HEPATIC DISPOSITION OF MPTP AND PESTICIDES ASSOCIATED WITH PARKINSON'S DISEASE

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Maria Chaesook Yang

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STATEMENT OF ORIGINALITY

The work presented in this thesis is original with an independent intellectual enterprise, except as acknowledged in the text, and has not been submitted, either in whole or in part, for a degree in the University of Sydney or any other University.

Maria Chaesook Yang

PUBLICATIONS

Yang MC, McLean AJ, Le Couteur DG. Cell membrane transport of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the liver and systemic bioavailability. *Biochem Biophy Res Comm* 2001, **289**, 130-136.

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ABSTRACT

Parkinson's disease (PD) is one of the most common neurological diseases in older people. The pathogenesis is unknown, but epidemiological evidence implicates environmental neurotoxins such as pesticides. The liver is the major organ for detoxification of xenobiotics and, therefore, the hepatic disposition of neurotoxins may influence susceptibility to PD. In this thesis, the hepatic disposition of three representative pesticides: paraquat, dichlorodiphenyltrichloroethane (DDT) and malathion, and the PD-producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were examined in the perfused rat liver using the multiple indicator-dilution (MID) technique and isolated hepatocytes.

First, the behavior of the above compounds in the intact liver was observed. Paraquat was not extracted by the liver (0% extraction), whereas MPTP was almost entirely extracted in the liver (98% extraction). The hepatic extraction of DDT and malathion was intermediate (68% and 89%, respectively). The permeability cell surface area products determined using the Goresky model indicate that transport of DDT and MPTP across cell membranes is by simple diffusion. However, there appeared to be a specific influx mechanism for malathion and a specific efflux mechanism for paraquat.

Second, the effects of pretreatment with pesticides on the hepatic disposition of pesticides and MPTP were observed because pesticides are used in combinations of compounds and are known to have effects on hepatic detoxification. Pretreatment with pesticides reduced the hepatic extraction of MPTP and malathion significantly. The fractional recovery of MPTP from the liver was at least doubled by pretreatment with paraquat, DDT or malathion, and the recovery of malathion was also increased to more than 170% of the control value by pretreatment with paraquat and DDT. However, pretreatment with pesticides had no effect on the recovery of sucrose, paraquat or DDT.

Next, the effect of ageing on the hepatic disposition of pesticides and MPTP was investigated. Old age is the major risk factor for PD. The recoveries of DDT, malathion

and MPTP were increased from the livers of the older rats to 253%, 134% and 258% of the values in young rat livers, respectively. The hepatic transport of DDT and malathion into hepatocytes was reduced with age, indicating that part of the impaired hepatic extraction of neurotoxins is secondary to an age-related barrier to influx.

The hepatocellular transport of MPTP was studied in the perfused liver and isolated hepatocytes because of the possibility that modulation of hepatocellular transport might influence susceptibility to parkinsonian neurotoxins. Most uptake of MPTP into hepatocytes was mediated by simple diffusion, however a small but significant proportion of uptake was via the organic cation transporter 1 (oct1) and possibly in isolated hepatocytes, via P-glycoprotein. Organic anion transporting polypeptide (oatp) is not involved in MPTP transport, and oct1 was a bidirectional transporter involved with efflux of MPTP in isolated rat hepatocytes. The results indicate that modulation of the hepatic transport of MPTP may also have dramatic effects on systemic availability.

Finally, the effect of cirrhosis on the hepatic disposition of malathion and MPTP was observed. Liver diseases influence the hepatic extraction of xenobiotics. Cirrhosis was induced using the phenobarbitone / carbon tetrachloride model and confirmed histologically. The extraction of malathion and MPTP was reduced in the cirrhotic liver and this reduction was associated with decreases in the hepatocellular sequestration for malathion and hepatocellular influx for MPTP. However, the transport parameters were not affected to the degree seen in ageing, indicating that the pathogenesis of functional changes in the liver with ageing are different from that in the cirrhotic liver.

From the above observations, it was concluded that there was considerable variability in the hepatic disposition of pesticides and MPTP. Pesticides, ageing, modulators of MPTP transport and liver disease could contribute to the risk of PD by altering hepatic detoxification and increasing systemic bioavailability of neurotoxins.

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALKP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotranferase
ATP	Adenosine 5'-triphosphate
AUC	Area under the curve
AUMC	Area under the first moment of the curve
BSA	Bovine serum albumin
C	Outflow concentration
cBST	Canalicular bile salt transporter
cMOAT	Canalicular multi-specific organic anion transporter
CYP	Cytochrome P450
DAT	Dopamine transporter
DDE	2,2-Bis(p-chlorophenyl)-1,1-dichloethylene
DDT	2,2-Bis(p-chlorophenyl)-1,1,1-trichloroethane
DOPAC	Dihydrophenylacetic acid
E	Hepatic extraction
FMO	Flavin-containing monooxygenases
GSH	Glutathione
GST	Glutathione transferase
H&E	Hematoxylin-eosin
i.p.	Intraperitoneal injection
k_1	Influx rate constant
k_2	Efflux rate constant
k_3	Sequestration rate constant
KH	Krebs-Henseleit bicarbonate
L-DOPA	Levodopa
Maneb	Ethylenebisdithiocarbamate
MAOB	Monoamine oxidase B

MDR	Multi-drug resistance gene product (mdr in mouse)
MID	Multiple indicator dilution
$MPDP^+$	1-methyl-4-phenyl-2,3-dihydropyridinium
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MR	Metabolic ratio
MRI	Magnetic resonance imaging
MRP	Multi-drug resistance-associated protein
MTT	Mean transit time
MTT_{sb}	Mean transit time of substrates
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NTCP	Sodium taurocholate co-transporter
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide (oatp in mouse)
OCT	Organic cation transporter (oct in mouse)
PD	Parkinson's disease
PM	Poor metaboliser
pO_2	Partial pressure of oxygen
PON	Paraoxonase
PS	Permeability cell-surface area
PS_{in}	Permeability cell-surface area for influx
PS_{out}	Permeability cell-surface area for efflux
PTP	4-Phenyl tetrapyridine
Q	Flow rate
R	Recovery
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
V	Volume of distribution
V_{sb}	Volume of distribution of substrates

V_{ext}	Volume of distribution for permeable solutes
V_{suc}	Volume of distribution for sucrose
ww	Wet weight

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INTRODUCTION

Parkinson's disease (PD), first described in 1817 by James Parkinson (Parkinson 1817), is a common neurodegenerative disorder leading to the onset of clinical features including bradykinesia, resting tremor, rigidity and postural imbalance (Calne *et al.* 1992). The disease affects 1% of the population over the age of 55 years (Tolwani *et al.* 1999) and the incidence of this disease is higher in men than in women (Dluzen & McDermott 2000). Even though the average age of diagnosis is around 65 years (Baldereschi *et al.* 2000), early-onset cases do exist.

Pathologically, this disease is linked to the destruction of dopaminergic neurons in the substantia nigra pars compacta, which are responsible for producing and transporting dopamine via the nigrostriatal tract to the striatum (Chase & Oh 2000; Crossman 2000). This loss of dopaminergic neurons leads to depletion of the neurotransmitter dopamine in the striatum and clinical signs of the disease appear when striatal dopamine is reduced by 80% (Koller 1992). This disease is also distinguished by Lewy bodies, which are intracellular neuronal inclusions found in the substantia nigra (Wakabayashi *et al.* 1998; Hattori *et al.* 2000). Lewy body formation is associated with α-synuclein aggregation (Baba *et al.* 1998; Wakabayashi *et al.* 1998) and over-expression of human α-synuclein in mice results in the loss of dopaminergic terminals in the basal ganglia and impaired motor function (Masliah *et al.* 2000). Additionally, Lewy bodies are also seen more frequently in patients with Alzheimer's disease (Hamilton 2000) and dementia (Parkkinen *et al.* 2001) than in the normal elderly population.

At present, there are no therapeutic treatments to halt or retard the progression of nigral cell pathology but dopamine replacement by levodopa (L-DOPA) can be administered to reduce symptoms of this disease. Levodopa crosses the blood-brain barrier, as opposed to dopamine, and is metabolised to dopamine by the enzyme DOPA decarboxylase (Young & Landsberg 1977). Levodopa does not prevent progression of the disease (Fuller & Tolbert 1991). Furthermore, patients on high doses of levodopa are also subject to severe side effects including dyskinesia (abnormal involuntary movements) (Fahn 2000). Therefore, there should be a deeper understanding of the cause of PD, not

only to prevent the disease, but also to develop therapeutic strategies to halt its progress. To date, the pathological mechanisms responsible for dopaminergic cell death remain unclear but the observation that poisoning with the chemical 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) leads to parkinsonism has accelerated studies of PD using experimental models.

1.1. MPTP-induced parkinsonism

In the 1980s, several narcotic users developed acute progressive and permanent parkinsonism after injecting synthetic mependine contaminated with a byproduct, MPTP (Davis *et al.* 1979; Langston & Ballard 1983a; Tetrud & Langston 1989). This observation led to the hypothesis that PD is secondary to exogenous environmental neurotoxins (Langston 1987; Tanner 1989). Later, intravenous administration of MPTP to nonhuman primates, such as rhesus monkeys, indicated that MPTP selectively destroys dopaminergic neurons in the nigrostriatal system and induces behavioral changes very similar to those of the human condition (Burns *et al.* 1983). MPTP-intoxicated humans and monkeys responded to levodopa similarly to PD patients, including the development of side effects seen after its chronic use (Olanow *et al.* 2000).

1.1.1. Mechanism of the toxicity of MPTP

MPTP is a highly lipophilic compound and, after systemic administration, is able to penetrate the blood-brain barrier. In the brain, MPTP is converted into its active metabolite, 1-methyl-4-phenylpyridinium (MPP^+), by monoamine oxidase B (MAOB) within non-dopaminergic neurons, such as glial cells and serotonergic neurons (Chiba *et al.* 1984). In both mice (Heikkila *et al.* 1984; Fuller & Hemrick-Luecke 1985) and monkeys (Langston *et al.* 1984), pretreatment with inhibitors of monoamine oxidase B prevented MPTP neurotoxicity. MPP^+ has a high affinity for plasma membrane dopamine transporter (DAT) and selective uptake of MPP^+ into dopaminergic neurons via DAT leads to its accumulation and accounts for its selective toxicity for dopaminergic cells. Many studies have shown that intracellular MPP^+ can be taken up and accumulated within mitochondria by an energy-dependent process (Ramsay *et al.* 1986a; Ramsay & Singer 1986b), where it blocks complex I of the electron transport chain (Nicklas *et al.* 1985). In mitochondria isolated from rat or mouse brains, MPP^+

interferes with NADH-linked oxidation of pyruvate or glutamate without affecting the oxidation of succinate, which indicates that the site of interaction with the respiratory process is at complex I (Nicklas *et al.* 1985). This in turn decreases the production of adenosine 5'-triphosphate (ATP) and increases the formation of free radicals such as superoxide (Nicklas *et al.* 1987; Przedborski *et al.* 1996). Glutamate-mediated excitotoxicity has also been implicated (Loschmann *et al.* 1994; Beal 1998).

Administration of MPTP results in the depletion of striatal dopamine and nigrostriatal cell death in a wide variety of animal species, such as mice (Tatton *et al.* 1992), dogs (Hineno *et al.* 1992), sheep (Baskin *et al.* 1994) and non-human primates (Burns *et al.* 1983). However, the degree of susceptibility among species varies on the basis of differences in the numbers of dopaminergic neurons (Muthane *et al.* 1994) and kinetic properties of MAOB (Inoue *et al.* 1999). For example, rats are resistant to MPTP unless it is administered directly into the substantia nigra. This resistance is because rats have more MAOB at the blood-brain barrier than other species, which can metabolise MPTP to MPP^+, which is hydrophilic and therefore can't cross the blood brain barrier (Kalaria *et al.* 1987).

Although the MPTP model is widely accepted as an experimental model for PD, it is not able to replicate some aspects of PD. First, PD is a slowly progressive disorder over several years, whereas MPTP toxicity in humans and other species is subacute (Kurlan *et al.* 1991; Earl *et al.* 1998). Second, Lewy bodies, which are regarded as a key feature of PD, have not been demonstrated in MPTP models (Langston 1996; Langston *et al.* 1999). Finally, MPTP has never been found in PD patients' brain or body fluids.

1.2. Etiology of PD

Many epidemiological studies have been performed to determine risk factors for PD. However, the definitive causes of PD are not known yet. The etiology of PD is likely to be diverse and complex (Figure 1.1). Exogenous factors might include environmental toxins. Endogenous toxins are more likely to be metabolic products of dopamine and

serotonin, such as, tetrahydroisoquinoline and beta-carbolinium, respectively, (Collins 1994; McNaught *et al.* 1998).

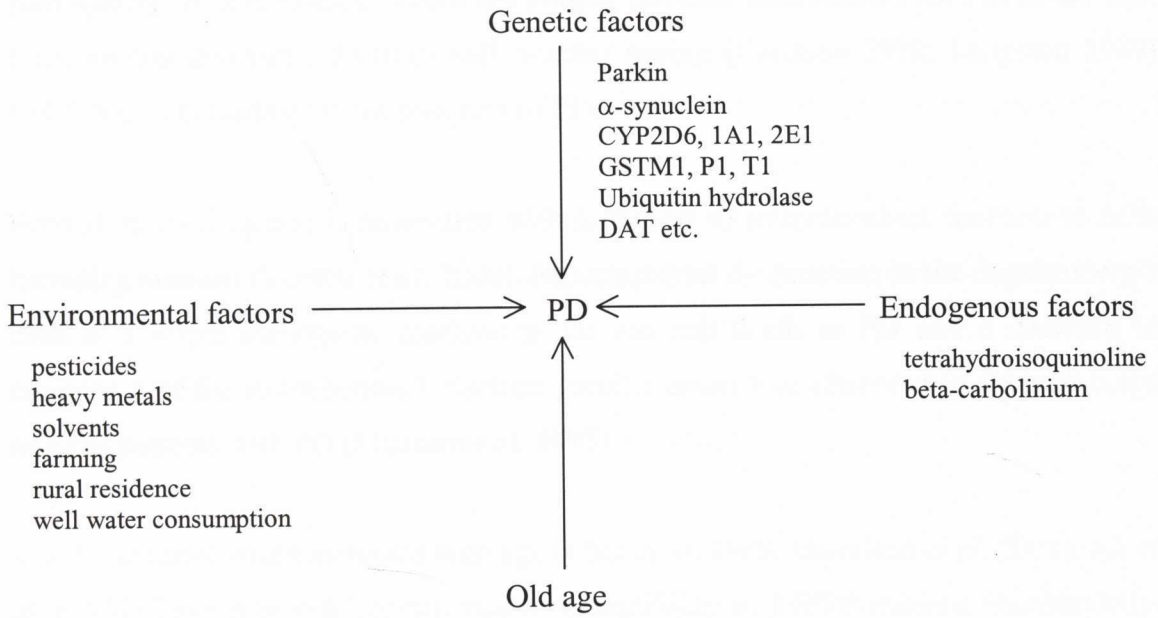

Figure 1.1. Summary of risk factors for PD. PD may be caused by a combination of any of the above genetic factors, environmental factors and old age.

Epidemiological studies have shown that ageing is the major risk factor for PD. Although many factors have been suggested to enhance the risk of PD, the only consistently accepted risk factor identified to date is increasing age (Mayeux *et al.* 1992; Langston 1998; Bower *et al.* 1999). Other risk factors of PD are family history (Hubble *et al.* 1993; McCann *et al.* 1998), pesticide exposure (Hubble *et al.* 1993; Seidler *et al.* 1996; Liou *et al.* 1997; Gorell *et al.* 1998), heavy metal and solvent exposure (Pezzoli *et al.* 1995; Seidler *et al.* 1996), farming (Liou *et al.* 1997; Gorell *et al.* 1998; Marder *et al.* 1998), rural residency (Koller *et al.* 1990; Liou *et al.* 1997; Marder *et al.* 1998; McCann *et al.* 1998) and well-water use (Koller *et al.* 1990; Marder *et al.* 1998). Cigarette smoking (Seidler *et al.* 1996; Liou *et al.* 1997), consumption of alcohol (Morano *et al.* 1994) and coffee (Benedetti *et al.* 2000) are suggested to be protective factors against PD.

1.2.1. Ageing as a risk factor of PD

The average onset of this disease is age 65 years. Despite the importance of ageing as a risk factor, it has been difficult to utilise this observation in order to understand the pathogenesis of this disease. There are several possible mechanisms for PD in old age. First, striatal dopamine declines with normal ageing (Carlsson 1978; Langston 1989) and this may contribute to the progress of PD.

Second, normal ageing is associated with a decline of mitochondrial function in cells including neurons (Toescu *et al.* 2000). Mitochondrial dysfunction in the doparminergic cells is a major pathogenic mechanism for the cell death in PD and a decrease in complex I of the mitochondrial electron transfer chain was observed in the substantia nigra of patients with PD (Mizuno *et al.* 1995).

Third, oxidative stress increases with age (Choi *et al.* 1998; Hamilton *et al.* 2001). Ali *et al.* (1994) have reported age-dependent susceptibility to MPTP-induced neurotoxicity. They observed that in older mice (C57/B6N), MPTP (40mg/kg, single i.p.) produced an increase of reactive oxygen species (ROS) that were associated with a subsequent decrease in dopamine concentration in the striatum. Younger mice were not affected significantly. Furthermore, dopamine recovery was impaired in mature mice (Saitoh *et al.* 1987).

Cytotoxic free radicals for an oxidative stress are generated by iron in the brain (Double *et al.* 1998) and the concentration of striatal iron is increased with advancing age (Martin *et al.* 1998). This could explain the increase in the prevalence of PD with age. With regards to gender differences, in the serum of people older than 60 years, males had lower levels of antioxidants than females (Mendoza-Nunez *et al.* 2001) and this finding may partially explain the higher prevalence of PD in men than in women (Dluzen & McDermott 2000).

Another possible mechanism for PD in the elderly is that brain MAOB activity increases with age (Desole *et al.* 1993). MAOB is responsible for metabolizing MPTP to its active

metabolite MPP^+. The high activity of MAOB with age was observed in humans (Fowler *et al.* 1997) and mice (Voitenko 1992).

Finally, old age is associated with marked changes in xenobiotic metabolism (Le Couteur & McLean 1998a) in addition to lifelong accumulation of exposure to neurotoxins with age. This finding provides a logical explanation of the predisposition of elderly people to Parkinson's disease and possibly other diseases of old age (Le Couteur *et al.* 1999a). The age-related impairment of xenobiotic detoxification in the liver may be secondary to many factors, including reductions of liver mass (Wynne *et al.* 1989), blood flow (Wynne *et al.* 1990) and phase I enzyme activity (Guo *et al.* 1993; Le Couteur & McLean 1998a). The ageing liver and impaired xenobiotic metabolism will be described in more detail in Chapter 1.3.4. Ageing liver and xenobiotic clearance.

1.2.2. Genetic risk factors of PD

Population-based studies and case reports have shown that the cause of PD may on occasion be purely genetic (Golbe *et al.* 1996) or a combination of genetic susceptibility and environmental risk factors (Le Couteur *et al.* 1999a; Giasson & Lee 2000). In such cases, the genetic component probably acts by increasing the vulnerability of dopamine containing neurons in the nigrostriatal system to injury by environmental risk factors. It has been reported that about 20% of people with PD have a positive family history and the risk for developing PD for a person who has a single affected relative is increased by approximately 2 to 3-fold (Le Couteur *et al.* 1999a). Langston (1998) has also shown that 17% of all patients with PD had at least one affected family member with the disease.

A large southern Italian family contains multiple family members with early onset parkinsonism linked to a locus on chromosome 4q21-23 (Polymeropoulos *et al.* 1996). The specific mutation was identified in the region encoding for a protein known as α-synuclein (Polymeropoulos *et al.* 1997). Synucleins belong to a diverse family of synaptic proteins that are expressed throughout the mammalian central nervous system (Maroteaux & Scheller 1991). The genetic defect is a single mutation at position 209,

from G to A in exon 4 (G209A), which results in an alanine to threonine substitution at position 53 (Ala53Thr) (Polymeropoulos *et al.* 1997). Histological studies have found that α-synuclein is a major component of Lewy bodies not only in PD but also in dementia with Lewy bodies and familial Alzheimer's disease (Lippa *et al.* 1998; Lippa *et al.* 1999; Spillantini *et al.* 1998). However, another group failed to find the same mutation as above in patients with younger onset PD, and they suggest that G209A mutation in the α-synuclein gene may rarely cause typical PD (Vaughan *et al.* 1998). It is not yet known whether the presence of the threonine residue in PD causes a loss of normal function but an abnormal gene product may activate a toxic cascade and dopaminergic neurons may be especially susceptible to this synuclein toxicity. In addition to genetic susceptibility, environmental factors such as oxidative stress (Paxinou *et al.* 2001) and pesticide exposure (Uversky *et al.* 2001) also contribute to the synuclein toxicity. Other genetic loci for familial forms of parkinsonism have been identified through studies of rare families exhibiting mendelian inheritance patterns of disease. These include the parkin gene (6q25.2-27) (Matsumine *et al.* 1997, Hatorri & Mizuno 1999), ubiquitin hydrolase gene (Wintermeyer *et al.* 2000) and unidentified genes on chromosome 17q21-22 (parkinsonism without tremor) (Foster et al 1997) and 19q13 (dystonia – parkinsonism) (Kramer *et al.* 1999).

In a twin study to determine the heredity of PD (Langston 1998), the risk ratio for concordance in homozygotic twins was compared to dizygotic twins. When the analysis was confined to twins in whom the disease began over the age of 50 years, the risk ratio for concordance was very low. This data indicates that sporadic PD with onset above the age of 50 years may not be an inherited disease.

On the other hand, when the analysis dealt with twins under the age of 50, all monozygotic twin pairs were concordant for this disease, while dizygotic twins were not, indicating that PD is inherited. Therefore, it was concluded that there is no genetic influence on patients with PD beginning after the age of 50 years but genetic influence is involved in younger-onset PD.

Many candidate genes for PD have been investigated, based on the knowledge of pathogenic mechanisms of the disease, including mitochondrial dysfunction and oxidative stress. In the mitochondrial complex I genes, Kosel *et al.* (2000) have found two missense mutations (ND2 4924 G/A, ND3 10192 C/T) from four pairs among five pairs of monozygotic twins with a longitudinal diagnosis of idiopathic PD. A mutation G-to-A at nucleotide position 11778 of the mitochondrial ND4 gene of complex I, which converts a highly conserved arginine to a histidine, has also been found from a family with maternally inherited adult-onset PD (Simon *et al.* 1999). These observations suggest that inherited mutations in mitochondrial DNA can contribute to the development of parkinsonism. On the other hand, Bandmann *et al.* (1997a) were unable to detect the previously described five polymorphisms at nucleotide 956-965 in Caucasian patients with PD (Shoffner *et al.* 1993) from a large scale study (100 cases of pathologically proven PD). These conflicting results may suggest that sporadic PD is caused by a combination of genetic and environmental factors and complex I inhibition represents only one such factor. Similarly, Tan *et al.* (2000) found that only four polymorphisms in 14 genes had a significant association with PD from 84 individual studies investigated.

Oxidative stress results from free-radical production or defects in antioxidant defenses. PD is associated with evidence for generating reactive oxygen species in the substantia nigra. Superoxide dismutase (SOD) normally prevents tissue damage from oxygen-derived free radicals and the activity of SOD is increased in the substantia nigra of PD patients (Marttila *et al.* 1988). Transgenic mice with increased SOD1 activity show resistance to the neurotoxin MPTP and abnormal SOD1 activity in PD could result in increased susceptibility of dopaminergic neurons to neurotoxins such as MPTP (Przedborski *et al.* 1992). However, Parboosingh *et al.* (1995) and Farin *et al.* (2001) failed to detect mutations in SOD1 and SOD2 genes in patients with idiopathic PD. Similarly, Bandmann *et al.* (1995) could not detect any changes in the entire coding region of SOD1 in 23 familial cases, and concluded that SOD gene variants do not contribute to PD. One additional gene involved in pathogenic mechanisms of PD is the dopamine transporter (DAT) gene. Le Couteur *et al.* (1997) reported that the frequency of the 11-copy allele of a variable number tandem repeat (VNTR) polymorphism in the

3'-untranslated region of the DAT gene was over-represented in PD patients compared to controls. Another group (Morino *et al.* 2000) also reported that frequency of the polymorphism (A1215G) in exon 9 of this gene was lower in PD patients than in the controls (Morino *et al.* 2000). However, Higuchi *et al.* (1995) have failed to detect any association between alleles of the dopamine receptor genes (D2, D3 or D4) or the DAT gene and PD.

Additionally, the frequency of known polymorphisms in genes involved in xenobiotic detoxification mechanisms has been compared between PD patients and control subjects. These genes include CYP2D6 (McCann *et al.* 1997; Checkoway *et al.* 1998), CYP1A1 (Takakubo *et al.* 1996) and N-acetyltransferase 2 (Bandmann *et al.* 1997b). Polymorphisms in glutathione transferase (GST) M1 (Stroombergen & Waring 1999), T1 (Stroombergen & Waring 1999) and P1 (Menegon *et al.* 1998) are also associated with PD. Genetic association in the xenobiotic metabolism of PD will be explained more in detail later in Chapter 1.2.3.1.2. Genetic influences on pesticide metabolism.

1.2.3. Environmental risk factors for PD

The discovery of MPTP led to the hypothesis that PD is secondary to exogenous environmental neurotoxins (Tanner 1989; Langston 1996) and epidemiological studies have identified environmental factors that increase the risk of PD. MPP^+, a metabolite of MPTP, has structural similarity to the herbicide paraquat and this led to epidemiological studies of PD and pesticides. Among many environmental risk factors (refer to Chapter 1.2. Etiology of PD), a significant association between pesticide exposure and PD was found (Ritz & Yu 2000). Many pesticides are neurotoxic and may directly affect various aspects of nervous system function. Among pesticides, organochlorines, organophosphates, herbicides (Stephenson 2000) and carbamates have been reported to be associated with PD. The organochlorine insecticide, dieldrin, was found in six of twenty PD postmortem brains although none was found in the control subjects (Fleming *et al.* 1994). A case study reported that five genetically related people who were exposed to a household organophosphate pesticide developed acute and reversible parkinsonism (Bhatt *et al.* 1999). Furthermore, Wistar rats administered paraquat showed dose-

dependent depletion of striatal dopamine. The monoamine oxidase B (MAOB) inhibitor selegiline attenuated paraquat-elicited dopaminergic toxicity (Liou *et al.* 2001). Often, pesticides are used as combinations of many compounds including solvents. In a study to investigate the effect of a combination of chemicals upon PD, paraquat and a fungicide manganese ethylenebisdithiocarbamate (maneb) were administered together to C57BL/6 mice. The combination of both compounds had a synergistic effect on the depletion of dopamine and its metabolite dihydrophenylacetic acid (DOPAC), compared with either compound administered alone (Thiruchelvam *et al.* 2000) (Figure 1.2).

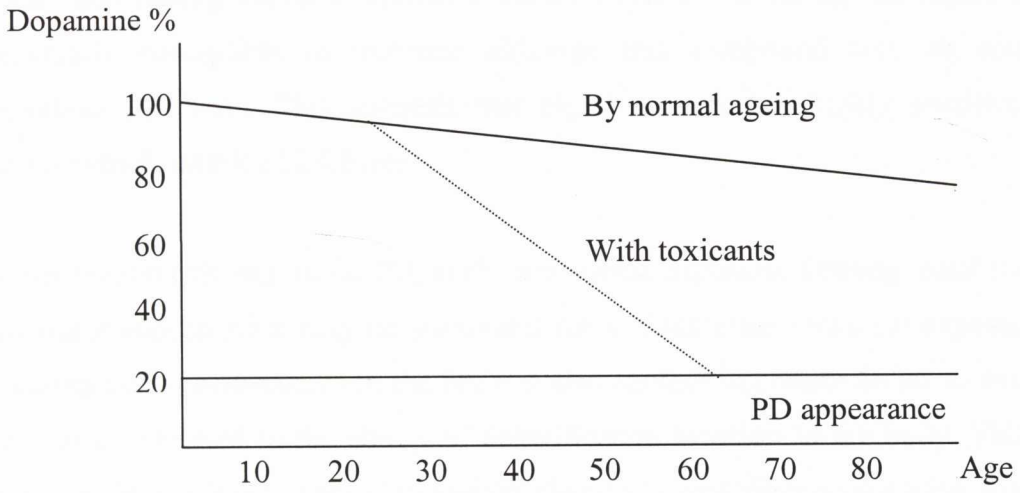

Figure 1.2. Schematic graph for the effect of environmental factors on PD. Symptoms of PD appear when the dopamine level in the striatum is reduced by 80% (Koller 1992) and environmental insults may trigger and/or accelerate the reduction of the striatal dopamine level.

A new animal model for PD, generated by chronic exposure to rotenone, recently provided evidence for the role of exposure to pesticides as a cause of PD (Betarbet *et al.* 2000; Giasson & Lee 2000). Rotenone is a plant-derived systemic complex I inhibitor that is widely used as a household insecticide and for eradicating fish. Unlike MPTP, rotenone binding is not specific for dopaminergic neurons. Because it is extremely hydrophobic, rotenone crosses plasma membranes and does not require DAT or any other transporter for access to the cytoplasm. Like MPTP, rotenone induces selective

cell death in the dopaminergic neurons of the substantia nigra by inhibition of complex I, however the mechanism of striatal neuron selectivity is not understood. Lewis rats infused with rotenone subcutaneously showed affinity of rotenone on complex I uniformly throughout the brain. The subsequent inhibition of complex I resulted in a loss of dopaminergic neuron markers, tyrosine hydrolase, DAT and vesicular monoamine transporter 2, which indicates a degeneration of nigrostriatal dopaminergic neurons. These animals also had cytoplasmic inclusions in nigral neurons that are similar to Lewy bodies in PD. Clinically, all animals with a dopaminergic lesion appeared hypokinetic and had unsteady movement and hunched posture. Some of them developed severe rigidity and resting tremor (Betarbet *et al.* 2000). It is interesting that nigral cells are particularly susceptible to rotenone although this compound acts on complex I throughout the brain. This suggests that nigral neurons are highly sensitive to the mitochondrial complex I inhibitor.

Environmental risk factors for PD, such as pesticide exposure, farming, rural living and well-water consumption may be surrogates for various other chemical exposures. The concentration of neurotoxins in the brain is also another important factor in developing PD, which is related to the ability of detoxification function in the body. This risk is because individuals are exposed to various chemicals, including neurotoxins, throughout their lifetime. High doses of rotenone induced nonspecific brain damage (Ferrante *et al.* 1997) and chronic exposure of low doses produced experimental PD (Betarbet *et al.* 2000). For this reason, detoxification of xenobiotics including pesticides will be important in determining the pharmacokinetic behaviors of neurotoxins and therefore the clinical outcomes.

1.2.3.1. Possible mechanisms for pesticide association with PD

1.2.3.1.1. Pesticides as neurotoxins

The pathogenesis of PD and MPTP neurotoxicity is known to involve inhibition of mitochondrial respiration and oxidative stress. Rotenone is a good example of mitochondrial impairment induced by pesticides. Rotenone is a systemic mitochondrial complex I inhibitor that produces parkinsonism in rats (Betarbet *et al.* 2000). Krueger *et*

al. (1990) have reported that rotenone binds at the same site on complex I as MPP^+. Many other pesticides produce mitochondrial dysfunction. For example, the organochlorine pesticide, endosulfan, produces T-cell apoptosis through disrupted mitochondrial transmembrane potential (Kannan *et al.* 2000). DDE (2,2-bis(p-chlorophenyl)-1,1-dichloethylene), a metabolite of DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane), also decreases mitochondrial respiration (complex II) and transmembrane potential (Ferreira *et al.* 1997). Dieldrin inhibits mitochondrial respiration by selectively depleting dopaminergic neurons (Sanchez-Ramos *et al.* 1998) and has been detected in some postmortem PD brains (Fleming *et al.* 1994). The herbicide paraquat depresses mitochondrial respiration through partial inhibition of complex III and IV (Palmeira *et al.* 1995). Paraquat has a similar chemical structure to MPTP and causes depletion of striatal dopamine (Liou *et al.* 2001). However, cellular death by this herbicide may be via redox cycles (Bus *et al.* 1976) because it is a strong oxidative agent.

Although many pesticides are associated with mitochondrial respiration inhibition and/or oxidative stress in the cells, they may also produce neurotoxicity through other mechanisms. For example, organophosphates and carbamates inhibit acetylcholine esterases, and organochlorines inhibit sodium channels (Ecobichon 1982). Although pesticides have many other pathogenic mechanisms in the brain in addition to mitochondrial complex I inhibition and oxidative stress in the substantia nigra, the nigrostriatal neurons may especially be vulnerable to many toxic and nonspecific insults.

1.2.3.1.2. Genetic influences on pesticide metabolism

Many candidate genes for PD have been studied that centre on enzymes that are involved in xenobiotic detoxification. If a genetic polymorphism leads to defective xenobiotic metabolism, then affected individuals might be more susceptible to neurotoxicity from pesticides and other putative neurotoxins.

Xenobiotic detoxification involves biotransformation of poorly excretable lipophilic substances to readily excretable water-soluble compounds. There are two major

pathways in this process (Sheweita 2000). Phase I pathways are involved in catalysis by oxidation and reduction of xenobiotics, and enzymes involved in this pathway include cytochrome P450 (CYP), flavin-containing monooxygenases (FMO) and esterases. Phase II pathways are involved in the conjugation of chemical compounds by enzymes, such as glutathione transferases (GST) and glucuronyl transferases. Pesticides are metabolized by many enzymes including CYP (Keseru 1998), FMO (Mushiroda *et al.* 2001), esterases (Karanth & Pope 2000), paraoxonase (Li *et al.* 2000) and GST (Prade *et al.* 1998).

Many xenobiotics undergo a two-step process of detoxification (Langston 1998). The first step is an activation step (phase I) that makes the xenobiotics more susceptible to conjugation. Conjugation (phase II) is the second step in this process and the conjugated products, in most cases, are nontoxic and/or easily excreted by the kidney. If there are abnormalities with genes in both phases of the detoxification process, there would be little compensation and such a combination of defects might therefore greatly enhance the risk of PD.

Defective enzyme production due to genetic polymorphism in the xenobiotic metabolizing enzymes may increase the risk of PD by influencing the disposition of pesticides. For example, CYP2D6 is involved in the detoxification of MPTP (Coleman *et al.* 1996), organophosphates and organochlorines (Hodgson & Levi 1996). Polymorphisms in this gene were more frequent in PD patients compared to controls (McCann *et al.* 1997; Ho *et al.* 1999; Morino *et al.* 2000; Harhangi *et al.* 2001). Another study has found an increased frequency of mutant CYP2D6 alleles in PD patients and these patients had a 2.5-fold increased risk of the disease (Smith *et al.* 1992). However, there are also conflicting observations where the association between polymorphisms of CYP2D6 and PD has not been detected (Diederich *et al.* 1996; Lo *et al.* 1998).

Other genes in the xenobiotic metabolising enzymes involved in PD are GST and paraoxonase (PON) genes. Polymorphisms in pi class GST may be more common in PD cases exposed to pesticides compared to similarly exposed control subjects (Menegon *et al.* 1998). Furthermore, at least one study reported that the deletion of the GSTM1 gene

apparently increased susceptibility to this disease (Stroombergen & Waring 1999). These polymorphisms may be associated with a decrease of total and reduced glutathione (GSH) level in the brain of PD patients. The loss of GSH is specific to the substantia nigra and not found in other brain areas (Perry *et al.* 1982; Sofic *et al.* 1992). Akhmedova *et al.* (2001) have shown that the frequency of the Met 54 allele of PON1 is significantly increased in patients with PD compared to the controls. They therefore estimated that the relative risk of PD in the Met 54 allele carriers is 2.3-fold higher than in controls for the L allele. Other studies have also reported the association of PON1 with PD (Kondo & Yamamoto 1998) and there are contradictory reports that there is no association between PON1 gene polymorphisms and susceptibility to Parkinson's disease (Taylor *et al.* 2000; Wang & Liu 2000).

1.2.3.1.3. Non-genetic modulation of xenobiotic metabolism

In addition to genetic factors, non-genetic factors can also alter xenobiotic metabolism, and therefore potentially alter the risk for PD. For example, ageing increases risk of PD (Tolwani *et al.* 1999) while cigarette smoking (Wechsler *et al.* 1991; Seidler *et al.* 1996; Liou *et al.* 1997), consumption of alcohol (Morano *et al.* 1994) and coffee (Benedetti *et al.* 2000) are known to decrease the risk, and all these factors can influence xenobiotics metabolism.

Old age is associated with impairment of xenobiotic metabolism (Le Couteur & McLean 1998a) and the liver is the major detoxification organ of the body. Le Couteur and McLean (1998a) have hypothesised that age-related changes in hepatic xenobiotic metabolism are secondary to impaired oxygenation of hepatocytes. This hypothesis states that ageing is associated with impaired oxygen diffusion into hepatocytes produced by oxidative injury to the cell membrane or to increased path length secondary to age-related swelling of hepatocytes. Later, it was found that the likely barrier to oxygen diffusion was the development of pseudocapillarisation of the sinusoidal endothelium, indicated by defenestration with reduced porosity, thickening of the endothelium and infrequent development of basal lamina (Le Couteur *et al.* 2001). Hypoxia causes a selective reduction in the activity of oxygen dependent enzymes such as CYP (Woodrooffe *et al.* 1995). Genetic polymorphism of CYP2D6 alters

susceptibility to PD (refer to Chapter 1.2.3.1.2. Genetic influences on pesticide metabolism). This finding supports the concept that impairment of xenobiotic detoxification metabolism in the aged liver is associated mechanistically with an increased risk of PD. Possible causes of impaired xenobiotic metabolism in the aged liver will be explained in more detail in Chapter 1.3.4. Ageing liver and xenobiotic clearance.

Epidemiological studies have shown that cigarette smoking (Seidler *et al.* 1996; Liou *et al.* 1997), alcohol and coffee intake (Morano *et al.* 1994) are protective against PD. These agents are known to be enzyme inducers (Koide *et al.* 1999; Kono *et al.* 2000; Smith *et al.* 2001) and the increased activity of liver detoxification enzymes may be associated with protection from PD. Many pesticides also modulate xenobiotic metabolising enzyme activity (Hodgson & Levi 1996). For example, organochlorine pesticides such as DDT induce CYP (Li *et al.* 1995) while organophosphate pesticides including parathion inhibit this enzyme (Butler & Murray 1997). Furthermore, GSTs are inhibited by the herbicide, tridiphane (Moody & Hammock 1987) and the fungicide, captofol (Di Ilio *et al.* 1996). Therefore, the modulation of xenobiotic metabolising enzyme activity by pesticides could influence the metabolism of exogenous or endogenous neurotoxins (Le Couteur & McCann 1998b) indirectly by influencing the disposition of these toxins. For example, the endogenous neurotoxin associated with PD, tetrahydroisoquinoline, is metabolised by CYP2D6 (Suzuki *et al.* 1992) and therefore inhibition of this enzyme by pesticides may increase its toxicity.

1.3. Hepatic detoxification of xenobiotics

1.3.1. Liver structure

The liver is positioned between the digestive tract and the systemic circulation. The size of this organ is approximately 2-5% of body weight in the adult man and 5% at birth (Meijer & Groothuis 1991). Because of its interposition, nutrients, drugs and other toxic xenobiotics that are absorbed in the gastrointestinal tract have to pass through this organ via the portal vein before they reach other tissues in the body, including the brain.

1.3.1.1. Blood supply

The liver receives 20 to 25% of cardiac output via the portal vein and hepatic artery (Dawson *et al.* 1979). The former accounts for 75-80% and the latter 20-25% of the total hepatic blood flow (Wallace *et al.* 1990). For blood supply to the liver (Figure 1.3), the portal vein receives venous blood from the intestines and spleen (Bombardieri & Conti 1997). It divides into interlobular branches that run in the connective tissues of the portal tract and branches into smaller terminal divisions that eventually become continuous with sinusoids (Rappaport 1980). Preterminal portal branches lie in small triangular portal tracts. Any nutrients and xenobiotics in the blood of sinusoids are absorbed into hepatocytes through the endothelium and metabolised in the cells. The sinusoidal endothelium acts as a sieve (Fraser *et al.* 1995). The metabolites in the hepatocytes are then drained into sinusoids or bile. The blood in the sinusoids is drained into central veins (Burkel & Low 1966) and these veins join to hepatic veins that empty into the inferior vena cava (Newell 1984).

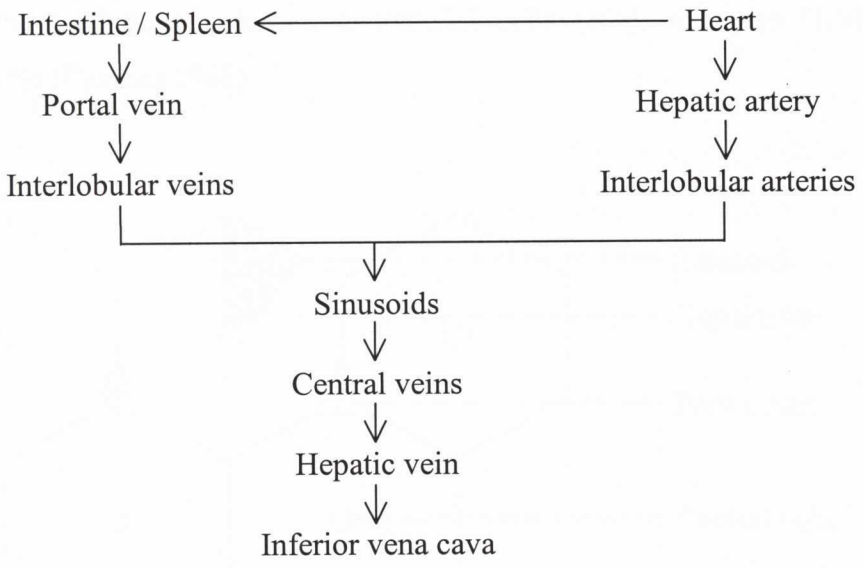

Figure 1.3. Organization of hepatic blood circulation

The hepatic artery that delivers oxygenated blood from the heart accompanies the portal vein (Ekataksin & Kaneda 1999). In their terminal portions, hepatic arteries branch to form arterioles and the arterial capillaries join the sinusoids directly (Rappaport 1980).

