough systematic investigation with low doses by oral and dermal route exposure were found to be scanty. Systematic exploration with different dose and time levels by dermal and oral route

exposure is importance since the use of PQ in agriculture is still in practice and exposure by means of oral and dermal routes are common. Hence the objectives of the present study were:

To elucidate the possible adverse toxic effects of low doses of PQ on different aspects of male reproductive system. More specifically we have aimed to investigate the toxic effect of PQ by means of oral and dermal routes on:

1. Body weight, reproductive organ weights – testes, epididymis, seminal vesicles and prostate.
2. Histopathological evaluation of the testes and epididymis – Qualitative changes like integrity of seminiferous epithelium, sloughing of epithelial cells, atrophy of germ cells if any. Quantitative estimation of seminiferous tubular diameter (STD), seminiferous epithelial height (SEH), epididymal epithelial height (EEH), count of Leydig cell number, count of spermatogonium, spermatocytes, spermatids and dividing cells.
3. Sperm analysis – Epididymal sperm count, sperm morphology, sperm motility and sperm mortality.
4. Estimation of testicular marker enzymes – serum levels of Acid Phosphatase (ACP) and Lactate dehydrogenase (LDH).
5. Estimation of hormones – serum levels of Follicle-Stimulating Hormone (FSH), Letuinizing Hormone (LH), Prolactin and Testosterone.

CHAPTER 2

REVIEW OF LITERATURE

2.1 General

Pests cause major problems and discomfort. Unfortunately, pests also invade fields and attack the crops cultivated for food and fiber. Large acreages of the same crops or concentrations of food-producing animals in a limited area favor buildup of insects, diseases, and certain weeds that must be controlled if usable food and fiber products are to be produced. For example, there are 80,000 to 100,000 diseases, 3,000 species of nematodes, 10,000 species of insects, and 1,800 species of weeds that damage the crop production process (Chambers 1992). Current estimates are that insects, diseases, and weeds destroy approximately one-third of the world food supply, even with the use of the most current pest management technology. Losses without this technology could soar to 60 to 80 percent.

For many years, pesticides have been used in conjunction with host plant resistance, cultural, mechanical, and biological tactics in an integrated pest management system to combat the battle against destructive pests. *The American Heritage Dictionary* (Davies 1979) defines pesticide as "a chemical that is used to control pests, especially insects, weeds and rodents". Pesticides may be naturally occurring substances or synthetic (man-made).

Pesticides can be categorized into various classes (Table 1) such as algicide, fungicides, herbicides, nematocides, insecticides, acarides and rodenticides.

2.2 The Role of Pesticides in Agriculture

The agricultural industry's use of pesticides amounts to approximately 83% of the whole pesticide manufacture (Robbins 1991). It utilizes pesticides in many ways. They are used during the crop growth as insecticides, herbicides and fungicides. It has been estimated that cereal crops receive approximately five to eight pesticide applications per growing season, while for high value crops, such as some vegetables and fruit, 10-15 applications are often the norm (Watterson 1989). After harvesting, during storage, most cereal, fruit and vegetable crops are dosed again with several pesticides to protect them from any storage diseases.

Consequently, even though the actual harvest may have been relatively uncontaminated with pesticides, this casual form of post-harvest storage treatment can add a considerable amount of pesticide residues to the finished product. Thus, pesticide residue levels on stored products can accumulate, as well as vary considerably from patch to patch (MAFF 1989). Pesticides are also used during livestock production, when they are either applied as 'animal medicines' such as sheep dips, lice/mange treatments, or as other 'veterinary pesticides' for controlling flies and other insects in livestock houses (Robbins 1991; BMA 1992).

2.3 General Properties of Paraquat (PQ)

Paraquat (1,1'-dimethyl, 4,4'-bipyridyllium dichloride) is a non selective contact herbicide. It is produced in several countries including China, Province of Taiwan, Italy, Japan, the United Kingdom and USA, and is used worldwide in approximately 130 countries. If not manufactured under strictly controlled conditions, it can contain impurities that are more toxic than the parent compound. It is almost exclusively used as a dichloride salt and is usually formulated to contain surfactant wetters.

