The Effect Of Subacute Mirex Administration On The Acute Inhalation Toxicity Of Parathion In The Rat

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ABSTRACT

THE EFFECT OF SUBACUTE MIREX ADMINISTRATION
ON THE ACUTE INHALATION TOXICITY
OF PARATHION IN THE RAT

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The inhalation toxicity of parathion in the rat decreased by a factor of 1.7 following subacute mirex administration. The protection afforded could not be explained by alterations in several biological parameters having potential to influence parathion toxicity. *In vitro* parathion conversion to paraoxon by liver microsomes approximately doubled following mirex treatment, with no change in paraoxon detoxification. Activity of microsomal enzymes involved in parathion activation to paraoxon increased. Parathion uptake and metabolism by the lung was unchanged with mirex treatment, as was competition of endogenous substrates with parathion and paraoxon for metabolic enzymes, and blood flow through the liver. Parathion and paraoxon storage in the liver, and activity of plasma and brain cholinesterases were also unchanged by mirex. Plasma detoxification of parathion and paraoxon decreased with mirex treatment but this change was in the wrong direction to explain the decrease in parathion toxicity. The possibility of hepatic parathion metabolism *in vivo* differing from that measured *in vitro* is discussed.

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1.0 INTRODUCTION

1.1 General introduction

The discovery of synthetic chlorinated hydrocarbon and organophosphate insecticides in the 1940s and 1950s began a period of rapid development of agricultural chemicals used for protecting agricultural products from pests. In the decades that followed, hundreds of synthetic chemicals were developed and put into widespread use to combat weeds, insects and fungi. Many of these chemicals were less than ideal as pesticides by virtue of their non-specificity for the target organism, their persistence in the environment or their high toxicity to wildlife and man. The hazard of many materials went unnoticed or was disregarded until serious damage was observed years later, as was the case with DDT contamination of the environment. An additional unrecognized hazard of widespread use of pesticides was realized in the 1960s and 1970s when it became evident that the toxicity of one pesticide could be modified by pre-exposure to a second pesticide. Research was initiated to elucidate the mechanisms of interaction and continues today because prediction of the effects of combinations of pesticides is still uncertain. The benefits to be gained from this work include recognition of the full potential hazard of present environmental contaminants and a contribution to the development of new pesticides through an ability to predict responses of target and non-target organisms.

1.2 Toxicity and metabolism of pesticides in mammals

1.2.1 General review

Organochlorine pesticides generally have low toxicity to mammals, however many organophosphate and carbamate insecticides have extremely high mammalian toxicity (88). The major hazard incurred to mammals and man from the use of these chemicals arises as a result of accident or improper use since little evidence suggests that pesticides used at normal dosages are grossly detrimental to wild mammals (88). Accidental exposure alone has been estimated to account for as many as 100,000 poisonings and 150 fatalities a year in the United States (88).

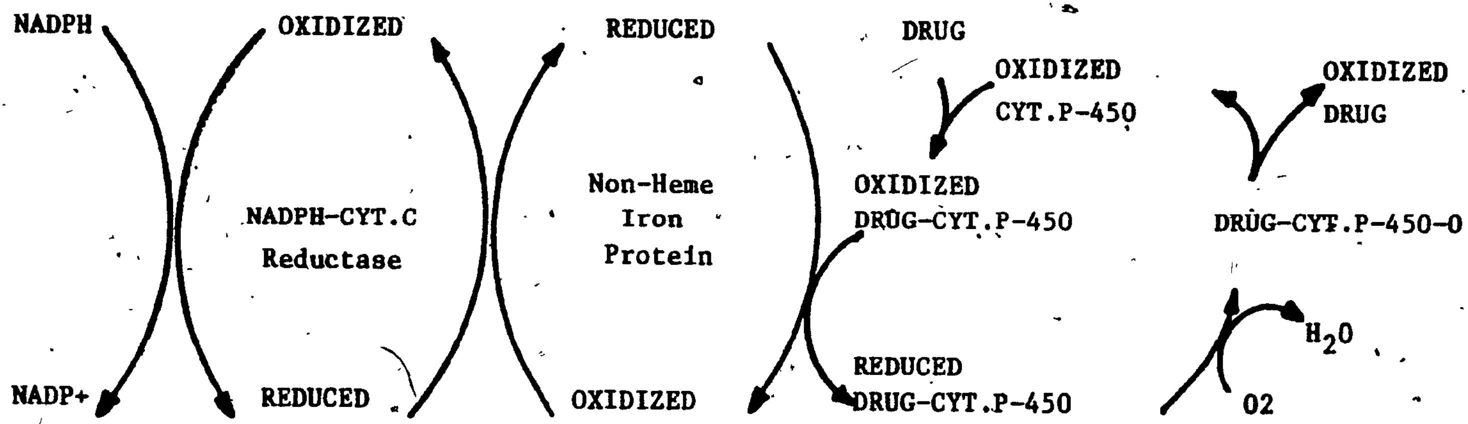
Some measure of protection against the toxicity of many pesticides is afforded mammals through endogenous detoxification mechanisms. The mammalian liver is the major site of metabolism of most drugs and chemicals, including pesticides, entering the body although other tissues such as kidney, lung, plasma, skin, nervous tissue and gut may play a role in the metabolism of some xenobiotics (56). The liver is ideally situated anatomically for its detoxification role in that ingested chemicals must pass first through the liver in portal venous blood before entering general circulation (56, 77). The essential action of hepatic drug metabolism occurs through a variety of reactions including oxidation, reduction and hydrolysis (75). The rate of these reactions is a governing factor in the rate of

toxicant elimination from the body (56). Oxidative reactions are quantitatively the most important (56) and they are generally catalyzed by hepatic microsomal enzymes located on the smooth endoplasmic reticulum (75). Reduction and hydrolysis reactions are catalyzed by both microsomal and non-microsomal enzymes (75). The oxidative drug metabolizing enzyme system is composed of cytochrome P-450 and two NADH or NADPH-dependent flavoproteins (44, 75) which together act to convert molecular oxygen to an "active" form which can be incorporated into a lipophilic molecule thereby increasing its polarity and water solubility. The involvement of these components in substrate oxidation is illustrated in Figure 1. Cytochrome P-450 has been shown to exist in multiple forms, each having selective but not specific substrate requirements (56). These various forms provide the animal with a mixed function oxidase (MFO) enzyme system capable of utilizing many substrates which are lipophilic and capable of penetrating into the endoplasmic reticulum (75). Since most metabolic intermediates are not MFO substrates by virtue of being polar compounds unable to penetrate into the endoplasmic reticulum (75), the MFO enzyme system is largely held in reserve for exogenous substrates including pesticides.

1.2.2 Toxicity and metabolism of parathion

Parathion (O,O-diethyl-O-p-nitrophenyl phosphorothionic acid) is an organophosphate pesticide which was widely used in North America for insect control by aerial spraying before being banned in the 1970s because of its high toxicity to mammals. Chemicals within the phos-