1.3.1.2. Hepatic lobule

The hepatic lobule is a region of parenchyma, typically hexagonal in cross section (Figure 1.4), which surrounds a central vein at its center (Ekataksin & Wake 1991). In most species including humans, connective tissue at the corners of the lobule contains arteries, veins, bile ducts and lymphatics (Vyrenkov 1983). Within the lobule, hepatocytes form radially around the central vein. The blood flows from the periphery of the lobule, from portal vein and hepatic artery, through sinusoids into central veins (McCuskey 2000). Each hepatocyte is exposed to the blood from two sinusoids representing at least up to 40% of the cell surface area (Blouin *et al.* 1977). The fenestrated endothelial lining (Fraser *et al.* 1995; Le Couteur *et al.* 2001) permits direct contact of blood constituents of limited size with the villous plasma membrane of the hepatocytes through the Space of Disse (Figure 1.5). Cells of the liver include Kupffer cells (2% of the total liver volume), endothelial cells (3%), lipocytes (1.5%) and hepatocytes (91%) (Campra 1988).

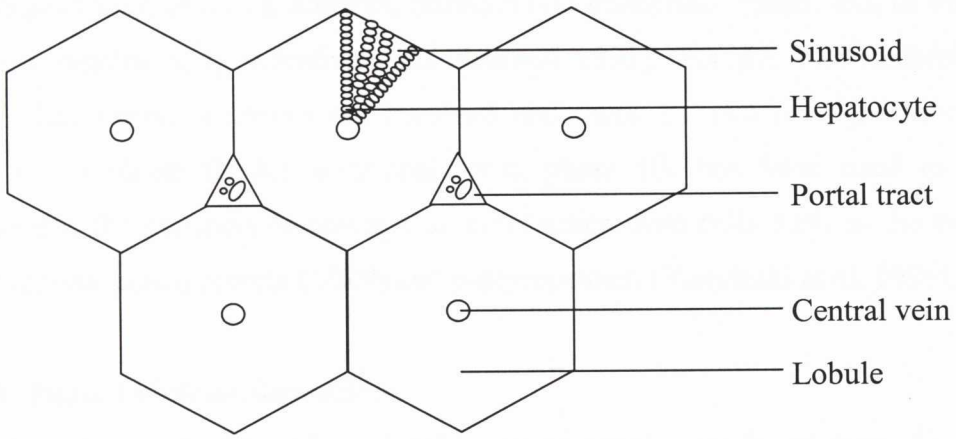

Figure 1.4. Schematic diagram of hepatic lobules. They are hexagonal in shape and portal tracts include interlobular portal veins and hepatic arteries, bile ducts and lymphatics.

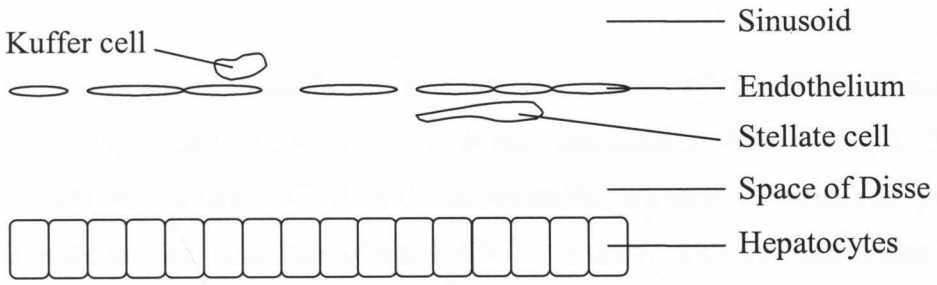

Figure 1.5. The microscopic structure of hepatic sinusoid and hepatocytes

1.3.2. Xenobiotic biotransformation in the liver

In addition to many hepatocyte functions (e.g. uptake of amino acids, carbohydrates, lipids and vitamins and their subsequent storage, metabolic conversion, and release into blood and bile), biotransformation is one of the most important functions of the liver (Smallwood 1990). After primary uptake of materials by the liver, metabolic degradation and biotransformation are performed in intracellular organelles such as the endoplasmic reticulum and lysosomes. These processes convert poorly excretable hydrophilic xenobiotics into readily excretable water-soluble compounds that can be excreted into bile or urine (Meyer 1996). Although the biotransformation system is also present in other organs such as lungs, kidneys, intestine and endocrine organs, that of the liver is the most important, quantitatively (Lindamood 1991). For the biotransformation of xenobiotics, various enzymes are involved and there are two principal mechanisms, phase I and phase II. An additional term, phase III, has been used to describe mechanisms for transport of conjugated xenobiotics from cells such as the multi-drug resistance-associated protein (MRP) and p-glycoprotein (Yamazaki *et al.* 1996).

1.3.2.1. Phase I biotransformation

Quantitatively, most of the phase I oxidation of xenobiotics is catalysed by the CYP monooxygenase enzyme system (Hodgson *et al.* 1995; Meyer 1996; Lang *et al.* 1997; Teyssier & Siess 2000). FMO (Lang & Rettie 2000), molybdenum hydroxylases

(Rashidi *et al.* 1997), epoxide hydrolase (Fennell & Brown 2001), alcohol and aldehyde dehydrogenases (Yao *et al.* 1997) are also involved in phase I biotransformation.

All tissues, except striated muscle and erythrocytes, possess CYP activity (Sligar 1999). CYP utilises oxygen as a substrate to metabolise xenobiotics (Snyder 2000). Briefly, a substrate binds to oxidised CYP and nicotinamide adenine dinucleotide phosphate (NADPH) reduces the substrate-oxidised CYP complex. The reduced complex then combines with molar oxygen and CYP is oxidised again; other products from this process are hydroxylated substrates (Modi *et al.* 1997) and water. These hydroxylated substrates are water soluble and, hence, are easily excreted from the body. CYP has been characterised into 34 families in the rat and 14 families in humans (Nebert *et al.* 1989a; Nebert *et al.* 1989b). Polymorphisms in CYP2D6 and CYP1A1 genes have been associated with PD (see Chapter 1.2.2. Genetic risk factors of PD). MPTP is metabolised by CYP2D6 (Di Monte *et al.* 1988; Coleman *et al.* 1996; Narimatsu *et al.* 1996; Modi *et al.* 1997), which suggests that individual susceptibility to PD may be associated with differences in Phase I activity. Decreased hepatic metabolism of xenobiotics would increase their delivery to the central nervous system and hence increase the likelihood of neurotoxic injury (Shahi *et al.* 1990).

The CYP2D6 gene codes for debrisoquine 4-hydroxylase which metabolises a wide range of compounds containing a basic nitrogen atom (Eichelbaum & Gross 1990; Tucker *et al.* 1994) including a variety of clinically important drugs. MPTP is also metabolised by this enzyme (Coleman *et al.* 1996). By the addition of NADPH and NADPH-CYP reductase, MPTP is metabolised to two products, 1-methyl–1,2,3,6-tetrahydropyridine and 1-methyl-4-(4'-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (Modi *et al.* 1997). The debrisoquine metaboliser phenotype is characterised by administration of a subtherapeutic dose of debrisoquine, and subsequent analysis of this compound and its 4-hydroxy metabolite after 8hrs in urine. The percentage recovery of debrisoquine divided by its metabolite provides a metabolic ratio (MR) and individuals with MRs of more than 12.6 are classified as poor metabolisers (PMs) of debrisoquine (Riedl *et al.* 1998). There is a close relationship between metabolic phenotype and genotype, that is, genetics affect the level of enzyme activity (Daly *et al.* 1996). The

distribution of CYP2D6 alleles varies across ethnic groups: 5-10% of the white population of Europe and the United States but less than 1% of Asians are PMs (Nakamura *et al.* 1985). Many studies have shown association between PD and mutant alleles in the CYP2D6 gene (Barbeau *et al.* 1985; Armstrong *et al.* 1992; Akhmedova *et al.* 1995; McCann *et al.* 1997). Smith *et al.* (1992) have reported that there is a 2.5-fold greater incidence of the CYP2D6 PM genotype in patients with PD compared to controls. Together, MPTP metabolism with CYP2D6 and polymorphisms in CYP2D6 PM genotype suggest that the activity of CYP2D6 may be a significant factor in the susceptibility to PD (Gilham *et al.* 1997; McCann *et al.* 1997). However, there are also contrasting reports that have failed to detect a link between PMs and PD (Gudjonsson *et al.* 1990; Kallio *et al.* 1991; Steiger *et al.* 1992; Yoshino *et al.* 1993), suggesting little influence of CYP2D6 gene polymorphisms on PD.

Another CYP gene possibly involved in PD is CYP1A1. CYP1A1 contains seven exons and Bennett (1994) has reported that a rare allele in exon 7 (A/G substitution in locus 2) was significantly involved in familial, but not sporadic, PD. A second sequence variant in this gene (dubbed m2) has also been reported to increase the risk for PD (Takakubo *et al.* 1996).

Organophosphate and organochlorine pesticides are substrates for CYP. For example, total body clearance of the organophosphate insecticide parathion was slower in rats pretreated with CYP inhibitors, SKF 525-A or ketoconazole, than control rats (Hurh *et al.* 2000). Incorporation of CYP2D6 and CYP3A4 inhibitors, quinidine and ketoconazole, respectively, reduced cholinesterase activity with parathion, diazinon and chlorpyrifos (Sams *et al.* 2000). The CYP system also metabolises malathion and produces the toxic metabolite malaoxon (Ehrich *et al.* 1984). An organochlorine pesticide DDT has been reported to induce hepatic CYP2B and CYP3A (Li *et al.* 1995; Nims *et al.* 1998). Furthermore, as an example of PD protective agents, alcohol induced mouse hepatocyte CYP2E1 up to 5-fold at the protein level (Yang *et al.* 2001) and two weeks of cigarette smoking induced rat liver CYP1A2 (3-fold) and CYP1A1 in the western immunoblotting analysis (Koide *et al.* 1999). Hepatic CYP1A2 was also induced in caffeine-treated mice (Casley *et al.* 1997). The level of protein expression

was the highest in the C3H/HeJ strain and females had lower 1,7-xanthine/caffeine ratios than male mice, which indicate that the induction of this enzyme is gender and strain-dependent. Activities of these enzymes are differentially regulated at the constitutive level and in response to xenobiotic exposure.

In addition to CYP-related enzymes, FMO is another active enzyme in phase I biotransformation (Iyer & Sinz 1999). It is localised in the endoplasmic reticulum and microsomes, and all tissues except muscle contain this enzyme. Liver, lung and kidney have the highest activity (Lindamood 1991). FMO catalyses the oxidation of nucleophilic nitrogen, sulfur and phosphate atoms (Hodgson & Levi 1992). A nitrogen-containing MPTP was metabolised to MPTP N-oxide by liver FMO (Chiba *et al.* 1988; Di Monte *et al.* 1988; Chiba *et al.* 1995) and its activity was the greatest in hepatic microsomes, as compared to kidney, lung and brain microsomes (Chiba *et al.* 1988). Some pesticides are also substrates for this enzyme. For example, methimazole, an inhibitor of FMO, partially suppressed the rat hepatocyte cytotoxicity produced by the herbicide, chlornitrofen-amino, which suggests that FMO plays an important role in the cytotoxicity induced by chlornitrofen-amino (Jinno *et al.* 1999). The sulfoxidation of methiocarb, an N-methylcarbamate insecticide, was catalyzed by rat liver microsomal FMO (Buronfosse *et al.* 1995).

1.3.2.2. Phase II biotransformation

Phase II biotransformation reactions form conjugates that serve as transport forms of compounds and allow selective uptake by target cells through specific transport systems that recognise the conjugating moiety. Major enzymes involved in the phase II biotransformation are glucuronyltransferases, GSTs, sulfotransferases, N-acetyltransferases and amino acid conjugation enzymes (Vereczkey *et al.* 1998). Among these, the enzymes most studied in relation to PD are the GSTs. Substrates for these enzymes can be xenobiotics with an electrophilic carbon, nitrogen, sulfur or oxygen atom (Morrow *et al.* 1998; Zhao *et al.* 1999; Mari & Cederbaum 2001). The reaction mechanism involves the formation of a thioether bond between a xenobiotic and glutathione. The conjugates are ultimately cleaved to cysteine derivatives in the kidney,

the cysteine moiety is acetylated and the compound is then excreted in the urine as the mercapturic acid derivative (Pickett & Lu 1989; Armstrong 1991).

Although GSH conjugation is, in general, a detoxification pathway for xenobiotic biotransformation, certain xenobiotics including diclofenac, an anti-inflammatory drug, are known to be bioactivated by this pathway, thus contributing to hepatotoxicity (Tang *et al.* 1999). Amongst pesticides, malathion induces GSTs (Hoshiya *et al.* 1993; Ito *et al.* 1994). Rats treated with malathion have induced activity of liver GSTs to 150% of the control value (Srikanth & Seth 1990). For the association of GST genes with PD, there have been contrasting reports. People with deletions of GSTM1 (Stroombergen & Waring 1999) and T1 (Tan *et al.* 2000) were found to be susceptible to PD. Furthermore, frequencies of the polymorphisms in GSTP1 were different between PD patients and controls who had been exposed to pesticides (Menegon *et al.* 1998). However, Rahbar *et al.* (2000) failed to find any differences in the frequencies of the homozygous deletion of GSTM1 and GSTT1 between PD patients and control groups. The inductions of the activities of phase I and phase II enzymes may have evolved as an adaptive mechanism to assure survival in response to toxicity from xenobiotics in the environment.

1.3.3. Hepatic membrane transport in xenobiotic elimination

As Figure 1.6 shows, xenobiotics are taken up by the liver from sinusoidal blood. The efficient delivery of these compounds occurs through fenestrations in the endothelial lining of the sinusoids (Fraser *et al.* 1995) into the extracellular space (Space of Disse). Proteins in the blood are excluded from entering hepatocytes and other components are taken up into the cells through the hepatocyte membrane. Molecular-based hepatocellular transport has been extensively reviewed (Evans 1996; Meier *et al.* 1997; Kamisako *et al.* 1999; Kullak-Ublick *et al.* 2000a). Figure 1.7 illustrates a summary of various hepatic membrane transport systems. For small, uncharged lipophilic molecules such as ethanol and urea, uptake into hepatocytes across the basolateral membrane occurs via passive diffusion mechanisms (Evans 1996). This transport system is so efficient that uptake is rate-limited by the delivery of the xenobiotics to the liver (e. g.

blood flow) rather than active membrane transport (Booth *et al.* 1996).

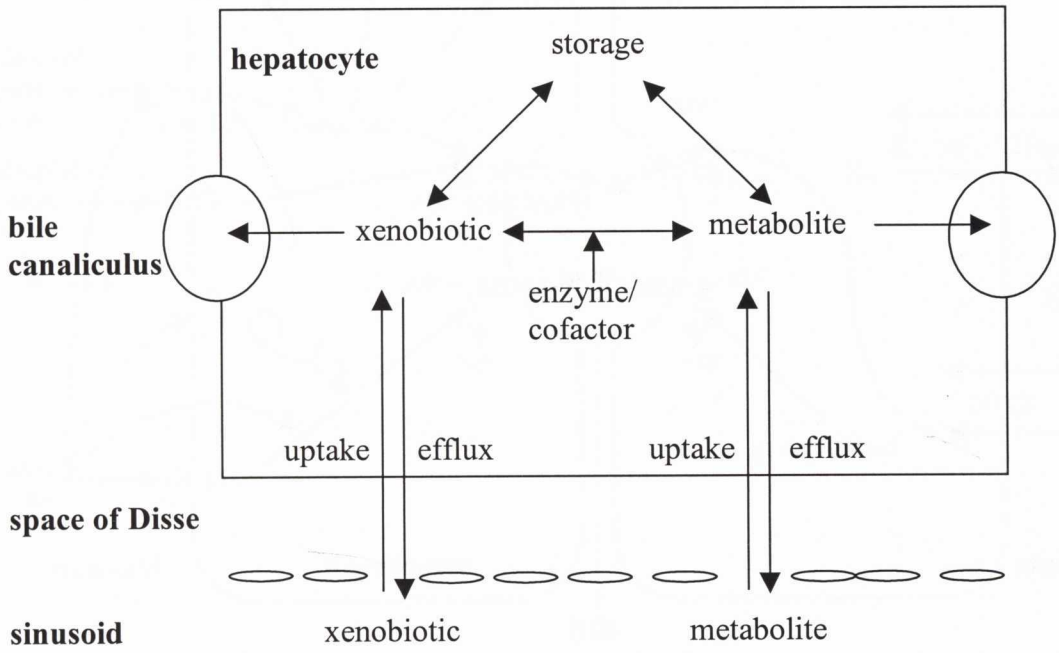

Figure 1.6. General routes of hepatic transport of xenobiotics.

For polar molecules such as organic cations and organic anions, hepatocellular uptake relies on carrier-mediated transport systems (Kamisako *et al.* 1999; Van Montfoort *et al.* 2001). For example, Martel *et al.* (1996a) have reported that MPP^+ is transported into hepatocytes by organic cation transporter 1 (OCT1). For these compounds, membrane transport may be dependent on the efficiency of hepatic elimination (Hisaka *et al.* 1999). Some metabolites, especially conjugated metabolites such as sulphates and glucuronides, are polar and they use carrier-mediated transport systems for sinusoidal efflux and biliary excretion (Evans 1996). However, most compounds are transported by multiple transport systems, passive diffusion with carrier-mediated transport systems (Burwen *et al.* 1992; Kullak-Ublick *et al.* 2000b), for both hepatocellular influx and efflux (Evans 1996) as shown in Figure 1.7.

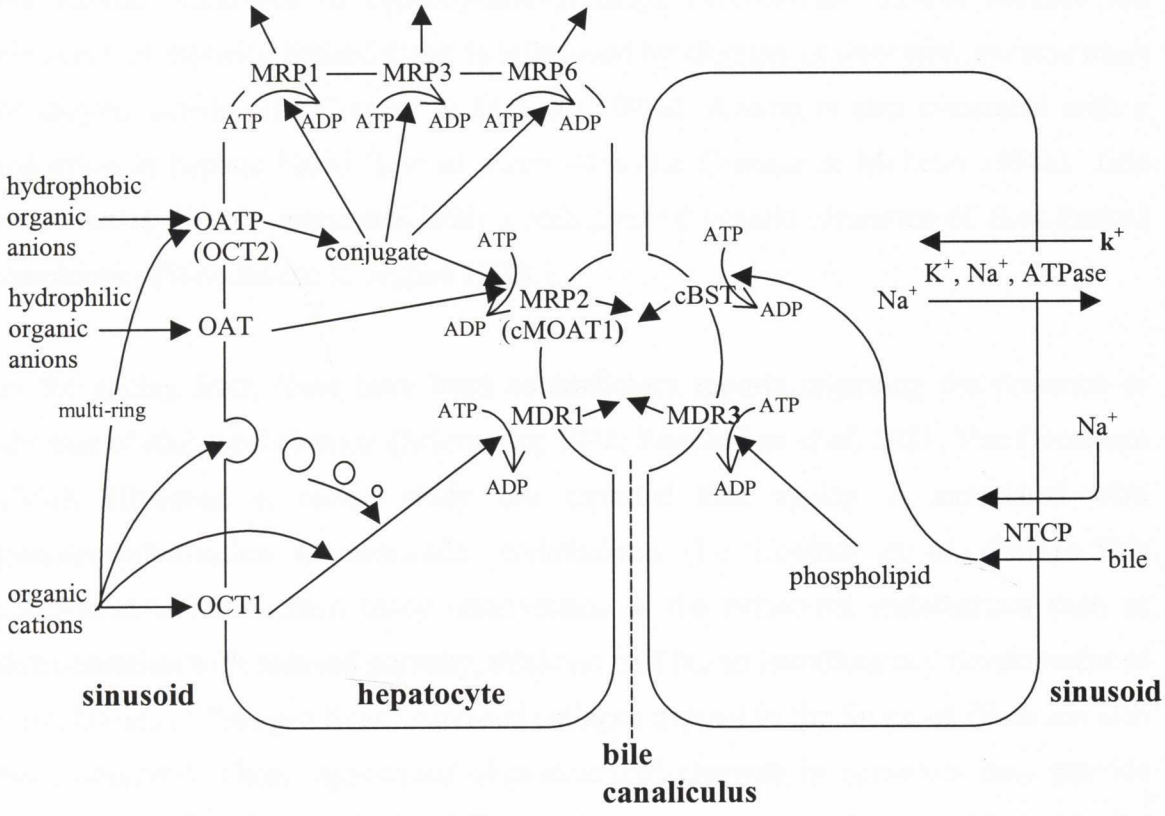

Figure 1.7. Schematic diagram of hepatocellular membrane transport. Cellular influx of compounds into hepatocytes is through various transport systems depending upon their chemical structures. Cellular efflux of some compounds and their metabolites is ATP dependent. ATP, adenosine triphosphate; ADP, adenosine diphosphate; cBST, canalicular bile salt transporter; cMOAT, canalicular multi-specific organic anion transporter; MDR, multi-drug resistance gene product; MRP, multi-drug resistance-associated protein; NTCP, sodium taurocholate co-transporter; OATP, organic anion transporting polypeptide; OAT, organic anion transporter; OCT, organic cation transporter.

1.3.4. Ageing liver and xenobiotic clearance

Xenobiotic clearance in the liver is reduced in older people (Le Couteur & McLean 1998a). With old age, major changes in the liver include reduction of cell mass and blood flow. With regard to liver mass, ageing is associated with a 20% reduction in

males and an 11% reduction in females (Boyd 1933). This reduction may be related to the reduced clearance of capacity-limited drugs (Woodhouse 1992a) because the clearance of capacity-limited drugs is influenced by changes in liver size, enzyme mass or enzyme activity (Le Couteur & McLean 1998a). Ageing is also associated with a reduction in hepatic blood flow of about 40% (Le Couteur & McLean 1998a). This reduction is directly associated with a reduction of hepatic clearance of flow-limited xenobiotics (Woodhouse & Wynne 1992b).

In the ageing liver, there have been contradictory reports regarding the presence or absence of structural changes (Schmucker 1978; Engelmann *et al.* 1981; Van Bezooijen 1984). However, a recent study has reported that ageing is associated with pseudocapillarisation of sinusoidal endothelium (Le Couteur *et al.* 2001). This conclusion is based upon many observations in the sinusoidal endothelium such as defenestration with reduced porosity, thickening of the endothelium and development of basal lamina in the aged liver. Increased collagen deposit in the Space of Disse has also been observed. These age-related ultra-structural changes in sinusoids may provide delays and/or interference in the delivery of components, including xenobiotics, in the sinusoidal blood into hepatocytes. In fact, age-related reduction in the hepatic uptake of drugs has previously been reported (Iwamoto *et al.* 1986a; Ohta *et al.* 1988; Ohta & Kitani 1990).

Ageing has variable effects on hepatic xenobiotic metabolism. Phase I enzyme activity is reduced, while phase II enzyme activity is maintained (Tarloff *et al.* 1991). MPTP and many pesticides are metabolised by CYP (Chapter 1.3.2.1. Phase I biotransformation) and Phase I enzymes use oxygen as a substrate to metabolise compounds. The age-related reduction of the phase I drug clearance may be secondary to impaired oxygen supply into hepatocytes by pseudocapillarisation of the sinusoidal endothelium (Le Couteur *et al.* 2001).

1.4. Aims of the study

The general hypothesis for this study is that modulation of hepatic xenobiotic metabolism will influence susceptibility to the effects of neurotoxins such as MPTP and pesticides.

The perfused rat liver was used as the main experimental model and the multiple indicator dilution (MID) technique was used to study hepatic disposition of compounds. Isolated hepatocytes from the liver were also used to study hepatocellular membrane transport in addition to the perfused rat liver.

In Chapter 2, the hepatic extraction and transport kinetics of MPTP and pesticides in the young rat liver was examined. MPTP and pesticides are known to be associated with PD. This study served as a preliminary study to examine differences in the disposition of these chemicals in the normal liver.

In Chapter 3, the effect of pretreatment of pesticides on the hepatic disposition of MPTP and pesticides was investigated. This study is based on the hypothesis that the combination of pesticides may reduce hepatic extraction and increase systemic bioavailability, as compared to when they are used individually. Most pesticides are used as a combination of compounds and people are often exposed to mixed chemicals. The combination of pesticides may reduce hepatic extraction by interacting with hepatocellular transporters or enzymes, or chemical interactions.

In Chapter 4, the effect of ageing upon the hepatic disposition of MPTP and pesticides was studied. Ageing is an important risk factor for PD. Therefore, the hypothesis is that increased PD in aged people may be due to decreased hepatic xenobiotic detoxification.

Chapter 5 investigated hepatocellular membrane transporters for MPTP transport into cells. This study was motivated by observations of hepatic extraction of MPTP in the previous chapters. In Chapter 2, MPTP was extracted in the liver extensively (98% of the injectate) in young rats. Pretreatment with pesticides in young rats (Chapter 3) and

aged rats (Chapter 4) reduced the hepatic extraction of MPTP, however it was still extracted extensively (more than 90% of the injectate) compared to other pesticides used. This study has been done in the perfused rat liver as well as in isolated hepatocytes.

In Chapter 6, hepatic disposition of MPTP and malathion in a diseased liver, i.e. the cirrhotic liver, was investigated and compared with that in the aged liver. This study is based on findings from Chapter 3 and Chapter 4. In Chapter 3, hepatic extraction of MPTP and malathion was reduced by pretreatment of rats with pesticides and hepatic extraction of these compounds was also reduced in the aged liver in Chapter 4. Therefore, a reduction of hepatic extraction of these compounds in the cirrhotic liver was hypothesised. Structural and functional differences between cirrhotic and aged livers will also be discussed in this Chapter.

2. HEPATIC DISPOSITION OF MPTP AND PESTICIDES

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2.1. INTRODUCTION

There are several groups of risk factors for idiopathic PD that have been determined using epidemiological methods (Chapter 1.2. Etiology of PD). These include advanced age, family history of PD and environmental factors, such as exposure to pesticides (Seidler *et al.* 1996), rural living and consumption of well water (Koller *et al.* 1990; Marder *et al.* 1998; Le Couteur *et al.* 1999a). The pivotal discovery that exposure to MPTP produces a Parkinsonian syndrome in man (Langston 1996) and animals (Tolwani *et al.* 1999) has lead to the hypothesis that PD is secondary to environmental or possibly endogenous neurotoxins with a mode of action similar to MPTP (Tanner & Ben-Shlomo 1999). The structural similarities between MPP^+, an active metabolite of MPTP, and paraquat and the epidemiological association between pesticide exposure and PD have raised the possibility that pesticides are causative neurotoxins in some cases of PD.

A correlation between the prevalence of PD and the volume of pesticide used was reported in Canada (Barbeau *et al.* 1987). In Israel, an association was detected between preparkinsonism and residence in areas where carbamates and organophosphates were used (Herishanu *et al.* 1998). Furthermore, case-control surveys from several continents have shown significant associations between pesticide exposure and PD (Le Couteur *et al.* 1999a). There are also a number of case reports of PD following pesticide exposure. A case was reported of a 41 year old farmer using numerous pesticides for years who developed PD (Bocchetta & Corsini 1986). There have also been other reports of PD developing after pesticide exposure including a 32 year old farmer who had worked with paraquat for 15 years (Sanchez-Ramos *et al.* 1987) and a 37 year old man with chronic exposure to the fungicide, maneb (manganese ethylene-bis-dithiocarbamate) (Meco *et al.* 1994). Together these community- and case-based studies provide persuasive evidence that pesticide exposure is associated with increased risk of PD.

Inhibition of mitochondrial function is thought to be a major cause of dopaminergic cell death in PD, although other mechanisms involving free radicals and excitatory

neurotransmitters have also been recognised (Fornai *et al.* 1997; Przedborski & Jackson-Lewis 1998). It is of note that mitochondrial impairment has been observed in the substantia nigra and other tissues in PD (Jenner & Olanow 1996). Although many pesticides have major actions unrelated to mitochondrial activity, there have been numerous reports that pesticides from various classes can inhibit mitochondrial respiration as an additional mode of action (Le Couteur *et al.* 1999a).

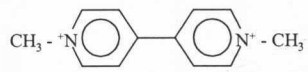
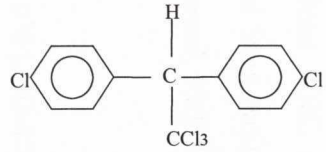
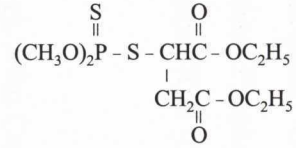
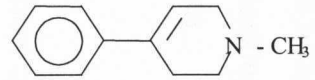
An organic pesticide, rotenone, that produces a model form of PD in rats, is a systemic mitochondrial complex I inhibitor (Betarbet *et al.* 2000). Dieldrin, an organochlorinated pesticide, which has been found in PD patient brains in the postmortem study, also inhibits mitochondrial respiration and selectively depletes dopaminergic neurons (Sanchez-Ramos *et al.* 1998). Furthermore, the herbicide paraquat depresses mitochondrial respiration through partial inhibition of complexes III and IV (Palmeira *et al.* 1995) even though cellular toxicity from this chemical may be through its redox cycles (Bus *et al.* 1976). Additionally, DDE, a metabolite of DDT, decreases mitochondrial respiration and transmembrane potential (Ferreira *et al.* 1997).

Investigation of the hepatic metabolism of pesticides and MPTP is important for understanding the pathogenesis of PD because the liver is the major detoxification organ in the body. To determine extraction and metabolism of xenobiotics in the liver, the multiple indicator dilution (MID) technique has been widely used in the perfused rat, dog and sheep livers (Goresky *et al.* 1989; Le Couteur *et al.* 1999b; Mellick & Roberts 1999b; Gow *et al.* 2000). However, rats are the most widely used among the above species and the preparation and application of isolated perfused rat liver has been explained in various reviews (Gores *et al.* 1986; Wolkoff *et al.* 1987).

The MID technique is an indirect approach to investigate cell entry, efflux from the cell and sequestration of substances in the liver (St-Pierre *et al.* 1989), hence it is also important for understanding the disposition of pesticides in the liver (Constantin *et al.* 1995; Marshall *et al.* 2001). A tracer of the substance to be studied is injected together

with a vascular, interstitial (vascular with Space of Disse) or intracellular marker into the liver and serial outflow samples are analysed for their activity in the liver (Goresky 1983). In this study, the hepatic disposition of MPTP and several classes of pesticides (Table 2.1) was investigated in the perfused rat liver using the MID technique.

Table 2.1. Chemical structure and classification of Malathion, paraquat, DDT and MPTP.

Name	Classification	Formula	Structure	MW
Paraquat	Bipyridyl herbicide	$C_{12}H_{14}N_2Cl_2$		257.2
DDT	Chlorinated hydrocarbon insecticide	$C_{14}H_9Cl_5$		354.5
Malathion	Organophosphate insecticide	$C_{10}H_{19}O_6PS_2$		330.4
MPTP	Neurotoxin	$C_{12}H_{15}N$		173.0

2.2. MATERIALS AND METHODS

2.2.1. Materials

Paraquat-methyl-^{14}C dichloride (32.3 mCi/mmol, 99.9%), 4,4'-DDT-RING-UL-^{14}C (12.8 mCi/mmol, 99.9%) and malathion-2,3-^{14}C (6.5 mCi/mmol, 99.9%) were purchased from Sigma (Missouri, USA) and methyl-4-phenyl-1,2,3,6-tetrahydropyridine,1-[methyl ^{3}H] (80 Ci/mmol, 99.9%) from ARC (Missouri, USA). [6,6'(n)-^{3}H]-Sucrose (14.8 Ci/mmol, 99.9%) was obtained from Amersham Pharmacia (Buckinghamshire, UK) and [U-^{14}C]-sucrose (462 mmCi/mmol, 99.9%) from ICN (Ca, USA).

2.2.2. Animals

Male Wistar rats (2-3 months old [240-400 g], John Curtin School of Medical Research, Canberra, Australia) maintained on standard rat food pellets and water *ad libitum* were used for study. The study was approved by the Australian National University Animal Experimentation Ethics Committee.

2.2.3. Liver perfusion

The perfused rat liver preparation used in this study has been described in detail previously (Le Couteur *et al.* 1994a; Le Couteur *et al.* 1999b). Rats were anaesthetised by intraperitoneal injection (i.p.) of sodium pentobarbitone (60 mg/kg, Nembutal, Abbott Ltd, Australia) and a midline laparatomy incision was made. Heparin (200 U, David Bull Laboratory, UK) was administered via the abdominal inferior vena cava. The portal vein was cannulated with an 18-gauge intravenous catheter (Johnson & Johnson Ltd, Italy) and the livers were perfused *in situ* via the portal vein cannula. The effluent buffer with blood in the liver was immediately released by incision of the inferior vena cava. More incisions were made for the thoracic inferior vena cava to be exposed. After the liver was free from its attachments to the diaphragm, the outflow cannula (10 cm length polyethylene tubing, ID 1.4 × OD 1.9 mm, Critchley Electrical Products, NSW, Australia) was inserted into the thoracic inferior vena cava to collect samples.

The perfusate consisted of Krebs-Henseleit bicarbonate (KH) buffer (132 mM NaCl, 3 mM KCl, 1.2 mM KH_2PO_4, 1.18 mM $MgSO_4.7H_2O$, 1.27 mM $CaCl_2.2H_2O$, 25 mM $NaHCO_3$ and 10 mM glucose, pH 7.4) saturated with 95% O_2 and 5% CO_2 (Linde, Canberra, Australia). The protein content in the perfusate was 2% (w/v) bovine serum albumin (BSA, Sigma, USA) and the temperature of the buffer was 37°C. The perfusate flow rate was maintained at 24-26 ml/min using a cartridge pump (Cole-Parmer Instruments, USA) in a non-recirculating (i.e. "single pass") system. The flow rates were determined volumetrically.

2.2.4. Liver viability

Liver viability was assessed by macroscopic appearance, oxygen consumption, portal venous pressure and assay of outflow samples for liver enzymes.

For oxygen consumption, inflow and outflow samples were collected in a 5 ml syringe before and after experiments to measure the partial pressure of oxygen (pO_2). pO_2 values were measured using an AVL Automatic Blood Gas System (AVL Medical Instruments, Vic, Australia). Oxygen consumption was calculated by the formula:

$$O_2\ consumption = 0.0227 \times 44.663 \times (pO_{2(\text{inf low})} - pO_{2(outflow)})Q \qquad (2.1)$$

In this relationship, pO2 is measured in mmHg, 0.0227 is the solubility of oxygen in KH buffer at 37°C expressed in ml/ml/atm (Christoforides & Hedley-Whyte 1969), 44.663 is a constant to convert 1 ml of oxygen to μmol, Q is the flow rate in ml/min/g liver wet weight (ww) and oxygen consumption is expressed in μmol/min/g liver ww (Le Couteur *et al.* 1993).

Portal venous pressure of rats was measured as the difference in the height of a vertical manometer attached to the portal venous cannula both in the presence and absence of the liver. The portal resistance was calculated by the difference in the manometer reading in cm H_2O divided by perfusate flow rate (ml/min/g liver) and it was expressed in cm

H_2O·min·g/ml (Le Couteur *et al.* 1993). For the liver enzyme assays, outflow samples were sent to the Biochemistry laboratory (The Canberra Hospital, ACT, Australia) to measure concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) (Le Couteur *et al.* 1999c).

2.2.5. Multiple indicator dilution experiments

The method of MID following the liver perfusion has also been described in detail previously (Gores *et al.* 1986; Goresky *et al.* 1989). Bolus injections (50 µl) contained 2 µCi of either ^{14}C-paraquat, ^{14}C-DDT, ^{14}C-malathion or ^{3}H-MPTP. ^{3}H-sucrose was included in the injectates for ^{14}C-paraquat, ^{14}C-DDT and ^{14}C-malathion and ^{14}C-sucrose for ^{3}H-MPTP. Sucrose was used as an extracellular marker. After preperfusion for 20 min for stabilization, the injection of indicators into the portal vein was performed in randomised order. Outflow samples from the liver were collected every 1.4 seconds (s) for 40 s and then one more collection at 60 s using a fraction collector (Universal Fraction Collector, Eldex Laboratories, USA). The outflow samples were kept at 4°C until ready for analysis. Outflow samples were analysed for ^{14}C or ^{3}H radioactivity by adding 5ml scintillation fluid (Starscint, Packard Bioscience Ltd, Netheland) in 50 µl samples and counted using a liquid scintillation counter (Packard Instruments, USA). Radioactivity in the outflow is unlikely to be obtained from metabolites of the chemicals injected because the time of collection is short. Bile was also collected until the experiment finished to check for biliary excretion of indicators, however, no radioactivity was detected in the bile (see 2.4. Discussion).

2.2.6. Data analysis

Analysis of the outflow concentration-time profile following bolus injection of a substance into the liver provides useful information regarding the disposition of the substance in the organ (Goresky 1983). Molecules that have intracellular access, such as water, appear in the outflow as a delayed wave relative to a molecule, such as sucrose, which is restricted to the extracellular space. Vascular components, such as red blood cells, are also excluded from the Space of Disse and therefore appear in the outflow prior

to sucrose (Goresky 1984). Intracellular metabolic sequestration and biliary secretion reduce the outflow recovery of returning compounds (Takenaka *et al.* 1995; Mellick & Roberts 1999b). By fitting outflow data, influx and efflux parameters and liver cellular space sizes for the substance can be estimated (Goresky 1983).

The hepatic outflow concentrations (C) were expressed as the fraction of the injected dose per ml of effluent perfusate. Therefore, a nonextracted substance such as sucrose injected into the liver should produce an outflow concentration-time with the area under the curve (AUC) equal to unity after multiplying by the perfusate flow rate (Q).

$$AUC_{0-60} = \sum_{t=0}^{t=60} C_{n+1}(t_{n+1} - t_n) \tag{2.2}$$

$$AUC \cdot Q = 1 \tag{2.3}$$

In this formula, t is the sample collection time (s). Hepatic extraction (E) of the indicators and their recovery (R) from the liver were determined from AUC and Q by the relationship:

$$E = 1 - R = 1 - AUC \cdot Q \tag{2.4}$$

The mean transit time (MTT, s) of the indicators through the liver was estimated from the ratio of the area under the first moment of the curve (AUMC) and AUC (St-Pierre *et al.* 1989):

$$AUMC_{0-60} = \sum_{t=0}^{t=60} C_{n+1} \frac{(t_{n+1} - t_n)(t_{n+1} + t_n)}{2} \tag{2.5}$$

$$MTT = \frac{AUMC}{AUC} \tag{2.6}$$

The MTT was corrected for the catheter transit time (t_o), estimated from the time of first appearance of radioactivity above background levels. The MTT is related to the volume of distribution (V, ml/g liver) (St-Pierre *et al.* 1989) for nonextracted substances including paraquat by the relationship:

$$V_{nonext} = MTT \cdot Q \tag{2.7}$$

The intercompartmental rate constants (s^{-1}) of the cellular influx and efflux (k_1 and k_2, respectively) and the rate constant for sequestration (k_3, s^{-1}) were estimated by regression of the outflow curve of the indicators according to the equation for the Goresky model (Goresky *et al.* 1973). This hepatocellular transport equation model is used for well-stirred two-compartment (extracellular and intracellular) cell systems. The rate of change of material in the hepatocytes is set equal to the difference between cellular influx, efflux and any sequestration rate in the cells (Goresky *et al.* 1973; Goresky & Nadeau 1974), and can be expressed in a simplified equation:

$$\frac{\partial z(x,t)}{\partial t} = k_1\, u(x,t) - k_2\, z(x,t) - k_3\, z(x,t) \tag{2.8}$$

In this formula, z(x,t) is the concentration of a substance in the hepatic cells at a distance x from the origin of sinusoid at a time t, and u(x,t) is the concentration both in the sinusoid and at the surface of the liver cells. This assumes a well-mixed condition between sinusoids and the Space of Disse.

The programs that use the above equation were written in Microsoft QuickBasic 2.0 by Rivory (1989) and calculations using the Goresky model were performed on a Dell OptiPlex GX1 computer (Dell Co., Penang, Malaysia).

It was found that paraquat was not extracted by the liver, therefore the barrier-limited model was used to determine k_1 and k_2. Malathion, DDT and MPTP were found to be

extracted by the liver, therefore the barrier-limited sequestration model was used to determine k_1, k_2 and k_3. In the barrier-limited model, the substance exchange across the hepatocyte membrane is dependant on the permeability and surface area of the membrane as well as the concentration in the plasma and the intracellular compartment on either side (Goresky 1984).