Both its herbicidal and toxicological properties are dependent on the ability of the parent cation to undergo a single electron addition to form a free radical which reacts with molecular oxygen to reform the cation and concomitantly produce a superoxide anion. This oxygen radical may directly or indirectly cause cell death. Paraquat can be detected because of its ability to form a radical. Numerous analytical procedures are available.

2.4 Identity and Properties

2.4.1 Identity

Paraquat is a non-selective contact bipyridylium herbicide. The term has been applied to 2 technical products: 1,1'-dimethyl-4,4'-bipyridylium dichloride ($C_{12}H_{14}N_2Cl_2$) and 1,1'-dimethyl-4,4'-bipyridylium dimethylsulphate ($C_{12}H_{14}[CH_3SO_4]_2$).

2.4.2 Physical and Chemical Properties

Pure paraquat salts are white and the technical products yellow. They are crystalline, odourless, hygroscopic powders with a relative molecular mass of 257.2 for paraquat dichloride and 408.5 for paraquat dimethylsulphate. The relative molecular mass of the paraquat ion is 186.2 (Summers 1980). Some of the other physical properties of paraquat dichloride, the salt most used for herbicide formulations, are listed in Table 1.

Paraquat is slightly soluble in alcohol and practically insoluble in organic solvents (Haley 1979). The chemical structure of paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride) is:

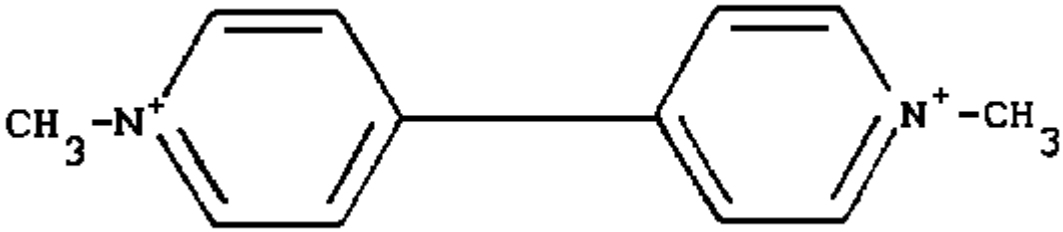

Table 2.1 Physical properties of Paraquat (Worthing 1979)

Specific gravity at 20°C	1.24 – 1.26
Melting point	175 - 180°C
Boiling point	Approximately 300°C with Decomposition
Solubility in water at 20°C	700g/litre
$_{P}$H of liquid formulation	6.5 – 7.5
Vapour pressure	Not measurable

Paraquat is non-explosive and non-flammable in aqueous formulations. It is corrosive to metals and imcompatible with alkylarylsulfonate wetting agents. It is stable in acid or neutral solutions but is readily hydrolysed by alkali. Paraquat readily undergoes a single-electron reduction to the cation radical. The redox potential for this reaction is 446mv. This chemical property led to its use as a redox indicator dye (methyl viologen) as early as 1933 (Summers 1980).

2.5 Environmental Distribution and Transformation-Environmental Effects

Paraquat deposits on plant surfaces undergo photochemical degradation to compounds that have a lower order of toxicity than the parent compound. On reaching the soil, paraquat becomes rapidly and strongly adsorbed to the clay minerals present. This process inactivates the herbicidal activity of the compound. While free paraquat is degraded by a range of soil microorganisms, degradation of strongly-adsorbed paraquat is relatively slow. In long-term studies, degradation rates were 5-10% per year. Strongly –bound paraquat has no adverse effects on soil microfauna or soil microbial process.