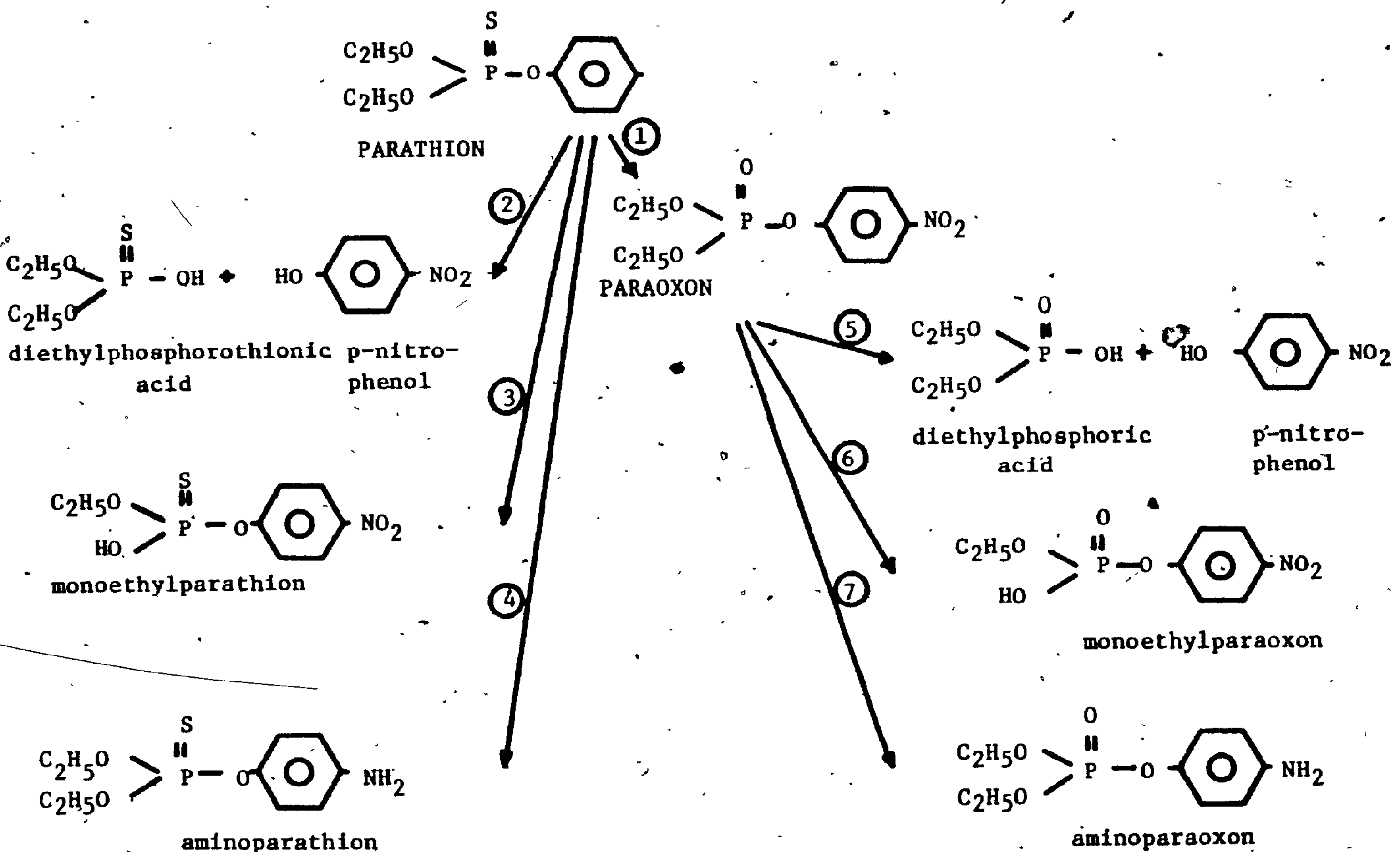
FIGURE 1 MAJOR COMPONENTS OF THE OXIDATIVE DRUG - METABOLIZING ENZYME SYSTEM IN MAMMALS (FROM REFERENCE 75)



phorothionic acid class of pesticides, including parathion, are exceptional in that they are relatively non-toxic in themselves, but are metabolized by insect and mammlian enzyme systems to oxygen analogs which are potent cholinesterase inhibitors (9, 15).

The metabolism of parathion by mammalian hepatic enzyme systems is complex (Figure 2). Parathion is converted to its toxic oxygen analog, paraoxon, by hepatic microsomal enzymes requiring reduced flavoproteins and O_2 (reaction 1) (3, 7, 31). Parathion can also be metabolized through competing pathways directly to non-toxic products by dearylation to diethylphosphorothionic acid and p-nitrophenol (reaction 2), O-deethylation to monoethyl parathion (reaction 3), or nitroreduction to aminoparathion (reaction 4) (7, 30, 31). Reactions 2 and 3 are catalyzed by NADH or NADPH-dependent microsomal fractions (7, 31) and reaction 4 by soluble and microsomal enzymes (30, 38). Paraoxon produced by reaction 1 can also undergo subsequent reactions resulting in detoxification. Paraoxon can undergo dearylation to diethylphosphoric acid and p-nitrophenol (reaction 5) by a NADPH-independent microsomal esterase (paraoxonase) or NADPH-dependent oxidases, O-deethylation to monoethyl paraoxon (reaction 6) by microsomal enzymes, and nitroreduction to aminoparaoxon (reaction 7) by hepatic soluble fractions (7, 15, 29, 30, 31, 47).

FIGURE 2 METABOLISM OF PARATHION BY MAMMALIAN HEPATIC ENZYME SYSTEMS



Although the liver is regarded as the most active organ in toxification and detoxification of parathion (9,15), other organs may participate in its metabolism. Parathion conversion to paraoxon has been reported in kidney, ileum, lung and brain but not in heart muscle, spleen, pancreas, serum, ovaries or skeletal muscle (34). Paraoxonase activity has been found in plasma, but not in brain, kidney or lung (15, 34, 48) and blood plasma and liver cytoplasmic proteins have been shown to inactivate low levels of paraoxon by non-enzymatic binding (29, 46, 54). The full toxicological significance of these extra-hepatic pathways of metabolism is uncertain.

The toxicity of parathion to animals has been shown to vary with factors such as age and sex. Young animals are more susceptible to parathion than mature animals since the affinity of microsomal detoxification enzymes for substrates is lower in the young than in the mature animal (9, 40). Males are less susceptible to parathion than females because males have higher levels of liver microsomal enzymes catalyzing a higher rate of detoxification (16, 17).

1.3 Alteration of pesticide toxicity and metabolism

1.3.1 Induction of the mixed function oxidase system

The hepatic mixed function oxidase enzyme system is subject to induction by a variety of chemicals including many chlorinated hydrocarbons (75). Enzyme induction has been observed following both acute and chronic exposure to inducers (5) and is believed to be associated with a proliferation of hepatic smooth endoplasmic reticulum (SER)

(2, 51). Enzyme inducers are lipid soluble and this property may allow them to mimic endogenous lipophilic substances which normally control synthesis of SER through specific RNA synthesis (2). All MFO inducers do not stimulate the same enzyme activities. Some, such as pentobarbital, are general while others, such as the polycylic hydrocarbons, are more specific (10). Their inducing capacity has been shown to correlate with storage in the body (5), and the degree of halogenation in the case of hydrocarbons (46) and the duration of induction generally continues until the inducing agent is eliminated from the body (75). These variables make it extremely difficult to predict the induction potential of chemicals and the effect of induction on the metabolism of pesticides.

1.3.2 Alteration of parathion toxicity and metabolism by MFO inducers

Chlorinated hydrocarbons have the largest potential as MFO inducers among the pesticides because of their extreme persistence in the environment and accumulation in wildlife and man (88). Many organochlorines have been found to decrease the toxicity of parathion from 1 to 6 fold (9, 13, 46, 48, 51, 54, 64). However, the exact mechanism by which inducer protection is afforded is not known (48, 51).