The apparent volume of distribution for permeable solutes (V_{ext}) such as DDT, malathion and MPTP was calculated from that of sucrose (V_{suc}) and k_1 and k_2 by the relationship:

$$V_{ext} = V_{suc}\left(\frac{k_1 + k_2}{k_2}\right) \tag{2.9}$$

The permeability cell-surface area (PS) product (ml/s/g) for cell membrane transfer was determined from the product of k_1 by multiplying the extracellular volume for the influx PS product, and from the product of k_2 by multiplying the intracellular volume for the efflux PS product (Miyauchi *et al.* 1993; Le Couteur *et al.* 1999c). The volume of distribution of sucrose (V_{suc}) was used for the extracellular volume, and the intracellular volume was assumed to be $1 - V_{suc}$.

All results were expressed as mean ± standard deviation (SD).

2.3. RESULTS

2.3.1. Liver viability

Table 2.2 shows values of perfusate flow rate, hepatic oxygen consumption, portal venous resistance and liver enzymes in the outflow buffer for paraquat, DDT, malathion and MPTP experiments. The values of the liver viability test results are consistent with previous observations (Le Couteur *et al.* 1993; Le Couteur *et al.* 1994b; Le Couteur *et al.* 1999c).

The average flow rate was maintained at 2.11 to 2.39 ml/min/g liver through all four injections during the experiment. This is above the normal hepatic blood flow of 1.03 ml/min/g liver (Tsukamoto & Xi 1989). Gores *et al.* (1986) have explained in their review that high flow rates are necessary to deliver enough oxygen to the liver and to maintain viability when KH buffer was used without an oxygen carrier such as red blood cells. The flow rates for adequate oxygenation in the liver used in this experiment were similar to those used by other workers (approximately 2 ml/min/g liver and 20 to 30 ml/min by Wolkoff *et al.* (1987) and Le Couteur *et al.* (1999c), respectively).

Oxygen consumption in the liver was at an average of 1.07 to 1.19 μmol/min/g before the experiment. These values are consistent with other reports that hepatic oxygen uptake during the liver perfusion was 1.0 μmol/min/g for 2-3 month old rats (Le Couteur *et al.* 1999b). Furthermore, there was no change in the oxygen consumption before and after the experiment (1.09 to 1.18 μmol/min/g after the experiment).

The portal resistance in the perfused rat liver was in the range of 1.07 to 1.40 cm H_20·min·g/ml for the four injections, with this value similar to other findings (1.4 ± 0.3 cm H_20·min·g/ml) (Le Couteur *et al.* 1993).

The activities of the hepatic enzymes in the effluent perfusate were 14.67 to 19.83 U/L, 1.25 to 3.20 U/L and 10.00 to 11.00 U/L for ALT, AST and ALKP, respectively, and

Table 2.2. Parameters of liver viability.

Compounds	n	Flow rate ($ml \cdot min^{-1} \cdot g^{-1}$ liver)	O_2 1 ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	O_2 2 ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal venous resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
paraquat	6	2.30 ± 0.66	1.10 ± 0.41	1.10 ± 0.37	1.40 ± 0.65	20 ± 2	3 ± 2	10 ± 0
DDT	4	2.11 ± 0.10	1.07 ± 0.16	1.09 ± 0.12	1.07 ± 0.24	16 ± 4	1 ± 1	11 ± 1
malathion	6	2.24 ± 0.25	1.19 ± 0.19	1.18 ± 0.19	1.13 ± 0.27	15 ± 1	1 ± 1	10 ± 1
MPTP	5	2.39 ± 0.69	1.17 ± 0.42	1.13 ± 0.40	1.25 ± 0.59	20 ± 2	3 ± 2	10 ± 0

Results were obtained from the number of rats indicated in each group (n), and were expressed as means ± SD.

O_2 1 and O_2 2 represent oxygen consumption before and after experiments, respectively.

ALT, AST and ALKP represent alanine transaminase, aspartate transaminase and alkaline phosphatase, respectively.

U represents units.

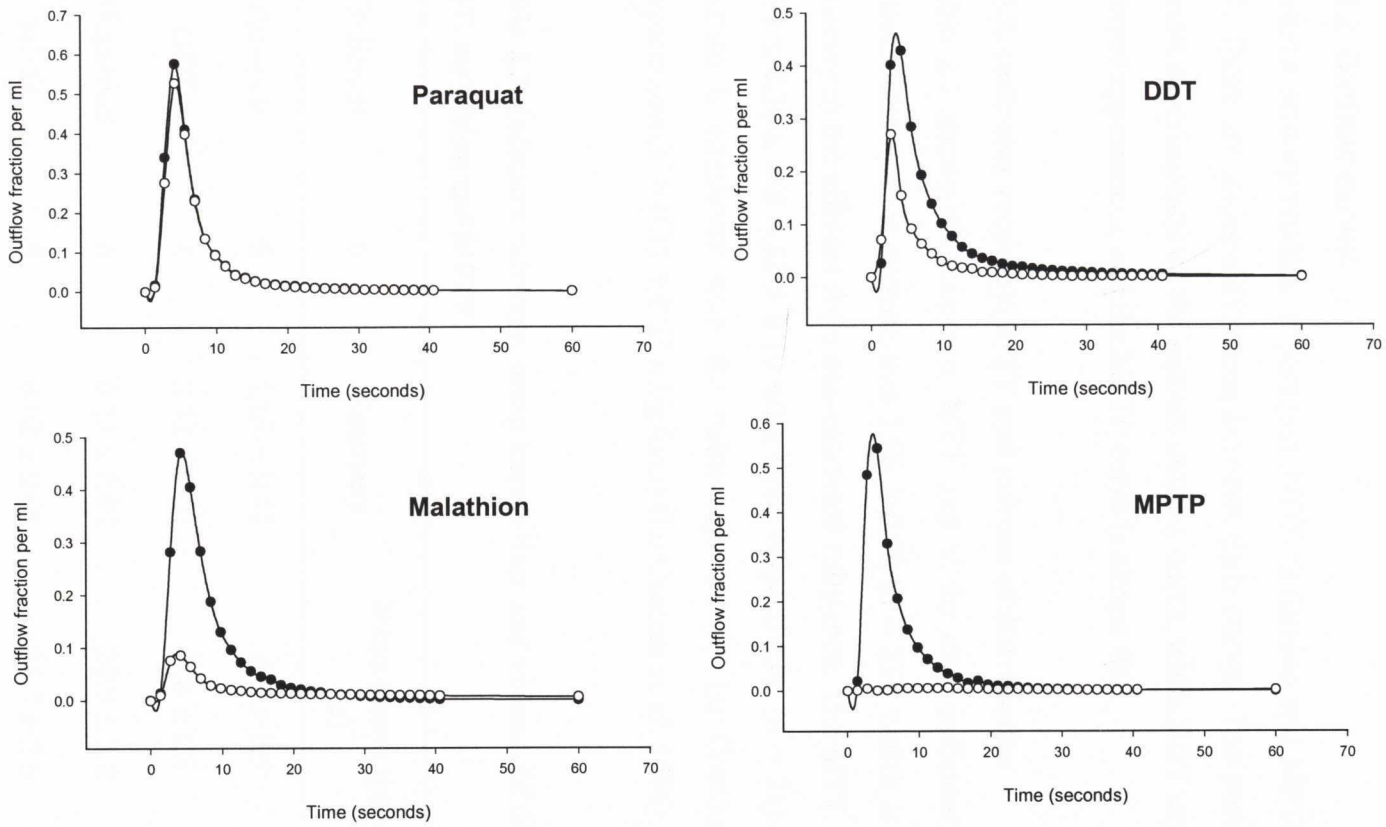


Figure 2.1. The outflow concentration-time profiles for paraquat, DDT, malathion and MPTP following bolus injection into the perfused rat liver. Each substance (open circle) was compared with an extracellular marker, sucrose (closed circle).

these results are also consistent with previous findings (ALT < 24 U/L, AST < 4 U/L and ASKP < 10 U/L) (Le Couteur *et al.* 1999c).

2.3.2. Outflow curves

Outflow activity profiles of paraquat, DDT, malathion and MPTP are shown in Figure 2.1. There are major differences between their curves. The paraquat outflow curve is almost superimposed on the sucrose outflow curve, while DDT and malathion have more delayed appearances, and the MPTP curve is almost flat.

2.3.3. Indicator recovery, MTT and volume of distribution

Table 2.3 shows the recovery, MTT and V for each indicator. The recovery of the extracellular marker sucrose was 1.06 ± 0.08 (n = 21), which is consistent with 100% recovery in the effluent for a non-extracted substance. The MTT and V of sucrose were 8.09 ± 2.34 s and 0.32 ± 0.10 ml/g liver, respectively, (n = 21) and the value of V of sucrose is consistent with the value reported by Le Couteur *et al.* with similar preparations (V = 0.31 ± 0.08 ml/g liver) (Le Couteur *et al.* 1996).

Table 2.3. Indicator recovery, mean transit time and volume of distribution of paraquat, DDT, malathion and MPTP.

Indicator	n	Recovery	Mean transit time (s)	Volume of distribution ($ml \cdot g^{-1}$ liver)
Paraquat	6	1.05 ± 0.12	6.5 ± 1.5	0.28 ± 0.13
DDT	4	0.32 ± 0.01	7.8 ± 1.5	0.69 ± 0.12
Malathion	6	0.11 ± 0.02	20.9 ± 3.2	3.30 ± 0.58
MPTP	5	0.02 ± 0.01	28.5 ± 2.6	5.10 ± 6.00

Note. Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

The recovery of paraquat was 1.05 ± 0.12, which indicates that paraquat is not sequestered in the liver during a single pass. On the other hand, the recoveries of the other indicators were significantly less than one: DDT, 0.32 ± 0.01, malathion, 0.11 ± 0.02 and MPTP, 0.02 ± 0.01.

The volume of distribution of paraquat was small (0.28 ± 0.13 ml/g liver) and similar to that of the extracellular marker, sucrose. The volumes of distribution of malathion and MPTP, on the other hand, exceeded the intracellular volume (3.30 ± 0.58 and 5.10 ± 6.00 ml/g liver, respectively). DDT had a volume of distribution of 0.69 ± 0.12 ml/g liver, intermediate to the other indicators.

2.3.4. Hepatocyte transmembrane transport and sequestration rate constants

The Goresky-based models appeared to fit the data reasonably well (Figure 2.2). The fit was least effective for MPTP reflecting the difficulty of fitting curves with very low isotopic activity in the outflow. The values of k_1, k_2, and k_3 for DDT, malathion and MPTP determined using this curve-fitting procedure are shown in Table 2.4. Only the values for k_1 and k_2 were determined for paraquat because it was not sequestered by the liver.

The rate constants for membrane transport were greatest for MPTP, followed by malathion, DDT and then paraquat. The similarity of the values for the PS_{influx} and PS_{efflux} products for DDT and MPTP suggest that transport is symmetrical for these indicators. The ratios of the PS_{influx} and PS_{efflux} products for malathion and paraquat suggest that the transmembrane transport of these indicators is asymmetrical and that there may be a specific transmembrane transport mechanism and/or selective drug binding on either side of the plasma membrane.

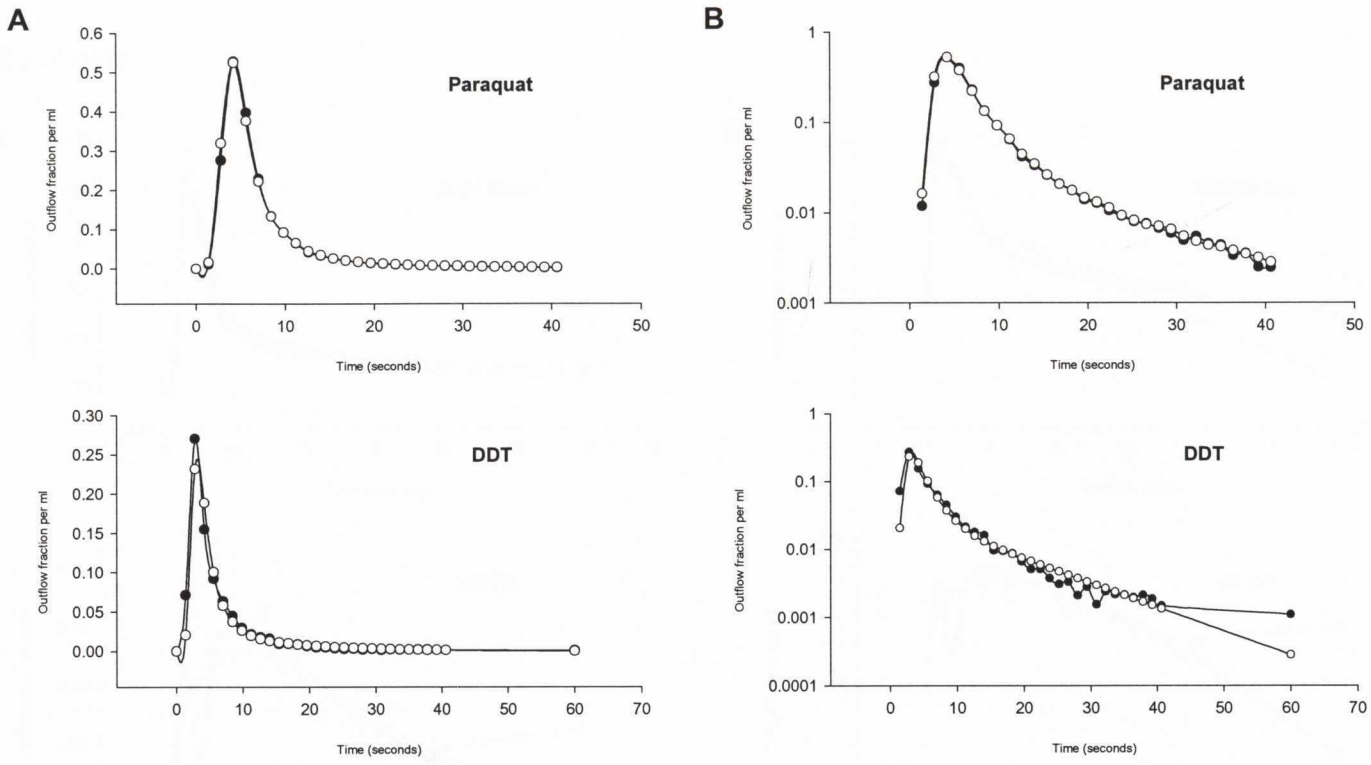


Figure 2.2. The Goresky model fitting for paraquat, DDT, malathion and MPTP. The outflow curves of each test indicator (closed circle) were compared to their Goresky models (open circle) in the normal scale (A) and the logarithmic scale (B).

Figure 2.2. Contd.

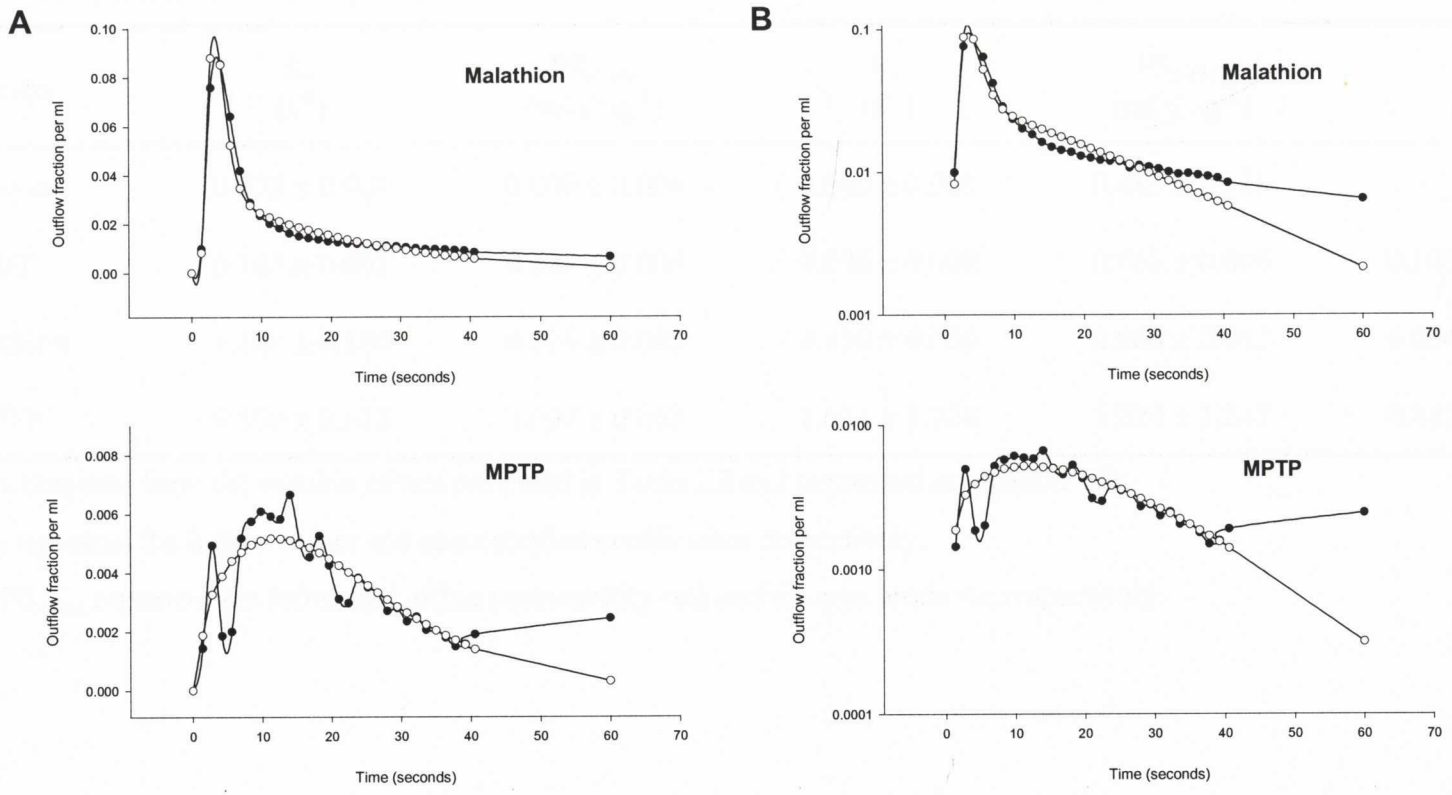


Table 2.4. Kinetic parameters for paraquat, DDT, malathion and MPTP.

Indicator	k_1 (s^{-1})	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$)	k_2 (s^{-1})	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$)	k_3[a] (s^{-1})
Paraquat	0.033 ± 0.004	0.009 ± 0.004	0.666 ± 0.060	0.485 ± 0.118	
DDT	0.103 ± 0.001	0.035 ± 0.006	0.098 ± 0.000	0.065 ± 0.006	0.100 ± 0.000
Malathion	1.140 ± 0.186	0.376 ± 0.049	0.130 ± 0.026	0.086 ± 0.012	0.084 ± 0.035
MPTP	9.590 ± 8.613	2.092 ± 0.863	1.634 ± 1.724	1.224 ± 1.247	0.443 ± 0.431

Results were obtained from the number of rats indicated in Table 2.2 and expressed as means ± SD.

k_1, k_2 and k_3 represent the influx, efflux and sequestration coefficients, respectively.

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

2.4. DISCUSSION

The liver plays a key role in the elimination of numerous endogenous substances and xenobiotics. In this study, the hepatic disposition of three representative pesticides and MPTP was examined using a MID technique in the perfused rat liver because of their potential involvement in the pathogenesis of PD. In addition, the behaviour of these compounds in the intact liver has not been examined previously.

In the perfused rat liver, viability is maintained for approximately 3 to 4 hours (h) under normal conditions (Gores *et al.* 1986) and the experiments with each rat took approximately 1 hour. The values of the liver viability test results were also consistent with previous reports (refer to Chapter 2.3.1. Liver viability).

The MID technique used in this study has been applied in many studies to investigate disposition of substances in the liver (Wolkoff *et al.* 1979; Goresky 1984; Le Couteur *et al.* 1996; Mellick & Roberts 1999b). The data were analysed for hepatocellular membrane transport using outflow concentration-time profiles based on the barrier-limited distribution modeled by Goresky (Goresky *et al.* 1973; Goresky 1983; Goresky 1984) as described in the Methods. Among many models including Roberts model (Roberts & Anissimov 1999) to analyse the hepatic disposition of substances after bolus injections into perfused livers, the Goresky model was widely used and the graphs generated from the experimental data fitted this model very well for paraquat, less well for MPTP and were intermediate for DDT and malathion. This indicates the difficulty in modelling substances that have very low isotope activities in the outflow.

There were marked differences in the hepatic disposition of these four compounds. The recovery of MPTP was only 2%, indicating that it is almost entirely extracted by the liver. Therefore, there will only be limited systemic exposure after a single oral dose of MPTP, but those factors that influence the hepatic uptake and metabolism of MPTP may have profound effects on its bioavailability. The hepatic transport mechanism of MPTP

has not been reported previously but an earlier study in the kidney has shown that the transport of MPTP in canine renal brush border membrane vesicles is mediated by the organic cation transporter (Sokol *et al.* 1987). However, the rapid transmembrane transport and almost symmetrical influx and efflux that were observed in the perfused rat liver suggest that the hepatic transmembrane transport of this uncharged lipophilic molecule is more likely to be mostly by simple diffusion.

The high extraction, large apparent volume of distribution and rapid k_3 of MPTP have several possible explanations including intracellular protein binding, lipophilicity or rapid metabolism. Most MPTP (90%) in hepatocytes is metabolised to MPP^+ by monoamine oxidase B (Di Monte *et al.* 1988). The transport of MPP^+ is thought to be mediated by the organic cation transporter 1 (Martel *et al.* 1996b) and P-glycoprotein, which is a biliary canalicular transporter (Martel *et al.* 1996a). The low recovery of MPTP in this experiment is compatible with rapid metabolism to MPP^+ and subsequent biliary excretion and/or accumulation of this metabolite in the cells. However, no radioactivity was detected in the bile and high radioactivity levels were detected in the liver (^3H count 0.02% and 91.5% of ^3H-MPTP injectate, respectively), indicating that MPTP and its metabolites accumulate in the liver. Overall, these results indicate that MPTP is rapidly taken up by the process of diffusion and extensively sequestered in the liver.

It was found that malathion has rapid but asymmetrical transmembrane uptake. This is consistent with transporter-mediated transport into cells, although the basis for such a mechanism is not clear. This result is in contrast to reported findings with another organophosphate insecticide, parathion, which is transported into hepatocytes by passive diffusion (Nakatsugawa 1992). Malathion was extensively sequestered in the liver, however, intracellular protein binding, biliary excretion and intracellular biotransformation of this molecule are poorly understood. Both MPTP and malathion are very lipophilic (Nakatsugawa 1992; Budavari *et al.* 1996; Przedborski & Jackson-Lewis 1998), therefore distribution into fatty tissues would be at least a partial explanation for

the very large apparent volumes of distribution and sequestration rate constants for these two indicators.

Paraquat was not extracted by the liver and did not enter liver cells to any extent. This finding may explain the observation that oral overdose by paraquat is associated with pulmonary and renal toxicity but not hepatotoxicity (Bairaktari *et al.* 1998; Bischoff *et al.* 1998). The bioavailability of paraquat can not be increased by inhibitors of liver activity, therefore such inhibitors will not have any effect on paraquat toxicity in overdose or putative susceptibility to parkinsonism associated with paraquat.

It is of note that the cellular efflux of paraquat was far greater than the influx. Although the data are difficult to interpret because the paraquat outflow curves were nearly superimposed upon the sucrose curves, this suggests that there may be a specific efflux transport mechanism for paraquat. Paraquat is a cationic herbicide transported by the organic cation transporter in a renal epithelial cell line, LLC-PK_1 (Chan *et al.* 1996a) and rat renal proximal tubular cells (Chan *et al.* 1996b). Additionally, paraquat is taken up into type II alveolar epithelium of the lung via the polyamine transport system (Hoet *et al.* 1995). The involvement of either of these transporters in paraquat transport in the liver is unknown.

Finally, DDT was moderately extracted by the liver with a recovery of about one third. The transmembrane transport of DDT was rapid and symmetrical. This suggests that the hepatic transport of this non-polar lipophilic molecule (Budavari *et al.* 1996) in the rat liver is by simple diffusion. The difference between the rate constants for the influx and efflux of MPTP and DDT may reflect the differences in their molecular weight and lipophilicity in addition to their plasma and intracellular protein bindings. The intracellular sequestration of DDT is consistent with the observation that cellular uptake of DDT in Chang liver cells was bi-directional but this compound was retained within the cells (Mangelsdorf *et al.* 1987). Furthermore, DDT is lipophilic and binds to an

intracellular protein, calmodulin (Warngard *et al.* 1988) thus providing potential mechanisms for this sequestration.

In conclusion, this study has shown that there is considerable variability in the hepatic disposition of putative neurotoxins such as MPTP and pesticides. Any factors that influence the hepatic disposition of neurotoxins may alter susceptibility to neurotoxic diseases even though the effects will be diverse. Furthermore, pesticide exposure may increase the risk of PD by altering the hepatic disposition of pesticides and other neurotoxins. Therefore, it was of interest to study the effects of pesticide exposure on the hepatic disposition of neurotoxins as reported in the following Chapter.

3. THE EFFECT OF PESTICIDES ON THE EXTRACTION OF MPTP AND PESTICIDES IN THE LIVER

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3.1. INTRODUCTION

The etiology of most cases of PD remains unknown even though some familial and early-onset cases of parkinsonism have been linked to rare sequence variants in α-synuclein (Polymeropoulos *et al.* 1997) and parkin genes (Kitada *et al.* 1998). Epidemiological studies suggest that there is a significant association between pesticide exposure and PD (Ritz & Yu 2000) and some studies have shown that the prevalence of PD is linked with the geographical distribution of pesticide usage (Barbeau *et al.* 1987) (refer to Chapter 2.1. Introduction). Other factors, including well water ingestion, rural residency and solvent exposure, may be considered surrogates for pesticide exposure, and linked to increased susceptibility to PD (Langston 1998).

Among pesticides, organochlorines, organophosphates, herbicides and carbamates have been reported to cause PD (Fleming *et al.* 1994; Bhatt *et al.* 1999; Stephenson 2000; Liou *et al.* 2001). An organochlorine pesticide dieldrin was found in PD patients' brains in a postmortem study (Fleming *et al.* 1994), and five genetically related people who were exposed to a household organophosphate pesticide developed acute and reversible parkinsonism (Bhatt *et al.* 1999). Furthermore, Wistar rats that were administered the herbicide paraquat showed dose-dependent depletion of striatal dopamine, and a MAOB inhibitor selegiline attenuated the paraquat-elicited dopaminergic toxicity (Liou *et al.* 2001). Despite the positive association of pesticides with PD, the mechanisms linking pesticide exposures to PD is poorly understood.

There are several possible mechanisms for the association of pesticides with PD. First, pesticides may be directly neurotoxic. PD is associated with impaired activity of complex I of the electron transfer chain of mitochondria within the substantia nigra and many pesticides have been shown to produce mitochondrial dysfunction as a primary or secondary toxicity (Ferreira *et al.* 1997; Sanchez-Ramos *et al.* 1998; Betarbet *et al.* 2000; Kannan *et al.* 2000). For example, pathogenesis of rotenone in PD is a good model of the mitochondrial impairment by pesticides (Betarbet *et al.* 2000). For other pesticides

associated with mitochondrial dysfunctions, DDE, a metabolite of DDT, decreases mitochondrial respiration (Ferreira *et al.* 1997) and dieldrin inhibits mitochondrial respiration by selectively depleting dopaminergic neurons (Sanchez-Ramos *et al.* 1998). Additionally, the herbicide paraquat depresses mitochondrial respiration through partial inhibition of complex III and IV (Palmeira *et al.* 1995).

Second, pesticides may simply be a confounding variable linked to other causative factors such as PD susceptible genes or chemicals. It has been reported that only about 20% of people with PD have a positive family history (Le Couteur *et al.* 1999a) and Menegon *et al.* (1998) proposed that PD may be caused by a combination of genetic susceptibility and environmental insults including pesticides. In such cases, environmental factors may trigger and/or accelerate the disease in individuals with genetic susceptibility, with the genetic component increasing the vulnerability of dopamine containing neurons in the nigrostriatal system to injury by environmental toxins. For the effect of combinations of different pesticides in PD, Thiruchelvam *et al.* (2000) have reported that the combination of paraquat and a fungicide manganese ethylenebisdithiocarbamate (maneb) had a synergistic effect on the depletion of dopamine and its metabolite DOPAC in C57BL/6 mice compared with either compound administered alone.

Finally, pesticides may modify PD risk by modulating the disposition of other neurotoxins (Le Couteur *et al.* 1999a), thus altering their systemic exposure. Many pesticides inhibit or induce xenobiotic metabolising enzymes (Li *et al.* 1995; Hodgson & Levi 1996; Butler & Murray 1997). For example, organochlorine pesticides such as DDT induce CYP (Li *et al.* 1995) while organophosphate pesticides including parathion inhibit CYP (Butler & Murray 1997). Furthermore, GSTs are inhibited by the herbicide tridiphane (Moody & Hammock 1987) and the fungicide captofol (Di Ilio *et al.* 1996). The possible importance of pesticide mechanisms on xenobiotic metabolising enzymes for PD is further supported by the observation that inhibition of FMO by administration

of N-methylmercaptoimidazole or thiobenzamide increases the toxicity of MPTP in mice (Chiba *et al.* 1988).

Although there is a significant association of pesticides with PD, the mechanism has not yet been clearly identified. Furthermore, the effect or mechanism of combinations of pesticides in PD has been poorly understood although pesticides are often used in combination. In the previous chapter, it was observed that there were major differences in the hepatic disposition of MPTP and pesticides. For example, MPTP was extracted in the liver extensively (98%) and the systemic bioavailability of this neurotoxin was only 2% of the injectate while paraquat was not extracted by the liver at all. This finding suggests that any factors that influence the hepatic uptake and metabolism of MPTP may have profound effects on its bioavailability, potentially altering MPTP-associated neurotoxicity. However, the bioavailability of paraquat can not be increased but may be able to be reduced by modulators of liver activity. Therefore, such modulators may reduce paraquat toxicity in overdose or any susceptibility to parkinsonism associated with paraquat.

In order to determine whether pesticides influence the hepatic disposition of putative neurotoxins, the effects of pretreatment with pesticides on the hepatic disposition of MPTP and pesticides were studied using the MID technique in the perfused rat liver.

3.2. MATERIALS AND METHODS

3.2.1. Pretreatment of animals

Male Wistar rats (2-3 months old, John Curtin School of Medical Research, Canberra, Australia) were administered a single dose of paraquat (30 mg/ 2 ml saline/ kg body weight, i.p.), DDT (180 mg/ 2 ml corn oil/ kg body weight, i.p.) or malathion (250 mg/ 2 ml corn oil/ kg body weight, i.p.), 24 h before liver perfusion. Such exposures to these chemicals have been used previously for pretreatments of animals in other studies (Bulusu & Chakravarty 1984; Sato 1991; Barros *et al.* 1994). These doses were also optimised for this study by observation of behaviours of the animals after pretreatment. These doses did not affect motor movements. Procedural controls included untreated, corn oil treated (2 ml/kg body weight) and saline treated (2 ml/kg body weight) animals. The study was approved by the Australian National University Animal Experimentation Ethics Committee.

3.2.2. Liver perfusion and MID technique

The liver perfusion and MID technique were used to determine the disposition of paraquat, DDT, malathion and MPTP in the perfused rat liver as described previously in Chapter 2.2. Briefly, the livers were perfused *in situ* via the portal vein with KH buffer in a single pass mode. The injectates (50 μl), containing either ^{14}C-paraquat, ^{14}C-DDT, ^{14}C-malathion or ^{3}H-MPTP, were administered via the portal vein cannula. Sucrose was used as the extracellular marker. ^{3}H-sucrose was included in each of the injectates that contained ^{14}C label, and ^{14}C-sucrose was included with ^{3}H-MPTP. Outflow samples from the liver were collected for 60 s after injection of the indicators and analysed with a scintillation counter.

To test liver viability, oxygen consumption was measured before and after the experiments and the outflow buffer was collected for hepatic enzyme assay. In addition, portal venous resistance was also measured during the experiments. When each experiment was finished, liver tissues from each lobe were cut and fixed with 4%

paraformaldehyde. They were sent to the Histology Department (The Canberra Hospital, Canberra) for hematoxylin-eosin (H&E) staining to allow light microscopic observation of the liver structures after pesticide pretreatment.

3.2.3. Data analysis

The data was analysed as described in Chapter 2.2.6. The hepatic outflow concentrations were expressed as a fraction of the injected dose per ml. The recovery of each test substance in the effluent was determined from the area under the outflow curve. The rate constant k_3, PS_{influx} and PS_{efflux} were determined using the physiological models developed by Goresky (1984).

Data were expressed as mean ± SD. Control and pretreatment groups were compared using the Student t-test and considered significant when $p \leq 0.05$.

3.3. RESULTS

3.3.1. Liver viability in control and pesticide pretreated rats

Table 3.1 shows parameters for liver viability of control and pesticide pretreated rats. The values of hepatic oxygen consumption for the paraquat, DDT, malathion and MPTP experiments in control rats were similar to those of the rats used in the previous Chapter (refer to Table 2.1). The hepatic oxygen consumption of paraquat, DDT and malathion pretreated rats was not different from that of the control rats. Furthermore, levels of oxygen consumption in the liver of control and pesticide pretreated rats were very similar before and after the experiments. Together, the above results indicate that the liver was viable as measured by the oxygen consumption, and that viability was maintained throughout the experiments. The values of portal venous resistance, ALT, AST and ALKP in the control rats were similar to those of rats used in Chapter 2 and these values are also similar to those of paraquat, DDT and malathion pretreated rats. These measurements indicate that the liver was viable as tested by portal resistance and assay of hepatic enzymes in the outflow buffer.

3.3.2. Liver structure after pretreatment with pesticides

The light microscopic liver structure of control and pesticide pretreated rats are shown in Figure 3.1. There were no differences observed in liver structure following pretreatment with a single dose of paraquat 30 mg/kg, DDT 180 mg/kg and malathion 250 mg/kg, 24 h prior to the experiments, when compared with the structure of control livers.

3.3.3. Fractional recovery changes by pretreatment with pesticides

The outflow recovery results are summarised in Figure 3.2 and Table 3.2. Illustrative outflow curves are shown in Figure 3.3. There were no differences between the three groups of control rats (untreated, corn oil treated, saline treated), hence these results were pooled. In the livers of control rats, the fractional recoveries of paraquat, DDT,

Table 3.1. Viability of livers from control and pesticide pretreated rats.

Pesticide pretreatment	n	Flow rate ($ml \cdot min^{-1} \cdot g^{-1}$)	O_2 1 ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	O_2 2 ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal venous resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat MID injection								
Control	14	1.81 ± 0.62	0.94 ± 0.34	0.92 ± 0.32	1.37 ± 0.78	18 ± 2	2 ± 2	11 ± 1
Paraquat	9	1.98 ± 0.23	1.04 ± 0.13	1.03 ± 0.13	1.22 ± 0.40	18 ± 2	1 ± 0	12 ± 1
DDT	8	1.56 ± 0.15	0.82 ± 0.08	0.81 ± 0.07	1.42 ± 0.71	18 ± 2	1 ± 0	11 ± 0
Malathion	6	1.50 ± 0.14	0.84 ± 0.05	0.84 ± 0.06	1.50 ± 0.72	18 ± 4	2 ± 1	12 ± 0
DDT MID injection								
Control	12	1.75 ± 0.36	0.93 ± 0.21	0.94 ± 0.21	1.44 ± 0.72	17 ± 3	2 ± 1	11 ± 1
Paraquat	8	1.98 ± 0.23	1.04 ± 0.13	1.03 ± 0.13	1.22 ± 0.40	18 ± 2	1 ± 0	12 ± 1
DDT	8	1.56 ± 0.15	0.82 ± 0.08	0.81 ± 0.07	1.42 ± 0.71	18 ± 2	1 ± 0	11 ± 0
Malathion	4	1.50 ± 0.15	0.84 ± 0.05	0.83 ± 0.05	1.63 ± 0.74	19 ± 4	2 ± 1	12 ± 1

Table 3.1. Contd.

Malathion MID injection								
Control	15	1.85 ± 0.40	0.99 ± 0.26	0.98 ± 0.26	1.20 ± 0.59	16 ± 2	2 ± 1	11 ± 1
Paraquat	9	1.98 ± 0.23	1.04 ± 0.13	1.03 ± 0.13	1.22 ± 0.40	18 ± 2	1 ± 0	12 ± 1
DDT	8	1.56 ± 0.15	0.82 ± 0.08	0.81 ± 0.07	1.42 ± 0.71	18 ± 2	1 ± 0	11 ± 0
Malathion	5	1.53 ± 0.14	0.85 ± 0.05	0.85 ± 0.06	1.35 ± 0.71	17 ± 3	2 ± 1	12 ± 0
MPTP MID injection								
Control	12	1.93 ± 0.62	1.00 ± 0.33	0.99 ± 0.32	1.38 ± 0.67	18 ± 2	2 ± 2	11 ± 1
Paraquat	9	1.98 ± 0.23	1.04 ± 0.13	1.03 ± 0.13	1.22 ± 0.40	18 ± 2	1 ± 0	12 ± 1
DDT	8	1.56 ± 0.15	0.82 ± 0.08	0.81 ± 0.07	1.42 ± 0.71	18 ± 2	1 ± 0	11 ± 0
Malathion	6	1.50 ± 0.14	0.84 ± 0.05	0.84 ± 0.06	1.50 ± 0.72	18 ± 4	2 ± 1	12 ± 0

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

O_2 1 and O_2 2 represent respectively oxygen consumption before and after MID experiments.

ALT, AST and ALKP represent alanine transaminase, aspartate transaminase and alkaline phosphatase, respectively.

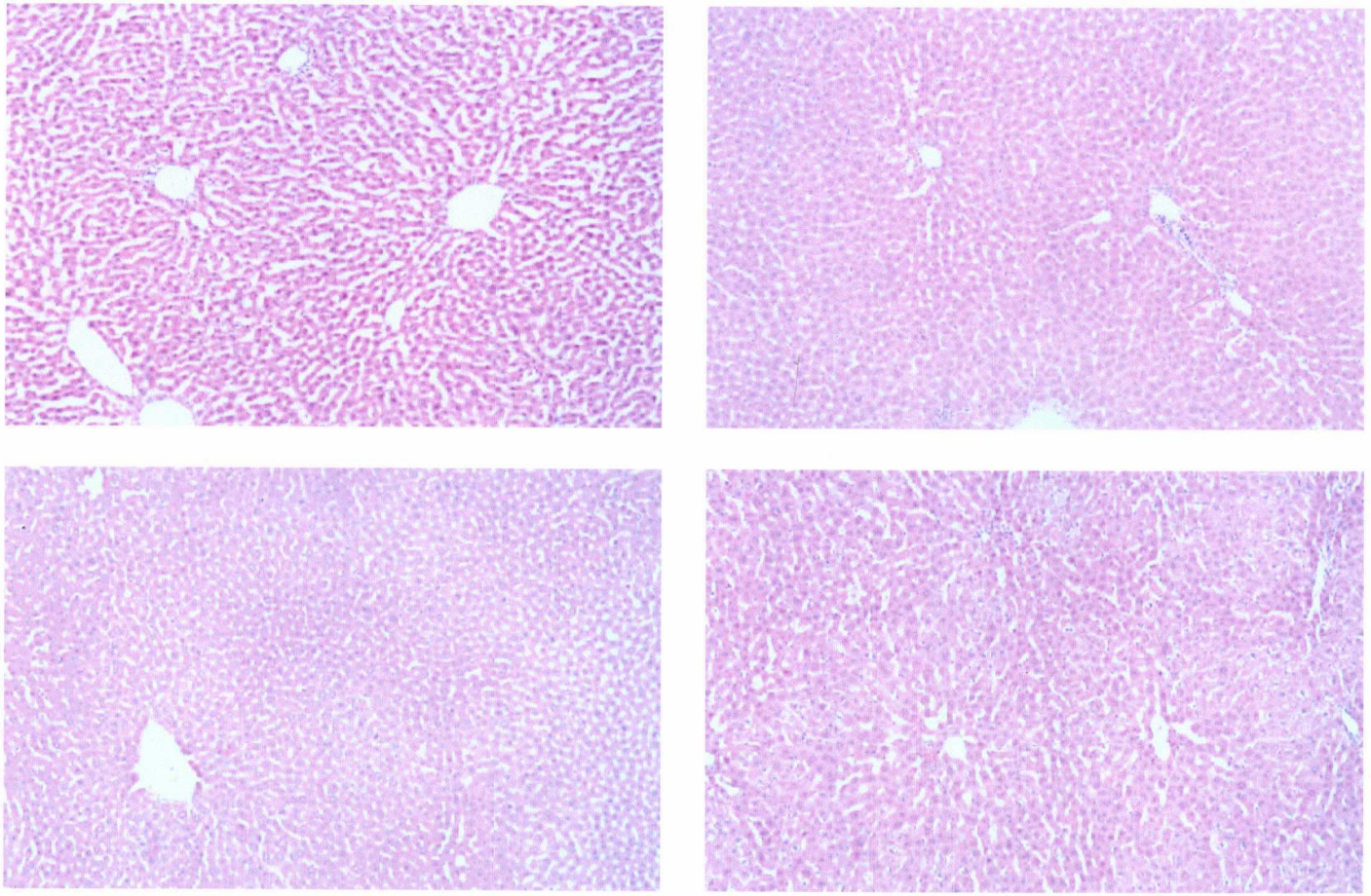

Figure 3.1. Light microscopic structures of control (top left) and pesticide pretreated rat livers. Pathological findings were not observed in the liver from paraquat (top right), DDT (bottom left) or malathion (bottom right) pretreatment. Magnification ×100 (H&E stain).

malathion and MPTP were 0.97 ± 0.10 (n = 14), 0.32 ± 0.07 (n = 12), 0.14 ± 0.05 (n = 15) and 0.02 ± 0.02 (n = 12), respectively, which is very similar to the values reported in Chapter 2.2.3 (refer to Table 2.3).