Paraquat residues disappear rapidly from water by adsorption on aquatic weeds and by strong adsorption to the bottom mud. The toxicity of paraquat for fish is low, and the compound is not cumulative. Normal applications of paraquat for aquatic weed control are not harmful to aquatic organisms. However, care should be taken when applying paraquat to water

containing heavy weed growth to treat only a part of the growth, since oxygen consumed by subsequent weed decay may decrease dissolved oxygen levels to an extent that may be dangerous for fish. Treated water should not be used for overhead irrigation for 10 days following treatment.

Paraquat is not volatile and following spraying the concentrations of airborne paraquat have been shown to be very low. Under normal working conditions, the exposure of workers in spraying and harvesting operations remains far below present TLVs and the exposure of passers-by or of persons living downwind of such operations is lower still. Normal paraquat usage has been shown not to have any harmful effects on birds. Finite paraquat residues are to be expected only when a crop is sprayed directly. Cattle allowed to graze on pasture for 4 hours after spraying at normal application rates did not suffer any toxic effects. Consequent residues in products of animal origin are very low.

2.6 Kinetics and Metabolism

Although toxic amounts of paraquat may be absorbed after oral ingestion, the greater part of the ingested paraquat is eliminated unchanged in the faeces. Paraquat can also be absorbed through the skin, particularly if it is damaged. The mechanism of the toxic effects of paraquat are largely the result of a metabolically catalyzed single-electron reduction-oxidation reaction, resulting in depletion of cellular NADPH and the generation of potentially toxic forms of oxygen such as the superoxide radical. Absorbed paraquat is

distributed via the bloodstream to practically all organs and tissues of the body, but no prolonged storage takes place in any tissues. The lung selectively accumulates paraquat from the plasma by an energy-dependent process. Consequently, this organ contains higher concentrations than other tissues. Since the removal of absorbed paraquat occurs mainly via the kidneys, an early onset of renal failure following uptake of toxic doses will have a marked effect on paraquat elimination and distribution and on its accumulation in the lung.

2.7 Effects on Experimental Animals

A characteristics dose-related lung injury reported to be induced in the rat, mouse, dog and monkey, but not in the rabbit, guinea-pig and hamster (Butler & Kleinerman 1971). The pulmonary toxicity is characterized by initial development of pulmonary edema and damage to the alveolar epithelium, which may progress to fibrosis. Exposure to high doses of paraquat may also cause less severe toxicity to other organs, primarily the liver and kidney. Minor toxic effects have been noted only at high doses in the nervous, cardiovascular, blood, adrenal and male reproductive systems.

Paraquat has not been found to be teratogenic or carcinogenic in long-term studies on rats and mice (Bus et al. 1974). *In vitro* mutagenicity studies have been inconclusive although generally suggestive of weak potential activity, while *in vivo* studies were negative (Bateman 1966).

2.7.1 Gastrointestinal Tract and Liver

Daniel and Gage (1966) studied the absorption of ^{14}C-paraquat following oral and subcutaneous single-dose administration to rats. About 76 – 90% of the oral doses were found in the faeces, and 11 – 20% in the urine; most of the subcutaneous dose (73 – 88%) was found in the urine and only 2 – 14.2% in the faeces.

The clinical signs of acute and chronic oral poisoning (Kimbrough & Gaines 1970; Murray & Gibson 1972) or of intraperitoneal injection (Butler & Kleinerman 1971) include transient diarrhoea and body weight loss, decreased food intake and dehydration. Some of the animals emited soon after paraquat administration. Residual skin contamination after dermal toxicity studies in rabbits (McElligott 1972) caused severe tongue ulceration and an unwillingness to eat. ALT and AST- levels, determined in monkeys (Macaca fascicularis) given a single toxic oral dose of paraquat (20 – 25 mg/kg cation), did not indicate liver dysfunction (Murray & Gibson 1972). In another study (Baynova & Anadoliiska 1969) albino rats given a single peroral dose of 50 mg/kg paraquat, developed metabolic acidosis, a disturbance that was attributed to both renal and liver toxicity. According to (Butler & Kleinermann 1971), the macroscopical and histological aspect of the liver of rabbits that were given single or repeated toxic paraquat doses, was normal. Based on (Clark et al. 1966), the histopathological appearance of the liver in rats, mice, rabbits given single or repeated intraperitoneal or oral doses of the herbicide. Small areas of centrolobular cell necrosis were also found in