Early work concentrated on alterations in hepatic metabolism as being the major mechanism of protection. However, a frequent lack of correlation between changes in hepatic enzyme activities and toxicity were observed (4, 18, 31, 50, 62). In addition, several

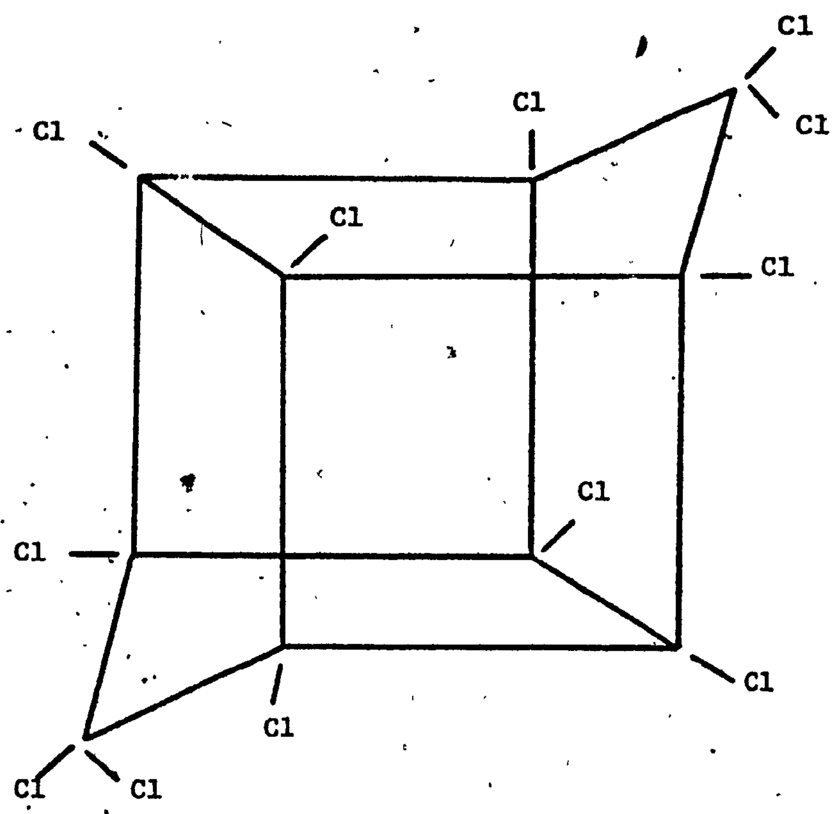
investigators have reported that the rate of paraoxon formation was increased with inducing agents (18, 31, 35, 51, 52, 62), in some cases more than the rate of parathion and/or paraoxon detoxification (31, 52). In a recent study it has been reported that paraoxon detoxification *in vivo* is unaltered by inducers (48). These findings suggest that alterations in hepatic metabolism may not be the only mechanism by which protection is provided (51). Extra-hepatic detoxification has also been implicated in protection against parathion (15, 48, 53). However, paraoxonase levels in plasma and other tissues is low (48, 51) and have not been observed to increase following organochlorine treatment (13, 46, 51). Increased paraoxon binding to plasma protein, resulting in decreased "free" paraoxon available to inactivate cholinesterases, has been suggested as another mechanism of protection against parathion (51, 53, 54). However, several investigators have reported that the expected decrease in paraoxon toxicity associated with increased paraoxon binding was not observed (46, 52).

In light of these conflicting reports, it may be that protection afforded by organochlorine inducers is mediated by more than one mechanism, or that the mechanism of protection varies with the inducer, given their known specificity in enzyme induction.

1.3.3 Mirex as an MFO inducer

Mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [c,d] pentalene) (Figure 3) is an organochlorine compound which was widely used to control fire ant populations in the southern United States and

FIGURE 3. CHEMICAL STRUCTURE OF MIREX
(FROM REFERENCE 1)



as a fire retardant in the plastic, paper and textile industries. Its use in the United States was banned in the 1970s because of its extreme persistence in the environment and its ability to accumulate in natural food chains. Mirex has been the subject of recent concern by environmentalists because of its contamination of the water and aquatic life of Lake Ontario (1).

Mirex has been described as the most potent of the known pesticide inducers (10) possibly because of its high degree of halogenation. It does not undergo any known biotransformation in mammals and insects (1, 10) and its half-life in the rat has been found to be in excess of 100 days (2). Subacute and chronic mirex administration has been found to increase liver size and weight through both stimulation of DNA synthesis leading to hyperplasia (2) and proliferation of the SER (2, 11). Dose dependent increases in MFO activity, including cytochrome P-450 levels, have been observed after as few as 2 weeks of mirex treatment (2, 11) and the higher dosage levels induced qualitative as well as quantitative changes in the cytochrome P-450 species found (49). The no-effect level for biochemical changes induced by mirex has been determined to be less than 1 ppm in chronic administration (2). In terms of its interaction with parathion metabolism, mirex has been found to increase dealkylation of parathion and paraoxon in vitro after as few as 14 days dietary administration at a dose level of 1 ppm (49). However, the effect of mirex treatment on competing pathways of parathion metabolism and on the acute toxicity of parathion have not been evaluated.

In view of the reported alterations in MFO activity and in vitro parathion and paraoxon detoxification resulting from mirex administration, it is reasonable to expect that mirex treatment may modify the acute toxicity of parathion. However, it is not possible to predict the magnitude of change which may be incurred nor the mechanisms responsible.

1.4 Purpose of this study

The present study was undertaken to determine the effect of subacute mirex administration on the acute inhalation toxicity of parathion in the male albino rat. The effect of mirex administration on biochemical, metabolic and physiological parameters capable of influencing parathion toxicity was investigated in an attempt to explain observed changes in parathion toxicity.

2.0 MATERIALS AND METHODS

2.1 Animals

Young adult male caesarian-derived Sprague-Dawley rats (Rattus norvegicus) were purchased from Charles River Canada Inc., (Canadian Breeding Farms and Laboratories Ltd.), St. Constant, Québec. Animals were housed individually in stainless steel mesh-bottomed cages and had ad libitum access to certified Purina Rodent Chow #5002 and fresh water via an automatic water sipper system. The housing environment was maintained at 22 $\pm$ 2°C, 50 $\pm$ 20% RH and a 12-hour light/12-hour dark photoperiod. All animals were acclimatized to the laboratory setting for a minimum of one week, and were used for experimentation at approximately 10-12 weeks of age.

2.2 Test substances

Mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [c,d] pentalene) was provided by Dr. Lawrence Kaminski, New York State Department of Health, Albany, New York.

Parathion (O,O-diethyl, O-p-nitrophenyl phosphorothionate) was supplied by the Monsanto Chemical Company, St. Louis, Missouri as technical grade (94.0% pure). High pressure liquid chromatographic analysis revealed the material to be essentially free of paraoxon.

2.3 Reagents

Other reagents used were purchased from the Sigma Chemical Company, St. Louis, Missouri, the Aldrich Chemical Company, Montreal, Quebec, or Canlab, Montreal, Quebec, and were of reagent grade quality or higher.

2.4 Mirex tolerance and MFO induction studies

2.4.1 Clinical observation, body and liver weights

Animals were treated by gavage with mirex dissolved in corn oil at dose levels of 7.5, 15, 30, 60 or 120 mg mirex/kg body weight/day for 4 days, for a total dose of 30, 60, 120, 240 or 480 mg mirex/kg body weight (dose volume - 5 mL/kg). A separate control group was treated with corn oil at a dose volume of 5 mL/kg body weight/day. Animals were examined daily for clinical signs of toxicity and body weight was measured before the first treatment and prior to sacrifice. Twenty-four hours after the last treatment, the animals were sacrificed by decapitation and the livers removed, weighed and frozen.

2.4.2 Hepatic microsomal enzyme activities

2.4.2.1 Hepatic microsome preparation

Livers from animals in each dose level were assayed for three microsomal mixed function oxidase enzymes and paraoxonase.

Hepatic microsomes were isolated by a modification of the method of Cinti (27). Liver was homogenized with ice-cold sucrose (0.25 M, pH 7.5) (1:4 w/w) in a Polytron homogenizer at half speed for 30 seconds. The homogenate was centrifuged in an IEC Model B20 refrigerated centrifuge at 600 g for 5 minutes to remove intact cells and debris and the supernatant was further centrifuged at 12,000 g for 10 minutes to sediment mitochondria. 16 mM $CaCl_2$, pH 7.5 (1:1 v/v) was added to the supernatant and the mixture was centrifuged at 27,000 g for 15 minutes. The pellet, consisting of Ca^{2+}-precipitated microsomes,

was washed by resuspending in 20 mL of phosphate buffer (0.067 M KH_2PO_4, pH 7.7) and resedimented at 27,000 g for 15 minutes. This microsomal pellet was resuspended in phosphate buffer for use in enzyme assays and its protein content was determined by the method of Lowry (74).