Pretreatment with paraquat and DDT increased the recovery of malathion to 0.24 ± 0.03 (n = 9, $p < 0.001$) and 0.25 ± 0.06 (n = 8, $p < 0.001$), respectively. Pretreatment with malathion did not affect the recovery of malathion.

After pretreatment with paraquat, DDT and malathion, the recovery of MPTP also increased approximately twofold to 0.05 ± 0.02 (n = 9, $p = 0.006$), 0.04 ± 0.01 (n = 8, $p = 0.02$) and 0.05 ± 0.02 (n = 6, $p = 0.013$), respectively.

The recoveries of sucrose, paraquat or DDT were not influenced by pretreatment with any of the pesticides used in these experiments.

3.3.4. Transport and sequestration parameters after pretreatment with pesticides

The results for PS_{influx}, PS_{efflux} and k_3 of malathion and MPTP are shown in Table 3.3. Pretreatment with paraquat and DDT was associated with a significant reduction in PS_{influx} and PS_{efflux} for malathion, and there was a trend towards reduction in k_3. Overall, pesticide pretreatment was associated with a trend for reductions in PS_{influx}, PS_{efflux} and k_3 for MPTP. However, as shown in Table 3.4, there were no significant changes in PS_{influx}, PS_{efflux} and k_3 of paraquat and DDT by pretreatment with pesticides.

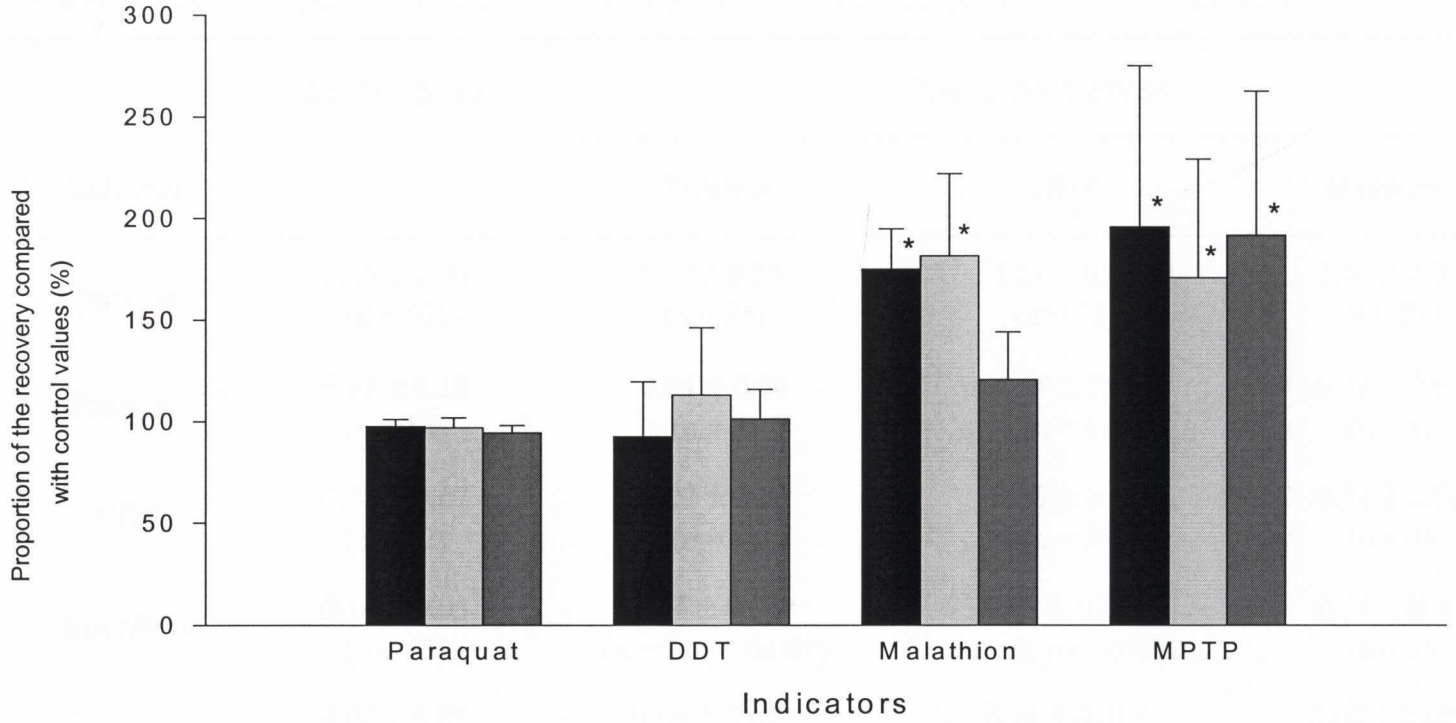

Figure 3.2. Effect of pesticide pretreatments on the recovery of paraquat, DDT, malathion and MPTP using the multiple indicator dilution technique. The recovery of each indicator in the effluent of liver after pretreatment with paraquat (black), DDT (light gray) and malathion (dark gray) was expressed as a proportion (%) of control value ± SD. * Statistically different from control values $p \leq 0.05$.

Table 3.2. Fractional recoveries of paraquat, DDT, malathion and MPTP determined using the multiple indicator dilution method in the perfused rat liver. Rats were pretreated with either paraquat, DDT or malathion.

Indicator	Control group	Pretreatment groups		
		Paraquat	DDT	Malathion
Sucrose	1.03 ± 0.06 (n = 53)	1.10 ± 0.04 (n = 35)	1.01 ± 0.03 (n = 32)	1.01 ± 0.03 (n = 21)
Paraquat	0.97 ± 0.10 (n = 14)	0.94 ± 0.04 (n = 8)	0.94 ± 0.05 (n = 8)	0.91 ± 0.04 (n = 6)
DDT	0.32 ± 0.07 (n = 12)	0.29 ± 0.09 (n = 8)	0.36 ± 0.11 (n = 8)	0.32 ± 0.05 (n = 4)
Malathion	0.14 ± 0.05 (n = 15)	0.24 ± 0.03* (n = 9, $p < 0.001$)	0.25 ± 0.06* (n = 8, $p < 0.001$)	0.16 ± 0.03 (n = 5)
MPTP	0.02 ± 0.02 (n = 12)	0.05 ± 0.02* (n = 9, $p = 0.006$)	0.04 ± 0.01* (n = 8, $p = 0.02$)	0.05 ± 0.02* (n = 6, $p = 0.013$)

Results were obtained from the number of rats (n) indicated in each group and were expressed as means ± SD.

p values refer to Student t-test.

* Statistically different from controls.

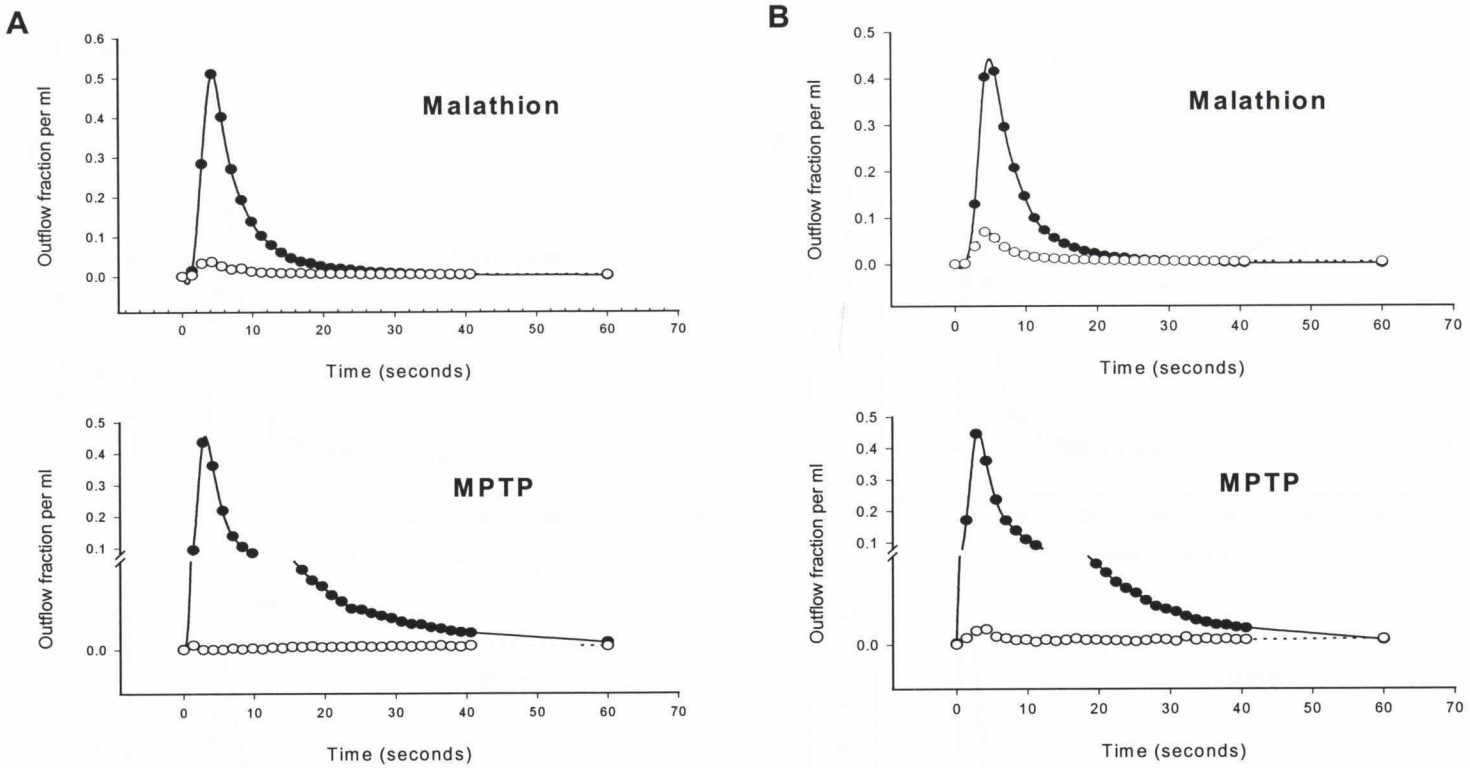

Figure 3.3. The effect of paraquat pre-treatment on the outflow of malathion, MPTP, paraquat and DDT in the perfused rat liver. Test substance (open circle) outflow concentration-time profiles were compared between the control (A) condition and after pre-treatment with paraquat (B). Sucrose (closed circle) was used as an extracellular marker.

Figure 3.3. Contd.

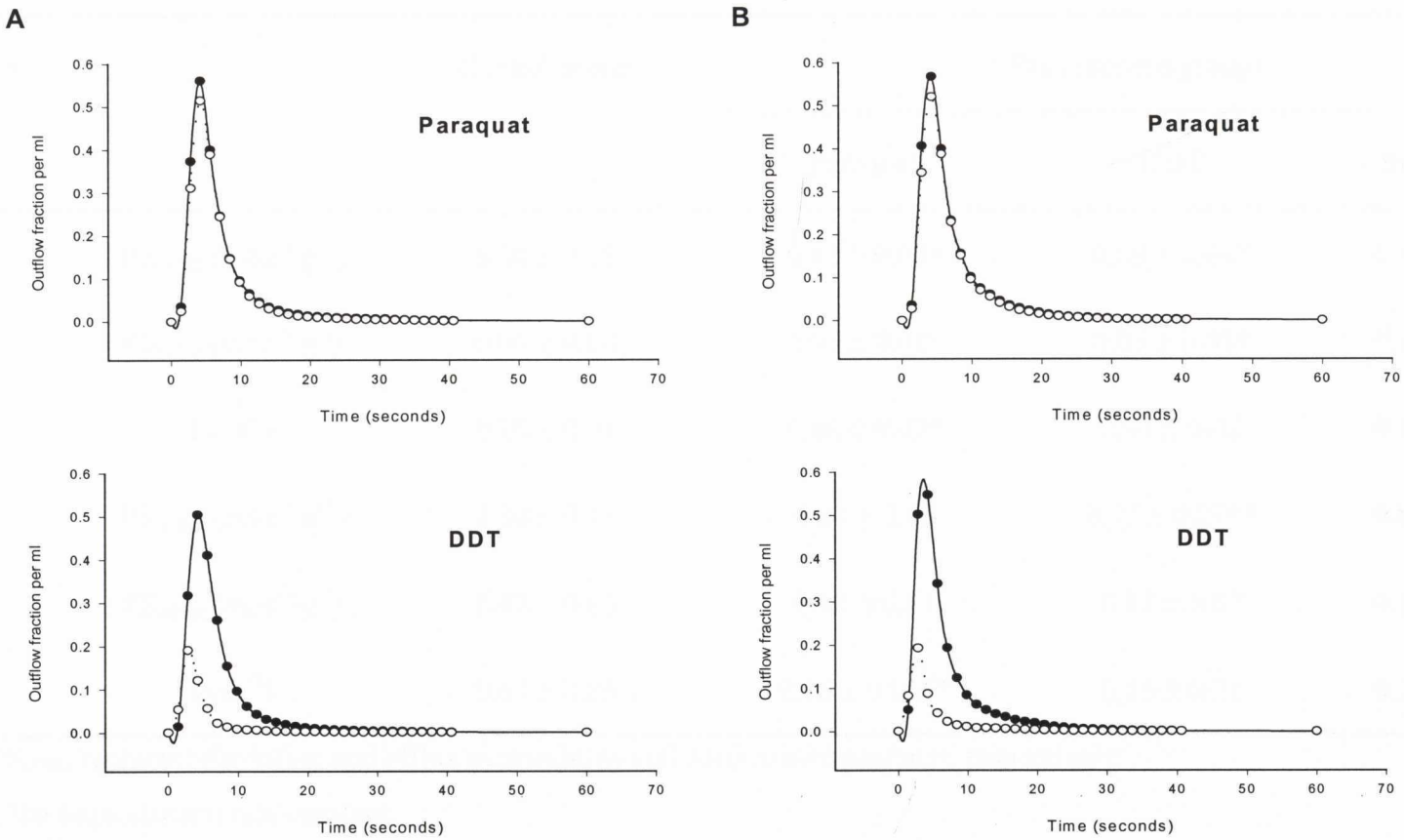

Table 3.3. Values for PS_{influx}, PS_{efflux} and k_3 of malathion and MPTP determined using the multiple indicator dilution technique in the perfused rat liver. Rats were pretreated with either paraquat, DDT or malathion.

Indicators		Control group	Pretreatment groups		
			paraquat	DDT	malathion
Malathion	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.30 ± 0.18	0.12 ± 0.03*	0.09 ± 0.04*	0.16 ± 0.04
	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.09 ± 0.04	0.04 ± 0.01*	0.05 ± 0.03*	0.11 ± 0.07
	k_3 (s^{-1})	0.10 ± 0.04	0.06 ± 0.02*	0.07 ± 0.02	0.10 ± 0.04
MPTP	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$)	1.30 ± 0.78	0.90 ± 0.69	0.77 ± 0.27**	0.80 ± 0.52
	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.47 ± 0.84	0.12 ± 0.11	0.13 ± 0.07	0.19 ± 0.14
	k_3 (s^{-1})	0.64 ± 0.85	0.06 ± 0.06**	0.15 ± 0.21	0.35 ± 0.61

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

k_3 represents the sequestration rate constant.

*Statistically different from controls $p < 0.05$, ** $0.05 < p < 0.08$.

Table 3.4. Values for PS_{influx}, PS_{efflux} and k_3 of paraquat and DDT determined using the multiple indicator dilution technique in the perfused rat liver. Rats were pretreated with either paraquat, DDT or malathion.

Indicators		Control group	Pretreatment groups		
			paraquat	DDT	malathion
Paraquat	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.01 ± 0.00	0.02 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.56 ± 0.12	0.46 ± 0.25	0.56 ± 0.06	0.48 ± 0.26
DDT	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.05 ± 0.02	0.08 ± 0.03	0.05 ± 0.02	0.05 ± 0.01
	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$)	0.03 ± 0.03	0.02 ± 0.02	0.04 ± 0.08	0.01 ± 0.01
	k_3 (s^{-1})	0.08 ± 0.03	0.08 ± 0.06	0.12 ± 0.18	0.07 ± 0.05

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

k_3 represents the sequestration rate constant.

3.4. DISCUSSION

The simplest explanation for the association of pesticides with PD is that pesticides are directly neurotoxic. Recently, it has been reported that chronic exposure of rats to rotenone produces systemic impairment of mitochondrial complex I, leading to selective nigrostriatal dopaminergic degeneration, hypokinesia and rigidity (Betarbet *et al.* 2000). Although the mitochondrial toxicity of many pesticides is recognised, the selective toxicity for dopaminergic neurons has not been explained except for MPP^+, which is taken up by the dopamine transporter (Kitayama *et al.* 1998).

An alternative explanation is that pesticides modify systemic exposure to neurotoxins through modulation of their metabolism (Le Couteur *et al.* 1999a). It is well recognised that pesticides can either induce or inhibit hepatic xenobiotic metabolism (Kolmodin *et al.* 1969; Moody & Hammock 1987; Anadon *et al.* 1995; Hodgson & Levi 1996; Berger & Sultatos 1997; Le Couteur *et al.* 1999a), and some enzymes, such as FMO and CYP2D6, are involved in detoxification of MPTP in the liver while MAOB metabolises MPTP to the PD-inducing metabolite MPP^+ (Chiba *et al.* 1984; Chiba *et al.* 1988).

In this study, the effect of pesticide exposure on the hepatic disposition of MPTP was examined because it is considered to be a prototypic neurotoxin that causes a parkinsonian syndrome in humans and animals (Langston 1985). Exposure to MPTP is very uncommon, but nevertheless substances with similar activity or structure to MPTP may be involved in the pathogenesis of idiopathic PD (Langston 1985). MPTP is highly extracted by the liver and only about 2% of MPTP entering the liver via the portal vein becomes systemically bioavailable (refer to Chapter 2). Therefore, the liver is likely to confer considerable protection against MPTP neurotoxicity or neurotoxicity of comparable pathogenic chemicals in the environment. The major mechanisms for the hepatic extraction of MPTP may include detoxification via FMO and CYPs (Chiba *et al.* 1988).

As shown in the results, pretreatment of rats with three pesticides, paraquat, DDT and malathion reduced the hepatic extraction of MPTP with a doubling of systemic bioavailability. This finding indicates that the risk of parkinsonism from MPTP might be increased when administered with these pesticides. Although this study does not give any mechanistic insight, candidate mechanisms for the reduced extraction in the liver include inhibitory effects on hepatocyte transport, xenobiotic detoxification enzymes or biliary excretion. A trend towards reduction in PS_{influx} and PS_{efflux} after pretreatment with the pesticides may be evidence for the inhibition of hepatic membrane transport. The trend towards reduction of k_3 may indicate inhibition of the activity of hepatic metabolising enzymes.

It is significant that the influence of pesticides on the hepatic disposition of pesticides was chemically selective. Pretreatment with paraquat and DDT inhibited hepatic extraction of malathion and significantly increased systemic bioavailability but it did not affect the recovery of paraquat and DDT. Furthermore, malathion pretreatment did not effect the hepatic extraction of either paraquat, DDT, malathion or MPTP. These results suggest that when certain pesticides are ingested in combination, they may be more bioavailable than when each chemical is administered alone. This finding is consistent with the report that a combination of paraquat and a fungicide maneb had a synergistic effect on the depletion of dopamine and its metabolite DOPAC in C57BL/6 mice, as compared with either compound administered alone (Thiruchelvam *et al.* 2000). This also indicates that the effect of pretreatment has not just a non-selective impairment of liver function.

Pretreatment with paraquat and DDT reduced the hepatic extraction of malathion and may be associated with inhibition of both hepatic membrane transport and hepatic enzyme activities. Malathion has not been linked directly to Parkinson's disease, however organophosphates are neurotoxic after acute exposure (He 2000). Furthermore, exposure to malathion in rats has been associated with lipid peroxidation in the brain, reduced motor activity (Haque *et al.* 1987) and inhibition of brain synthesis of nitric

oxide synthase (Rao *et al.* 1999). Additionally, old male rats (18 months old) treated with malaoxon, an active metabolite of malathion, showed severe neuronal injury in the cortex, hippocampus and subcortical structures (Hirvonen *et al.* 1993). In studies to investigate the effect of malathion on xenobiotic metabolising enzymes, hepatic glutathione S-transferase activity was increased in both rats (Srikanth & Seth 1990) and mice (Chhabra *et al.* 1993) after pre-treatment with malathion. Furthermore, hepatic glutathione content was also reduced in these pesticide pretreated rats (Srikanth & Seth 1990).

Analysis of the outflow curves using the physiological models of Goresky and colleagues, as described in Chapter 2, suggested that the effect upon hepatic extraction is mediated by modulation of both transport and sequestration. The outflow concentration-time curves for paraquat fitted the Goresky model very well, and the curves for DDT and malathion fitted moderately well. However, it should be noted that outflow concentrations in the MPTP experiments were low, which reduces the accuracy of the curve fitting procedures used to determine these values.

The overall effect of pretreatment of these three pesticides on MPTP extraction is of considerable interest for the pathogenesis of PD. The results suggest that the association between pesticides and PD could be contributed to by the effects of pesticides on the hepatic disposition of neurotoxins such as MPTP. This type of mechanism has been suggested for the protective association between cigarette smoking and PD. Smoking reduces the risk of PD (Seidler *et al.* 1996; Liou *et al.* 1997) and this reduction is thought to be mediated in part by the inhibition of monoamine oxidase B, the enzyme that catalyses the conversion of MPTP to its toxic metabolite MPP^+ (Mellick *et al.* 1999a). In fact, many of the risk factors for PD that have been identified using epidemiological methods may be hepatic metabolism modulators, with positive risk factors tending to be inhibitors of xenobiotic metabolism and protective factors tending to be inducers of xenobiotic metabolism (Le Couteur *et al.* 1999a).

In conclusion, pretreatment with pesticides reduced the hepatic extraction of MPTP and malathion. Modulation of the hepatic disposition of environmental neurotoxins with modulation of systemic exposure may explain in part the association of pesticides with neurological diseases such as PD.

4. EFFECT OF AGEING ON THE HEPATIC DISPOSITION OF MPTP AND PESTICIDES

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4.4. DISCUSSION 89

4.1. INTRODUCTION

Parkinson's disease is one of the most common neurodegenerative diseases affecting older people (Graeber *et al.* 1998; Giasson & Lee 2000). The disease affects approximately 1-2% of the population over the age of 50 (Tolwani *et al.* 1999; Smeyne *et al.* 2001). Even though early-onset cases have been found, the average age of diagnosis is around 65 years (Baldereschi *et al.* 2000). Although the etiology of Parkinson's disease is unknown, one of the major risk factors is old age. There are several possible mechanisms for PD in old age.

First, striatal dopamine declines with normal ageing (Carlsson 1978; Langston 1989) and this age-related decrease may contribute to the progress of PD. Pathologically, this disease is associated with the destruction of dopaminergic neurons in the substantia nigra pars compacta, which are responsible for producing and transporting dopamine via the nigrostriatal tract to the striatum (Chase & Oh 2000; Crossman 2000). It has been reported that the number of dopamine containing neurons in the substantia nigra of humans decline by 5-10% per decade from ageing (Naoi & Maruyama 1999).

Second, normal ageing is associated with a gradual decline of mitochondrial function in various cell types including neurons (Toescu *et al.* 2000). A decrease in complex I of the mitochondrial electron transfer chain was observed in the substantia nigra of patients with PD (Mizuno *et al.* 1995). Mitochondrial dysfunction in dopaminergic cells is a major pathogenic mechanism for cell death in PD. Degeneration of the mitochondrial structure was also seen ultrastructurally in the hippocampal nerve cells of aged Wistar rats (Lolova 1990). Furthermore, Harmon *et al.* (1987) have reported that the amount of cytochrome c decreases approximately 50% with increasing age in synaptic mitochondria isolated from Fischer 344 rats.

Third, oxidative stress increases with age (Choi *et al.* 1998; Hamilton *et al.* 2001). In MPTP neurotoxicity, ROS are generated after blockade of complex I (Mizuno *et al.* 1995) and dopamine oxidation (Nicklas *et al.* 1987; Przedborski *et al.* 1996). Age-

dependent susceptibility to MPTP-induced neurotoxicity has been reported by Ali *et al.* (1994). They have observed that in older mice (C57/B6N), a single dose of injection of MPTP produced an increase of ROS that was associated with a subsequent decrease in dopamine concentration in the striatum. However, younger mice were not significantly affected, suggesting that old age may be associated with an increased sensitivity to neurotoxins. Furthermore, the striatal dopamine content depleted by MPTP was found to be partially recovered within 3 months of injection in young mice only, thus indicating impaired dopamine recovery in mature mice (Saitoh *et al.* 1987).

Additionally, cytotoxic free radicals resulting in oxidative stress are generated by iron in the brain (Double *et al.* 1998), and the concentration of striatal iron is increased with advancing age (Martin *et al.* 1998). Amongst people over 60 years, males have lower serum levels of antioxidants than females (Mendoza-Nunez *et al.* 2001), which may partially explain why the incidence of PD is higher in men than in women (Dluzen & McDermott 2000).

A further possible pathogenic mechanism for PD in the elderly is that brain MAOB activity increases with age (Desole *et al.* 1993). This increase was observed in humans (Fowler *et al.* 1997) and mice (Voitenko 1992). MAOB is responsible for metabolizing MPTP to its active metabolite MPP^+. Walsh and Wagner (Walsh & Wagner 1989) have reported that the increased sensitivity to MPTP in aged animals may be attributed to the increase in MAOB activity in older animals, by showing significant correlation between levels of MAOB activity and degrees of lesion induced by MPTP treatment in the brain of Swiss-Webster male mice. However, in humans the concentration of MPTP in the brain is usually very low, which suggests that changes in MAOB activity would only have limited effects.

Finally, old age is associated with marked changes in xenobiotic metabolism (Le Couteur & McLean 1998a) and lifelong accumulation of exposure and risk of exposure to neurotoxins. These changes provide an alternative and logical explanation of the predisposition of elderly people to Parkinson's disease and possibly other diseases of old

age (Le Couteur *et al.* 1999a), as the liver is the major organ for the detoxification of xenobiotics. The age-related impairment of xenobiotic detoxification in the liver may be secondary to many factors, including reduction of liver mass (Wynne *et al.* 1989), blood flow (Wynne *et al.* 1990), phase I enzyme activity (Guo *et al.* 1993; Le Couteur & McLean 1998a) and pseudocapillarisation of the hepatic sinusoidal endothelium (Le Couteur *et al.* 2001).

In this chapter, the association between ageing and altered hepatic disposition of neurotoxins, such as MPTP and pesticides, was investigated. These experiments were designed to explore this potential association of old age with Parkinson's disease.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Paraquat-methyl-^{14}C dichloride, 4,4'-DDT-RING-UL-^{14}C and malathion-2,3-^{14}C were purchased from Sigma (Mi, USA) and methyl-4-phenyl-1,2,3,6-tetrahydropyridine,1-[methyl ^{3}H] from ARC (Mi, USA). Isotopic [6,6'(n)-^{3}H]-sucrose was obtained from Amersham Pharmacia (Buckinghamshire, UK) and [U-^{14}C]-sucrose from ICN (Ca, USA).

4.2.2. Animals

Male Fischer F344 rats were maintained on standard rat food pellets and water *ad libitum.* Rats aged 3 and 18 months were studied as young and old rats, respectively. The study was approved by the Australian National University Animal Experimentation Ethics Committee.

4.2.3. Liver perfusion

The multiple indicator dilution technique was used to determine the disposition of paraquat, DDT, malathion and MPTP in the perfused rat liver as described in Chapter 2.2.3. Briefly, rats were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and a laparotomy incision was made. The livers were perfused *in situ* via the portal vein in a single pass mode. The perfusate was KH bicarbonate buffer saturated with 95% O_2 and 5% CO_2, with addition of 2% (w/v) BSA. The flow rate of the perfusate was maintained at 19-21 ml/min using a cartridge pump. The outflow cannula was inserted into the thoracic inferior vena cava to collect samples.

4.2.4. Liver viability

Liver viability was assessed by macroscopic appearance, oxygen consumption before and after experiments, and assay of liver enzymes in the outflow samples, as described in Chapter 2.2.4.

4.2.5. Light microscopy of liver structure

When each experiment was completed, liver tissue from each lobe was cut and fixed in 4% paraformaldehyde. Liver tissue was then sent to the Histology Department (The Canberra Hospital, Canberra) for H&E staining for observation of liver structures by light microscopy.

4.2.6. Multiple indicator dilution experiments

Bolus injections (50 μl) contained ^{3}H-sucrose with either ^{14}C-paraquat, ^{14}C-DDT or ^{14}C-malathion, or ^{14}C-sucrose with ^{3}H-MPTP. Sucrose was used as an extracellular marker. After injection of the bolus into the portal vein cannula, outflow samples were collected over 60 s. The samples were then analysed with a liquid scintillation counter (see Chapter 2.2.6).

4.2.7. Data analysis

The hepatic outflow concentrations were expressed as the fraction of the injected dose per ml. Data analysis for hepatic extraction (E), recovery (F), mean transit time (MTT) and volume of distribution (V) of the indicators were determined as described in Chapter 2.2.6.

The rate constants for sequestration (k_3) and the permeability-surface area products for the hepatocellular influx (PS_{influx}) and efflux (PS_{efflux}) were determined using the physiological models developed by Goresky (1992) and programmed in Microsoft QuickBasic 2.0, as described previously in Chapter 2.2.6.

Data were expressed as mean ± SD. Results from young and aged rat livers were compared using the Student t-test and considered significant when $p \leq 0.05$.

4.3. RESULTS

4.3.1. Liver viability

The oxygen consumption, portal venous resistance and assay of liver enzymes in young and old rat livers are shown in Table 4.1. The baseline oxygen consumption in young rat livers (0.91 ± 0.13 to 1.00 ± 0.11 μmol/min/g) was similar to that in the control rat livers of Chapter 3 at similar flow rates of the perfusate (refer to Table 3.1), despite strain differences. The oxygen consumption was unchanged at completion of the experiment (0.91 ± 0.12 to 0.99 ± 0.11 μmol/min/g). These values are also similar to the observation of oxygen uptake in fed young rat livers (1.0 ± 0.2 μmol/min/g) as reported by Le Couteur *et al.* (1994b).

The baseline values of oxygen consumption in the old rat livers were slightly lower than those in the young rat livers (0.86 ± 0.06 to 0.86 ± 0.07 μmol/min/g). This reduction may be caused by the lower flow rates used in the perfusion of the old rat livers. The oxygen consumption is flow rate dependent (Le Couteur *et al.* 1993) and the low flow rates are due to the larger livers of old rats (i.e. the absolute flow rate was constant at 19 to 21 ml/min in both young and old rats, refer to Appendixes 4.1 to 4.4 for the liver weights of young and old rats). However, the oxygen consumption in the old rat livers was constant throughout the experiment (oxygen uptake after experiments was 0.84 ± 0.06 μmol/min/g).

The portal venous resistance in old rat livers (1.16 ± 0.52 to 1.16 ± 0.51 cm H_2O·min·g/ml) was similar to that in young rat livers (1.12 ± 0.21 to 1.22 ± 0.15 cm H_2O·min·g/ml). These values were consistent with those of the control rats in Chapter 3 (refer to Table 3.1). The values for ALT, AST and ALKP were similar between old and young rat livers. These values were also similar to those of the control rat livers in Chapter 3.3.1.

Overall, the parameters of liver viability in young rats are similar to those in the control rats of Chapter 3.3.1, despite the difference in rat strains. This similarity remained in the livers of old rats, except for the values of oxygen consumption as detailed above.

Table 4.1. Liver viability of young and old rats.

Indicators	n	Flow rate ($ml \cdot min^{-1} \cdot g^{-1}$)	O_2 uptake before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	O_2 uptake After exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal venous resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat								
Young	6	1.85 ± 0.18	1.00 ± 0.11	0.99 ± 0.11	1.12 ± 0.21	20 ± 1	1 ± 0	10 ± 0
Old	7	1.56 ± 0.12	0.86 ± 0.07	0.84 ± 0.06	1.16 ± 0.52	17 ± 3	1 ± 0	11 ± 1
DDT								
Young	4	1.97 ± 0.08	0.97 ± 0.11	0.97 ± 0.11	1.21 ± 0.14	20 ± 1	1 ± 0	10 ± 0
Old	7	1.55 ± 0.11	0.86 ± 0.06	0.84 ± 0.06	1.16 ± 0.51	17 ± 3	1 ± 0	11 ± 1
Malathion								
Young	6	1.84 ± 0.18	0.99 ± 0.10	0.99 ± 0.10	1.13 ± 0.21	20 ± 1	1 ± 0	10 ± 0
Old	7	1.55 ± 0.12	0.86 ± 0.07	0.84 ± 0.06	1.16 ± 0.52	17 ± 3	1 ± 0	11 ± 1
MPTP								
Young	4	1.95 ± 0.09	0.91 ± 0.13	0.91 ± 0.12	1.22 ± 0.15	20 ± 1	1 ± 0	10 ± 0
Old	7	1.55 ± 0.12	0.86 ± 0.07	0.84 ± 0.06	1.16 ± 0.52	17 ± 3	1 ± 0	11 ± 1

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

ALT, AST and ALKP represent alanine transaminase, aspartate transaminase and alkaline phosphatase, respectively.

4.3.2. Liver structure

Macroscopically, no visible differences in liver morphology were detected between young and old rats. By light microscopy (Figure 4.1), there were no significant differences observed with H&E stains except finer textures in the tissue of the young liver.

4.3.3. Recoveries

As shown in Figure 4.2, the recoveries of DDT, malathion and MPTP were increased to 253%, 134% and 258%, respectively, with age. Compared to young rat livers, however, there were no differences in the recovery of sucrose and paraquat between young and old rat livers (sucrose recovery 1.024 ± 0.033 and 0.997 ± 0.049 from livers of young and old rats, respectively). Figure 4.3 shows outflow concentration-time curves for each of the indicators from the livers of young and old rats.

4.3.4. Hepatic extraction, mean transit time and volume of distribution

Table 4.2 shows the results for extraction, mean transit time and volume of distribution of the indicators in the livers of young and old rats. In young rats (6 rats with 20 injections), 100% of the sucrose was recovered and its MTT and volume of distribution were 7.901 ± 1.952 s and 0.246 ± 0.061 ml/g, respectively. These values were similar to those observed in the previous chapters (Chapter 2 and 3) and they were unaffected by age change (0.997 ± 0.049, 8.160 ± 1.404 s and 0.209 ± 0.037 ml/g for the recovery, MTT and volume of distribution, respectively, in aged rat livers generated from 7 rats with 28 injections).

Ageing was associated with a reduced extraction of DDT, malathion and MPTP in the liver while the hepatic extraction of paraquat was unaffected by age. The MTT of DDT and MPTP was reduced and the MTT of malathion showed a trend towards reduction in old rats, compared to young rats. The volume of distribution of malathion was reduced in livers from old rats, although the volumes of paraquat, DDT and MPTP were unaffected by age.

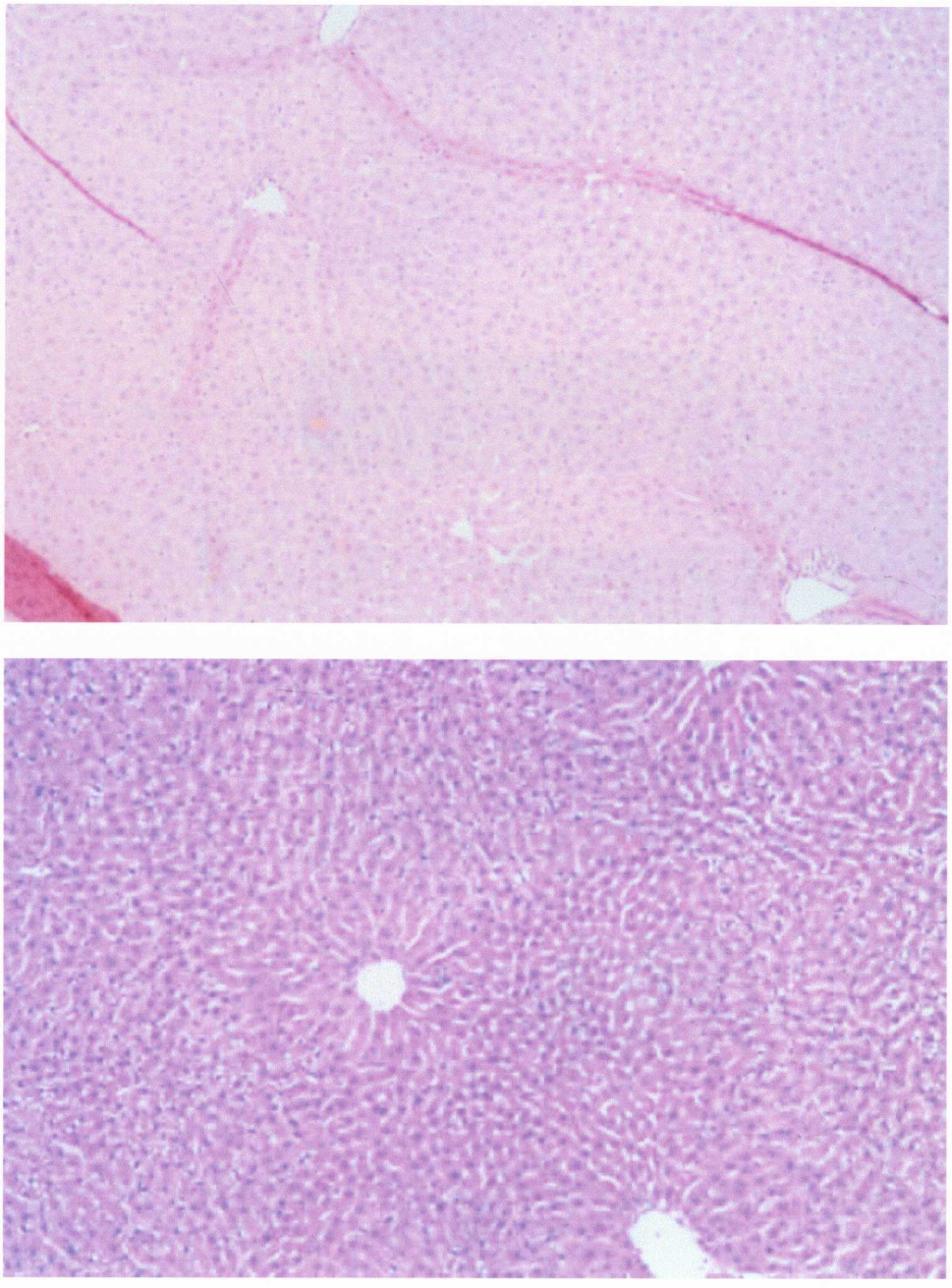

Figure 4.1. Comparison of liver tissues from young (top) and old (bottom) rats. Magnification ×100 (H&E stain).

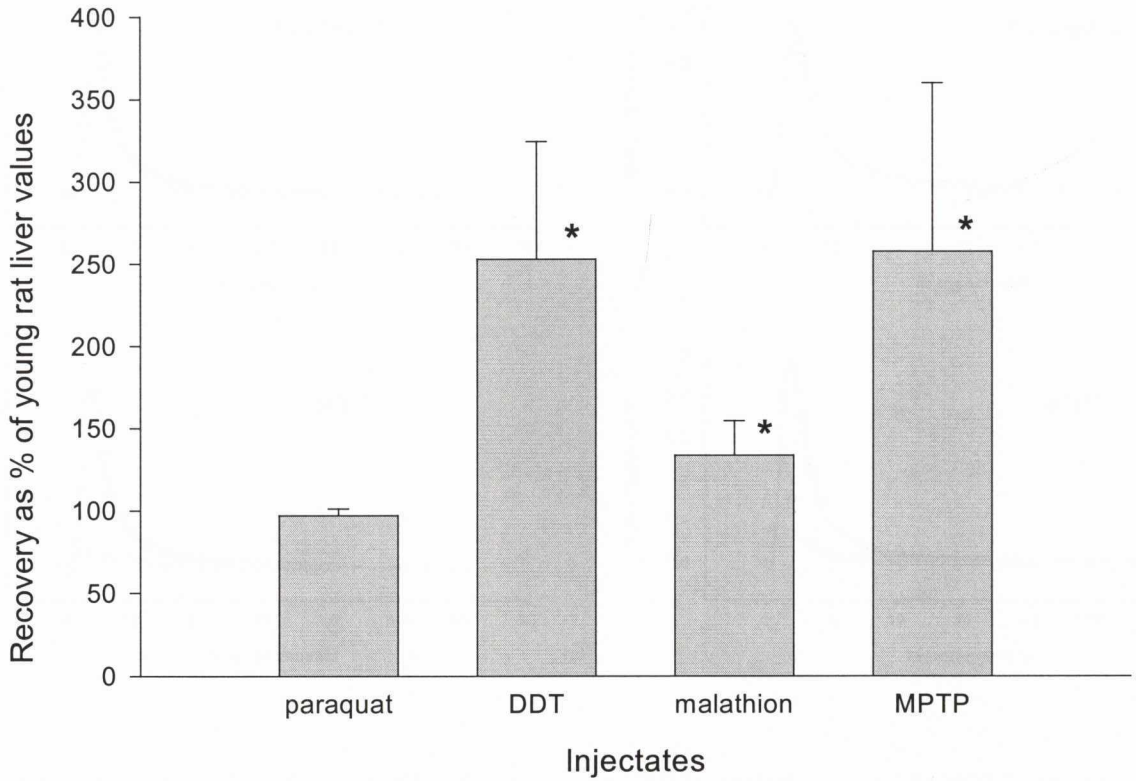

Figure 4.2. The effect of ageing on the recoveries of paraquat, DDT, malathion and MPTP. * Statistically different from young rat livers $p \leq 0.05$.