another study (Murray & Gibson 1972) in which rats were given a single oral dose of 143 mg/kg of paraquat cation and were sacrified from 1 to 14 days after the administration.

After intravenous administration of paraquat (27 mg/kg) to Sprague-Dawley rats, the histopathological appearance of the liver was normal 1 to 2 days after the administration. However, in animals surviving 3 days, a prominent loss of glycogen was noted near the central hepatic vein and interpreted as a possible effect of food intake reduction (Fisher et al. 1973a). There have been several reports of liver damage following exposure to high doses of paraquat (Clark et al. 1966; Bainova 1969a; Murray & Gibson 1972). Centrilobular necrosis of hepatocytes with proliferation of the Kupffer cells and bile canals have been described. In general, liver damage in experimental animals has not been severe compared with lung and kidney damage. Serum enzyme activities (SGOT & SGPT) only increased when large amounts of paraquat were given (Giri et al. 1979).

This, together with the absence of marked biliary excretion, evidence of poor absorption along the gut. This low rate of absorption was confirmed by Litchfield et al. (1973) and Conning et al. (1969). Rats, guinea-pigs, and monkeys orally administered LD50 doses of ^{14}C-paraquat had low peak serum concentrations (2.1 – 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 (Bennett et al. 1976). After an oral dose of 0.12 mg/kg body weight, 46 – 66% was absorbed in 6 hours. For doses of 2 – 5 mg/kg, only 22 – 38% and 25 – 28% of the dose was absorbed, respectively. Dose-dependent data from dogs and whole body autoradiography suggest that absorption is facilitated in the small intestine. Some non-ionic surfactants (0.001%) increased ^{14}C-paraquat transport through isolated gastric mucosa models, but histological evaluation suggested that this was due to damage of the epithelial cell membranes (Walter et al. 1981).

2.7.2 Pulmonary Absorption

Absorption of paraquat following instillation and inhalation in the lung has been described in several studies (Gage 1968a, Kimbrough & Gaines 1970, Seidenfeld et al. 1978, Popenoe 1979). The uptake of ^{14}C-paraquat after an intratracheal injection of 1.86 nmol/lung was investigated in the isolated perfused rat lung by Charles et al. (1978). The efflux of ^{14}C-paraquat was biphasic with a rapid phase half-life of 2.65 minutes and a slow phase half-life of 356 minutes. Various doses of ^{3}H-paraquat (10^{-5} – 10^{-12} g) in 0.1 ml saline were introduced directly into the left bronchus of rats (Wyatt et al. 1981). Fifteen minutes after instilling 10^{-8} of ^{3}H-paraquat, 90% of the ion could accounted in the tissues and urine, 50% being present in the lung.

Zavala and Rhodes (1978) reported that the lung of the rabbit was highly sensitive to paraquat intrabronchial instillation in doses ranging from 0.1 g – 1 pg; moderately sensitive to intravenously administered paraquat (25

mg/kg body weight); resistant to the herbicide when given intraperitoneally or subcutaneously (25 mg/kg body weight). Clark et al. (1966) reported that, in rats in the earlier stages after a single toxic oral dose of paraquat, breathing was gasping or deep and fast, but some days after a single or repeated toxic doses, the respiration became increasingly laboured, and the hairs around the mouth and nares were soiled with a brownish liquid.