2.4.2.2 Cytochrome P-450 activity

Microsomal cytochrome P-450 was measured by the method of Omura and Sato (25). The difference spectra produced by the absorption of carbon monoxide-bound reduced cytochrome P-450 relative to non-CO-bound cytochrome P-450 was determined using a dual beam Unicam Model SP820 Series 2 spectrophotometer. The difference in absorption at 450 nm (due to cytochrome P-450) relative to 480 nm was measured with sample and reference cells both containing 5 mL of phosphate buffer, 1 mL of microsomal preparation and several crystals of sodium dithionite as reducing agent but with the sample cell medium saturated with carbon monoxide. Cytochrome P-450 was expressed as the change in absorption (450 nm-480 nm) per milligram of microsomal protein.

2.4.2.3 NADPH cytochrome c reductase activity

NADPH cytochrome c reductase was determined by a modification of the method of Masters (22). The rate of NADPH- dependent cytochrome c reduction was measured in a reaction mixture containing 1.5 mL of phosphate buffer, 0.4 mL of 0.36 mM cytochrome c, 0.4 mL of 1 mM NADPH and 0.05 mL of microsomal preparation. An identical reference mixture was used, except that NADPH was replaced with phosphate buffer. The

reaction was initiated by the addition of the NADPH and was followed at 550 nm during the linear portion of the reaction. NADPH cytochrome c reductase activity was expressed as units per milligram microsomal protein per minute where 1 unit is defined as an absorbance change of 1.0 per minute at 25°C.

2.4.2.4 NADH Cytochrome C Reductase activity

NADH cytochrome c reductase was assessed similar to NADPH cytochrome c reductase except that NADH replaced NADPH in the reaction mixture, and 0.01 mL of microsomal preparation was used instead of 0.05 mL.

2.4.2.5 Paraoxonase activity

Microsomal paraoxonase activity was measured spectrophotometrically by the method of Main (14). Five mL of 0.1 mM paraoxon in phosphate buffer was incubated at 37°C for 15 minutes with 0.4 mL of microsomal protein from liver. The reaction was stopped by the addition of 4.6 mL of ethanol reagent. The mixture was adjusted to pH 9.2 and the absorbance read at 400 nm against a reference solution containing buffer in place of substrate. Non-enzymatic hydrolysis of paraoxon was measured in a mixture containing substrate without protein preparation and was subtracted from enzymatic hydrolysis. p-Nitrophenol produced was quantified by comparing the absorbance values of the reaction media with values of known concentrations of p-nitrophenol, and was expressed as umoles per mg protein per 15 minutes.

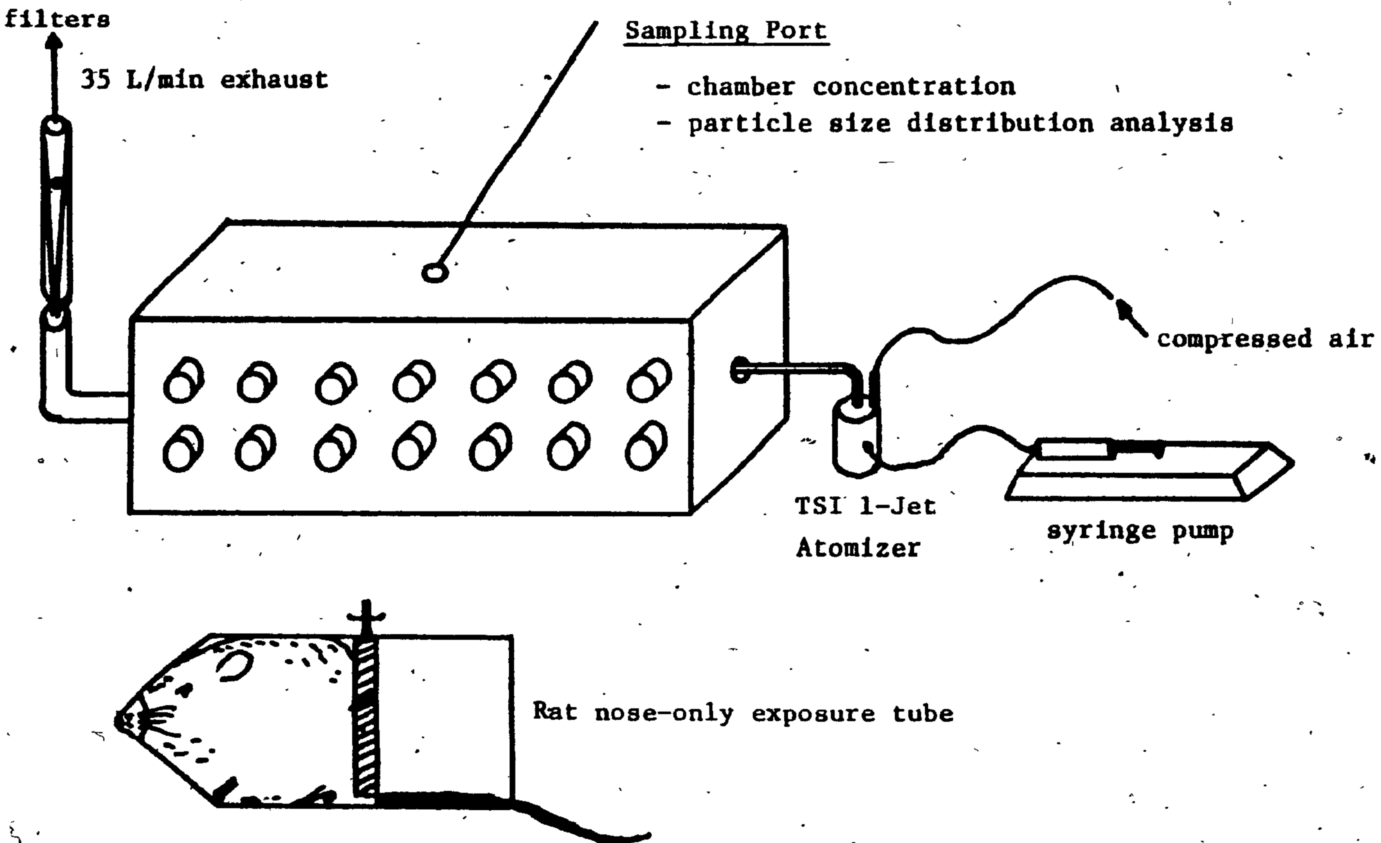
2.5 Parathion LC50 evaluation in control and mirex-treated animals

One hundred and forty male rats were randomly assigned to 2 subsets, each consisting of 7 groups of 10 rats per group. Subset 1 (Groups No. 1, 2, 3, 4, 5, 6 and 7) was treated by gavage with corn oil (5 mL/kg/day) daily for 4 days. Subset 2 (Groups no. 10, 20, 30, 40, 50, 60 and 70) was treated by gavage with mirex (15 mg/kg/day) in corn oil daily for 4 days. The start of treatment of successive groups within each subset was offset by one day so that the large number of inhalation exposures scheduled to follow the last gavage treatment could be performed at the correct time relative to the end of gavage treatment. Twenty-four hours after the last gavage treatment, each group was exposed to an aerosol of parathion via the nose-only inhalation route. Of the 7 groups in each subset, only 6 were required to complete the parathion LC50 evaluations. The remaining group from each subset was used for other studies.

2.5.1 Description of inhalation exposure equipment

Several rectangular nose-only rodent exposure chambers (volume equal to 60 L) were utilized (Figure 4). Each chamber was constructed so that up to 32 restraint cones could be inserted into the sides along the long axis. During treatment, rats were restrained individually in 3-inch diameter plexiglas tubes with a tapered, open end through which the animal's nares protruded into the chamber. Thus, the animals were permitted to breath the atmosphere within the chamber without otherwise being exposed to parathion, such as via the oral or percutaneous routes.