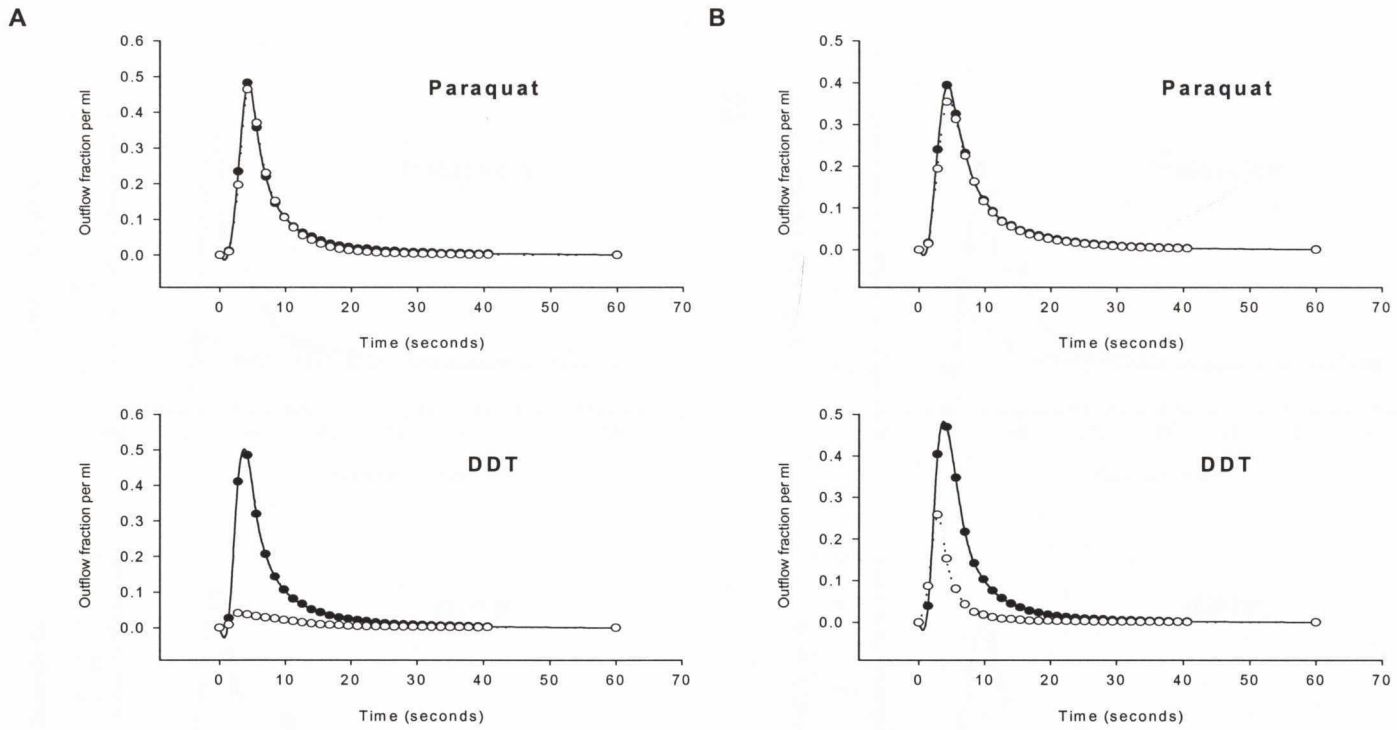

Figure 4.3. Comparison of outflow concentration-time profiles for paraquat, DDT, malathion and MPTP in the young (A) and old (B) rat livers using the multiple indicator dilution technique. In the livers of old rats, there are significant increases in the areas under the curve of DDT, malathion and MPTP, but not paraquat. Test substances and sucrose (extracellular marker) are marked as open circles and closed circles, respectively.

Figure 4.3. Contd.

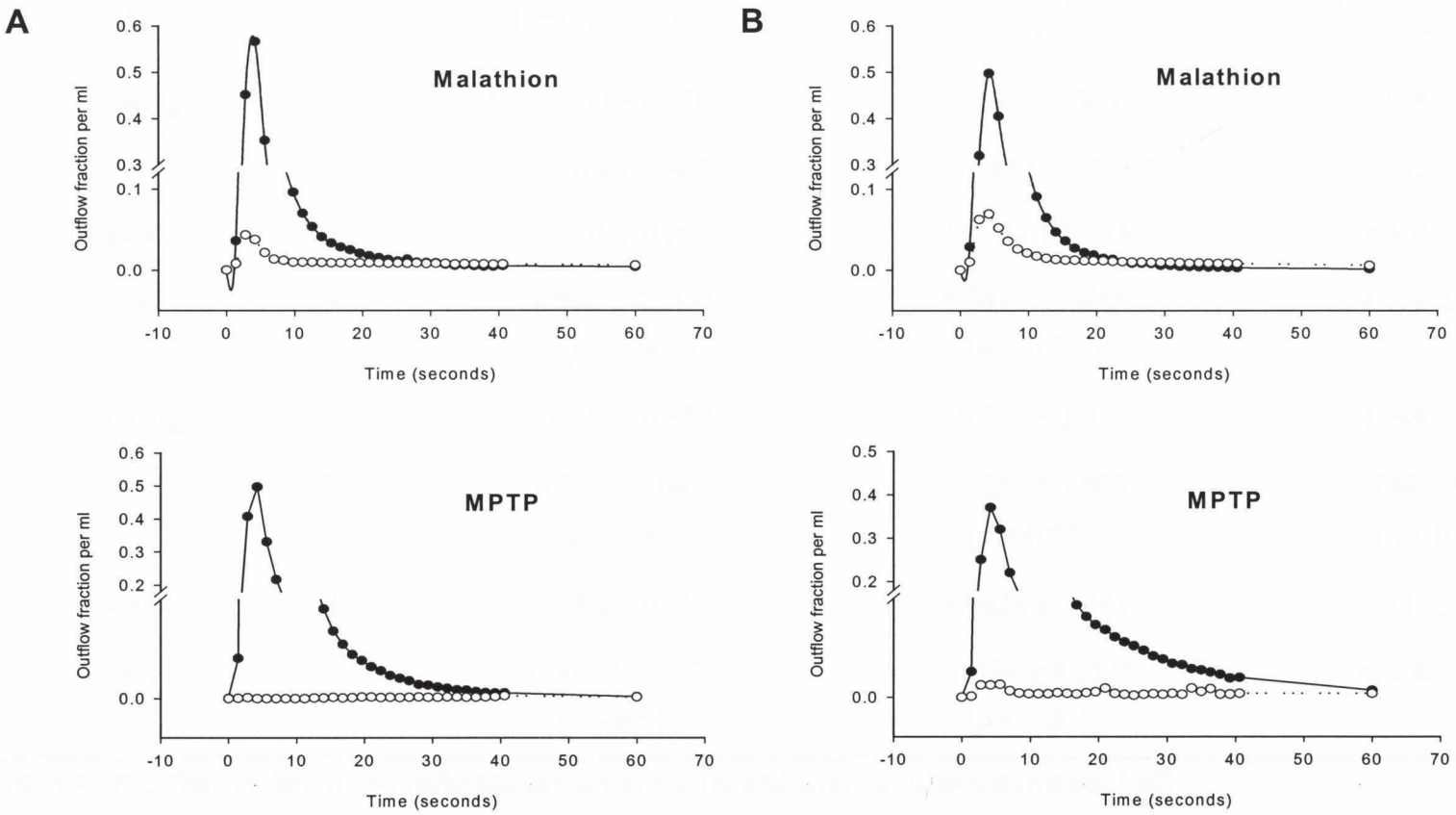

Table 4.2. Hepatic extraction, mean transit time and volume of distribution of paraquat, DDT, malathion and MPTP in the livers of young and old rats.

Substrate	Age	n	Hepatic extraction	Mean transit time (s)	Volume of distribution ($ml \cdot g^{-1}$ liver)
Paraquat	young	6	0.069 ± 0.026	6.392 ± 1.384	0.195 ± 0.034
	old	7	0.096 ± 0.037	7.549 ± 0.779	0.195 ± 0.023
DTT	young	4	0.835 ± 0.053	11.680 ± 1.913	1.462 ± 0.257
	old	7	0.582 ± 0.119* ($p = 0.003$)	5.841 ± 0.821* ($p < 0.001$)	1.552 ± 1.510
Malathion	young	6	0.793 ± 0.022	20.730 ± 2.580	4.298 ± 0.773
	old	7	0.724 ± 0.044* ($p = 0.005$)	18.281 ± 1.962 ($p = 0.078$)	2.082 ± 0.701* ($p < 0.001$)
MPTP	young	4	0.988 ± 0.002	35.628 ± 2.447	5.453 ± 1.497
	old	7	0.970 ± 0.012* ($p = 0.017$)	27.636 ± 4.551* ($p = 0.011$)	4.708 ± 2.572

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

* Statistically different from young rat livers $p \leq 0.05$.

4.3.5. Hepatic transport and sequestration parameters

Table 4.3 shows PS_{influx}, PS_{efflux} and k_3 for each of the indicators. For DDT, PS_{influx}, PS_{efflux} and k_3 were all significantly reduced in aged rat livers. For malathion, PS_{influx} was reduced in aged rat livers and there was an increase in k_3.

Table 4.3. Rates of influx, efflux and sequestration of paraquat, DDT, malathion and MPTP in livers of young and old rats.

Substrate	Age	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)	k_3 (s^{-1})
Paraquat	young	0.009 ± 0.009	0.353 ± 0.321	
	old	0.006 ± 0.002	0.519 ± 0.051	
DDT	young	0.199 ± 0.031	0.119 ± 0.015	0.181 ± 0.023
	old	0.031 ± 0.016* ($p < 0.001$)	0.051 ± 0.036* ($p = 0.006$)	0.087 ± 0.018* ($p < 0.001$)
Malathion	young	0.184 ± 0.036	0.035 ± 0.010	0.029 ± 0.005
	old	0.101 ± 0.017* ($p < 0.001$)	0.045 ± 0.009 ($p = 0.084$)	0.044 ± 0.009* ($p = 0.004$)
MPTP	young	1.166 ± 0.599	0.187 ± 0.111	0.363 ± 0.372
	old	0.870 ± 0.488	0.225 ± 0.197	0.085 ± 0.045 ($p = 0.072$)

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

k_3 represents the sequestration coefficient.

* Statistically different from young rat livers $p \leq 0.05$.

4.4. DISCUSSION

Ageing is associated with impaired hepatic clearance of many xenobiotics and this can be related to disease states. Age-related reductions in hepatic drug clearance (Woodhouse & Wynne 1992b; Le Couteur & McLean 1998a) determine adverse drug reactions which are prevalent in older people. Similarly, the increased susceptibility of older people to some diseases may be secondary to age-related impairment of the hepatic clearance of pathogenic xenobiotics. In particular, it has been suggested that exposure to pesticides and other neurotoxins is implicated in the pathogenesis of Parkinson's disease, a neurodegenerative disease common in older people (Le Couteur *et al.* 1999a; Betarbet *et al.* 2000; Priyadarshi *et al.* 2000). Together, these observations led to an investigation of whether there are any age-related differences in the hepatic disposition of pesticides and other neurotoxins putatively associated with Parkinson's disease.

As seen in the previous chapters (Chapters 2 and 3) and the results of this experiment, there are marked differences in the hepatic extraction of these potentially neurotoxic compounds. MPTP is almost entirely extracted by the liver, whereas paraquat is poorly metabolized. Malathion and DDT are partially extracted by the liver. Consequently, factors influencing hepatic clearance such as blood flow, intrinsic enzyme activity, protein binding, ageing and liver disease will have profoundly different effects on the systemic exposure to each of these substrates.

Ageing is associated with a reduction in hepatic blood flow in the order of 40-50% (Le Couteur & McLean 1998a). This reduction is associated with the reduced clearance of flow-limited drugs (Woodhouse & Wynne 1992b). In a study of the effect of blood flow on the hepatic clearance of drugs, a reduction of the flow by 50% (20 ml/min) led to a large reduction in hepatic clearance of verapamil (Frink *et al.* 1990). The extraction of MPTP in the liver is very high, and therefore, its hepatic clearance is flow-limited. Any age-related reduction in hepatic blood flow will alone increase the systemic exposure to MPTP, regardless of age-related changes in hepatic enzyme activity.

Liver volume declines with age. Le Couteur and McLean (1998a) have reported that the reduction of liver size is in the order of 25 to 30% with age, which may contribute to the reduced clearance of capacity-limited drugs (Woodhouse & Wynne 1992b; Zeeh 2001). The clearance of capacity-limited drugs is influenced by changes in liver size, enzyme mass or enzyme activity (Le Couteur & McLean 1998a). Reduced hepatic extraction of DDT, malathion and MPTP in old rats in this experiment might also be due to the reduction in the liver volume from age. However, it has also been reported that not all capacity-limited drug clearance is reduced in old age, since some drugs such as digitoxin are unaffected by age (Le Couteur & McLean 1998a).

Binding to plasma proteins reduces hepatic clearance of drugs that are highly protein bound and undergo capacity-limited metabolism (e.g. warfarin, phenytoin) (Goresky 1983). Ageing is associated with a decrease of albumin concentration (Wingerd & Sponzilli 1977) and an increase in the unbound fraction of some drugs (Boudinot *et al.* 1993). However, age-related effects on the protein binding have less clinical significance compared to other factors, such as hepatic blood flow and enzyme activity (Wallace & Verbeeck 1987). In aged male Fischer 344 rats, plasma protein binding of ketoprofen, an anti-inflammatory drug, was reduced due to decreases in albumin concentration and binding affinity with age. However, changes in the plasma clearance of this drug in aged rats were insignificant when total plasma concentrations were examined (Satterwhite & Boudinot 1992).

In the studies reported here, there are age-related reductions in the hepatic extraction of DDT, malathion and MPTP of sufficient magnitude to produce clinically relevant increases in systemic bioavailability and, possibly, neurotoxicity. The mechanisms for the reduction in the hepatic extraction of these substrates appeared to be related to changes in hepatocellular influx, efflux and sequestration.

The PS_{influx} products for DDT and malathion were decreased, which indicates that transport of these pesticides into hepatocytes is reduced with old age. Recently, it has been shown that the liver sinusoidal endothelium is thickened and numbers of

fenestrations in the endothelium are reduced, with increased collagen deposits within the space of Disse (Le Couteur *et al.* 2001). These changes in the ultrastructure of the liver may inhibit delivery of xenobiotics into the hepatocytes.

There were inconsistent results for the effects of age on sequestration (reduced for DDT, trend towards reduction for MPTP, and increased for malathion). Sequestration represents a number of processes including metabolism, biliary excretion and intracellular binding. Ageing has variable effects on hepatic xenobiotic metabolism but overall, phase I enzyme activity is reduced, whereas phase II activity is maintained (Tarloff *et al.* 1991). It has been reported that the activity of CYP isoenzymes is affected by ageing, especially in men (Woodhouse & Wynne 1992b). This finding may partially explain the age-related reduction of MPTP sequestration in this experiment, since MPTP is a substrate for phase I enzymes such as CYP2D6, FMO and MAOB (Modi *et al.* 1997). DDT is also a substrate for phase I enzymes such as CYP2B and 3A (Li *et al.* 1995; Nims *et al.* 1998). Malathion is a substrate for both phase I and phase II pathways (e.g. CYP and GST) (Ketterman *et al.* 1987). The difference in the effects of ageing on the hepatic sequestration of these substrates may reflect differences in their hepatic handling.

In conclusion, there was a significant difference found in the hepatic disposition of DDT, malathion and MPTP in the liver of old rats, manifesting as altered distribution, transport, extraction and sequestration. These alterations may have implications for greater systemic bioavailability and neurotoxicity of these toxins with age and may provide a mechanism for the association of ageing with Parkinson's disease. Further investigations on hepatic membrane transport and enzyme metabolic mechanisms will be useful for determining the overall effects of age on hepatic xenobiotic metabolism.

5. CELL MEMBRANE TRANSPORT OF MPTP IN THE LIVER AND SYSTEMIC BIOAVAILABILITY

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5.4. DISCUSSION 108

5.1. INTRODUCTION

MPTP exposure is an established experimental model for PD. MPTP is a cyclic tertiary allylamine that has been shown to produce a parkinsonian syndrome and dopaminergic cell death in humans (Langston *et al.* 1983b), non-human primates (Burns *et al.* 1983) and various rodents (Heikkila & Sonsalla 1987). The mechanism of action and disposition of MPTP has been extensively investigated (Lyden-Sokolowski *et al.* 1988; Przedborski & Jackson-Lewis 1998; Fabre *et al.* 1999).

MPTP administered systemically is detoxified, presumably primarily in the liver, by CYP and FMO enzymes. MPTP is metabolised to MPTP N-oxide by CYP2D6 (Coleman *et al.* 1996; Gilham *et al.* 1997) and to 4-phenyl tetrapyridine (PTP) by FMO (Cashman & Ziegler 1986; Di Monte *et al.* 1988). MPTP that escapes systemic metabolism can cross the blood-brain barrier because it is lipophilic (Riachi *et al.* 1989). MPTP is a pro-toxin that is bio-activated by MAOB to 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) and this intermediate undergoes further oxidation to MPP^+ (Trevor *et al.* 1987) within nondopaminergic neurons, such as glial cells and serotonergic neurons (Chiba *et al.* 1984; Singer & Ramsay 1990). MPP^+ has a high affinity for DAT on the plasma membrane (Storch *et al.* 1999) and it is selectively transported into dopaminergic cells by DAT. Speciale *et al.* (1998) have observed that systemic administration of low doses of [^{3}H]-MPTP in mice caused a significant accumulation of [^{3}H]-MPP^+ and a large increase in the amount of vesicular monoamine transporter within monoaminergic neurons, including dopaminergic neurons. There was also a positive relationship between the density of [^{3}H]-MPP^+ label and the density of vesicular monoamine transporter immunoreactivity in the neurons. Furthermore, a mutation in the DAT 11th hydrophobic putative transmembrane domain has been found to increase affinity for MPP^+ (Hagenbuch *et al.* 2000).

Intracellular MPP^+ is taken up and accumulated within mitochondria by an energy-dependent process (Ramsay *et al.* 1986a; Ramsay & Singer 1986b). Then MPP^+ binds complex I of the mitochondrial electron transfer chain, producing cell death as a result of

ATP depletion and oxidative stress (Ali *et al.* 1994; Przedborski *et al.* 1996). An interaction of MPP^+ with mitochondrial complex I has been reported by Nicklas *et al.* (1985). In mitochondria isolated from rat and mouse brains, MPP^+ interferes with NADH-linked oxidation of pyruvate or glutamate without affecting the oxidation of succinate, which indicates that the site of interaction with the respiratory process is at complex I. Glutamate-mediated excitotoxicity has also been implicated (Beal 1998).

The effect of the blood brain barrier is critical because MPP^+, which is unable to penetrate the blood-brain barrier, is only neurotoxic when administered via an intracerebral route (Altar *et al.* 1986; Rollema *et al.* 1989), whereas MPTP causes neurotoxicity when administered systemically (Freyaldenhoven *et al.* 1997; Speciale *et al.* 1998). Variation in the systemic disposition of MPTP is thought to influence susceptibility to MPTP-induced neurotoxicity by affecting the amount of MPTP that is delivered to the blood-brain barrier. Modulation of hepatic metabolism of MPTP by inhibitors of CYP and FMO alters susceptibility to MPTP neurotoxicity (Chiba *et al.* 1988; Tanner 1991), and furthermore, frequencies of the genetic polymorphisms in CYP2D6 (McCann *et al.* 1997) and other xenobiotic metabolising enzymes influence the risk of PD in humans (Bandmann *et al.* 1997b; Menegon *et al.* 1998).

In Chapter 2, it has been shown that the liver extensively extracts MPTP (98% of the injectate) from the portal vein. Therefore small changes in hepatic extraction of MPTP will have profound effects on systemic exposure and hence, neurotoxicity. For example, as shown in Chapters 3 and 4, pesticide pretreatment and ageing respectively reduce hepatic extraction and increase the systemic bioavailability of MPTP approximately twofold in rats. This indicates a significant increase in potential neurotoxicity.

Although the hepatic metabolism of MPTP has been investigated, there are few reports on the hepatocellular transport of MPTP. In the previous study (Chapter 3 and 4), increases in the systemic bioavailability of MPTP by pesticide pretreatment and ageing was associated with changes in hepatocellular influx, efflux and sequestration. Because of the possibility that modulators of hepatic cellular transport of MPTP might also

influence susceptibility to MPTP-induced neurotoxicity in addition to modulations in the MPTP metabolism in hepatocytes, transport mechanisms for MPTP were studied in the intact perfused rat liver and in isolated hepatocytes.

5.2. MATERIALS AND METHODS

5.2.1. Animals

Male Wistar rats (2-3 months old [250-480 g], John Curtin School of Medical Research, Canberra, Australia) maintained on standard rat food pellets and water *ad libitum* were studied. The study was approved by the Australian National University Animal Experimentation Ethics Committee.

5.2.2. Chemicals

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine,1-[methyl ^{3}H] was purchased from ARC (Missouri, USA) and [U-^{14}C]-sucrose and daunomycin from ICN (CA, USA). Amiloride, d-tubocurarine chloride, rifamycin and collagenase were obtained from Sigma (MO, USA).

5.2.3. Disposition of ^{3}H-MPTP in the perfused rat liver

The liver perfusion and MID technique described previously in Chapter 2 were used to determine the disposition of MPTP in the perfused rat liver. Briefly, the livers were perfused *in situ* via the portal vein cannula with KH buffer containing 1% BSA in a single pass mode. The perfusate flow rate was maintained at 19-21 ml/min and the injectate (50 μl), containing ^{3}H-MPTP and ^{14}C-sucrose (0.5 μCi), was administered as a bolus through the portal vein catheter without any pretreatments of the liver for a control experiment. ^{14}C-sucrose was the extracellular marker. Outflow samples from the thoracic inferior vena cava were collected for 60 s after the injection and analysed with a scintillation counter. An additional experiment was performed in the same liver after ten minutes pretreatment with either 1 mM amiloride, 200 μM tubocurarine, 100 μM daunomycin or 10 μM rifamycin. Amiloride and tubocurarine are inhibitors of organic cation transporter 1 (OCT1 in humans, oct1 in rats) (Martel *et al.* 1996a). Daunomycin is an inhibitor of a multi-drug resistance gene product (MDR in humans, mdr in rats) also known as P-glycoprotein (Martel *et al.* 1996a). Rifamycin is an inhibitor of organic anion transporting polypeptide (OATP in humans, oatp in rats) 1 & 2 (Fattinger *et al.* 2000).

5.2.4. Data analysis of multiple indicator-dilution experiments

The hepatic outflow concentrations were expressed as the fraction of the injected dose per ml. The recovery of ^{3}H-MPTP in the effluent was determined from the area under the outflow curve (AUC). The rate constants for cellular influx, efflux and sequestration (k_1, k_2 and k_3, respectively), and the permeability-surface area products for the hepatocellular influx (PS_{influx}) and efflux (PS_{efflux}) were determined using the physiological models developed by Goresky (1984) as described previously in Chapter 2. The volume of distribution for ^{3}H-MPTP in the liver was calculated from that of sucrose, k_1 and k_2 by the relationship:

$$V_{ext} = V_{suc}\left(\frac{k_1 + k_2}{k_2}\right)$$

Data were expressed as mean ± SD. Control and test groups were compared using the Student t-test and considered significant when $p \leq 0.05$.

5.2.5. Preparation of rat hepatocytes

Hepatocytes were isolated according to a procedure described previously by Berry and Phillips (2000) with modification. Livers were perfused for two minutes with calcium-free KH buffer (132 mM NaCl, 3 mM KCl, 1.2 mM KH_2PO_4, 1.18 mM $MgSO_4.7H_2O$, 25 mM $NaHCO_3$ and 10 mM glucose, pH7.4) saturated with 95% O_2 / 5% CO_2 at a flow rate of 40 ml/min. The perfusion was continued for 8 min with collagenase added (100 mg in 150 ml buffer). The liver was removed and, after breaking its capsule, it was digested at 37°C for 5 min in the collagenase buffer containing 1 mM $CaCl_2.2H_2O$. The cells were sieved and washed three times at 4°C with calcium containing KH buffer (refer to Chapter 2.2.3) with 1% BSA. Cell viability was determined by cell count using Trypan Blue (0.16%). Preparations containing more than 90% viable cells were used to assess ^{3}H-MPTP uptake into hepatocytes.

5.2.6. Uptake of ^{3}H-MPTP into hepatocytes

Cells (2×10^{6} cells/ml) in triplicate were pre-incubated for 5 min at 37°C in KH buffer containing 1% BSA and equilibrated with 95% O_2 / 5% CO_2, then incubated for a further 5 min with either 1 mM amiloride, 200 μM tubocurarine, 100 μM daunomycin or 10 μM rifamycin. ^{3}H-MPTP (0.3 μCi) was added to the cell suspensions and incubation continued for another 5 min. The cells were washed three times at 4°C with incubation buffer and lysed in perchloric acid (0.2 M $HClO_4$, Sigma, USA). Scintillation fluid (Starscint 5ml) was added to the lysates and ^{3}H radioactivity was counted using a liquid scintillation counter. The effect of each agent on MPTP uptake was calculated as a proportion of the radioactivity in each sample relative to that in the control samples (i.e. no cell membrane transporter inhibitors added). Experiments were replicated four times. Baseline control experiments established that 5 min incubation of cells with the above concentrations of the inhibitors had no affect on the viability of the hepatocytes.

5.2.7. Statistics

All data were presented as mean ± SD. The Student t-test was used to compare results and considered significant when $p \leq 0.05$.

5.3. RESULTS

5.3.1. Liver viability

Table 5.1 shows results of the viability of perfused rat livers. The oxygen consumption in the livers before experiments were started was similar to that established for control rat livers in Chapter 3 at similar flow rates of the perfusion buffer. This did not change over the course of the experiments. Furthermore, portal venous resistances during the perfusions, and ALT, AST and ALKP activities in the effluent were consistent with previous reports (Le Couteur *et al.* 1993; Le Couteur *et al.* 1999c) and similar to findings detailed in Chapter 2. Overall, the viability of rat livers used in these experiments was good and the results were constant throughout the experiments.

5.3.2. Recovery and volume of distribution of ^{3}H-MPTP in the perfused rat livers

Figure 5.1 shows the effect of cell membrane transporter inhibitors on the recovery of ^{3}H-MPTP in the effluent of perfused rat livers. The oct1 inhibitors, amiloride and tubocurarine, increased the recovery of ^{3}H-MPTP in the outflow by 253 ± 78% (P = 0.01) and 283 ± 64% (P = 0.004), respectively. The P-glycoprotein inhibitor, daunomycin, and the oatp1 & 2 inhibitor, rifamycin, did not have any significant effects on the recovery of ^{3}H-MPTP (92 ± 11% and 107 ± 29%, respectively).

Figure 5.2 shows representative outflow concentration-time profiles before and after the addition of hepatocyte membrane transport inhibitors to the perfusate. The increase in the area under the curve of the ^{3}H-MPTP after the addition of amiloride and tubocurarine is clearly evident. However, addition of daunomycin and rifamycin did not change the area under the curve of ^{3}H-MPTP.

The values for recoveries and volumes of distribution are shown in Table 5.2. The recovery of the extracellular marker sucrose was approximately 100% (98.7 ± 3.3%, n = 17) and its volume of distribution was 0.203 ± 0.053 ml/g liver in control experiments, which is consistent with the findings detailed in previous chapters (Chapters 2, 3 and 4). Furthermore, they were not influenced by the addition of membrane transport

Table 5.1. Liver viability of perfused rats before and after pretreatment with hepatocellular membrane transporter inhibitors.

Inhibitors	n	Flow rate ($ml \cdot min^{-1} \cdot g^{-1}$)	O_2 uptake before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	O_2 uptake after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Amiloride								
Control	6	1.52 ± 0.06	0.88 ± 0.04	0.88 ± 0.05	1.37 ± 0.33	16 ± 2	1 ± 0	10 ± 0
Test	5	1.51 ± 0.06	0.87 ± 0.04	0.87 ± 0.06	1.39 ± 0.37	16 ± 2	1 ± 0	10 ± 0
Tubocurarine								
Control	4	1.50 ± 1.04	0.86 ± 0.02	0.86 ± 0.02	1.34 ± 0.28	17 ± 2	1 ± 0	10 ± 0
Test	3	1.51 ± 0.06	0.86 ± 0.03	0.86 ± 0.03	1.21 ± 0.17	17 ± 3	1 ± 0	10 ± 0
Daunomycin								
Control	3	1.64 ± 0.14	0.91 ± 0.07	0.88 ± 0.10	1.23 ± 0.33	20 ± 0	1 ± 0	12 ± 1
Test	3	1.64 ± 0.14	0.91 ± 0.07	0.88 ± 0.10	1.23 ± 0.33	20 ± 0	1 ± 0	12 ± 1
Rifamycin								
Control	4	2.03 ± 0.34	0.96 ± 0.07	0.96 ± 0.07	0.94 ± 0.17	20 ± 1	1 ± 0	13 ± 1
Test	4	2.03 ± 0.34	0.96 ± 0.07	0.96 ± 0.07	0.94 ± 0.17	20 ± 1	1 ± 0	13 ± 1

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

ALT, AST and ALKP represent alanine transaminase, aspartate transaminase and alkaline phosphatase, respectively.

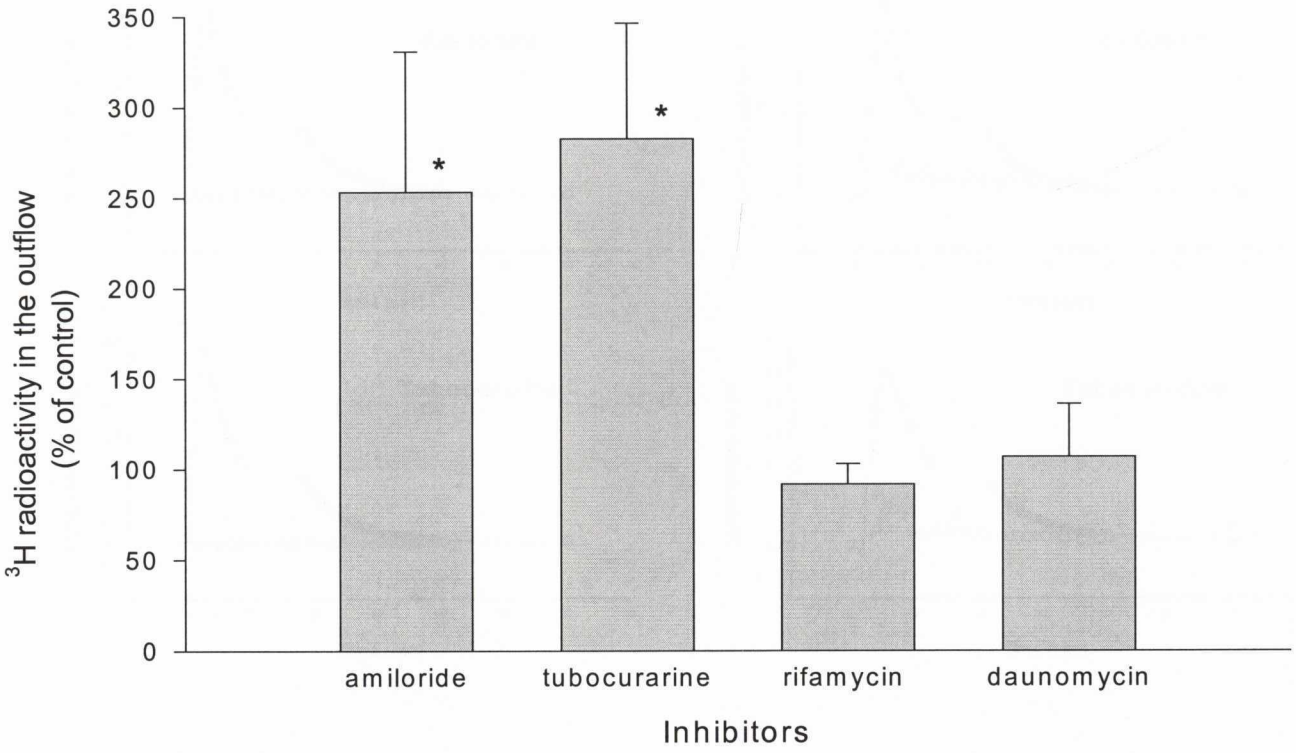

Figure 5.1. The effect of hepatocyte membrane transporter inhibitors on the recovery of MPTP in the perfused rat liver. Results are shown as a percentage of the control (100%) value (mean ± SD). *Significantly different from control ($p \leq 0.05$).

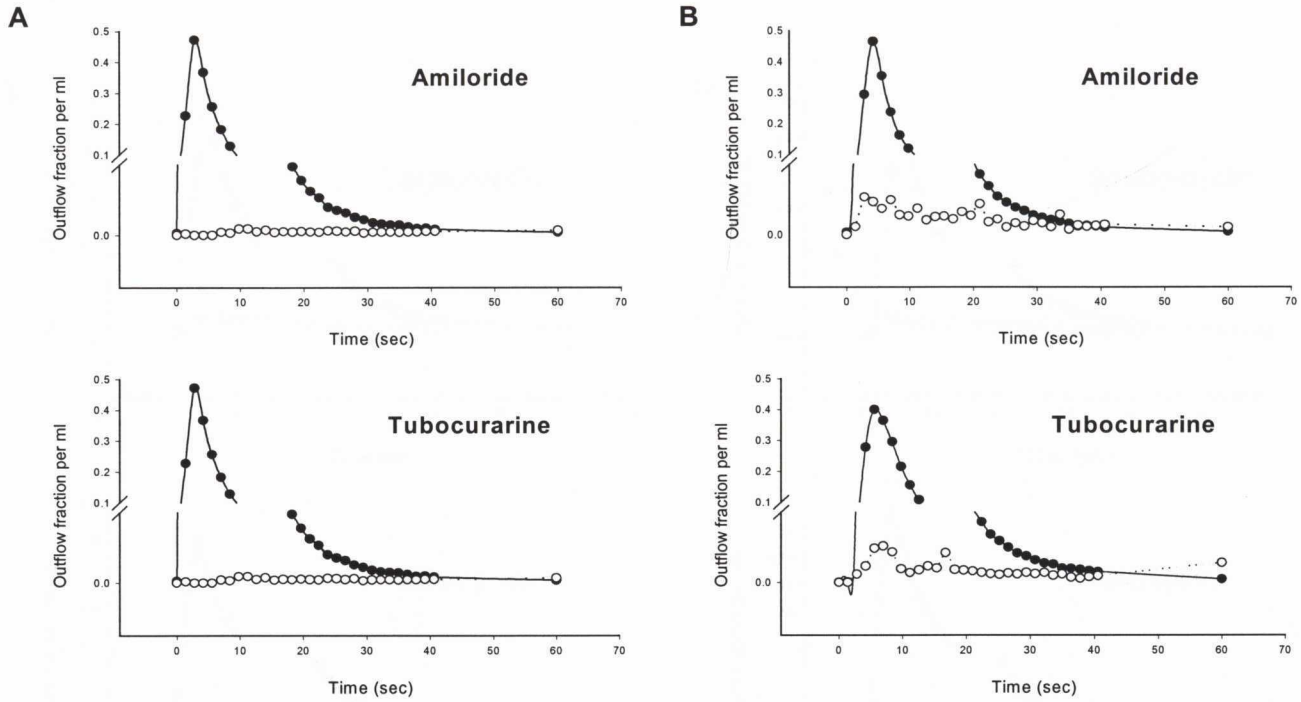

Figure 5.2. The effect of hepatocyte membrane transport inhibitors on the outflow curves of MPTP in the perfused rat liver. The injectate consisted of ^{3}H-MPTP (open circles) and ^{14}C-sucrose (closed circles). The experiments were performed in the absence (A) and presence (B) of inhibitors in the same liver.

Figure 5.2. Contd.

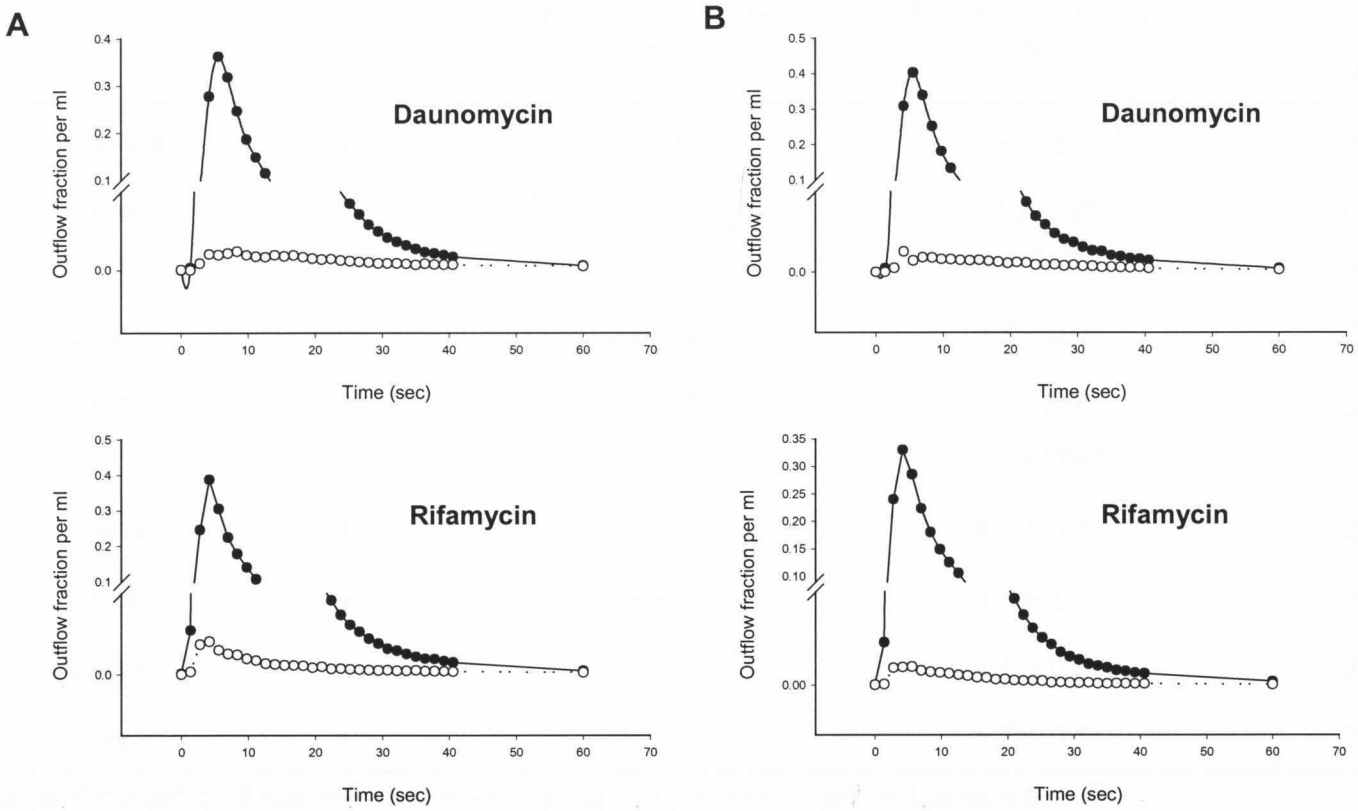

Table 5.2. Fractional recoveries of sucrose and MPTP, and volume of distribution of MPTP in the perfused rat liver before and after treatment with membrane transport inhibitors.

Inhibitors		n	Recovery of sucrose (%)	Recovery of MPTP (%)	Volume of distribution of MPTP ($ml \cdot g^{-1}$ liver)
Amiloride	Control	6	98 ± 4	3.7 ± 3.1	3.84 ± 1.63
	Test	5	96 ± 4	9.2 ± 2.8* ($p = 0.01$)	1.70 ± 1.47* ($p = 0.05$)
Tubocurarine	Control	4	100 ± 3	2.5 ± 0.9	2.15 ± 0.24
	Test	3	104 ± 2	7.2 ± 1.6* ($p = 0.004$)	2.25 ± 0.57
Daunomycin	Control	3	98 ± 2	4.2 ± 0.2	2.73 ± 0.23
	Test	3	98 ± 3	4.5 ± 1.2	2.59 ± 0.83
Rifamycin	Control	4	100 ± 3	4.1 ± 0.3	3.09 ± 0.24
	Test	4	99 ± 1	3.7 ± 0.5	2.25 ± 0.62

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

* Statistically different from controls $p \leq 0.05$.

inhibitors (98.6 ± 3.7% for recovery and 0.208 ± 0.038 ml/g liver for volume of distribution, respectively, n = 15 in test experiments). The recovery of ^{3}H-MPTP in control experiments was 2.5-4.1%, indicating that the hepatic extraction of MPTP is extremely efficient and this value is consistent with the recovery of ^{3}H-MPTP reported in Chapter 2 (average 2%) and in control experiments of Chapter 3 (average 2%). The volume of distribution of MPTP was approximately 3 ml/g of liver and this was reduced significantly after treatment with amiloride (3.84 ml/g in control versus 1.70 ml/g liver after addition of amiloride, $p = 0.05$) but not the other agents.