The extensive alveolar edema observed in severe intoxication was responsible for the development of hypoxia, cyanosis, and dyspnea. The progressive development of pulmonary fibrosis was accompanied by difficulty in breathing, gasping and hyperpnea (Smith et al. 1973). Exposure of rats to high concentration of respirable paraquat aerosols was accompanied by shallow respiration. Within 2 – 3 hours, the test animals became dyspnoeic, cyanotic, and inactive and there were signs of local eye and nose irritation (Gage 1968a).

2.7.3 Dermal and eye administration

Paraquat absorption through animal and human skin has been studied using an *in vitro* technique (Walker et al. 1983). 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 animal skins tested (including rat, rabbit, and guinea-pig). In mice and rats, the application of 5 – 20 gm paraquat/ liter solutions in single and 21 days repeated dermal toxicity tests provoked dose-related toxic dermatitis

with erythema, edema, desquamation and necrosis (Bainova 1969b). No skin sensitization was observed in studies on guinea-pigs when paraquat was applied (Bainova 1969b; Fodri et al. 1977).

The instillation of dilutions of paraquat (up to 500 gm/liter) in rabbit eye induced inflammation within 24 hours and continued for 96 hours (Clark et al. 1966). Sinoi & Wei (1973) introduced 62.5, 125, 250, 500, and 1000 gm paraquat/liter into the rabbit eye. Concentration of 62.5 and 125 gm/liter caused severe conjunctival reactions; higher levels (250 – 500 gm/liter) provoked iritis and pannus, while at the 500 gm/liter concentration there was corneal opacification, iritis and conjunctivitis. All rabbits receiving 0.2 ml of paraquat at 1000 gm/liter in one eye or 0.2 ml of a concentration of 500 gm/liter in both eyes died within 6 days of application (Sinow & Wei 1973).

There are no *in vivo* studies on the rate of absorption of paraquat through the skin. However, observations of dose-related dermal toxicity in experimental animals and human percutaneous poisoning have provided some qualitative information concerning the dermal absorption of paraquat.

2.7.4 Renal System

In paraquat toxicity, kidney damage often precedes signs of respiratory distress (Clark et al. 1966; Butler & Kleinerman 1971; Murray & Gibson 1972). Paraquat is excreted mainly via the urine and the concentration of the herbicide in the kidneys are relatively high. BUN-measurements in

experimental animals poisoned by paraquat were performed only in one study (Murray & Gibson 1972): in monkeys (*Macaca fascicularis*) given single toxic doses of paraquat cation ranging from 20 to 25 mg/kg, BUN-levels were normal. Gross pathological and histological examinations of paraquat-poisoned rats, guinea-pigs, rabbits, and dogs revealed vacuolation of the convoluted renal tubules and proximal tubular necrosis (Bainova 1969a; Murray & Gibson 1972; Tsutsui et al 1976). The generation of the proximal tubular cells has also been confirmed by electron-optical studies (Fowler & Brooks 1971; Marek et al. 1981). Paraquat is actively secreted by the kidney base transport system. 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).

2.7.5 Effects on Reproductive System

2.7.5.1 Testes

Some histological changes in the testes have been reported in a few paraquat toxicity studies. Butler & Kleinerman (1971) found multinucleated giant cells in rabbit testicular tubules. When paraquat was orally administrated at 4 mg/kg body weight to male rats for 60 days and the 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 that of the controls, nor

did quantitative and qualitative histological examination of the testicular tubule cells reveal any abnormality (Butler & Kleinerman 1971).

Degeneration of the seminiferous tubules with the formation of small multinucleated giant cells lying free in their lumen was observed in the testes of two of five rats that had been given a single large 200mg/kg or few repeated small peroral or peritoneal doses of paraquat (Clark et al. 1966). Other studies (Butler & Kleinermann 1971) also reported on the histological appearance of the testes of immature New Zealand rabbits that had been given doses of paraquat ranging from 2 to 100 mg/kg, administered in 1 – 5 intraperitoneal injections. Seven of the 20 animals receiving 50 mg/kg of paraquat or more, had multinucleated giant cells in the lumen of their testicular tubules. A decrease in route of spermatogenesis was also observed in rabbits that were given a single toxic dermal dose of 70 to 500 mg paraquat cation/kg during 24 hours (McElligott 1972). Impairment of spermatogenesis has also been detected with the dominant lethal test in male mice that were given a single intraperitoneal dose of 25mg/kg paraquat or diquat (Pasi et al. 1974; Pasi & Embree 1975).