FIGURE 4 INHALATION EXPOSURE EQUIPMENT



The chamber was operated dynamically at an airflow of 35 liters/minute and under a slight negative pressure with respect to atmosphere. Decontamination of the chamber air was accomplished by passing the air through a glass fiber filter trap and a charcoal filter.

2.5.2 Generation of the Parathion Atmosphere

An aerosol of parathion was generated using a TSI Inc. Single-Jet Constant Output Atomizer supplied with predried compressed air. Parathion was delivered to the atomizer using a Sage syringe pump. The aerosolized parathion was delivered horizontally into one end of the inhalation chamber and the chamber was exhausted via the opposite end. The various chamber concentrations of parathion were achieved by adjusting the rate of parathion delivery to the atomizer.

2.5.3 Exposure procedure

All groups of rats from subsets 1 and 2 were treated with an aerosol of parathion for a single period of 4 hours. At the end of this time, the aerosol generator was turned off and the chamber allowed to equilibrate with room air for 15 minutes prior to removal of the animals. An air control group from each subset was restrained and handled identically to the parathion-treated groups, except that uncontaminated room air only was allowed to flow through the chamber.

2.5.4 Measurement of chamber concentration and particle size distribution

Chamber concentrations of parathion aerosol were determined gravimetrically by collecting chamber air samples on high efficiency glass fiber filters hourly during exposure. The time-weighted average chamber concentration for each 4-hour exposure period was calculated.

The parathion aerosol particle size distribution was measured in preliminary work by withdrawing a sample of chamber atmosphere at a rate of 28.4 L/min through a 9-stage Andersen cascade impactor. The mass collected on each stage (% of total collected) was calculated and the cumulative percent on the various stages was plotted against the cutoff diameter (um) of the various stages using log-probability graph paper. The mass median particle diameter (um) was read from the line of best fit and its geometric standard deviation was calculated as the square root of the 84% size divided by the 16% size.

2.5.5 Observations and calculations

Following inhalation exposure to parathion, all animals were observed daily until symptoms of cholinesterase inhibition ceased. Percent mortality of the groups within each subset was plotted against parathion dose level (mg/L) and the LC50 value and its 95% confidence interval were calculated separately for the corn oil control and mirex-treated subsets by the method of Litchfield and Wilcoxon (55). Only one 0% or 100% mortality dose level was used in the calculations. The two dose-mortality curves were tested for parallelism and the toxicity of parathion to mirex-treated animals relative to corn oil controls was calculated by the method of Litchfield and Wilcoxon (55). All calculations were performed using the computer programs of Tallorida and Murray (36).

2.6 Microsomal metabolism of parathion and paraoxon

Parathion and paraoxon stock solutions (10 mM) were prepared by dissolving 29.1 mg of parathion or 27.5 mg of paraoxon in 2 mL of absolute ethanol and diluting with phosphate buffer to a volume of 10 mL. One mL of this 10 mM stock solution was diluted to 100 mL with phosphate buffer to prepare the 0.1 mM substrates used.

0.6 umoles of parathion or paraoxon (i.e., 6 mL of 0.1 mM substrate) were incubated at 37°C with 1 mL of microsomal preparation (prepared as described earlier, except that the liver was homogenized with phosphate buffer in place of 0.25 M sucrose) and 0.8 mL of phosphate buffer, 0.8 mL of 1 mM NADH or 0.8 mL of 1 mM NADPH. The reaction was stopped at 15 minutes by the addition of 5 mL of ethanol reagent (prepared by adding 6.25 mL of 2N NH_3OH to 400 mL of 95% ethanol and diluting to 500 mL with water). The mixture was cooled to 4°C, centrifuged for 10 minutes at 2000 g to sediment precipitated protein and assayed for parathion, paraoxon and p-nitrophenol content by high pressure liquid chromatography. A Waters Associates High Pressure Pump (Model M45), WISP Sample Programmer (Model 710B) and UV/Visible Variable Wavelength Detector (Model 480) or Perkin Elmer Model LC75 UV/Visible Detector and a Hewlett Packard Model 3380A Recording Integrator were used. Aliquots of the reaction media were injected onto a Waters C_{18} Radial Pak A Reverse Phase column, eluted by pumping an acetonitrile: water mobile phase through the column at a flow rate of 1 to 2 mL per minute into the detector set at an analytical wavelength of 274 nm. The proportion of acetonitrile to

water in the mobile phase varied from 40:60 to 60:40 depending on the separation required for quantitation of the components of interest. Parathion, paraoxon and p-nitrophenol in the reaction media were identified and quantified by comparing column retention times and peak areas, respectively, with retention times and peak areas of known concentrations of parathion, paraoxon and p-nitrophenol.

Parathion metabolism by microsomes was determined by measuring parathion disappearance from the reaction medium. This metabolism was attributed to activation to paraoxon (reaction 1 - Figure 2) and detoxification (reactions 2, 3 and 4 - Figure 2) including non-enzymatic binding to protein. Parathion conversion to paraoxon was determined by measuring paraoxon production in the reaction medium (reaction 1 - Figure 2). Paraoxon metabolism was determined by measuring paraoxon disappearance from the reaction medium and was attributed to detoxification (reactions 5, 6 and 7 - Figure 2) including non-enzymatic binding to protein.

Background hydrolysis of parathion and paraoxon under the conditions of assay was measured in blank solutions identical to the reaction media except without the microsomal protein and these values were subtracted from microsomal metabolism. The rates of substrate utilization and product formation by microsomal enzymes were expressed as umoles per mg microsomal protein per 15 minutes.

2.7 Endogenous substrate competition with parathion and paraoxon for metabolic enzymes

Livers from corn oil control and mirex-treated rats were homogenized in phosphate buffer (1:4 w/w) and the homogenate centrifuged at 1200 g for 10 minutes. The supernatant (1 mL) was incubated at 37°C with 0.6 umoles of parathion or paraoxon (6 mL of 0.1 mM substrate), and 0.8 mL of 1 mM NADPH. The reaction was stopped at 15 minutes with 5 mL of ethanol reagent. The mixture was cooled to 4°C, centrifuged to sediment precipitated protein and assayed for parathion, paraoxon and p-nitrophenol by HPLC. Substrate utilization and product formation were expressed as umoles per mL supernatant per 15 minutes.

2.8 Parathion uptake via the lung

Parathion uptake via the lung was determined indirectly by measuring respiratory rate. The respiratory rate of corn oil control and mirex-treated rats was measured by whole-body pletheismography. The restraint tube used for nose-only exposure was modified by inserting a 3-inch circle of rubber dental dam with a 1-inch center hole across the inside of the restraint tube, approximately 2 inches from the nose opening. A hole was drilled into the tubular section of the restraint tube and tapped to accomodate a hose nipple. A rat was placed in the cone with his head projecting through the center hole in the dental dam thereby creating a partial seal around his neck. The open end of the tube behind the rat was closed with a stopper and the hose nipple connected to a Statham Model P23 AC pressure transducer. Pressure changes inside the tube with respect to atmosphere caused by expansion and

contraction of the animal's thorax during respiration were detected by the pressure transducer, and amplified and recorded on a Grass Instruments Model 7 polygraph.

2.9 Lung metabolism of parathion and paraoxon

Parathion and paraoxon conversion to p-nitrophenol by soluble and microsomal fractions of lung and liver tissue were measured spectrophotometrically by the method of Main (14). Lung and liver microsomal proteins were prepared as described earlier. The supernatant remaining after the Ca^{2+} precipitation of the microsomal fraction was used as the soluble protein fraction.