5.3.3. Hepatic transport and sequestration parameters in perfused livers after pretreatment of cell membrane transport inhibitors

Table 5.3 shows the values for k_1, k_2, k_3, PS_{influx} and PS_{efflux} of ^{3}H-MPTP in the perfused liver. The oct1 inhibitors, amiloride and tubocurarine reduced the value of k_1 and PS_{influx} significantly, indicating that these agents increased the recovery of MPTP by inhibiting influx. PS_{efflux} was reduced by tubocurarine and although this was not statistically significant, it may indicate that MPTP efflux also occurs via oct1. Neither agent significantly influenced k_3. Daunomycin and rifamycin had no significant effects on k_1, k_2, k_3, PS_{influx} and PS_{efflux} of ^{3}H-MPTP.

5.3.4. Disposition of ^{3}H-MPTP in isolated rat hepatocytes

In the control experiments, 78% of the total radioactivity was taken up by isolated hepatocytes. Figure 5.3 shows the effects of the transporter inhibitors on the uptake of ^{3}H-MPTP into the hepatocytes. The results are quite different from those seen in the perfused liver. Amiloride and tubocurarine increased hepatic uptake of ^{3}H-MPTP (123 ± 12%, $p = 0.08$ and 106 ± 2% of control values, respectively, $p = 0.002$). Daunomycin reduced hepatocyte uptake to 78 ± 8% (P = 0.001) of the control value and rifamycin did not have any effect.

Table 5.3. The effects of membrane transport inhibitors on k_1, k_2, k_3, PS_{influx}, and PS_{efflux} in the perfused rat liver.

Inhibitors		k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)
Amiloride	Control	4.3 ± 2.5	0.3 ± 0.2	0.2 ± 0.3	0.69 ± 0.36	0.22 ± 0.21
	Test	1.4 ± 0.6* (P = 0.03)	0.4 ± 0.3	0.2 ± 0.2	0.27 ± 0.11* (P = 0.04)	0.29 ± 0.21
Tubocurarine	Control	6.2 ± 3.8	0.5 ± 0.3	0.1 ± 0.05	0.97 ± 0.50	0.42 ± 0.23
	Test	1.4 ± 0.4 (P = 0.09)	0.1 ± 0.04 (P = 0.06)	0.1 ± 0.03	0.23 ± 0.05* (P = 0.05)	0.10 ± 0.04 (P = 0.07)
Daunomycin	Control	2.4 ± 0.6	0.2 ± 0.1	0.1 ± 0.02	0.55 ± 0.21	0.17 ± 0.05
	Test	2.1 ± 0.4	0.2 ± 0.1	0.1 ± 0.1	0.45 ± 0.05	0.16 ± 0.07
Rifamycin	Control	1.7 ± 0.6	0.2 ± 0.1	0.2 ± 0.1	0.48 ± 0.22	0.12 ± 0.06
	Test	2.0 ± 0.6	0.3 ± 0.2	0.2 ± 0.1	0.53 ± 0.19	0.22 ± 0.11

Statistically different from controls $p \leq 0.05$.

k_1, k_2 and k_3 represent the influx, efflux and sequestration coefficients, respectively.

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

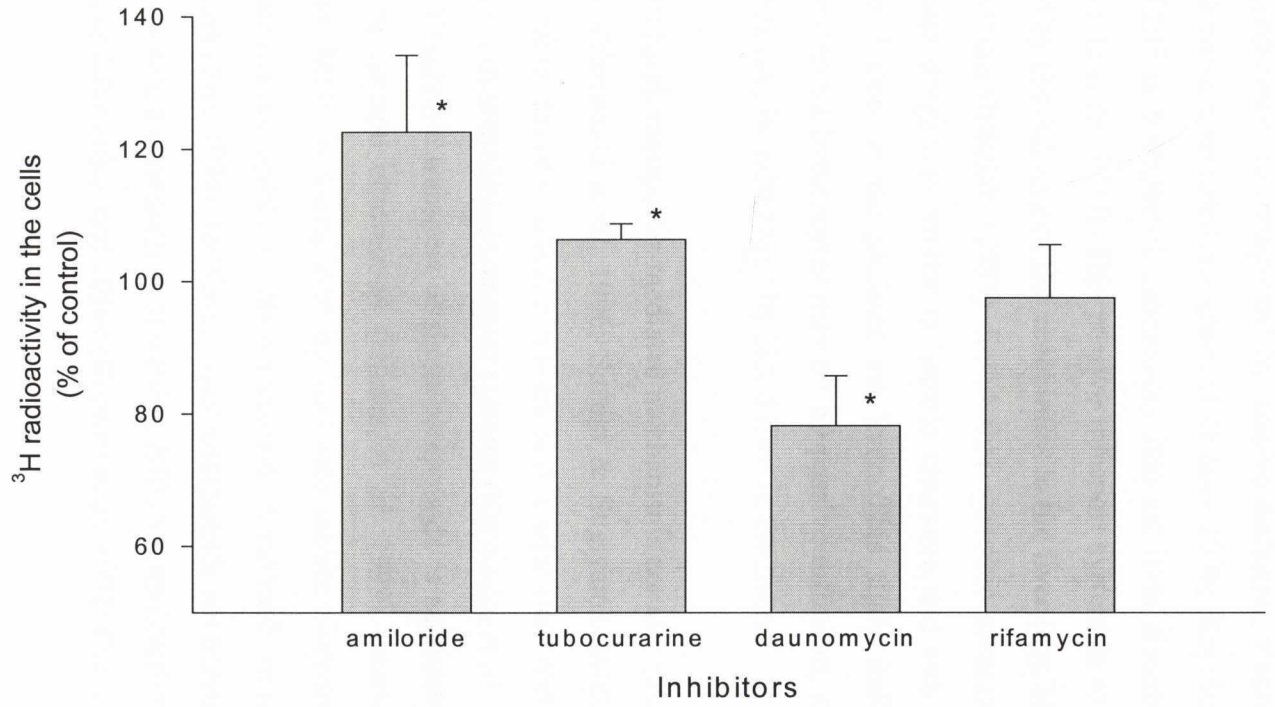

Figure 5.3. The effect of cell membrane transporter inhibitors on the uptake of MPTP in isolated hepatocytes. Results are shown as a percentage of the control (100%) value (mean ± SD). *Significantly different from control ($p \leq 0.05$).

5.4. DISCUSSION

The transport of xenobiotics across the plasma membrane of hepatocytes is an important determinant of hepatic elimination (Evans 1996; Keppler & Konig 2000). Uncharged lipophilic molecules are transported by passive diffusion, where substances move into the cells down a concentration gradient (Evans 1996; Keppler & Konig 2000). For example, GSH is transported osmotically into rat liver sinusoidal plasma membrane vesicles (Inoue *et al.* 1984). This passive transport system is so efficient that uptake is rate-limited by the delivery of the xenobiotics to the liver (e.g. blood flow) (Booth *et al.* 1996). Szymura-Oleksiak (1989) found that systemic clearance of imipramine (an antidepressant drug) was similar to hepatic clearance and was affected by changes in hepatic blood flow in the perfused rat liver. This study indicates that transport of imipramine into rat hepatocytes may be by passive diffusion, and its hepatic transport and clearance may be influenced by blood flow in the liver.

On the other hand, transporter-mediated mechanisms are also present for other classes of xenobiotics (Yamazaki *et al.* 1996; Suzuki & Sugiyama 2000). For polar molecules, such as organic cations, organic anions and conjugated metabolites, hepatocellular uptake is by carrier-mediated transport systems (Kamisako *et al.* 1999; Van Montfoort *et al.* 2001). Membrane transport of these compounds is saturable and dependent on the efficiency of hepatic elimination (Hisaka *et al.* 1999). Martel *et al.* (1996a) have reported that MPP^+ is transported by oct1 into isolated hepatocytes. It has also been reported that hepatic uptake of phenol red was a saturable process in the perfused rat liver (Nishida *et al.* 1989). However, most compounds are transported by multi-specific transport systems, a combination of passive diffusion and carrier-mediated transport, for both hepatocellular influx and efflux (Burwen *et al.* 1992; Evans 1996; Kullak-Ublick *et al.* 2000a).

In this chapter, the hepatic transport of MPTP was investigated because this neurotoxin is implicated in the pathogenesis of PD, and because of the possibility that modulation of

hepatic transporters might influence susceptibility to PD by altering hepatic extraction and hence systemic exposure to the neurotoxin.

As shown in the results (refer to Table 5.2 and Figure 5.3), MPTP was extensively taken up by both the perfused rat liver and isolated hepatocytes. The extraction of MPTP by the perfused liver was greater than 95% and uptake from media for isolated hepatocytes was nearly 80%. As mentioned previously in Chapter 2, it was considered likely that MPTP, which is a lipophilic molecule, is mostly taken up by the liver via simple diffusion. However, it was found that inhibitors of hepatocyte membrane transporters also influenced uptake to a significant extent, indicating that other mechanisms apart from diffusion are available. The difference in the uptake by isolated hepatocytes and the perfused liver might represent the influence of non-parenchymal cells, such as Kupffer cells, sinusoidal endothelial cells and stellate cells.

The findings shown in this experiment imply that oct1 is involved in MPTP transport into rat hepatocytes (Figure 5.1 and Table 5.2). There are two types of oct in the sinusoidal membrane of the hepatocyte. Type 1 oct is involved in the uptake of relatively small monovalent organic cations such as MPP^+ (Martel *et al.* 1996a) and procainamide (Yabuuchi *et al.* 1999), while the type 2 oct accepts organic cations with bulky ring structures, such as vecuronium (Zhang *et al.* 1997). The inhibitors of oct1, amiloride and tubocurarine, increased the recovery of MPTP from the perfused rat liver from about 3-4% to about 7-9% (Table 5.2). Furthermore, this was associated with a substantial reduction in the values for PS_{influx}, confirming that this effect was mediated by inhibition of the inward transport of MPTP. Overall, the results suggest that approximately 5% of MPTP uptake by the intact liver occurs via oct1. Although this is a small fraction of the total uptake, inhibition of oct1 with amiloride and tubocurarine increased the recovery by 250-300% (Figure 5.1), which indicates a profound effect on systemic exposure to MPTP. By contrast, amiloride and tubocurarine were associated with an increased accumulation of MPTP in isolated hepatocytes, indicating that oct1 is involved in MPTP transport from the cells.

The observation that oct1 mediates MPTP influx in the intact rat liver and efflux in isolated rat hepatocytes is apparently paradoxical. It is possible that in the isolated hepatocytes, the duration of the experiment is long enough for MPTP to be transformed by hepatic MAOB to MPP^+, which is effluxed by oct1. There is thought to be little leakage of MPP^+ from hepatocytes (Di Monte *et al.* 1988). It is also possible that the effects of P-glycoprotein on MPTP transport, which is only seen in isolated hepatocytes, influences the action of oct1. Finally it is also conceivable that there may be MPTP transport in non-parenchymal cells via oct1 in the perfused liver. Sokol *et al.* (1987) have observed that in brush border membranes of canine kidney cortex, MPTP undergoes bidirectional transport via OCT1. In isolated rat hepatocytes, it has been shown that MPP^+ undergoes substantial uptake via oct1 (Martel *et al.* 1996a). In this experiment, the results of the perfused rat liver and isolated hepatocytes show that oct1 mediates the influx, and possibly bidirectional transport, of a small fraction of total MPTP uptake into hepatocytes.

In the isolated hepatocyte experiment (Figure 5.3), it was found that MPTP was transported into the cells by P-glycoprotein. P-glycoprotein or MDR is a member of a super family of ATP-dependent canalicular membrane transporters (Kamisako *et al.* 1999; Kullak-Ublick *et al.* 2000a). P-glycoprotein in the liver is found on the canalicular surface of the hepatocytes, and is involved with the transport of various substrates (e.g. organic cations (Smit *et al.* 1998), glutathione conjugates (Paulusma *et al.* 1999) and glucuronides (Kwon *et al.* 1996)) from the hepatocyte into the bile. Daunomycin, an inhibitor of p-glycoprotein, did not have any effect on MPTP recovery from the perfused rat liver. This may be because the canalicular membrane is not exposed to P-glycoprotein inhibitors delivered by the portal vein in the perfused liver experiment. However, when P-glycoprotein was exposed directly to daunomycin in isolated hepatocytes, the uptake of MPTP into the hepatocytes was reduced by 20% of the control experiment. This indicates that P-glycoprotein is involved in the MPTP transport into hepatocytes and that this transport may be unidirectional. Again, this is an apparently paradoxical result because P-glycoprotein is generally considered to be an efflux pump (Kullak-Ublick *et al.* 2000a). However, it has been reported that MPP^+ is

taken up into isolated rat hepatocytes by P-glycoprotein, with a reduction in the accumulation of MPP^+ to 1% of control values by daunomycin (Martel *et al.* 1996a). In addition, daunomycin did not have any effect on MPP^+ transport in the perfused liver (Martel *et al.* 1996a), which might reflect limited access.

Finally, it was found that MPTP transport into rat hepatocytes is not mediated by oatp. Oatp1 is a sodium-independent multispecific transporter that mediates hepatocellular uptake of bromosulphophthalein (Hagenbuch *et al.* 2000) and bile salts (Eckhardt *et al.* 1999). Oatp2 is a close homologue of oatp1 and also transports bile salts and steroid conjugates with partially selective substrate specificities (Reichel *et al.* 1999). Both oatp1 & 2 are localised to the basolateral membranes of hepatocytes (Bergwerk *et al.* 1996; Reichel *et al.* 1999). Rifamycin, an inhibitor of oatp 1 & 2, had no effect on the uptake of MPTP either in the perfused rat liver or isolated hepatocytes and did not influence the parameters for influx or efflux of MPTP in the perfused liver.

In contrast to MPTP transport, the transport of MPP^+, the neurotoxic metabolite of MPTP, has been well studied (Sokol *et al.* 1987; Martel *et al.* 1996a). MPP^+ is a small organic cation and carrier-mediated active transport systems are necessary for its penetration into cells. Oct1 and P-glycoprotein are involved in MPP^+ transport into rat hepatocytes (Martel *et al.* 1996a). MPP^+ is also taken up into canalicular rat liver plasma membrane vesicles by an organic cation/H^+ exchanger (Moseley *et al.* 1997). However, from the point of view of the pathogenesis of PD, the study of MPTP is more important given that MPP^+ is unable to cross the blood brain barrier and variability in systemic exposure to MPP^+ would not be expected to influence neurotoxicity in the substantia nigra.

In summary, MPTP is extracted extensively by the liver. Most uptake into rat hepatocytes appears to be mediated by simple diffusion, however, a small but potentially significant proportion of uptake is mediated via oct1 and, in isolated hepatocytes, via P-glycoprotein. Oatp is not involved in MPTP transport, and oct1 may be a bidirectional transporter involved with efflux of MPTP in isolated hepatocytes. Although only a small

fraction of MPTP uptake is mediated by transporters, with extensive hepatic extraction even minor modulation of MPTP transport will have dramatic effects on systemic exposure to MPTP, and hence susceptibility to neurotoxicity.

6. DISPOSITION OF MALATHION AND MPTP IN THE CIRRHOTIC LIVER

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6.1. INTRODUCTION

In previous chapters, the effects of modulators of hepatic metabolism and transporters and ageing on neurotoxin disposition have been investigated. Liver disease is clearly another factor that influences hepatic clearance of xenobiotics including neurotoxins (refer to Chapter 4.4). In patients with chronic liver disease, the parkinsonism-producing neurotoxin manganese has been detected in the basal ganglia by magnetic resonance imaging (MRI) and was found to correlate with PD-associated neurological symptoms (Misselwitz *et al.* 1995). Manganese is primarily cleared by the liver (Hauser *et al.* 1994). Free manganese that enters the systemic circulation is able to cross the blood-brain barrier (Lockman *et al.* 2001) and causes neurotoxicity (Misselwitz *et al.* 1995). For example, occupational exposure to manganese is suspected to be associated with PD (Gorell *et al.* 1999a; Gorell *et al.* 1999b).

Cirrhosis is one of the most common chronic liver diseases (Sherman & Williams 1994). In the earlier stages of the disease, the liver may be enlarged. In advanced cases, it may be smaller than normal, with a tan or orange-yellow color with fine to gross nodularity. Splenomegaly is usually present, as well as ascites when portal hypertension complicates cirrhosis. Increased mesenteric fat lobulation and lymph node hyperplasia frequently occur (Perez Tamayo 1983). Microscopically, hepatic fibrosis progresses as the precursor lesion in the development of cirrhosis. The fibrotic process commences in the perivenular region of the lobule. A central role in this process is played by hepatic stellate cells, which are found in the perisinusoidal Space of Disse and regulate the composition of intracellular matrix (Sherman & Williams 1994). They undergo transformation into myofibroblasts and produce type 1 and type 3 collagen (Burt 1992). At the level of the sinusoid, there is defenestration of endothelial cells and formation of basement membrane (capillarisation). This change results in impaired delivery of nutrients and xenobiotics from the portal venous system to hepatocytes (Cardoso *et al.* 1994).

The most common cause of cirrhosis is chronic alcoholism, especially in western

countries (Lelbach 1976), even though there are many other causes, such as chronic viral hepatitis, Wilson's disease, hemochromatosis and biliary atresia. The latter three diseases are inherited diseases. About 8 to 30% of long-term alcohol abusers develop alcoholic cirrhosis (Grant *et al.* 1988). Wilson's disease and hemochromatosis result in free copper (Johnson 2001) and iron (Johnson 2000) accumulation, respectively, in the liver and other organs, including the brain. Patients with these diseases may present with parkinsonian syndromes, possibly secondary to oxidative damage in the substantia nigra by free radicals produced after an accumulation of copper (Johnson 2001) or iron (Nielsen *et al.* 1995). However, parkinsonism caused by these two diseases may be due to altered copper and iron metabolism rather than dysfunction of the liver itself. In biliary atresia, the bile duct is blocked and biliary excretion of toxins is inhibited (Ohi *et al.* 1984; Nietgen *et al.* 1992). Impaired hepatic biotransformation of phenazone, an otic medication, has been reported in animals with bile duct obstruction (Wojcicki *et al.* 1996). Thus liver damage is expected to result in the accumulation of toxic substrates.

There have been a few reports of PD directly associated with cirrhosis. Spahr *et al.* (2000) have found parkinsonian signs related to basal ganglia alterations detected by MRI and proton spectroscopy in patients with cirrhosis (age 44 to 65 years). After liver transplantation, the symptoms reduced and the signal alterations on the MRI disappeared, which suggests that there may be brain metabolic abnormalities in patients with cirrhosis. The authors also suggested that parkinsonian symptoms found in cirrhosis may be associated with manganese overload. This is because MRI pallidal changes share similarities with chronic manganese intoxification (Hauser *et al.* 1994) and abnormally high tissue concentrations of manganese are present in the areas (pallidum) of hyperintensities (Pomier-Layrargues *et al.* 1995). Furthermore, whole blood manganese levels correlate with the intensity of pallidal signal abnormality in patients with cirrhosis (Pomier-Layrargues *et al.* 1995; Spahr *et al.* 1996). Hauser *et al.* (1994) have also observed a significant elevation of blood manganese concentration in patients with hepatic cirrhosis who exhibited neuronal dysfunction and characteristic abnormal signal hyperintensity in the substantia nigra via MRI.

Impaired drug elimination in the cirrhotic liver has been studied extensively (Reichen *et al.* 1987; May *et al.* 1992; Wensing *et al.* 1993). The clearance of drugs such as antipyrine (Wensing *et al.* 1993) and dapsone, an antituberculosis drug (May *et al.* 1992), appears to be impaired in cirrhotic livers. Furthermore, n-demethylation of aminopyrine was reduced in cirrhotic rat livers compared to control livers (Reichen *et al.* 1987). Systematic review (Morgan & McLean 1991; Morgan & McLean 1995) showed that drugs subject to CYP metabolism have reduced clearance, whilst drugs subject to conjugation reactions prior to clearance are unaffected. These studies suggest that if people with cirrhosis are exposed to PD-producing neurotoxins, high systemic availability of the toxins may be expected due to the dysfunction of the detoxification system in the liver, followed by damage to the dopaminergic neurons in the substantia nigra.

In this chapter, the hepatic disposition of a parkinsonism-inducing neurotoxin MPTP and pesticide malathion was investigated in cirrhotic rat livers. A multiple indicator dilution technique was used to determine the effect of chronic liver disease on neurotoxin disposition.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Malathion-2,3-^{14}C was purchased from Sigma-Aldrich Co. (MO, USA) and methyl-4-phenyl-1,2,3,6-tetrahydropyridine,1-[methyl ^{3}H] from American Radiolabeled Chemicals, Inc. (MO, USA). [6,6'(n)-^{3}H]-Sucrose was obtained from Amersham Pharmacia Biotech Ltd. (Buckinghamshire, UK) and [U-^{14}C]-sucrose from ICN Pharmaceuticals, Inc. (Ca, USA).

6.2.2. Induction of cirrhosis

Male Wistar rats (1 month old, 80–100 g) were obtained from John Curtin School of Medical Research (Canberra, Australia). The induction of cirrhosis has been described in detail previously (Proctor & Chatamra 1982; Le Couteur *et al.* 1999d). The study protocol was approved by the Australian National University Animal Experimentation Ethics Committee. Rats were administered carbon tetrachloride (CCl_4, Sigma, USA) in 1ml corn oil (5-80% v/v) by oral gavage for 8 to 10 weeks, and pentobarbitone sodium (Sigma, USA) was added to their drinking water (440 mg/l); commencing 2 weeks prior to CCl_4 treatment. The initial dose of CCl_4 was 5% v/v and the next dose was determined by the weight loss of the rats at 48 h. If the rats lost less than 5% of their weight, the concentration of CCl_4 was increased by 10% v/v for the next dose. If the weight loss was 5-10%, the CCl_4 concentration was increased by 5% v/v, and if the weight loss was 10-15%, the same concentration of CCl_4 was repeated for the next dose. Control rats were treated in parallel with corn oil and pentobarbitone sodium. When the treatment protocol was completed (or as a result of disease markers such as lethargy and enlarged abdomen due to ascites), rats were allowed at least 2 weeks on standard rat food pellets and water *ad libitum* without CCl_4 or pentobarbitone sodium.

6.2.3. Liver perfusion

Liver perfusions were performed as described in Chapter 2.2.3. Briefly, rats were anaesthetised and a laparotomy incision was made. The livers were perfused *in situ* via the portal vein with a non-recirculatory system. The perfusate was KH bicarbonate

buffer saturated with 95% O_2 and 5% CO_2, with addition of 2% w/v BSA. The flow rate of the perfusate was maintained at 19-23 ml/min using a cartridge pump. The outflow cannula was inserted into the thoracic inferior vena cava to collect samples. After 10 min of preperfusion, MID experiments were performed.

6.2.4. Liver viability

Liver viability of control and cirrhotic rats was assessed by macroscopic appearance, oxygen consumption before and after experiments, portal venous resistance and assay of liver enzymes in the outflow samples as described in Chapter 2.2.4.

6.2.5. Light microscopy of liver structure

After each experiment was completed, liver tissue was sampled and fixed in 4% paraformaldehyde. Tissue was stained with H&E and Masson trichrome stains for light microscopic examination of liver structures. Cirrhotic livers were identified by the presence of bridging fibrosis and nodular regeneration (Abou-Shady *et al.* 2000; Simile *et al.* 2001).

6.2.6. Multiple indicator dilution experiments

Bolus injections (50 µl) contained ^{3}H-sucrose with ^{14}C-malathion or ^{14}C-sucrose with ^{3}H-MPTP. Sucrose was used as an extracellular marker. After injection of the bolus into the portal vein cannula, outflow samples were collected every 1.4 s for 40 s and at 60 s. The samples were analysed with a liquid scintillation counter.

6.2.7. Data analysis

The data analysis for hepatic extraction (E), recovery (F), mean transit time (MTT) and volume of distribution (V) of sucrose, malathion and MPTP was determined as described in Chapter 2.2.6. The hepatic outflow concentrations were expressed as the fraction of the injected dose per ml.

The rate constants for cellular influx, efflux and sequestration (k_1, k_2 and k_3, respectively) and the permeability-surface area products for the hepatocellular influx and

efflux (PS_{influx} and PS_{efflux}, respectively) were determined as described previously in Chapter 2.2.6.

Data were expressed as mean ± SD. Results from control and cirrhotic rat livers were compared using the Student t-test and considered significant when $p \leq 0.05$.

6.3. RESULTS

6.3.1. Induction of cirrhosis

Macroscopically, most of the CCl_4-treated rats used for experiments had ascites and their livers were a little larger than those of the controls. Livers showed a firm texture and an orange-yellow color with gross nodulation. Splenomegaly was also observed (see Table 6.1). Portal veins in cirrhotic rats were large and fragile compared to controls. Microscopically, bridging fibrosis and nodulations were well developed in CCl_4-treated rat livers, as detected with both H&E and Masson trichrome stains (Figures 6.1a and 6.1b, respectively). However, these features were clearer with Masson trichrome stain than with H&E stain.

6.3.2. Liver viability

The parameters of control and cirrhotic rat livers is shown in Table 6.1. The rats were 4 to 6 months old before cirrhosis developed. The body weight and liver sizes of cirrhotic rats were slightly smaller and larger, respectively, compared to control rats but not significantly different. However, spleens in cirrhotic rats were more than double the size of controls.

The oxygen consumption of control rat livers was lower than that of previous experiments (refer to Chapters 2 and 3). This reduction is due to lower flow rates as compared to previous experiments, as a consequence of larger liver size. The flow rate in this experiment was 19 to 23 ml/min regardless of the liver size. However, the partial pressure of oxygen was 470 mmHg to 500 mmHg in inflow, and less than 100 mmHg in outflow of the perfusate. Furthermore, oxygen consumption in the liver was constant throughout the experiment, indicating good liver viability. The oxygen consumption of cirrhotic livers was slightly lower than that of control livers, but it was not significantly different and was also constant throughout the experiment. The portal venous resistance in cirrhotic rats was much higher than that of controls. Among liver enzymes tested,

Table 6.1. Liver viability of control and cirrhotic rats.

	control (n = 6)	cirrhosis (n = 5)	*p* value
Body weight (g)	459 ± 37	446 ± 65	
Liver weight (g)	15.20 ± 1.04	17.59 ± 3.25	
Spleen weight (g)	1.35 ± 0.17	3.20 ± 0.59*	<0.001
Flow rate ($ml \cdot min^{-1} \cdot g^{-1}$)	1.35 ± 0.09	1.23 ± 0.24	
O_2 uptake before experiment ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	0.75 ± 0.06	0.68 ± 0.14	
O_2 uptake after experiment ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	0.75 ± 0.05	0.68 ± 0.15	
Portal venous resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	1.36 ± 0.40	2.62 ± 0.62*	0.003
ALT (U/L)	20 ± 0	21 ± 1	0.09
AST (U/L)	1 ± 0	3 ± 2	0.06
ALKP (U/L)	14 ± 0	13 ± 2	

Results were expressed as means ± SD.

ALT, AST and ALKP represent alanine transaminase, aspartate transaminase and alkaline phosphatase, respectively.

*Statistically different from controls $p \leq 0.05$.

ALT and AST were slightly higher in cirrhotic rats than in controls, but these differences were not significantly different.

6.3.3. Recovery, mean transit time and volume of distribution in cirrhotic rat livers

Fractional recoveries, MTT and volume of distribution of malathion and MPTP in the perfused liver of control and cirrhotic rats are shown in Table 6.2, and their outflow profiles in Figure 6.2. The recovery of the extracellular marker sucrose was almost 100% in the effluent of control rat livers, which is consistent with results in previous experiments (Chapters 2,3,4 and 5), and it was not changed by cirrhosis. The MTT of sucrose was increased, but its volume of distribution unchanged, by cirrhosis.

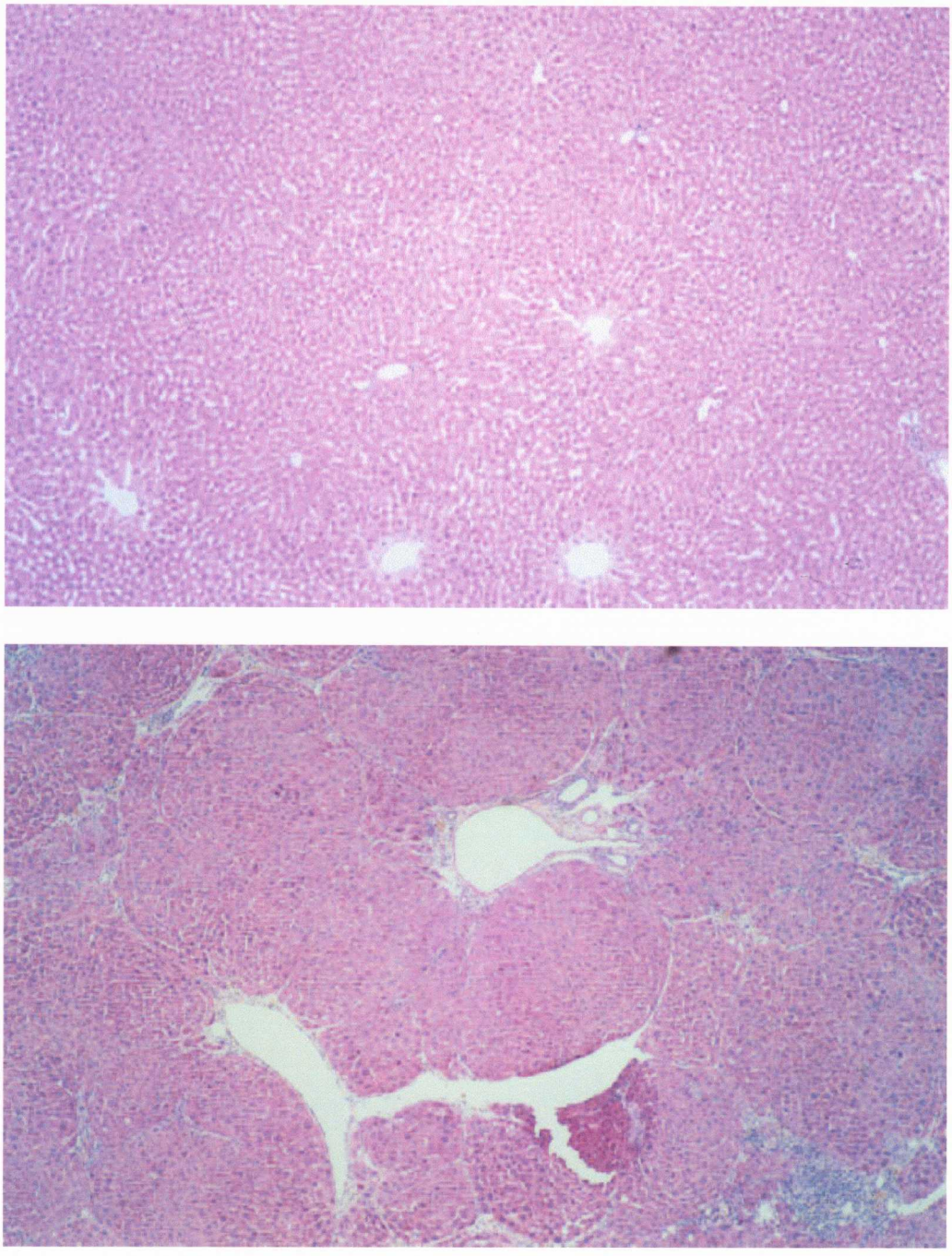

Figure 6.1a. Immunohistochemistry of healthy (top) and cirrhotic (bottom) rat livers. In the CCl_4-induced cirrhotic liver, normal hepatic architecture was disrupted. Magnification ×50 (H&E stain).

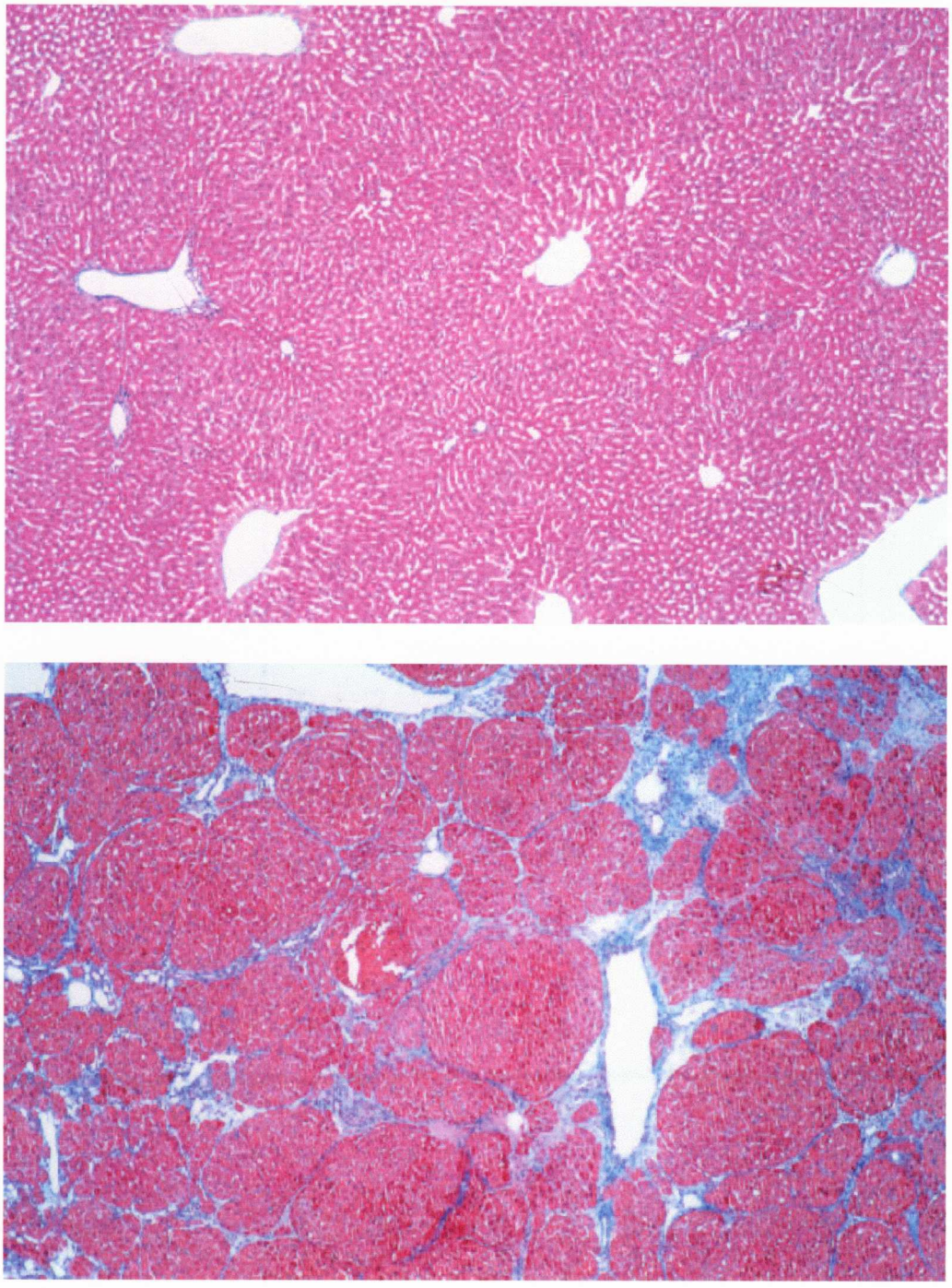

Figure 6.1b. Masson trichrome stain of healthy (top) and cirrhotic (bottom) rat livers. The micronodules and fibrosis are characteristics CCl_4-induced cirrhotic liver. Magnification ×50.

Table 6.2. Fractional recoveries, mean transit time and volume of distribution of sucrose, malathion and MPTP in the perfused liver of control and cirrhotic rats.

Indicators		n	Recovery	Mean transit time (s)	Volume of distribution ($ml \cdot g^{-1}$ liver)
Sucrose	Control	12	0.989 ± 0.025	8.783 ± 1.723	0.179 ± 0.041
	Cirrhosis	10	0.982 ± 0.023	10.578 ± 1.609* ($p = 0.02$)	0.217 ± 0.068
Malathion	Control	6	0.175 ± 0.015	18.290 ± 1.922	1.683 ± 0.435
	Cirrhosis	5	0.216 ± 0.032* ($p = 0.02$)	21.790 ± 0.737* ($p = 0.004$)	2.703 ± 0.538* ($p = 0.007$)
MPTP	Control	6	0.066 ± 0.022	24.851 ± 6.133	1.874 ± 0.625
	Cirrhosis	5	0.098 ± 0.015* ($p = 0.02$)	23.450 ± 7.532	2.894 ± 0.740* ($p = 0.04$)

Results were obtained from the number of rats indicated in each group (n) and were expressed as means ± SD.

* Statistically different from controls $p \leq 0.05$.

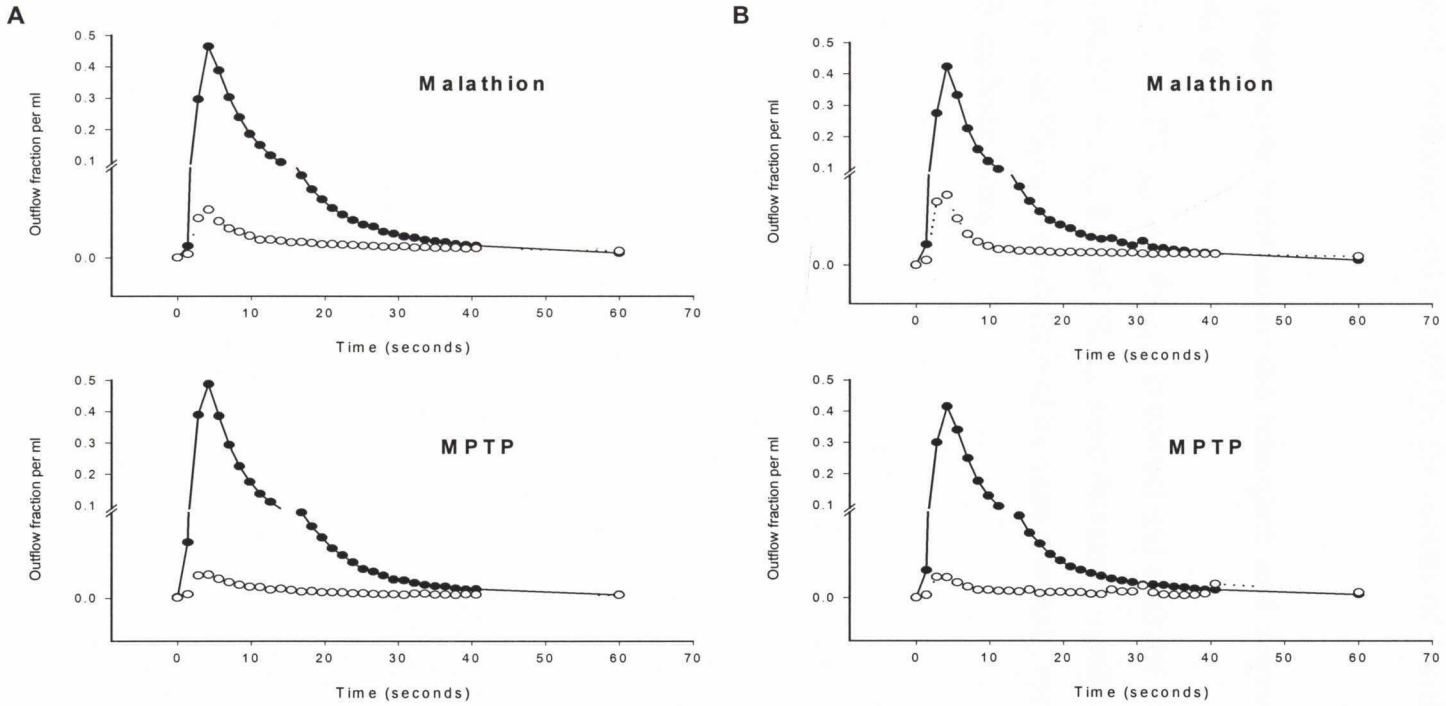

Figure 6.2. Outflow profiles of malathion and MPTP in control (A) and cirrhotic (B) rat livers by multiple indicator dilution techniques. Sucrose (closed circles) is an extracellular marker and its recovery was almost 100% in both control and cirrhotic livers. Test indicators were expressed as open circles. There were visible differences in the area under the curve of malathion in control versus cirrhotic livers.

Recoveries of malathion and MPTP were increased to 123% and 149% of control values, respectively, in cirrhotic livers. For malathion, there were increases in MTT and volume of distribution, and for MPTP, the volume of distribution was increased (Table 6.2).

6.3.4. Hepatocyte transmembrane transport and sequestration rate constants of cirrhotic livers

The k_1, k_2, k_3, PS_{influx} and PS_{efflux} in control and cirrhotic rat livers are shown in Table 6.3. In malathion, k_2, k_3 and PS_{efflux} were decreased significantly in cirrhotic livers. For MPTP, k_2 and PS_{efflux} were decreased by more than half, and k_1 and PS_{influx} tended to be lower in cirrhotic livers.

Table 6.3. The effects of cirrhosis on k_1, k_2, k_3, PS_{influx}, and PS_{efflux} in the perfused rat liver.

Indicators		k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	PS_{influx} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)	PS_{efflux} ($ml \cdot s^{-1} \cdot g^{-1}$ liver)
Malathion	Control	0.617 ± 0.115	0.082 ± 0.021	0.072 ± 0.024	0.116 ± 0.018	0.066 ± 0.018
	Cirrhosis	0.536 ± 0.106	0.047 ± 0.010* (p = 008)	0.024 ± 0.008* (p = 0.002)	0.115 ± 0.028	0.036 ± 0.007* (p = 0.007)
MPTP	Control	1.751 ± 1.009	0.159 ± 0.039	0.096 ± 0.049	0.276 ± 0.125	0.133 ± 0.032
	Cirrhosis	0.846 ± 0.259 (P = 0.09)	0.066 ± 0.020* (p < 0.001)	0.062 ± 0.025	0.167 ± 0.022 (P = 0.09)	0.051 ± 0.010* (p < 0.001)

Results were obtained from the number of rats indicated in Table 6.2 and were expressed as means ± SD.

k_1, k_2 and k_3 represent the influx, efflux and sequestration coefficients, respectively.