On the other hand, the testes of rats, mice, guinea-pig, rabbits and dogs that had been exposed to paraquat aerosols concentrations of up to 0.4 μgm/liter for periods of up to three weeks were histologically normal (Gage 1968a). Also morphologically normal were the testes of a 15 year old boy (Matthew et al. 1968) and two adults (Nienhaus & Ehrenfeld 1971; Bronkhorst et al. 1968) fatally poisoned by paraquat as were epididymes, seminal

vesicles and prostate. A three generation reproductive study has been carried out in rats treated with paraquat ion at 100 mg/kg diet (FAO/WHO 1973). There were no significant abnormalities in fertility, fecundity and neonatal morbidity or mortality, nor were there any signs of gonadotoxicity or structural or functional lesions.

2.7.5.2 Ovaries

In 97 cases of paraquat poisoning reported in the literature there were no observations on the function and morphology of the ovaries. Experimental observations on the action of paraquat on this organ are also scarce. According to Molnar (1971), after the single peroral administration of 160 mg/kg paraquat on the 9^{th} day of pregnancy, the ovaries and Fallopian tubes of rats, did not show any significant macroscopical or microscopical lesions.

2.7.6 Embryotoxicity and Teratogenicity

Oral and intraperitoneal administration of high doses of paraquat to mice and rats on various days of gestation produced significant maternal toxicity, evidenced by increased mortality rates (Bainova & Vulcheva 1974; Bus et al. 1975). Examination of the fetuses from the higher-dose groups revealed a reduction in fetal body weights, and increased resorption rate in mice, as a result of the maternal intoxication. The absence of a specific embryotoxic action of paraquat has also been observed and reported in other

studies in rats (Khera et al. 1968; Luty et al. 1978), mice (Selypes et al. 1980), and rabbits (FAO/WHO 1973).

In a perinatal toxicity study, Bus & Gibson (1975) administered paraquat at 50 or 100 mg/liter in the drinking water to pregnant mice beginning on day 8 of gestation, with continued treatment of the litters up to 42 days after birth. Paraquat treatment did not alter postnatal growth rate, although the mortality rate in the 100 mg/liter-treated mice increased to 33% during the first 7 days after birth. It was also noted that paraquat at 100 mg/liter significantly increased the sensitivity of the pups to oxygen toxicity on days 1, 28, and 42 after birth.

2.7.7 Mutagenicity and Genotoxicity

PQ has been found to have minimal to no genotoxic activity when evaluated in a variety of *in vitro* and *in vivo* test systems. In studies producing weakly positive results (Moody & Hassan 1982; Parry 1973; Bignami & Grebelli 1979), which were limited to *in vitro* studies, paraquat genotoxicity was accompanied by high cytotoxicity. These results were best explained by Moody & Hassan (1982), showed that the mutagenicity of paraquat in bacterial test systems (*Salmonella typhimurium* TA98 and TA100) was mediated by the formation of superoxide.

The genotoxic potential of the herbicide PQ was comparatively tested in various genotoxicity tests with V79 Chinese hamster cells. PQ clearly

induced cytotoxicity and chromosome aberrations but did not induce gene mutations at the HPRT locus or increased DNA migration in the comet assay under the same treatment conditions. Speit and co-workers (1998) suggest that PQ does not significantly induce DNA lesions relevant for HPRT gene mutations in cultivated V79 cells. Furthermore, paraquat was not mutagenic when evaluated in human leucocytes and in *in vivo* cytogenetic tests on mouse bone marrow (Selypes & Paldy 1978) and dominant lethal tests on mice (Pasi et al. 1974; Anderson et al. 1976).