Five mL of 0.1 mM parathion or 0.1 mM paraoxon was incubated at 37°C for 15 minutes with 0.2 mL of 1 mM NADPH or 1 mM NADH and 0.4 mL of soluble or microsomal fraction from lung or liver. The reaction was stopped by the addition of 4.4 mL of ethanol reagent. The mixture was adjusted to pH 9.2 and the absorbance read at 400 nm against a reference solution containing buffer in place of substrate. Non-enzymatic hydrolysis of parathion and paraoxon was measured in a mixture containing substrate without protein preparation and was subtracted from enzymatic hydrolysis. p-Nitrophenol produced was quantified by comparing the absorbance values of the reaction media with values of known concentrations of p-nitrophenol. Protein content in the soluble and microsomal fractions was measured by the method of Lowry (74). p-Nitrophenol production was expressed as umoles per mg protein per 15 minutes.

2.10 Plasma metabolism of parathion and paraoxon

Blood was collected using heparin as anticoagulent from the abdominal aorta of ether-anesthetized control and mirex-treated rats and centrifuged at 2000 g for 10 minutes to sediment red blood cells. Plasma (0.5 mL) was incubated at 37°C for 15 minutes with 0.1 umole of parathion or 0.6 umoles of paraoxon (1 mL and 6 mL of 0.1 mM substrates, respectively) and the reaction was stopped by the addition of ethanol reagent (volume the same as the substrate volume). The mixture was cooled to 4°C, centrifuged to sediment precipitated protein and assayed for parathion, paraoxon and p-nitrophenol content by HPLC as previously described. Substrate utilization and product formation were expressed as μmoles per mg protein per 15 minutes.

2.11 Liver histopathology

Livers from 6 control and 6 mirex-treated animals were fixed in neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically for pathological changes.

2.12 Hepatic blood flow

Bromosulfophthalein (BSP - 25 mg/mL in 0.9% NaCl solution) was injected into the lateral tail vein of control and mirex-treated rats at a dose volume of 1 mL/kg body weight. Blood samples were collected in heparin from the abdominal aorta of ether-anesthetized animals 10 minutes after injection and assayed for BSP content. Additional untreated animals were injected with BSP and were bled 4, 6 and 10 minutes later to determine the half-life of BSP in the naive rat. All

blood samples were processed as follows: Red blood cells were sedimented at 2000 g for 10 minutes and 1 mL of plasma was added to 4.5 mL of alkaline buffer (prepared by dissolving 12.92 g of Na_2HPO_4, 3.54 g of Na_3PO_4 and 6.4 g of sodium p-toluenesulfonate in 1 L of water and adjusting the pH to 10.6). Absorbance of the resulting purple colored solution was measured at 580 nm against water. 0.2 mL of 2 M NaH_2PO_4 was added to the solution to convert BSP to the colorless form, and the absorbance was read again. BSP content of the plasma was determined by comparing the difference in the above-measured absorbance values with the absorbance values of known concentrations of BSP in alkaline solution. BSP was expressed as % retention, where 100% retention was equivalent to 1.0 mg BSP/mL plasma (this theoretical value is based on 25 mg BSP having been injected into a 1 kg rat with a plasma volume of 25 mL).

2.13 *In vivo* levels of parathion and paraoxon in plasma and liver

Corn oil control and mirex-treated animals were exposed by inhalation to an aerosol of parathion until clinical signs of toxicity were observed (all animals were exposed to parathion at a concentration of approximately 0.15 mg/L for exactly 3 hours). At the end of exposure, blood samples were collected under anesthesia and the livers removed. All samples were kept at 4°C during processing to preclude enzymatic conversion of the components of interest. Blood was centrifuged at 1000 g to sediment RBCs and 3 mL of plasma was removed and washed with 1 mL of hexane to extract parathion and paraoxon. 5.0 g of liver was homogenized with 10 mL of distilled water and centrifuged at 12,000 g for 10 minutes to sediment debris and mitochondria and the supernatant

was washed with 1 mL of hexane. 200 uL of each plasma and liver hexane extract was evaporated to dryness, redissolved in 200 uL of acetonitrile: water (60:40) and assayed for parathion and paraoxon by HPLC. Tissue content is expressed as uM/mL plasma and uM/g liver.

2.14 Plasma and brain cholinesterases

Plasma and brain cholinesterases were measured by the method of Ellman and Courtney (23). Blood was collected in EDTA from ether-anesthetized corn oil control and mirex-treated rats and brains were removed following exsanguination of the animals. Blood was prepared for assay by centrifugation to sediment RBCs. One gram of brain tissue was homogenized with 11 mL of Sorensen phosphate buffer (pH 7.4) in a Polytron homogenizer. Plasma (0.05 mL) or brain homogenate (0.1 mL) was added to 0.1 mL of 0.01 M DTNB (5,5'-dithiobis-2-nitrobenzoic acid), 3.4 mL of Sorensen phosphate buffer and 0.4 mL of 0.01 M substrate (propionylthiocholine iodide was used as the substrate for plasma cholinesterase, and acetylthiocholine iodide was used as the substrate for brain cholinesterase). The linear portion of the reaction was followed for 1 minute at 412 nm in a temperature controlled (37°C) Unicam Model SP820 spectrophotometer against a blank containing the above components and buffer replacing the substrates. The difference in absorbance between the start and stop of the 1 minute time scan was compared with absorbance values of known concentrations of reduced glutathione, to determine the amount of substrate reduced. Plasma and brain cholinesterases were expressed as umoles of substrate reduced per mg plasma protein per minute and umoles of substrate reduced per gram brain tissue per minute, respectively.

3.0 RESULTS

3.1 Mirex tolerance and MFO induction

3.1.1 Clinical observations, body weight and liver weight changes

Rats were treated for 4 days with mirex at dose levels of 0, 7.5 15, 30, 60 and 120 mg/kg/day as per Materials and Methods (Section 2.4.1) to assess the rats tolerance to mirex.

All animals treated at 120 mg/kg/day and 1 of 3 rats treated at 60 mg/kg/day died. Clinical signs preceding death consisted of weakness, ataxia and loose feces containing bloody mucus, and gross pathological examination revealed dark, discolored livers with white foci on all lobes. Slightly loose feces was observed in animals treated with 30 mg/kg/day and no clinical signs of toxicity were observed at lower dose levels.

Slight body weight loss (generally less than 10%) was observed in some animals of the 15 mg/kg/day and 30 mg/kg/day dose groups and greater weight loss was seen in the 60 mg/kg/day dose group (Table 1).

Absolute and relative liver weights tended to increase with dose level (Table 1 and Figure 5) and significant increases in both parameters compared to controls were seen at the 15, 30 and 60 mg/kg/day dose levels. (A comparison of each treated group to the control was performed using Student's t-test only when an analysis of variance revealed a significant F value.)