PS_{influx} and PS_{efflux} represent the influx and efflux permeability cell surface area products, respectively.

* Statistically different from controls $p \leq 0.05$.

6.4. DISCUSSION

Overall, the hepatic clearance of xenobiotics is influenced by many factors, such as liver volume (Reichen *et al.* 1987), hepatocyte mass (Iwamoto *et al.* 1986b), blood flow (Iwamoto *et al.* 1986b), hepatic metabolism (Beckett *et al.* 1987; Renton 2000) and liver disease (Wensing *et al.* 1993). Impaired hepatic clearance of many drugs has been reported in cirrhosis (Reichen *et al.* 1987; Wensing *et al.* 1993; Froomes *et al.* 1999) and, as a result, plasma drug concentrations are increased in cirrhotic patients (Bozkurt *et al.* 1996).

In this experiment, the increased spleen weight and portal resistance of cirrhotic rats were consistent with previous observations (Le Couteur *et al.* 1999d). Oxygen consumption tended to be lower in cirrhotic rats compared to control rats but did not reach significant levels. The trend towards reduced oxygen consumption in the cirrhotic liver might be due to inhibition of oxygen delivery into hepatocytes by structural changes, such as micronodular regeneration and capillarisation of sinusoidal endothelium (Gaudio *et al.* 1997).

In the cirrhotic liver, it is interesting that MTT of the extracellular marker sucrose increased (8.78 s to 10.58 s, Table 6.2), although the recovery was unchanged. These increased MTTs for sucrose suggest a trend towards larger extracellular volumes in cirrhotic rats. This may reflect the effects of portal hypertension.

There were increased recoveries of malathion and MPTP in the cirrhotic livers. This finding indicates a higher bioavailability of these xenobiotics systemically in cirrhotic patients and, hence, a greater risk of toxicity to target organs. However, the magnitude of the increase in the recovery of MPTP in cirrhosis is less than in the aged rat liver (149% and 258% of control values, respectively). This difference may explain why ageing is a greater risk factor for PD than cirrhosis.

In the cirrhotic liver, the increase in the recovery of MPTP is associated with reduced

hepatocellular influx, although PS_{influx} of control versus cirrhotic rats was not significantly different. The reduced extraction of malathion is associated with decreased cellular sequestration. Mechanisms for the increased recovery of both indicators, with reduced hepatocellular efflux, may be secondary to reduced sequestration or efflux of their metabolites.

The cellular influx of both indicators showed a trend towards reduction in cirrhosis but they were not significantly different compared to controls. It is difficult to explain the mechanisms for cellular transport of these chemicals in cirrhosis because structural changes, such as capillarisation of sinusoidal endothelium, results in a decrease in the uptake of substances into hepatocytes. For example, both aged and cirrhotic livers undergo capillarisation of sinusoids (Popper 1986; Le Couteur *et al.* 2001). In old rat livers (Chapter 4), hepatic extraction of malathion was reduced compared to young rat livers, which is a similar observation to that in cirrhosis. However, mechanisms for the reduced extraction of this pesticide in the livers of old rats were related to decreased cellular influx. This decrease was not, however, observed in the cirrhotic liver.

Another notable difference between cirrhotic and aged livers is that the sequestration rate constant of malathion was increased by ageing, whereas it was reduced by cirrhosis.

Cirrhotic livers undergo a range of pathophysiological changes that may alter drug and xenobiotic disposition (Hall *et al.* 1991; Froomes *et al.* 1999). Intrahepatic shunts (Mori *et al.* 1987) and capillarisation of sinusoids (Huet *et al.* 1985), secondary to cell necrosis and deposition of collagen (Vollmar *et al.* 1998) may result in decreased hepatic elimination of xenobiotics, including drugs. This finding is supported by much evidence that hepatic clearance of drugs is impaired in cirrhosis (Cardoso *et al.* 1994; Froomes *et al.* 1999).

Richter *et al.* (2000) have reported that total hepatic blood flow was significantly reduced due to diminished portal venous blood flow in CCl_4-induced cirrhotic rats. Changes in the microcirculation due to collagen deposits, are directly associated with an

increase in portal venous resistance (Blendis & Wong 2001). As a result, a decrease in the portal venous blood flow (Tochio *et al.* 2001) suggests impaired hepatic elimination of flow-limited xenobiotics. In fact, increasing portal blood flow in cirrhotic rats has improved hepatic elimination of taurocholate and propranolol without deleterious effects on liver viability (Cardoso *et al.* 1994). MPTP is also a xenobiotic whose elimination is dependent on hepatic blood flow (refer to Chapter 5). However, the impaired clearance of this neurotoxin in the cirrhotic liver in these experiments may be caused by other factors, such as reduced hepatic influx, rather than blood flow because the flow rate of the perfusate was maintained at 19-23 ml/min in both control and cirrhotic livers.

A reduced number of hepatocytes due to advanced fibrosis, which is also a feature of the cirrhotic liver (Wood *et al.* 1979; Imamura *et al.* 1991), results in a reduction of hepatic clearance of capacity-limited xenobiotics. Reichen *et al.* (1987) have observed that N-demethylation of aminopyrin was reduced in rats with liver cirrhosis (2.08 ± 0.77 μmol/min and 1.00 ± 0.81 μmol/min in control and cirrhotic rats, respectively). They concluded that this reduction was due to a loss of liver cell volume resulting from a reduction in hepatocellular volume with no change in N-methylase activity per ml. On the other hand, significantly decreased ratios of adenine nucleotide content, protein content and ornithine carbamoyltransferase activity per fractional hepatic area have also been observed in cirrhotic livers, which indicates impaired hepatocyte metabolism (Matsui *et al.* 1994). The decrease in the biochemical parameters may result not only from the loss of hepatocyte volume but also from impairment of hepatocyte metabolism.

It has been suggested that sinusoidal capillarisation may contribute to impaired drug elimination in cirrhosis by affecting the uptake of drugs and cofactors, including oxygen required for drug metabolism (Reichen *et al.* 1988; Morgan & McLean 1991). Activities of some CYP enzymes (i.e. oxygen dependent phase I enzymes) are known to be reduced in cirrhosis (Bastien *et al.* 2000). For example, the hepatic clearance of theophylline was significantly reduced by 54% in cirrhotic patients compared with controls, and oxygen supplementation improved plasma clearance of this drug by 34% (Froomes *et al.* 1999). This result indicates that impaired hepatic oxygenation in

cirrhosis may contribute to a reduction of oxidative drug metabolism, and that this drug metabolism can be improved by oxygen supplementation. This is because the hepatic metabolism of theophylline is catalysed by CYP1A1, CYP1A2 and CYP2E1 (Sarkar & Jackson 1994; Zhang & Kaminsky 1995). Furthermore, MPTP is detoxified by hepatic CYP2D6 and FMO, and malathion by CYP (i.e. enzymes which require oxygen as a substrate). Hence, the reduction of hepatic extraction of these chemicals may be also, in part, due to the impaired oxygen delivery into hepatocytes by sinusoidal capillarisation.

In conclusion, hepatic extraction of malathion and MPTP was reduced in cirrhosis, indicating an increase in the systemic bioavailability of these toxins. This reduction of hepatic extraction is associated with reduced hepatocellular sequestration for malathion and hepatocellular influx for MPTP. The changes seen in the disposition of malathion and MPTP were different quantitatively, and qualitatively, to those seen with ageing.

FINAL COMMENTS

Many studies investigating the etiology of PD have focused on neurological observations because of the pathological site of the disease. However, the disease may depend on the availability of neurotoxins for dopaminergic neurons in the substantia nigra. As explained in previous chapters (Chapter 2 to 6), hepatic detoxification of PD-associated neurotoxins should be studied because of the effects on availability in the brain.

In these studies, it has been found that the hepatic disposition of neurotoxins (pesticides and MPTP) varies considerably and can be influenced greatly by old age, exposure to pesticides, liver disease and inhibitors of hepatic transporters.

Therefore, the liver and xenobiotic metabolism in general may have a pivotal role in susceptibility to PD and other diseases with toxic pathogenesis.

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APPENDIX

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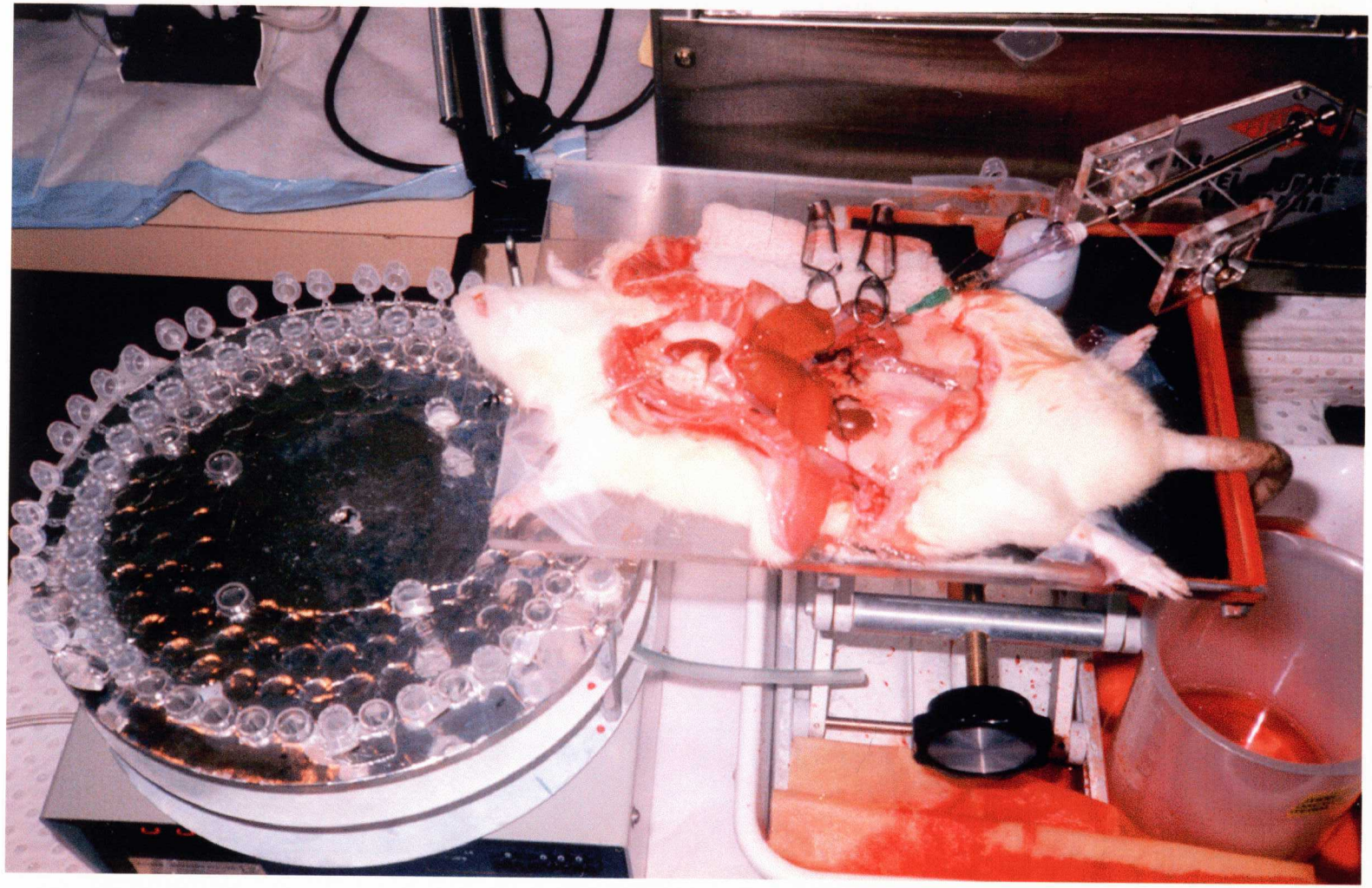
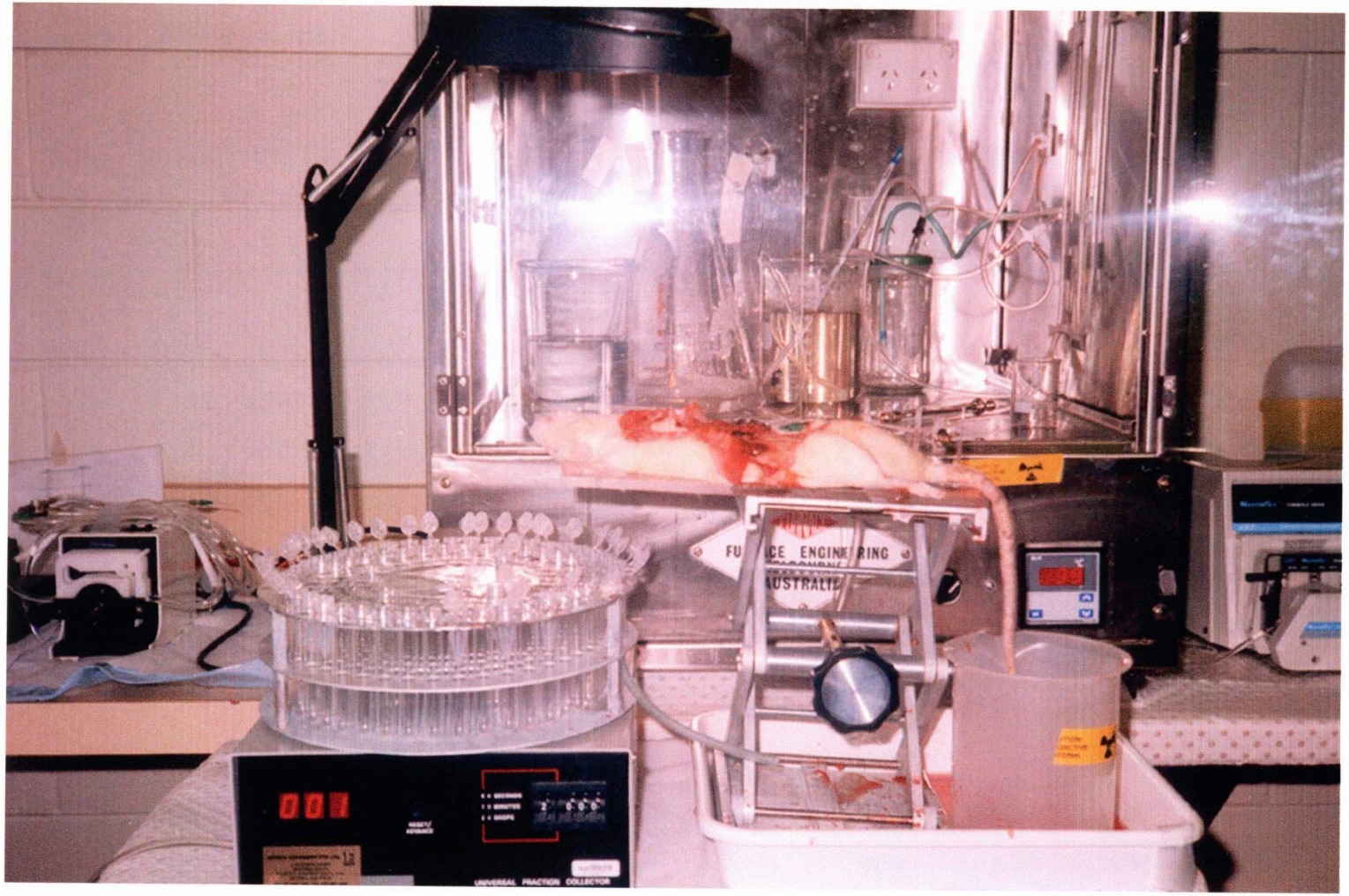
APPENDIX 1. THE ISOLATED RAT LIVER PERFUSION PREPARATION.



Appendix 1. Contd.



APPENDIX 2. EXPERIMENTAL DATA

Chapter 2.1. Liver viability of rats

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1}$)	F.R/liver ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat													
1	397.20	14.24	26.00	1.83	434.40	71.10	0.88	80.50	0.86	1.10	22.00	5.00	10.00
2	341.20	13.74	25.50	1.86	472.30	138.20	0.83	125.80	0.86	2.16	19.00	2.00	10.00
3	240.00	7.62	26.00	3.41	512.50	110.10	1.83	114.20	1.81	0.59	16.00	1.00	10.00
4	276.30	9.12	25.50	2.80	505.10	150.20	1.32	194.40	1.16	1.43	21.00	6.00	10.00
5	365.00	14.03	26.00	1.85	416.50	94.20	0.80	62.20	0.88	2.16	20.00	1.00	10.00
6	315.00	12.06	25.00	2.07	458.40	121.60	0.93	92.80	1.01	0.96	21.00	2.00	10.00
DDT													
1	312.90	11.34	25.50	2.25	526.00	123.70	1.21	130.20	1.19	0.89	14.00	1.00	12.00
2	330.80	11.61	24.50	2.11	433.30	106.60	0.92	92.40	0.96	1.42	14.00	1.00	12.00
3	315.00	12.06	25.00	2.07	458.40	121.60	0.93	92.80	1.01	0.96	21.00	2.00	10.00
4	270.20	12.67	25.50	2.01	513.00	61.90	1.21	68.10	1.19	0.99	13.00	1.00	10.00

Chapter 2.1. Contd.

Malathion

1	265.00	11.88	24.50	2.06	440.90	72.30	1.01	80.10	0.99	1.45	14.00	2.00	10.00
2	259.30	11.63	24.50	2.11	490.70	79.90	1.15	85.40	1.14	1.42	16.00	2.00	10.00
3	253.50	11.47	24.50	2.14	439.10	104.60	0.95	100.80	0.96	0.94	16.00	1.00	10.00
4	270.20	12.67	25.50	2.01	513.00	61.90	1.21	68.10	1.19	0.99	13.00	1.00	10.00
5	238.30	10.13	26.00	2.57	486.00	79.90	1.39	80.90	1.39	0.78	14.00	1.00	11.00
6	235.10	10.22	26.00	2.55	508.00	84.60	1.44	87.30	1.43	1.18	15.00	1.00	11.00

MPTP

1	315.00	12.06	25.00	2.07	458.40	121.60	0.93	92.80	1.01	0.96	21.00	2.00	10.00
2	397.20	14.24	26.00	1.83	434.40	71.10	0.88	80.50	0.86	1.10	22.00	5.00	10.00
3	341.20	13.74	25.50	1.86	472.30	125.80	0.86	138.20	0.83	2.16	19.00	2.00	10.00
4	240.00	7.62	26.00	3.41	512.50	110.10	1.83	114.20	1.81	0.59	16.00	1.00	10.00
5	276.30	9.12	25.50	2.80	505.10	150.20	1.32	194.40	1.16	1.43	21.00	6.00	10.00

Chapter 2.2. Recovery, hepatocellular transport and sequestration of paraquat, DDT, malathion and MPTP

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat											
1	1.120	1.033	0.039	0.710		4.894	5.113	0.147	0.153	0.006	0.606
2	1.278	1.183	0.033	0.666		5.764	5.823	0.173	0.175	0.006	0.551
3	1.097	1.011	0.032	0.581		7.373	7.045	0.413	0.395	0.013	0.341
4	1.042	0.955	0.028	0.709		8.214	8.34	0.378	0.384	0.011	0.441
5	0.980	0.928	0.037	0.605		7.315	5.68	0.255	0.163	0.009	0.451
6	1.142	1.216	0.029	0.723		7.214	8.024	0.202	0.408	0.006	0.577
DDT											
1	1.017	0.329	0.102	0.099	0.100	8.206	8.92	0.383	0.200	0.039	0.061
2	1.031	0.306	0.102	0.099	0.100	9.442	8.817	0.390	0.204	0.040	0.060
3	1.041	0.326	0.105	0.098	0.100	8.816	5.763	0.256	0.135	0.027	0.073
4	1.200	0.335	0.105	0.098	0.100	10.97	7.766	0.318	0.168	0.033	0.067
Malathion											
1	0.993	0.091	1.165	0.095	0.052	9.206	24.434	0.313	1.025	0.365	0.065

Chapter 2.2. Contd.

2	1.038	0.093	1.278	0.138	0.058	10.527	24.193	0.358	1.318	0.457	0.089
3	1.041	0.128	1.325	0.110	0.071	7.711	20.324	0.270	1.007	0.358	0.080
4	0.975	0.099	1.216	0.121	0.072	8.174	21.752	0.270	0.938	0.328	0.088
5	1.047	0.106	0.814	0.156	0.147	9.879	17.813	0.415	1.046	0.338	0.091
6	1.015	0.125	1.046	0.164	0.106	9.401	16.781	0.395	1.242	0.413	0.099
MPTP											
1	0.996	0.001	not fit			8.507	12.658	0.357			
2	1.202	0.018	19.218	1.279	0.408	5.254	29.455	0.158	8.400	3.029	1.077
3	0.990	0.020	6.937	3.509	0.891	9.218	25.537	0.277	7.511	1.918	2.539
4	1.008	0.031	2.616	0.116	0.030	9.07	30.385	0.508	3.608	1.329	0.057
5	0.992	0.008	not fit			8.17	41.394	0.464			

Chapter 3.1. Liver viability of control rats

Exp. No	Treatment conc	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat injection													
1	C. oil 2ml/kg	366.10	14.41	1.42	434.40	72.70	0.69	73.20	0.69	0.35	19.00	3.00	11.00
2	C. oil 2ml/kg	356.60	14.46	1.35	455.90	121.80	0.60	130.30	0.59	0.74	14.00	2.00	10.00
3	C. oil 2ml/kg	302.50	10.62	1.93	482.10	86.30	1.02	86.90	1.02	0.26	16.00	1.00	12.00
4	C. oil 2ml/kg	302.00	11.62	1.81	529.00	84.50	1.07	66.40	1.12	1.66	16.00	1.00	11.00
5	C. oil 2ml/kg	392.00	14.81	1.35	452.00	71.60	0.69	74.30	0.68	1.48	15.00	2.00	12.00
6	C. oil 2ml/kg	369.20	11.99	1.71	578.00	92.10	1.11	93.50	1.10	1.76	18.00	3.00	12.00
7	C. oil 2ml/kg	345.50	14.86	1.35	498.60	107.70	0.70	113.40	0.69	2.60	19.00	1.00	11.00
8	Saline 2ml/kg	249.80	12.08	1.74	473.80	70.80	0.93	80.10	0.91	1.15	16.00	1.00	12.00
9	Saline 2ml/kg	333.40	18.24	1.07	479.60	96.30	0.55	102.50	0.54	2.81	20.00	3.00	12.00
10	No treat	295.00	12.07	1.70	510.60	119.30	0.89	124.70	0.87	1.18	19.00	1.00	11.00
11	No treat	397.20	14.24	1.83	434.40	71.10	0.88	80.50	0.86	1.10	22.00	5.00	10.00
12	No treat	341.20	13.74	1.86	472.30	138.20	0.83	125.80	0.86	2.16	19.00	2.00	10.00
13	No treat	240.00	7.62	3.41	512.50	110.10	1.83	114.20	1.81	0.59	16.00	1.00	10.00

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14	No treat	276.30	9.12	2.80	505.10	150.20	1.32	194.40	1.16	1.43	21.00	6.00	10.00
DDT injection													
1	C. oil 2ml/kg	302.50	10.62	1.93	482.10	86.30	1.02	86.90	1.02	0.26	16.00	1.00	12.00
2	C. oil 2ml/kg	302.00	11.62	1.76	529.00	84.50	1.05	66.40	1.09	1.70	16.00	1.00	11.00
3	C. oil 2ml/kg	392.00	14.81	1.28	452.00	71.60	0.65	74.30	0.65	1.56	15.00	2.00	12.00
4	C. oil 2ml/kg	369.20	11.99	1.71	578.00	92.10	1.11	93.50	1.10	1.76	18.00	3.00	12.00
5	C. oil 2ml/kg	345.50	14.86	1.35	498.60	107.70	0.70	113.40	0.69	2.60	19.00	1.00	11.00
6	Saline 2ml/kg	249.80	12.08	1.74	473.80	70.80	0.93	80.10	0.91	1.15	16.00	1.00	12.00
7	Saline 2ml/kg	333.40	18.24	1.07	479.60	96.30	0.55	102.50	0.54	2.81	20.00	3.00	12.00
8	No treat	295.00	12.07	1.70	510.60	119.30	0.89	124.70	0.87	1.18	19.00	1.00	11.00
9	No treat	270.20	12.67	2.01	513.00	61.90	1.21	68.10	1.19	0.99	13.00	1.00	10.00
10	No treat	312.90	11.34	2.25	526.00	123.70	1.21	130.20	1.19	0.89	14.00	1.00	12.00
11	No treat	330.80	11.61	2.11	433.30	106.60	0.92	92.40	0.96	1.42	14.00	1.00	12.00
12	No treat	315.00	12.06	2.07	458.40	121.60	0.93	92.80	1.01	0.96	21.00	2.00	10.00

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Malathion injection

1	C. oil 2ml/kg	366.10	14.41	1.42	434.40	72.70	0.69	73.20	0.69	0.35	19.00	3.00	11.00
2	C. oil 2ml/kg	356.60	14.46	1.35	455.90	121.80	0.60	130.30	0.59	0.74	14.00	2.00	10.00
3	C. oil 2ml/kg	302.50	10.62	1.93	482.10	86.30	1.02	86.90	1.02	0.26	16.00	1.00	12.00
4	C. oil 2ml/kg	302.00	11.62	1.81	529.00	84.50	1.07	66.40	1.12	1.66	16.00	1.00	11.00
5	C. oil 2ml/kg	392.00	14.81	1.28	452.00	71.60	0.65	74.30	0.65	1.56	15.00	2.00	12.00
6	C. oil 2ml/kg	369.20	11.99	1.71	578.00	92.10	1.11	93.50	1.10	1.76	18.00	3.00	12.00
7	C. oil 2ml/kg	345.50	14.86	1.35	498.60	107.70	0.70	113.40	0.69	2.60	19.00	1.00	11.00
8	Saline 2ml/kg	249.80	12.08	1.74	473.80	70.80	0.93	80.10	0.91	1.15	16.00	1.00	12.00
9	Saline 2ml/kg	295.00	12.07	1.70	510.60	119.30	0.89	124.70	0.87	1.18	19.00	1.00	11.00
10	No treat	265.00	11.88	2.06	440.90	72.30	1.01	80.10	0.99	1.45	14.00	2.00	10.00
11	No treat	259.30	11.63	2.11	490.70	79.90	1.15	85.40	1.14	1.42	16.00	2.00	10.00
12	No treat	253.50	11.47	2.14	439.10	104.60	0.95	100.80	0.96	0.94	16.00	1.00	10.00
13	No treat	270.20	12.67	2.01	513.00	61.90	1.21	68.10	1.19	0.99	13.00	1.00	10.00
14	No treat	238.30	10.13	2.57	486.00	79.90	1.39	80.90	1.39	0.78	14.00	1.00	11.00
15	No treat	235.10	10.22	2.55	508.00	84.60	1.44	87.30	1.43	1.18	15.00	1.00	11.00

Chapter 3.1. Contd.

MPTP injection

1	C. oil 2ml/kg	302.50	10.62	1.93	482.10	86.30	1.02	86.90	1.02	0.26	16.00	1.00	12.00
2	C. oil 2ml/kg	302.00	11.62	1.76	529.00	84.50	1.05	66.40	1.09	1.70	16.00	1.00	11.00
3	C. oil 2ml/kg	392.00	14.81	1.28	452.00	71.60	0.65	74.30	0.65	1.56	15.00	2.00	12.00
4	C. oil 2ml/kg	369.20	11.99	1.71	578.00	92.10	1.11	93.50	1.10	1.76	18.00	3.00	12.00
5	Saline 2ml/kg	249.80	12.08	1.74	473.80	70.80	0.93	80.10	0.91	1.15	16.00	1.00	12.00
6	Saline 2ml/kg	333.40	18.24	1.10	479.60	96.30	0.56	102.50	0.55	2.74	20.00	3.00	12.00
7	No treat	295.00	12.07	1.70	510.60	119.30	0.89	124.70	0.87	1.18	19.00	1.00	11.00
8	No treat	315.00	12.06	2.07	458.40	121.60	0.93	92.80	1.01	0.96	21.00	2.00	10.00
9	No treat	397.20	14.24	1.83	434.40	71.10	0.88	80.50	0.86	1.10	22.00	5.00	10.00
10	No treat	341.20	13.74	1.86	472.30	125.80	0.86	138.20	0.83	2.16	19.00	2.00	10.00
11	No treat	240.00	7.62	3.41	512.50	110.10	1.83	114.20	1.81	0.59	16.00	1.00	10.00
12	No treat	276.30	9.12	2.80	505.10	150.20	1.32	194.40	1.16	1.43	21.00	6.00	10.00

Chapter 3.2. Liver viability of paraquat pretreated rats

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat injection												
1	276.10	11.20	1.83	499.60	96.30	0.98	95.30	0.99	2.19	19.00	2.00	12.00
2	284.00	11.48	1.74	480.50	104.70	0.87	116.20	0.85	1.44	15.00	1.00	11.00
3	287.30	11.87	1.73	480.00	99.30	0.88	103.80	0.87	0.87	17.00	1.00	11.00
4	218.40	9.01	2.22	476.70	125.60	1.04	129.80	1.03	0.90	17.00	1.00	12.00
5	214.00	9.98	1.90	484.80	76.30	1.04	80.20	1.03	1.05	19.00	1.00	12.00
6	245.40	11.48	1.74	497.00	73.00	0.99	79.50	0.97	1.15	21.00	1.00	12.00
7	225.00	9.32	2.25	515.50	91.50	1.27	102.90	1.24	1.11	17.00	1.00	11.00
8	250.90	9.50	2.21	497.20	105.01	1.16	103.70	1.16	1.13	18.00	1.00	12.00
9	254.60	9.98	2.21	489.70	97.40	1.15	100.50	1.15	1.13	19.00	1.00	12.00
DDT injection												
1	276.10	11.20	1.83	499.60	96.30	0.98	95.30	0.99	2.19	19.00	2.00	12.00

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2	284.00	11.48	1.74	480.50	104.70	0.87	116.20	0.85	1.44	15.00	1.00	11.00
3	287.30	11.87	1.73	480.00	99.30	0.88	103.80	0.87	0.87	17.00	1.00	11.00
4	218.40	9.01	2.22	476.70	125.60	1.04	129.80	1.03	0.90	17.00	1.00	12.00
5	245.40	11.48	1.74	497.00	73.00	0.99	79.50	0.97	1.15	21.00	1.00	12.00
6	225.00	9.32	2.25	515.50	91.50	1.27	102.90	1.24	1.11	17.00	1.00	11.00
7	250.90	9.50	2.21	497.20	105.01	1.16	103.70	1.16	1.13	18.00	1.00	12.00
8	254.60	9.98	2.21	489.70	97.40	1.15	100.50	1.15	1.13	19.00	1.00	12.00
Malathion injection												
1	276.10	11.20	1.83	499.60	96.30	0.98	95.30	0.99	2.19	19.00	2.00	12.00
2	284.00	11.48	1.74	480.50	104.70	0.87	116.20	0.85	1.44	15.00	1.00	11.00
3	287.30	11.87	1.73	480.00	99.30	0.88	103.80	0.87	0.87	17.00	1.00	11.00
4	218.40	9.01	2.22	476.70	125.60	1.04	129.80	1.03	0.90	17.00	1.00	12.00
5	214.00	9.98	1.90	484.80	76.30	1.04	80.20	1.03	1.05	19.00	1.00	12.00
6	245.40	11.48	1.74	497.00	73.00	0.99	79.50	0.97	1.15	21.00	1.00	12.00
7	225.00	9.32	2.25	515.50	91.50	1.27	102.90	1.24	1.11	17.00	1.00	11.00
8	250.90	9.50	2.21	497.20	105.01	1.16	103.70	1.16	1.13	18.00	1.00	12.00

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9	254.60	9.98	2.21	489.70	97.40	1.15	100.50	1.15	1.13	19.00	1.00	12.00
MPTP injection												
1	276.10	11.20	1.83	499.60	96.30	0.98	95.30	0.99	2.19	19.00	2.00	12.00
2	284.00	11.48	1.74	480.50	104.70	0.87	116.20	0.85	1.44	15.00	1.00	11.00
3	287.30	11.87	1.73	480.00	99.30	0.88	103.80	0.87	0.87	17.00	1.00	11.00
4	218.40	9.01	2.22	476.70	125.60	1.04	129.80	1.03	0.90	17.00	1.00	12.00
5	214.00	9.98	1.90	484.80	76.30	1.04	80.20	1.03	1.05	19.00	1.00	12.00
6	245.40	11.48	1.74	497.00	73.00	0.99	79.50	0.97	1.15	21.00	1.00	12.00
7	225.00	9.32	2.25	515.50	91.50	1.27	102.90	1.24	1.11	17.00	1.00	11.00
8	250.90	9.50	2.21	497.20	105.01	1.16	103.70	1.16	1.13	18.00	1.00	12.00
9	254.60	9.98	2.21	489.70	97.40	1.15	100.50	1.15	1.13	19.00	1.00	12.00

Chapter 3.3. Liver viability of DDT pretreated rats

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat injection												
1	345.20	13.18	1.59	489.70	82.60	0.87	90.30	0.85	1.57	22.00	2.00	12.00
2	313.00	13.84	1.52	495.10	107.70	0.78	115.90	0.77	2.64	17.00	1.00	11.00
3	292.50	11.80	1.78	451.30	73.90	0.90	86.30	0.87	1.40	17.00	1.00	11.00
4	296.10	11.52	1.74	488.60	82.90	0.94	90.70	0.92	1.15	17.00	2.00	11.00
5	338.20	12.63	1.58	493.90	111.20	0.81	105.80	0.82	0.63	18.00	1.00	11.00
6	356.00	15.23	1.35	493.20	102.10	0.70	112.40	0.68	2.23	18.00	1.00	11.00
7	360.20	14.64	1.43	486.30	76.90	0.78	82.60	0.77	1.05	19.00	1.00	11.00
8	330.00	13.44	1.49	481.20	97.30	0.76	93.80	0.77	0.67	19.00	1.00	11.00
DDT injection												
1	345.20	13.18	1.59	489.70	82.60	0.87	90.30	0.85	1.57	22.00	2.00	12.00
2	313.00	13.84	1.52	495.10	107.70	0.78	115.90	0.77	2.64	17.00	1.00	11.00
3	292.50	11.80	1.78	451.30	73.90	0.90	86.30	0.87	1.40	17.00	1.00	11.00

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4	296.10	11.52	1.74	488.60	82.90	0.94	90.70	0.92	1.15	17.00	2.00	11.00
5	338.20	12.63	1.58	493.90	111.20	0.81	105.80	0.82	0.63	18.00	1.00	11.00
6	356.00	15.23	1.35	493.20	102.10	0.70	112.40	0.68	2.23	18.00	1.00	11.00
7	360.20	14.64	1.43	486.30	76.90	0.78	82.60	0.77	1.05	19.00	1.00	11.00
8	330.00	13.44	1.49	481.20	97.30	0.76	93.80	0.77	0.67	19.00	1.00	11.00
Malathion injection												
1	345.20	13.18	1.59	489.70	82.60	0.87	90.30	0.85	1.57	22.00	2.00	12.00
2	313.00	13.84	1.52	495.10	107.70	0.78	115.90	0.77	2.64	17.00	1.00	11.00
3	292.50	11.80	1.78	451.30	73.90	0.90	86.30	0.87	1.40	17.00	1.00	11.00
4	296.10	11.52	1.74	488.60	82.90	0.94	90.70	0.92	1.15	17.00	2.00	11.00
5	338.20	12.63	1.58	493.90	111.20	0.81	105.80	0.82	0.63	18.00	1.00	11.00
6	356.00	15.23	1.35	493.20	102.10	0.70	112.40	0.68	2.23	18.00	1.00	11.00
7	360.20	14.64	1.43	486.30	76.90	0.78	82.60	0.77	1.05	19.00	1.00	11.00
8	330.00	13.44	1.49	481.20	97.30	0.76	93.80	0.77	0.67	19.00	1.00	11.00

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MPTP injection

1	345.20	13.18	1.59	489.70	82.60	0.87	90.30	0.85	1.57	22.00	2.00	12.00
2	313.00	13.84	1.52	495.10	107.70	0.78	115.90	0.77	2.64	17.00	1.00	11.00
3	292.50	11.80	1.78	451.30	73.90	0.90	86.30	0.87	1.40	17.00	1.00	11.00
4	296.10	11.52	1.74	488.60	82.90	0.94	90.70	0.92	1.15	17.00	2.00	11.00
5	338.20	12.63	1.58	493.90	111.20	0.81	105.80	0.82	0.63	18.00	1.00	11.00
6	356.00	15.23	1.35	493.20	102.10	0.70	112.40	0.68	2.23	18.00	1.00	11.00
7	360.20	14.64	1.43	486.30	76.90	0.78	82.60	0.77	1.05	19.00	1.00	11.00
8	330.00	13.44	1.49	481.20	97.30	0.76	93.80	0.77	0.67	19.00	1.00	11.00

Chapter 3.4. Liver viability of malathion pretreated rats

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat injection												
1	300.00	12.09	1.70	486.00	88.70	0.90	87.00	0.90	0.59	16.00	1.00	12.00
2	385.30	14.41	1.42	495.00	75.30	0.80	81.50	0.79	2.11	14.00	2.00	12.00
3	350.30	12.57	1.63	497.00	79.40	0.91	75.20	0.92	0.61	17.00	1.00	12.00
4	387.60	15.08	1.36	505.00	66.90	0.79	68.40	0.79	1.84	16.00	3.00	12.00
5	396.00	12.64	1.54	470.00	60.80	0.84	61.10	0.84	1.62	22.00	1.00	12.00
6	444.00	15.12	1.36	507.00	59.90	0.81	62.60	0.80	2.21	23.00	2.00	11.00
DDT injection												
1	300.00	12.09	1.70	486.00	88.70	0.90	87.00	0.90	0.59	16.00	1.00	12.00
2	385.30	14.41	1.42	495.00	75.30	0.80	81.50	0.79	2.11	14.00	2.00	12.00
3	396.00	12.64	1.54	470.00	60.80	0.84	61.10	0.84	1.62	22.00	1.00	12.00
4	444.00	15.12	1.36	507.00	59.90	0.81	62.60	0.80	2.21	23.00	2.00	11.00

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Malathion injection												
1	300.00	12.09	1.70	486.00	88.70	0.90	87.00	0.90	0.59	16.00	1.00	12.00
2	385.30	14.41	1.42	495.00	75.30	0.80	81.50	0.79	2.11	14.00	2.00	12.00
3	350.30	12.57	1.63	497.00	79.40	0.91	75.20	0.92	0.61	17.00	1.00	12.00
4	387.60	15.08	1.36	505.00	66.90	0.79	68.40	0.79	1.84	16.00	3.00	12.00
5	396.00	12.64	1.54	470.00	60.80	0.84	61.10	0.84	1.62	22.00	1.00	12.00
MPTP injection												
1	300.00	12.09	1.70	486.00	88.70	0.90	87.00	0.90	0.59	16.00	1.00	12.00
2	385.30	14.41	1.42	495.00	75.30	0.80	81.50	0.79	2.11	14.00	2.00	12.00
3	350.30	12.57	1.63	497.00	79.40	0.91	75.20	0.92	0.61	17.00	1.00	12.00
4	387.60	15.08	1.36	505.00	66.90	0.79	68.40	0.79	1.84	16.00	3.00	12.00
5	396.00	12.64	1.54	470.00	60.80	0.84	61.10	0.84	1.62	22.00	1.00	12.00
6	444.00	15.12	1.36	507.00	59.90	0.81	62.60	0.80	2.21	23.00	2.00	11.00

Chapter 3.5. Recovery and pharmacokinetics of control rats

Exp. No.	Treatment Conc.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat injection												
1	C. oil 2ml/kg	0.958	0.864	0.03	0.621		9.686	9.732	0.232	0.234	0.007	0.477
2	C. oil 2ml/kg	0.981	0.9	0.021	0.519		12.92	12.89	0.284	0.284	0.006	0.371
3	C. oil 2ml/kg	1.005	0.905	0.027	0.713		10.081	10.091	0.323	0.323	0.009	0.483
4	C. oil 2ml/kg	1.094	0.996	0.027	0.765		7.779	7.738	0.233	0.232	0.006	0.586
5	C. oil 2ml/kg	1.002	0.859	0.035	0.88		8.076	5.827	0.186	0.134	0.007	0.717
6	C. oil 2ml/kg	1.001	0.923	0.033	0.689		5.867	5.815	0.164	0.163	0.005	0.576
7	C. oil 2ml/kg	0.988	0.866	0.036	0.798		8.612	8.283	0.189	0.182	0.007	0.647
8	Saline 2ml/kg	1.072	0.977	0.028	0.768		6.433	6.274	0.187	0.182	0.006	0.697
9	Saline 2ml/kg	1.013	0.922	0.029	0.78		6.228	5.802	0.112	0.104	0.005	0.625
10	No treat	1.034	0.943	0.044	0.798		4.515	4.784	0.126	0.134	0.003	0.693
11	No treat	1.12	1.033	0.039	0.710		4.894	5.113	0.147	0.153	0.006	0.606
12	No treat	1.278	1.183	0.033	0.666		5.764	5.823	0.173	0.175	0.006	0.551
13	No treat	1.097	1.011	0.032	0.581		7.373	7.045	0.413	0.395	0.013	0.341
14	No treat	1.042	0.955	0.028	0.709		8.214	8.34	0.378	0.384	0.011	0.441