2.7.8 Carcinogenicity

A carcinogenicity study was performed on mice at dietary levels of 25, 50 and 75 mg/kg per day for 80 weeks (FAO/WHO 1973). There were rediced weight gains among the animals receiving paraquat, but deaths during the study were associated with respiratory disease. Clinical and histopathological examination determined that paraquat was not tumorigenic in mice.

A two year study of exposure of rats to 1.3 and 2.6 mg/liter daily, in the drinking-water provoked histopathological changes in the lung, liver, kidney and myocardium. The lung lesion were dose-related; inflammation, atelectasis, reactive proliferation of the epithelium, pulmonary fibrosis, and pulmonary adenomatosis were noted, but no sign of tumour growth or atypism (Bainova & Vulcheva 1977). Nor was any increased tumour incidence reported in rats in a 2-year study with a maximum dietary level of 250 mg/kg diet (12.5 mg/kg body weight per day) (FAO/WHO 1971)

Bainova & Vulcheva (1977) did not discover any indication of tumourigenicity in a 2-year study in rats receiving paraquat at 1.3 or 2.6 mg/liter in their drinking-water. While testing the carcinogenicity of urethane in mice, Bojan et al. (1968), also attempted to evaluate the influence of paraquat on urethane-induced lung tumourigenesis. It is felt that the results of this study are not of relevance for the assessment of the carcinogenic potential of paraquat.

2.8 Clinical Signs of Paraquat Poisoning in Experimental Animals

2.8.1 1st Phase of Paraquat Intoxication: Latency & First Clinical Signs

According to (Molnar-Sebestyen 1971), the first clinical signs appeared 12 hours after the peroral administration of a saline solution of paraquat dichloride to adult albino rats in doses ranging from 80 – 320 mg cation/kg body weight. The treated animals looked sick, were hypoactive and their food intake was decreased.

After intraperitoneal administration of paraquat, the latency time of appearance of first clinical signs was surprisingly and unexplainably as long as 48 hours (Molnar-Sebestyen 1971), although administered doses were comparable or larger than the peroral doses mentioned in the previous study. In another study (Roujeau et al. 1974), however, performed on Wistar rats, latency time was considerably shorter.

Intravenous administration of a single toxic dose (27 mg/kg) of paraquat to rats, first clinical signs appeared as expected after a latency time of few hours (Fisher et al. 1970). In subhuman primates (Macaca fascicularis) given the chemical by gavage, early signs of paraquat poisoning included tachycardia and hyperpnea (Murray & Gibson 1972). Lethargy (possibly a central nervous system effect) as one of the first signs was observed in rats that had received a single oral dose of 143 mg/kg of paraquat (Murray & Gibson 1972). In mice that had received 200 ppm of paraquat with the drinking water for a period of 4 weeks, first signs included immobility, fur irregularities and avoidance of food and water intake. Respiratory difficulties appeared only at a level of 300 ppm (Brooks 1971).

2.8.2 2nd and 3rd Phase of Paraquat Intoxication

In both intrpertitoneally and orally treated rats, the first intoxication phase (Phase I) lasted for about 48 hours. Then (Phase II), a period of apparent remission occurred in both groups and continued for 1 – 10 days in the intraperitoneally treated animals and for only 16 hours in the orally treated rats. Subsequently, (Phase III) an abrupt onset of respiratory difficulty occurred in both orally and intraperitoneally treated rats. The animals became again hypoactive, their eyes remained closed and the respiratory rate was increased. White foam appeared around their mouths and this became bloody; blood was also noticed around the eyes and on the paws. Terminally animals exhibited an increased tendency to turn away from light. They all died apparently from respiratory distress (Molnar-Sebestyen 1971).