TABLE I — EFFECT OF INCREASING MIREX DOSAGE ON LIVER WEIGHTS

Total Mirex Dose	Animal No.	Body Weight (g) Initial	Body Weight (g) Presacrifice	Liver Weight Absolute (g)	Liver Weight Relative (g/kg)
Corn Oil Control	5001	287	291	10.6	36.4
	5002	286	284	11.1	39.1
	5003	280	286	9.1	31.8
	5017	322	330	13.3	40.3
	5018	222	229	10.9	36.5
Mean		297.4	284.0	11.0	36.8
± S.D.		36.09	36.03	1.51	3.27
Mirex 7.5 mg/kg/day	5016	300	318	15.2	47.8
Mean		300	318.0	15.2	47.8
± S.D.		0	0	0	0
Mirex 15 mg/kg/day	5014	317	320	20.3	63.4
	5015	310	287	21.9	76.3
Mean		313.5	303.5	21.1[B]	69.9[B]
± S.D.		4.95	23.33	1.13	9.12

[B] $P < .01$

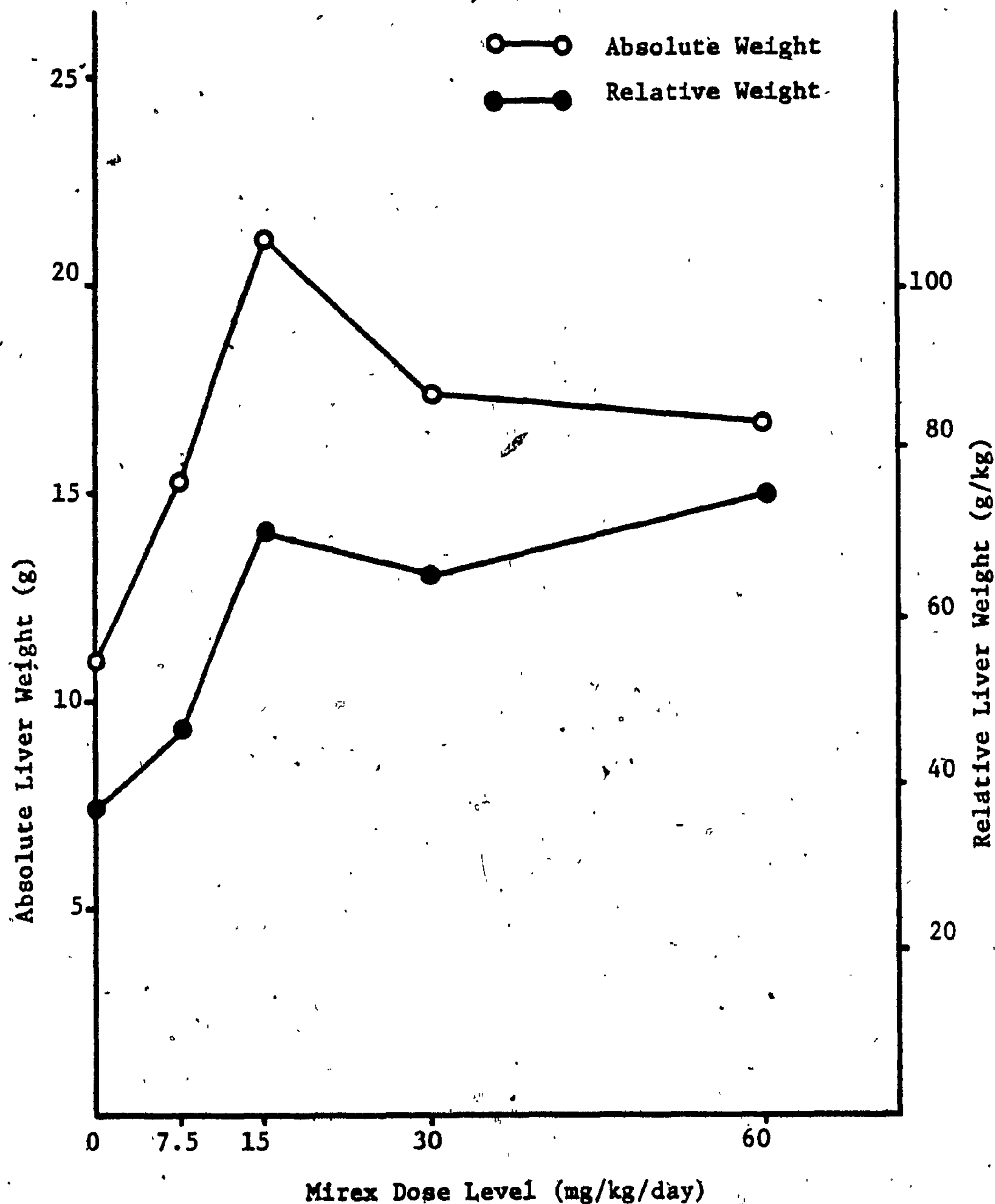
TABLE 1 (CONT'D)

EFFECT OF INCREASING MIREX DOSAGE ON LIVER WEIGHTS

Total Mirex Dose	Animal No.	Body Weight (g) Initial	Body Weight (g) Presacrifice	Liver Weight Absolute (g)	Liver Weight Relative (g/kg)
Mirex 30 mg/kg/day	5004	264	241	16.4	68.0
	5005	312	290	19.6	67.6
	5006	292	218	12.4	56.9
	5013	290	300	20.0	66.7
Mean		289.5	262.3	17.1[B]	64.8[B]
± S.D.		19.69	39.18	3.52	5.29
Mirex 60 mg/kg/day	5007	285	259	21.4	82.6
	5008	254	Died	-	-
	5009	277	177	11.9	67.2
Mean		272.0	218.0	16.7	74.9[B]
± S.D.		16.09	57.98	6.72	10.89
Mirex 120 mg/kg/day	5010	282	Died	-	-
	5011	315	Died	-	-
	5012	310	Died	-	-

B $P < .01$

FIGURE 5 EFFECT OF INCREASING MIREX DOSAGE ON LIVER WEIGHTS



These findings indicate that mirex is well tolerated by the rat in a subacute dosing regime at levels of up to 30 mg/kg/day and that dose levels of 15 mg/kg/day and above induce an increase in liver weight. Consequently, a dosing regime of 15 mg mirex/kg/day for 4 days (i.e., 60 mg total dose) was selected for a more detailed examination of body and liver weight changes (Table 2). Reduced body weight gain was observed in treated animals compared to controls by the second day of treatment and weight loss averaging about 5% was observed by the fifth day. Absolute and relative liver weights both increased significantly compared to controls ($p < .01$), and the magnitude of increase was approximately 30% and 48%, respectively. The larger percentage increase in relative liver weight was due in part to a body weight loss in treated animals.

3.1.2 Microsomal enzyme activities

Livers of animals treated with mirex at dose levels of 0, 7.5, 15, 30 and 60 mg/kg/day were assayed for activity of several MFO enzymes according to Materials and Methods (Sections 2.4.2 to 2.4.5).

3.1.2.1 Cytochrome P-450 activity

Cytochrome P-450 activity increased at all dose levels compared to controls and significant increases ($p < .01$) were seen at the 15, 30 and 60 mg/kg/day dose levels (Table 3, Figure 6). The magnitude of increase ranged from 82-123%.

TABLE 2

EFFECT OF MIREX TREATMENT ON BODY AND LIVER WEIGHTS

Treatment		Animal No.	Body Weight (g) Day 1	Day 2	Day 3	Day 4	Sacrifice	Absolute Liver Weight (g)	Relative Liver Weight (g/kg)
Corn Oil Control		101	330	333	337	338	335	14.04	41.91
		102	334	337	340	342	348	12.13	34.86
		103	286	287	290	289	294	10.48	35.54
		104	328	328	331	331	335	13.15	39.25
		105	299	300	301	302	311	10.84	34.86
		106	312	317	317	324	329	10.66	32.40
		107	329	333	336	339	341	12.16	35.66
		108	319	320	323	327	328	12.38	37.74
		109	285	291	294	297	308	10.45	33.93
		110	320	324	332	331	338	13.81	40.86
	Mean		314.2	317.0	320.1	322.0	326.7	12.01	36.70
	± S.D.		18.23	18.12	18.75	19.00	16.98	1.366	3.116
Mirex 15 mg/kg/day		201	273	276	266	254	251	13.78	54.90
		202	304	302	291	276	268	15.80	58.96
		203	314	319	319	318	308	15.21	49.38
		204	326	331	315	307	306	16.77	54.80
		205	315	315	318	313	306	15.99	52.25
		206	319	316	304	294	291	17.78	61.10
		207	276	279	279	278	283	13.38	49.01
		208	287	287	276	266	273	15.34	56.19
		209	324	326	316	301	313	16.70	53.34
		210	309	306	311	310	313	17.27	55.18
	Mean		304.7	305.7	299.5	291.7	291.2	15.80[B]	54.51[B]
	± S.D.		19.39	19.38	19.87	21.91	21.66	1.428	3.797