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DDT injection												
1	C. oil 2ml/kg	0.997	0.291	0.153	0.013	0.059	11.781	9.409	0.377	4.814	0.058	0.008
2	C. oil 2ml/kg	1.046	0.264	0.258	0.01	0.046	8.492	7.153	0.246	6.600	0.064	0.008
3	C. oil 2ml/kg	0.999	0.393	not fit			11.77	9.289	0.247			
4	C. oil 2ml/kg	0.997	0.451	0.319	0.019	0.074	5.306	3.923	0.149	2.643	0.047	0.016
5	C. oil 2ml/kg	1.023	0.242	0.41	0.027	0.112	6.344	5.506	0.140	2.259	0.057	0.023
6	Saline 2ml/kg	0.998	0.256	0.311	0.002	0.044	6.299	5.118	0.183	28.588	0.057	0.002
7	Saline 2ml/kg	0.979	0.381	0.246	0.009	0.028	6.188	6.251	0.111	3.156	0.027	0.008
8	No treat	1.034	0.238	0.499	0.014	0.084	4.88	4.338	0.137	5.007	0.068	0.012
9	No treat	1.017	0.329	0.102	0.099	0.1	13.206	8.92	0.383	0.778	0.039	0.061
10	No treat	1.031	0.306	0.102	0.099	0.1	13.442	8.817	0.390	0.791	0.040	0.060
11	No treat	1.041	0.326	0.105	0.098	0.1	8.816	5.763	0.256	0.530	0.027	0.073
12	No treat	1.2	0.335	0.105	0.098	0.1	10.97	7.766	0.318	0.659	0.033	0.067
Malathion injection												
1	C. oil 2ml/kg	0.996	0.081	0.938	0.294	0.165	11.608	19.08	0.279	1.167	0.261	0.212
2	C. oil 2ml/kg	0.996	0.103	0.624	0.239	0.116	15.588	21.237	0.343	1.238	0.214	0.157

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3	C. oil 2ml/kg	0.99	0.142	0.792	0.203	0.06	14.642	38.483	0.426	2.087	0.337	0.117
4	C. oil 2ml/kg	0.989	0.147	0.442	0.088	0.098	11.219	32.614	0.326	8.009	0.761	0.067
5	C. oil 2ml/kg	0.988	0.142	0.596	0.132	0.12	9.768	16.845	0.205	1.131	0.122	0.105
6	C. oil 2ml/kg	1.122	0.205	0.799	0.077	0.095	6.234	15.991	0.175	1.986	0.139	0.064
7	C. oil 2ml/kg	1.104	0.154	0.856	0.085	0.083	7.546	18.11	0.166	1.838	0.142	0.071
8	Saline 2ml/kg	1.016	0.206	0.57	0.035	0.054	6.038	14.734	0.175	3.027	0.094	0.071
9	Saline 2ml/kg	1.019	0.225	0.616	0.084	0.134	5.421	13.764	0.152	1.265	0.100	0.029
10.	No treat	0.993	0.091	1.165	0.095	0.052	9.206	24.434	0.313	4.151	0.365	0.065
11	No treat	1.038	0.093	1.278	0.138	0.058	10.527	24.193	0.358	3.673	0.457	0.089
12	No treat	1.041	0.128	1.325	0.11	0.071	7.711	20.324	0.270	3.521	0.358	0.080
13	No treat	0.975	0.099	1.216	0.121	0.072	8.174	21.752	0.270	2.981	0.328	0.088
14	No treat	1.047	0.106	0.814	0.156	0.147	9.879	17.813	0.415	2.580	0.338	0.091
15	No treat	1.015	0.125	1.046	0.164	0.106	9.401	16.781	0.395	2.913	0.413	0.099
MPTP injection												
1	C. oil 2ml/kg	1.013	0.015	1.725	0.063	0.386	13.305	38.483	0.426	12.083	0.734	0.036
2	C. oil 2ml/kg	1.091	0.019	2.331	0.099	0.029	11.252	32.614	0.326	8.009	0.761	0.067

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3	C. oil 2ml/kg	0.995	0.021	2.862	0.18	0.013	14.282	35.654	0.286	4.827	0.818	0.129
4	C. oil 2ml/kg	0.985	0.053	7.696	0.225	1.481	6.87	29.8	0.192	6.772	1.480	0.182
5	Saline 2ml/kg	0.977	0.025	4.253	0.011	2.493	8.534	34.236	0.247	5.935		
6	Saline 2ml/kg	1.016	0.033	2.886	0.18	0.048	11.28	31.117	0.203	3.458	1.053	0.008
7	No treat	1.01	0.048	not fit			7.451	29.596	0.209		0.586	0.143
8	No treat	0.996	0.0005	not fit			10.507	12.658	0.357			
9	No treat	1.202	0.018	19.218	1.279	0.408	5.254	29.455	0.158	2.526	3.029	1.077
10	No treat	0.99	0.020	6.937	3.509	0.891	9.218	25.537	0.277	0.823	1.918	2.539
11	No treat	1.008	0.031	2.616	0.116	0.03	9.07	30.385	0.508	11.962	1.329	0.057
12	No treat	0.992	0.008	not fit			11.17	41.394	0.514			

Chapter 3.6. Recovery and pharmacokinetics of paraquat pretreated rats

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat injection											
1	1.042	0.950	0.029	0.697		7.634	7.492	0.229	0.225	0.085	0.013
2	1.069	0.968	0.032	0.739		7.767	7.694	0.225	0.223	0.007	0.573
3	1.012	0.926	0.032	0.709		6.381	6.264	0.179	0.175	0.006	0.582
4	0.987	0.900	0.031	0.736		7.813	7.623	0.289	0.282	0.009	0.523
5	1.002	0.927	0.026	0.025		5.379	5.773	0.161	0.173	0.004	0.021
6	1.019	0.925	0.035	0.749		6.008	5.871	0.168	0.164	0.006	0.623
7	1.081	0.992	0.032	0.754		5.586	5.542	0.201	0.200	0.006	0.602
8	0.999	0.912	0.034	0.785		5.625	5.552	0.203	0.200	0.007	0.626
9	1.093	1.000	0.030	0.762		6.468	6.419	0.220	0.218	0.007	0.594
DDT injection											
1	1.046	0.213	0.372	0.017	0.056	7.634	7.492	0.229	5.241	0.085	0.013
2	0.996	0.439	0.177	0.011	0.053	7.755	6.454	0.217	3.711	0.038	0.009
3	1.063	0.377	0.241	0.011	0.117	6.490	4.148	0.188	4.312	0.045	0.009
4	0.993	0.253	0.491	0.090	0.207	5.517	6.845	0.204	1.318	0.100	0.072

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5	1.005	0.355	0.233	0.006	0.057	6.684	4.725	0.187	7.455	0.044	0.005
6	1.046	0.247	0.427	0.004	0.015	5.882	5.222	0.224	24.084	0.095	0.003
7	1.092	0.198	0.525	0.022	0.080	5.841	6.424	0.216	5.373	0.113	0.017
8	1.096	0.266	0.406	0.014	0.053	5.868	5.024	0.211	6.337	0.086	0.011
Malathion injection											
1	1.001	0.234	0.497	0.061	0.071	7.518	16.563	0.226	2.063	0.112	0.047
2	0.990	0.184	0.597	0.054	0.072	7.082	14.567	0.205	2.476	0.123	0.043
3	1.033	0.228	0.604	0.054	0.078	5.692	14.155	0.159	1.942	0.096	0.045
4	0.972	0.250	0.640	0.044	0.037	6.633	16.474	0.239	3.712	0.153	0.033
5	0.987	0.276	0.564	0.038	0.016	10.779	18.110	0.302	4.781	0.170	0.027
6	0.974	0.250	0.560	0.078	0.088	6.234	15.409	0.175	1.428	0.098	0.064
7	0.961	0.240	0.613	0.052	0.065	5.576	14.882	0.201	2.567	0.123	0.042
8	1.066	0.263	0.625	0.055	0.076	5.323	13.581	0.192	2.369	0.120	0.044
9	0.999	0.219	0.543	0.036	0.054	6.052	14.749	0.188	3.017	0.102	0.029

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MPTP injection											
1	0.939	0.089	2.219	0.265	0.226	10.359	40.371	0.311	2.913	0.690	0.183
2	1.007	0.026	6.993	0.431	0.052	10.671	35.059	0.309	5.330	2.164	0.298
3	1.009	0.035	7.190	0.404	0.049	9.317	32.604	0.261	4.904	1.876	0.299
4	1.022	0.048	2.846	0.096	0.046	8.711	28.535	0.305	9.343	0.868	0.067
5	0.996	0.065	1.455	0.057	0.038	9.625	27.451	0.270	7.149	0.392	0.042
6	0.993	0.045	1.753	0.050	0.031	9.710	31.726	0.272	9.804	0.477	0.036
7	0.993	0.042	2.514	0.092	0.038	8.449	30.209	0.304	8.616	0.765	0.064
8	1.003	0.040	2.909	0.112	0.055	7.937	28.415	0.286	7.707	0.831	0.080
9	1.004	0.035	0.206	0.065	0.023	8.832	33.253	0.318	1.326	0.065	0.044

Chapter 3.7. Recovery and pharmacokinetics of DDT pretreated rats.

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat injection											
1	0.992	0.882	0.036	0.671		9.650	9.877	0.261	0.267	0.009	0.496
2	1.000	0.913	0.035	0.731		6.182	6.430	0.155	0.161	0.005	0.618
3	1.004	0.953	0.020	0.726		6.134	6.399	0.184	0.192	0.004	0.592
4	1.027	0.990	0.019	0.613		6.800	7.435	0.190	0.208	0.004	0.496
5	0.992	0.935	0.018	0.624		7.659	7.722	0.199	0.201	0.004	0.500
6	1.001	0.910	0.031	0.678		7.798	7.630	0.172	0.168	0.005	0.562
7	1.005	0.908	0.030	0.775		7.090	6.902	0.163	0.159	0.005	0.649
8	1.039	1.018	0.010	0.623		4.670	4.759	0.112	0.114	0.001	0.553
DDT injection											
1	0.983	0.233	0.381	0.080	0.201	8.227	8.017	0.214	1.233	0.081	0.063
2	1.063	0.254	0.506	0.248	0.506	5.662	4.660	0.142	0.430	0.072	0.213
3	1.000	0.470	0.184	0.002	0.027	6.620	4.279	0.199	18.470	0.037	0.002
4	1.003	0.432	0.133	0.006	0.019	10.913	6.656	0.306	7.079	0.041	0.004

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5	0.999	0.386	0.204	0.001	0.029	7.361	5.063	0.191	39.234	0.039	0.001
6	1.002	0.509	not fit			5.125	3.780	0.113			
7	1.025	0.322	0.259	0.002	0.032	6.929	5.342	0.166	21.702	0.043	0.002
8	0.998	0.263	0.534	0.009	0.041	3.929	3.941	0.098	5.926	0.052	0.008
Malathion injection											
1	0.996	0.175	0.509	0.179	0.097	13.029	20.182	0.326	1.252	0.166	0.121
2	1.007	0.252	0.641	0.060	0.048	6.056	17.260	0.151	1.769	0.097	0.051
3	0.970	0.305	0.401	0.040	0.065	6.763	10.108	0.203	2.237	0.081	0.032
4	1.001	0.277	0.273	0.049	0.066	10.646	14.178	0.298	1.959	0.081	0.034
5	0.999	0.313	0.403	0.039	0.061	5.784	11.151	0.150	1.704	0.061	0.033
6	1.002	0.176	0.651	0.071	0.087	7.317	15.841	0.161	1.637	0.105	0.060
7	0.989	0.206	0.353	0.039	0.075	8.589	12.962	0.198	1.986	0.070	0.031
8	0.974	0.270	0.521	0.045	0.081	4.574	10.783	0.110	1.381	0.057	0.040
MPTP injection											
1	1.109	0.044	4.117	0.158	0.024	10.203	36.061	0.275	7.454	1.134	0.114

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2	1.031	0.047	4.803	0.324	0.080	7.187	27.528	0.180	2.843	0.863	0.266
3	1.003	0.046	4.216	0.179	0.511	7.682	38.007	0.230	5.659	0.972	0.138
4	0.999	0.068	1.811	0.091	0.025	11.001	30.513	0.308	6.438	0.558	0.063
5	1.003	0.042	1.435	0.077	0.049	10.902	28.086	0.273	5.352	0.391	0.056
6	0.997	0.018	3.907	0.112	0.011	11.363	36.484	0.250	8.970	0.977	0.084
7	1.011	0.031	1.941	0.210	0.443	10.126	35.885	0.233	2.386	0.452	0.161
8	1.012	0.035	4.394	0.154	0.029	7.377	35.352	0.177	5.229	0.778	0.127

Chapter 3.8. Recovery and pharmacokinetics of malathion pretreated rats.

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat injection											
1	1.077	0.986	0.015	0.014		6.680	6.860	0.187	0.192	0.003	0.011
2	0.995	0.908	0.027	0.629		9.320	9.421	0.224	0.226	0.006	0.488
3	1.003	0.899	0.035	0.764		6.770	6.516	0.183	0.176	0.006	0.624
4	1.01	0.898	0.035	0.872		6.915	6.899	0.159	0.159	0.006	0.733
5	0.986	0.911	0.023	0.569		12.344	12.691	0.296	0.305	0.007	0.400
6	0.983	0.884	0.03	0.795		8.283	7.736	0.191	0.178	0.006	0.644
DDT injection											
1	0.992	0.385	0.191	0.006	0.027	7.192	5.866	0.201	6.612	0.038	0.005
2	1.005	0.278	0.21	0.039	0.141	10.405	6.869	0.250	1.594	0.052	0.029
3	1.012	0.321	0.171	0.01	0.049	10.802	7.597	0.259	4.692	0.044	0.007
4	0.996	0.302	0.184	0.006	0.052	10.420	6.657	0.240	7.589	0.044	0.005

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Malathion injection											
1	1.094	0.206	1.137	0.203	0.11	6.746	15.821	0.189	1.247	0.215	0.165
2	0.991	0.153	0.688	0.246	0.157	10.147	14.035	0.244	0.925	0.168	0.186
3	0.971	0.12	0.473	0.054	0.07	11.211	17.617	0.303	2.954	0.143	0.038
4	1.002	0.163	0.419	0.06	0.072	10.642	18.691	0.245	1.954	0.103	0.045
5	0.978	0.18	0.834	0.159	0.098	9.656	17.641	0.232	1.447	0.193	0.122
MPTP injection											
1	0.999	0.029	7.41	0.418	0.075	7.748	26.522	0.217	4.063	1.608	0.327
2	0.95	0.024	5.25	0.042	1.586	7.949	31.014	0.191	24.038	1.002	0.034
3	0.99	0.059	3.312	0.246	0.015	11.738	36.626	0.317	4.584	1.050	0.168
4	0.995	0.066	1.578	0.524	0.123	10.779	31.760	0.248	0.995	0.391	0.394
5	1.007	0.044	0.705	0.108	0.215	12.919	19.670	0.310	2.334	0.219	0.075
6	1.009	0.056	2.425	0.161	0.064	9.645	19.698	0.222	3.563	0.538	0.125

Chapter 4.1. Liver viability of young rats

Exp. No.	Age (months)	B.W. (g)	L.W. (g)	F.R/liver ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat													
1	3	292.20	12.53	1.61	490.20	87.70	0.87	83.90	0.87	1.24	20.00	1.00	10.00
2	3	265.40	9.96	1.96	468.00	75.60	1.02	75.90	1.02	1.28	19.00	1.00	10.00
3	3	287.50	10.69	1.90	480.00	85.10	1.00	85.80	1.00	1.32	20.00	1.00	10.00
4	3	259.10	9.58	2.13	503.90	88.60	1.18	90.20	1.18	1.17	20.00	1.00	10.00
5	3	288.90	11.95	1.71	476.50	72.10	0.92	77.30	0.91	0.88	21.00	1.00	10.00
6	3	281.00	10.79	1.82	483.20	75.40	0.99	78.30	0.98	0.83	20.00	1.00	10.00
DDT													
1	3	298.80	10.28	1.97	456.00	65.10	1.02	66.30	1.02	1.02	20.00	1.00	10.00
2	3	313.00	10.71	1.88	389.70	69.50	0.80	68.60	0.80	1.33	19.00	1.00	10.00
3	3	299.70	9.77	2.07	429.00	58.60	1.02	60.70	1.02	1.21	20.00	1.00	10.00
4	3	298.80	10.28	1.97	456.00	65.10	1.02	66.30	1.02	1.27	20.00	1.00	10.00

Chapter 4.1. Contd.

Malathion

1	3	292.20	12.53	1.60	490.20	87.70	0.86	83.90	0.86	1.25	20.00	1.00	10.00
2	3	265.40	9.96	1.96	468.00	75.60	1.02	75.90	1.02	1.28	19.00	1.00	10.00
3	3	287.50	10.69	1.91	480.00	85.10	1.01	85.80	1.00	1.31	20.00	1.00	10.00
4	3	259.10	9.58	2.09	503.90	88.60	1.16	90.20	1.15	1.20	20.00	1.00	10.00
5	3	288.90	11.95	1.69	476.50	72.10	0.91	77.30	0.90	0.89	21.00	1.00	10.00
6	3	281.00	10.79	1.82	483.20	75.40	0.99	78.30	0.98	0.83	20.00	1.00	10.00

MPTP

1	3	298.80	10.28	1.97	456.00	65.10	1.02	66.30	1.02	1.02	20.00	1.00	10.00
2	3	313.00	10.71	1.88	389.70	69.50	0.80	68.60	0.80	1.33	19.00	1.00	10.00
3	3	299.70	9.77	2.07	429.00	58.60	1.02	60.70	1.02	1.21	20.00	1.00	10.00
4	3	313.00	10.71	1.88	389.70	69.50	0.80	68.60	0.80	1.33	20.00	1.00	10.00

Chapter 4.2. Liver viability of old rats

Exp. No.	Age (months)	B.W. (g)	L.W. (g)	F.R/liver ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Paraquat													
1	18	419.10	13.42	1.45	509.00	66.90	0.86	80.00	0.83	2.06	20.00	1.00	12.00
2	18	381.10	12.35	1.63	468.00	63.00	0.88	74.00	0.86	1.54	20.00	2.00	12.00
3	18	437.80	13.15	1.55	499.70	71.00	0.89	88.00	0.85	0.65	20.00	1.00	12.00
4	18	426.60	13.27	1.51	503.00	82.00	0.85	86.40	0.84	1.33	17.00	1.00	12.00
5	19	452.20	14.02	1.49	481.00	75.60	0.81	87.30	0.78	0.67	12.00	1.00	10.00
6	19	450.70	13.72	1.46	472.00	77.70	0.77	79.10	0.77	1.02	16.00	1.00	10.00
7	19	433.80	11.15	1.79	490.00	77.70	0.99	92.00	0.95	0.84	16.00	1.00	10.00
DDT													
1	18	419.10	13.42	1.45	509.00	66.90	0.86	80.00	0.83	2.06	20.00	1.00	12.00
2	18	381.10	12.35	1.64	468.00	63.00	0.89	74.00	0.86	1.52	20.00	2.00	12.00
3	18	437.80	13.15	1.55	499.70	71.00	0.89	88.00	0.85	0.65	20.00	1.00	12.00
4	18	426.60	13.27	1.51	503.00	82.00	0.85	86.40	0.84	1.33	17.00	1.00	12.00

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6	19	450.70	13.72	1.46	472.00	77.70	0.77	79.10	0.77	1.02	16.00	1.00	10.00
7	19	433.80	11.15	1.75	490.00	77.70	0.96	92.00	0.93	0.86	16.00	1.00	10.00
Malathion													
1	18	419.10	13.42	1.45	509.00	66.90	0.86	80.00	0.83	2.06	20.00	1.00	12.00
2	18	381.10	12.35	1.62	468.00	63.00	0.87	74.00	0.85	1.54	20.00	2.00	12.00
3	18	437.80	13.15	1.54	499.70	71.00	0.88	88.00	0.84	0.65	20.00	1.00	12.00
4	18	426.60	13.27	1.51	503.00	82.00	0.85	86.40	0.84	1.33	17.00	1.00	12.00
5	19	452.20	14.02	1.48	481.00	75.60	0.80	87.30	0.78	0.68	12.00	1.00	10.00
6	19	450.70	13.72	1.46	472.00	77.70	0.77	79.10	0.77	1.02	16.00	1.00	10.00
7	19	433.80	11.15	1.79	490.00	77.70	0.99	92.00	0.95	0.84	16.00	1.00	10.00
MPTP													
1	18	419.10	13.42	1.45	509.00	66.90	0.86	80.00	0.83	2.06	20.00	1.00	12.00
2	18	381.10	12.35	1.62	468.00	63.00	0.87	74.00	0.85	1.54	20.00	2.00	12.00
3	18	437.80	13.15	1.54	499.70	71.00	0.88	88.00	0.84	0.65	20.00	1.00	12.00
4	18	426.60	13.27	1.51	503.00	82.00	0.85	86.40	0.84	1.33	17.00	1.00	12.00

Chapter 4.2. Contd.

5	19	452.20	14.02	1.48	481.00	75.60	0.80	87.30	0.78	0.68	12.00	1.00	10.00
6	19	450.70	13.72	1.46	472.00	77.70	0.77	79.10	0.77	1.02	16.00	1.00	10.00
7	19	433.80	11.15	1.79	490.00	77.70	0.99	92.00	0.95	0.84	16.00	1.00	10.00

Chapter 4.3. Recovery, transport and sequestration kinetics of indicators in young rat livers

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat											
1	1.060	0.950	0.018	0.872		9.719	8.890	0.261	0.239	0.005	0.644
2	0.987	0.922	0.024	0.770		5.024	4.899	0.164	0.160	0.004	0.644
3	1.043	0.959	0.095	0.101		7.385	5.819	0.234	0.184	0.022	0.077
4	0.975	0.886	0.009	0.905		7.910	6.605	0.281	0.234	0.003	0.651
5	1.016	0.931	0.004	0.032		8.065	6.582	0.227	0.185	0.001	0.025
6	1.033	0.939	0.094	0.101		7.013	5.554	0.212	0.168	0.020	0.080
DDT											
1	1.025	0.152	0.682	0.182	0.148	7.297	11.169	0.239	1.135	0.163	0.138
2	1.054	0.159	0.718	0.166	0.185	8.295	10.227	0.259	1.382	0.186	0.123
3	0.975	0.239	0.500	0.202	0.200	14.133	14.490	0.470	1.634	0.235	0.107
4	1.019	0.112	0.811	0.146	0.191	8.067	10.834	0.259	1.698	0.210	0.108
Malathion											
1	1.041	0.224	0.678	0.075	0.033	11.341	19.052	0.302	3.028	0.205	0.052

Chapter 4.3. Contd.

2	1.067	0.209	0.696	0.040	0.033	7.623	19.896	0.249	4.575	0.173	0.030
3	1.073	0.195	0.885	0.041	0.019	7.473	23.610	0.238	5.370	0.210	0.031
4	1.059	0.185	0.929	0.051	0.031	6.529	22.615	0.226	4.343	0.210	0.039
5	1.039	0.241	0.538	0.031	0.031	7.679	16.830	0.216	3.971	0.116	0.024
6	1.052	0.188	0.793	0.044	0.028	7.813	22.378	0.237	4.500	0.188	0.034
MPTP											
1	0.992	0.011	3.536	0.102	0.805	6.710	37.971	0.214	7.646	0.758	0.080
2	0.995	0.011	8.027	0.353	0.053	6.952	37.386	0.216	5.137	1.737	0.277
3	0.982	0.011	2.541	0.133	0.535	6.504	32.880	0.216	4.350	0.550	0.104
4	0.987	0.015	8.100	0.361	0.058	6.482	34.274	0.200	4.681	1.618	0.289

Chapter 4.4. Recovery, transport and sequestration kinetics of indicators in old rat livers

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Paraquat											
1	1.006	0.917	0.031	0.567		8.736	8.567	0.212	0.207	0.007	0.447
2	0.969	0.889	0.028	0.609		8.613	8.384	0.236	0.230	0.007	0.465
3	1.052	0.947	0.029	0.705		7.334	6.726	0.190	0.174	0.005	0.571
4	1.021	0.949	0.023	0.627		6.709	6.531	0.169	0.164	0.004	0.521
5	0.983	0.893	0.033	0.638		7.619	7.512	0.189	0.187	0.006	0.517
6	0.942	0.843	0.032	0.733		8.216	7.875	0.201	0.192	0.006	0.586
7	1.004	0.891	0.043	0.671		7.586	7.251	0.221	0.211	0.010	0.523
DDT											
1	0.983	0.371	0.104	0.099	0.101	8.201	6.125	0.196	0.401	0.020	0.080
2	0.991	0.352	0.245	0.012	0.078	6.736	5.333	0.182	3.904	0.045	0.010
3	1.005	0.676	0.100	0.099	0.100	6.988	5.274	0.181	0.363	0.018	0.081
4	0.936	0.376	0.100	0.099	0.101	7.062	4.657	0.175	0.351	0.017	0.082
5	0.936	0.350	0.199	0.015	0.067	8.048	5.891	0.197	2.812	0.039	0.012
6	0.959	0.447	0.102	0.099	0.101	8.733	6.549	0.210	0.426	0.021	0.078

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7	0.970	0.355	0.224	0.024	0.059	8.702	7.059	0.252	2.609	0.057	0.018
Malathion											
1	1.021	0.299	0.426	0.048	0.044	7.556	17.001	0.183	1.807	0.078	0.039
2	1.065	0.321	0.436	0.052	0.041	7.670	17.641	0.207	1.943	0.090	0.041
3	0.957	0.198	0.676	0.035	0.031	6.924	16.608	0.177	3.600	0.120	0.029
4	1.033	0.265	0.539	0.063	0.055	7.075	18.081	0.173	1.656	0.093	0.052
5	1.041	0.242	0.615	0.064	0.034	8.355	22.350	0.206	2.181	0.126	0.051
6	1.022	0.304	0.415	0.071	0.053	9.121	19.042	0.223	1.524	0.092	0.055
7	0.999	0.304	0.426	0.048	0.044	8.438	17.241	0.244	1.865	0.109	0.051
MPTP											
1	1.009	0.040	1.079	0.091	0.111	12.703	21.304	0.304	3.915	0.329	0.063
2	1.000	0.035	1.379	0.075	0.094	11.675	21.722	0.315	6.109	0.435	0.051
3	1.166	0.024	6.019	0.271	0.041	8.255	31.674	0.211	4.904	1.272	0.214
4	1.008	0.050	1.318	0.035	0.007	10.428	27.929	0.252	9.723	0.332	0.026
5	0.931	0.024	6.421	0.576	0.120	7.813	28.608	0.189	2.290	1.211	0.467

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6	0.961	0.025	8.207	0.617	0.088	7.653	33.076	0.185	2.646	1.518	0.503
7	0.943	0.014	4.692	0.315	0.134	7.342	29.140	0.212	3.368	0.994	0.248

Chapter 5.1. Liver viability of control rats.

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Amiloride - control												
1	472.00	14.66	1.43	495.00	51.20	0.85	59.40	0.83	1.40	18.00	1.00	10.00
2	391.20	13.75	1.49	481.00	57.40	0.84	66.10	0.83	1.01	13.00	1.00	10.00
3	396.20	13.08	1.61	493.50	56.00	0.94	54.00	0.94	1.25	14.00	1.00	10.00
4	394.80	13.23	1.59	488.00	57.30	0.91	52.70	0.92	1.26	15.00	1.00	10.00
5	313.10	13.95	1.51	485.50	66.40	0.84	66.60	0.84	1.99	18.00	1.00	10.00
6	350.20	13.34	1.51	515.00	70.10	0.90	54.50	0.93	1.32	20.00	1.00	10.00
Tubocurarine - control												
1	409.10	14.99	1.47	520.60	79.60	0.86	77.90	0.87	1.70	16.00	1.00	10.00
2	405.90	14.96	1.47	509.40	59.00	0.88	61.10	0.88	1.02	21.00	1.00	10.00
3	402.50	14.86	1.48	496.30	76.20	0.83	75.50	0.83	1.35	16.00	1.00	10.00
4	374.60	14.37	1.57	488.50	69.80	0.88	70.60	0.88	1.27	15.00	1.00	10.00

Chapter 5.1. Contd.

Daunomycin - control												
1	342.50	13.47	1.49	484.50	56.80	0.85	81.70	0.80	1.35	20.00	1.00	12.00
2	326.30	11.93	1.68	472.00	72.10	0.89	94.40	0.84	1.49	20.00	1.00	12.00
3	334.00	10.82	1.76	486.00	63.70	0.99	62.20	0.99	0.85	20.00	1.00	13.00
Rifamycin - control												
1	319.90	10.98	1.82	454.50	70.50	0.93	65.70	0.94	0.82	19.00	1.00	12.00
2	333.20	12.27	1.71	451.70	63.90	0.89	65.60	0.88	1.17	20.00	1.00	12.00
3	259.20	9.49	2.11	432.00	84.40	0.98	85.20	0.97	0.95	20.00	1.00	13.00
4	257.20	7.85	2.47	410.20	90.40	1.05	91.30	1.05	0.81	21.00	1.00	13.00

Chapter 5.2. Liver viability of perfused rats after pretreatment of cell membrane transport inhibitors.

Exp. No.	B.W. (g)	L.W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Amiloride												
1	472.00	14.66	1.43	495.00	51.20	0.85	59.40	0.83	1.40	18.00	1.00	10.00
2	391.20	13.75	1.49	481.00	57.40	0.84	66.10	0.83	1.01	13.00	1.00	10.00
3	396.20	13.08	1.61	493.50	56.00	0.94	54.00	0.94	1.25	14.00	1.00	10.00
4	313.10	13.95	1.51	485.50	66.40	0.84	66.60	0.84	1.99	18.00	1.00	10.00
5	350.20	13.34	1.51	515.00	70.10	0.90	54.50	0.93	1.32	20.00	1.00	10.00
Tubocurarine												
1	405.90	14.96	1.47	509.40	59.00	0.88	61.10	0.88	1.02	21.00	1.00	10.00
2	402.50	14.86	1.48	496.30	76.20	0.83	75.50	0.83	1.35	16.00	1.00	10.00
3	374.60	14.37	1.57	488.50	69.80	0.88	70.60	0.88	1.27	15.00	1.00	10.00
Daunomycin												
1	342.50	13.47	1.49	484.50	56.80	0.85	81.70	0.80	1.35	20.00	1.00	12.00

Chapter 5.2. Contd.

2	326.30	11.93	1.68	472.00	72.10	0.89	94.40	0.84	1.49	20.00	1.00	12.00
3	334.00	10.82	1.76	486.00	63.70	0.99	62.20	0.99	0.85	20.00	1.00	13.00
Rifamycin												
1	319.90	10.98	1.82	454.50	70.50	0.93	65.70	0.94	0.82	19.00	1.00	12.00
2	333.20	12.27	1.71	451.70	63.90	0.89	65.60	0.88	1.17	20.00	1.00	12.00
3	259.20	9.49	2.11	432.00	84.40	0.98	85.20	0.97	0.95	20.00	1.00	13.00
4	257.20	7.85	2.47	410.20	90.40	1.05	91.30	1.05	0.81	21.00	1.00	13.00

Chapter 5.3. Recovery, transport and sequestration of MPTP in control rat livers.

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Amiloride - control											
1	0.945	0.016	4.394	0.158	0.030	8.142	35.601	0.179	5.161	0.787	0.130
2	1.004	0.02	6.777	0.345	0.797	6.887	29.172	0.172	3.554	1.167	0.286
3	0.948	0.012	4.586	0.169	0.048	7.257	31.961	0.189	5.309	0.865	0.137
4	0.923	0.022	6.941	0.629	0.101	7.296	33.775	0.117	1.405	0.810	0.556
5	1.038	0.06	2.009	0.072	0.013	7.614	30.339	0.190	5.502	0.382	0.058
6	0.997	0.089	1.581	0.082	0.056	7.034	27.255	0.176	3.566	0.278	0.068
Tubocurarine - control											
1	0.959	0.021	10.551	0.744	0.179	5.967	33.929	0.125	1.902	1.322	0.651
2	1.000	0.016	7.892	0.67	0.088	9.108	27.468	0.179	2.287	1.413	0.55
3	1.019	0.036	4.608	0.43	0.084	8.285	32.624	0.171	2.007	0.789	0.356
4	1.022	0.029	1.813	0.159	0.092	8.411	14.098	0.194	2.405	0.352	0.128
Daunomycin - control											
1	0.998	0.040	2.169	0.192	0.094	8.357	21.343	0.201	2.467	0.435	0.153

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2	0.972	0.042	1.937	0.160	0.115	7.936	18.168	0.216	2.834	0.419	0.125
3	0.953	0.043	3.054	0.303	0.074	8.913	25.625	0.261	2.890	0.797	0.224
Rifamycin - control											
1	1.037	0.040	1.565	0.142	0.116	9.421	18.440	0.286	3.437	0.447	0.101
2	0.994	0.037	1.282	0.109	0.136	8.191	16.144	0.234	2.982	0.300	0.084
3	0.976	0.041	1.543	0.131	0.167	6.700	16.752	0.235	3.006	0.363	0.100
4	0.995	0.044	2.56	0.306	0.233	7.575	14.105	0.312	2.923	0.799	0.211

Chapter 5.4. Recovery, transport and sequestration of MPTP in rat livers after treatment with cell membrane transport inhibitors.

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Amiloride											
1	0.952	0.073	0.841	0.342	0.356	8.859	13.029	0.195	0.674	0.164	0.275
2	0.915	0.080	1.557	0.716	0.291	9.276	11.548	0.204	0.648	0.318	0.570
3	0.935	0.064	2.303	0.160	0.069	7.446	30.741	0.194	2.980	0.446	0.129
4	0.984	0.117	1.255	0.498	0.402	6.852	19.727	0.171	0.603	0.215	0.413
5	1.000	0.128	1.182	0.064	0.039	7.402	32.585	0.185	3.603	0.219	0.052
Tubocurarine											
1	1.028	0.083	1.591	0.160	0.109	7.504	29.210	0.146	1.598	0.232	0.137
2	1.031	0.053	1.581	0.120	0.064	8.418	20.273	0.176	2.492	0.278	0.099
3	1.055	0.079	0.944	0.075	0.061	8.349	20.946	0.195	2.654	0.184	0.060
Daunomycin											
1	1.004	0.032	2.089	0.168	0.101	7.628	19.571	0.189	2.536	0.394	0.136
2	0.973	0.057	2.522	0.304	0.201	7.187	16.810	0.193	1.792	0.486	0.245

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3	0.952	0.045	1.788	0.143	0.068	8.732	23.743	0.256	3.450	0.457	0.106
Rifamycin											
1	0.983	0.035	2.450	0.367	0.207	8.227	15.000	0.250	1.916	0.612	0.275
2	0.982	0.042	1.409	0.179	0.240	8.039	13.648	0.227	2.016	0.320	0.138
3	0.988	0.032	1.686	0.145	0.159	7.238	14.889	0.252	3.177	0.424	0.109
4	1.001	0.039	2.647	0.476	0.326	6.996	11.287	0.285	1.872	0.755	0.340

Chapter 5.5. ^{3}H-MPTP DPM count in hepatocytes

	Negative control	Positive control	Amiloride	Tubocurarine	Daunomycin	Rifamycin
	25.27	133816.60	157358.40	146121.60	103098.60	134836.50
Exp. 1	23.95	140467.30	153033.60	150084.90	96442.72	123861.30
	16.23	134854.60	167065.30	141345.60	98915.30	126395.70
	26.18	83196.70	130292.40	110048.50	81730.40	104203.70
Exp. 2	59.39	109293.60	135969.40	102728.30	85080.20	105498.00
	21.40	96246.10	131072.50	103008.60	83385.90	103660.10
	16.30	82264.29	88019.59	105909.30	79780.22	95414.30
Exp. 3	22.33	110155.30	116201.90	109236.70	75872.98	83501.00
	32.77	96209.80	115768.40	88905.40	82065.27	102985.10
	23.40	74311.20	99322.86	78742.99	62780.95	75343.97
Exp. 4	18.94	86787.80	101874.40	81113.98	47834.51	69519.89
	21.85	75156.20	94178.08	85258.83	56460.86	67319.15

Chapter 6.1. Liver viability of control and cirrhotic rats.

Exp. No.	B.W. (g)	L.W. (g)	Spleen W. (g)	F.R. ($ml \cdot min^{-1} \cdot g^{-1}$)	pO_2 inflow before exp. (mmHg)	pO_2 outflow before exp. (mmHg)	O_2 consump. before exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	pO_2 outflow after exp. (mmHg)	O_2 consump. after exp. ($\mu mol \cdot min^{-1} \cdot g^{-1}$)	Portal resistance ($cm\ H_2O \cdot min \cdot g \cdot ml^{-1}$)	ALT (U/L)	AST (U/L)	ALKP (U/L)
Control rats													
1	504.20	15.88	1.22	1.32	498.00	94.60	0.71	82.70	0.73	0.76	20.00	1.00	13.00
2	480.40	16.04	1.47	1.31	488.00	69.90	0.73	73.30	0.72	1.91	20.00	1.00	14.00
3	485.00	15.40	1.41	1.30	480.00	61.90	0.72	64.50	0.72	1.54	20.00	1.00	14.00
4	444.50	15.40	1.56	1.30	496.00	75.70	0.73	76.30	0.73	1.54	20.00	1.00	14.00
5	437.90	15.28	1.33	1.37	497.00	77.20	0.77	80.00	0.76	1.10	19.00	1.00	14.00
6	402.70	13.18	1.11	1.53	489.00	65.70	0.86	66.90	0.86	1.31	20.00	1.00	14.00
Cirrhotic rats													
1	543.50	20.19	2.23	1.14	491.000	63.300	0.65	64.000	0.65	1.76	22.00	1.00	10.00
2	461.80	19.35	3.83	1.14	475.900	69.800	0.62	68.700	0.62	2.64	20.00	2.00	10.00
3	420.00	19.89	3.25	1.01	482.000	95.900	0.52	101.100	0.51	3.48	20.00	3.00	14.00
4	437.50	15.85	3.47	1.26	488.000	69.200	0.71	61.000	0.72	2.77	20.00	2.00	14.00
5	366.60	12.69	3.23	1.62	483.000	69.000	0.90	62.900	0.91	2.46	21.00	7.00	15.00

Chapter 6.2. Recovery, hepatocellular transport and sequestration of malathion and MPTP in control and cirrhotic rat livers.

Exp. No.	Recovery (sucrose)	Recovery (substrates)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	MTT_{suc} (s)	MTT_{sb} (s)	V_{su} ($ml \cdot g^{-1}$)	V_{sb} ($ml \cdot g^{-1}$)	PS_{in} ($ml \cdot s^{-1} \cdot g^{-1}$)	PS_{out} ($ml \cdot s^{-1} \cdot g^{-1}$)
Malathion - control											
1	0.980	0.179	0.524	0.084	0.091	10.167	15.640	0.220	1.591	0.115	0.066
2	1.013	0.161	0.594	0.095	0.080	11.391	17.808	0.241	1.751	0.143	0.072
3	0.950	0.187	0.493	0.047	0.042	9.629	20.001	0.208	2.394	0.103	0.037
4	1.004	0.166	not fit			8.987	17.695	0.196			
5	1.007	0.159	0.750	0.082	0.053	9.928	21.017	0.129	1.309	0.097	0.071
6	1.036	0.196	0.722	0.102	0.096	6.679	17.580	0.169	1.369	0.122	0.085
Malathion – cirrhosis											
1	0.982	0.220	0.446	0.035	0.016	10.898	20.937	0.207	2.844	0.092	0.028
2	0.976	0.260	0.710	0.051	0.017	8.419	22.830	0.160	2.381	0.113	0.043
3	0.981	0.224	0.562	0.048	0.029	9.908	21.329	0.156	1.983	0.088	0.041
4	1.020	0.173	0.469	0.040	0.027	12.662	21.692	0.266	3.389	0.125	0.029
5	0.937	0.203	0.495	0.061	0.033	12.298	22.164	0.320	2.915	0.158	0.041

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MPTP - control											
1	0.995	0.075	1.664	0.216	0.108	9.163	26.421	0.197	1.716	0.328	0.173
2	0.987	0.101	1.115	0.136	0.070	9.736	23.695	0.200	1.842	0.223	0.109
3	0.975	0.042	1.831	0.140	0.064	8.963	23.491	0.194	2.731	0.355	0.113
4	0.959	0.043	3.702	0.193	0.048	5.631	36.115	0.123	2.485	0.456	0.169
5	1.003	0.064	1.162	0.110	0.105	8.873	20.756	0.111	1.287	0.129	0.098
6	0.963	0.073	1.031	0.159	0.183	6.250	18.625	0.158	1.184	0.163	0.134
MPTP - cirrhosis											
1	0.965	0.089	1.111	0.055	0.038	9.486	35.301	0.180	3.819	0.200	0.045
2	1.000	0.089	0.891	0.050	0.058	9.471	18.431	0.180	3.378	0.160	0.041
3	0.973	0.086	1.071	0.052	0.038	8.618	26.693	0.137	2.963	0.147	0.045
4	0.979	0.105	0.612	0.080	0.082	11.539	18.439	0.248	2.142	0.152	0.060
5	1.001	0.120	0.546	0.095	0.094	12.479	18.386	0.321	2.168	0.175	0.064