[B] $P < .01$

TABLE 3

EFFECT OF INCREASING MIREX DOSAGE ON MICROSOMAL ENZYME ACTIVITIES

Mirex Dose	Animal No.	Cytochrome P-450 Activity (ΔO.D./mg Protein)	NADPH Cytochrome C Reductase (Units/mg prot./min)	NADH Cytochrome C Reductase (Units/mg prot./min)	Paraoxonase (mMpNP/mg prot./15 min)
Corn Oil	5001	0.0091	0.751	5.000	0.0039
Control	5002	0.0048	0.968	7.419	-
	5003	0.0089	1.250	8.600	0.0022
	5017	0.0074	0.735	5.735	0.0015
	5018	0.0088	0.765	4.706	0.0007
Mean		0.0078	0.894	6.292	0.0021
± S.D.		0.00181	0.2206	1.6652	0.00136
Mirex 7.5 mg/kg/day	5016	0.0161	1.331	4.000	0.0010
Mean		0.0161	1.331	4.000	0.0010
± S.D.		0	0	0	0
Mirex	5014	0.0156	1.438	3.906	0.0023
15 mg/kg/day	5015	0.0128	1.222	4.000	0.0028
Mean		0.0142[B]	1.330	3.953	0.0025
± S.D.		0.00198	0.1527	0.0665	0.0035

B $P < .01$

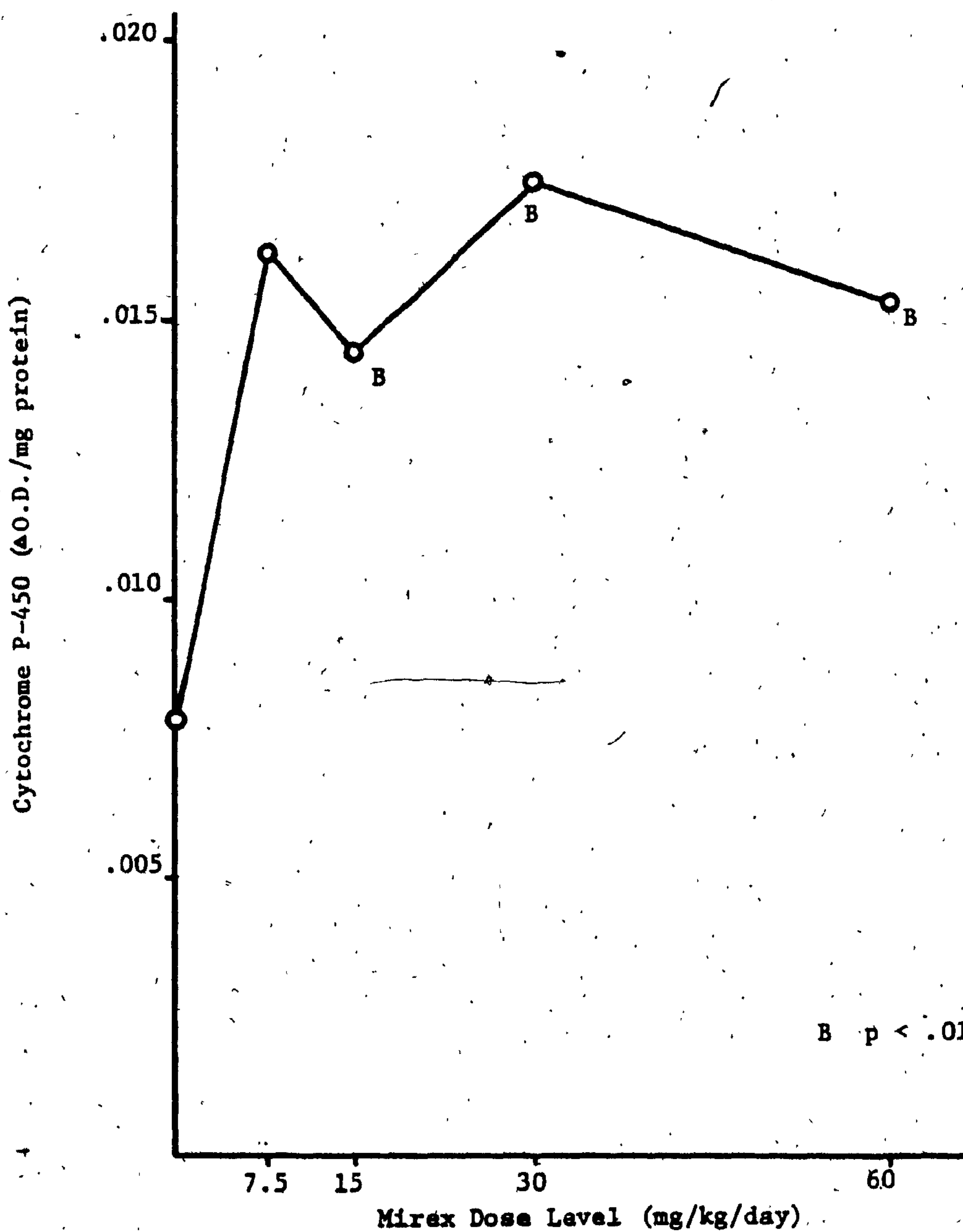
TABLE 3
(CONT'D)

EFFECT OF INCREASING MIREX DOSAGE
ON MICROSOMAL ENZYME ACTIVITIES

Mirex Dose	Animal No.	Cytochrome P-450 Activity (Δ O.D./mg Protein)	NADPH Cytochrome C Reductase (Units/mg prot./min)	NADH Cytochrome C Reductase (Units/mg prot./min)	Paraoxonase (mMpNP/mg prot./15 min)
Mirex	5004	0.0163	1.442	2.692	0.0024
30 mg/kg/day	5005	0.0145	1.452	2.581	0.0032
	5006	0.0208	1.415	2.075	-
	5013	0.0180	1.014	2.321	-
Mean		0.0174^B	1.331^A	2.417^B	0.0031
± S.D.		0.00268	0.2117	0.2761	0.00070
Mirex	5007	0.0153	1.667	5.278	0.0032
60 mg/kg/day	5009	0.0151	1.512	1.860	0.0035
Mean		0.0152^B	1.590^B	3.569	0.0034
± S.D.		0.00014	0.1096	2.4169	0.00021

A $P < .05$
B $P < .01$

FIGURE 6 EFFECT OF INCREASING MIREX DOSAGE ON CYTOCHROME P-450 ACTIVITY



3.1.2.2 NADPH cytochrome c reductase activity

NADPH cytochrome c reductase activity increased at all dose levels compared to controls and significant increases were observed at the 30 and 60 mg/kg/day dose levels ($p < .05$, $p < .01$, respectively) (Table 3, Figure 7). The magnitude of increase ranged from 49 to 78%.

3.1.2.3 NADH cytochrome c reductase activity

NADH cytochrome c reductase activity decreased at all dose levels compared to controls and a significant decrease ($p < .01$) was observed at 15 mg/kg/day dose level (Table 3, Figure 8). The magnitude of decrease ranged from 36 to 43%.

3.1.2.4 Paraoxonase activity

Microsomal paraoxonase activity increased slightly with mirex treatment (Table 3 and Figure 9), however, no significant changes were observed compared to controls, possibly because of the small number of assays performed.

Subsequent studies were performed with animals treated at a dose level of 15 mg/kg/day for 4 days (60 mg total dose) because significant changes were observed in liver weight and microsomal enzyme activities following this dosage regime.

3.2 Parathion LC50 evaluation in control and mirex-treated animals

An inhalation LC50 evaluation was performed in control and mirex-treated animals as previously described (Section 2.5).

Particle size distribution analysis of the chamber atmosphere revealed the parathion aerosol to have an aerodynamic equivalent mass

FIGURE 7 EFFECT OF INCREASING MIREX DOSAGE ON NADPH CYTOCHROME C REDUCTASE ACTIVITY

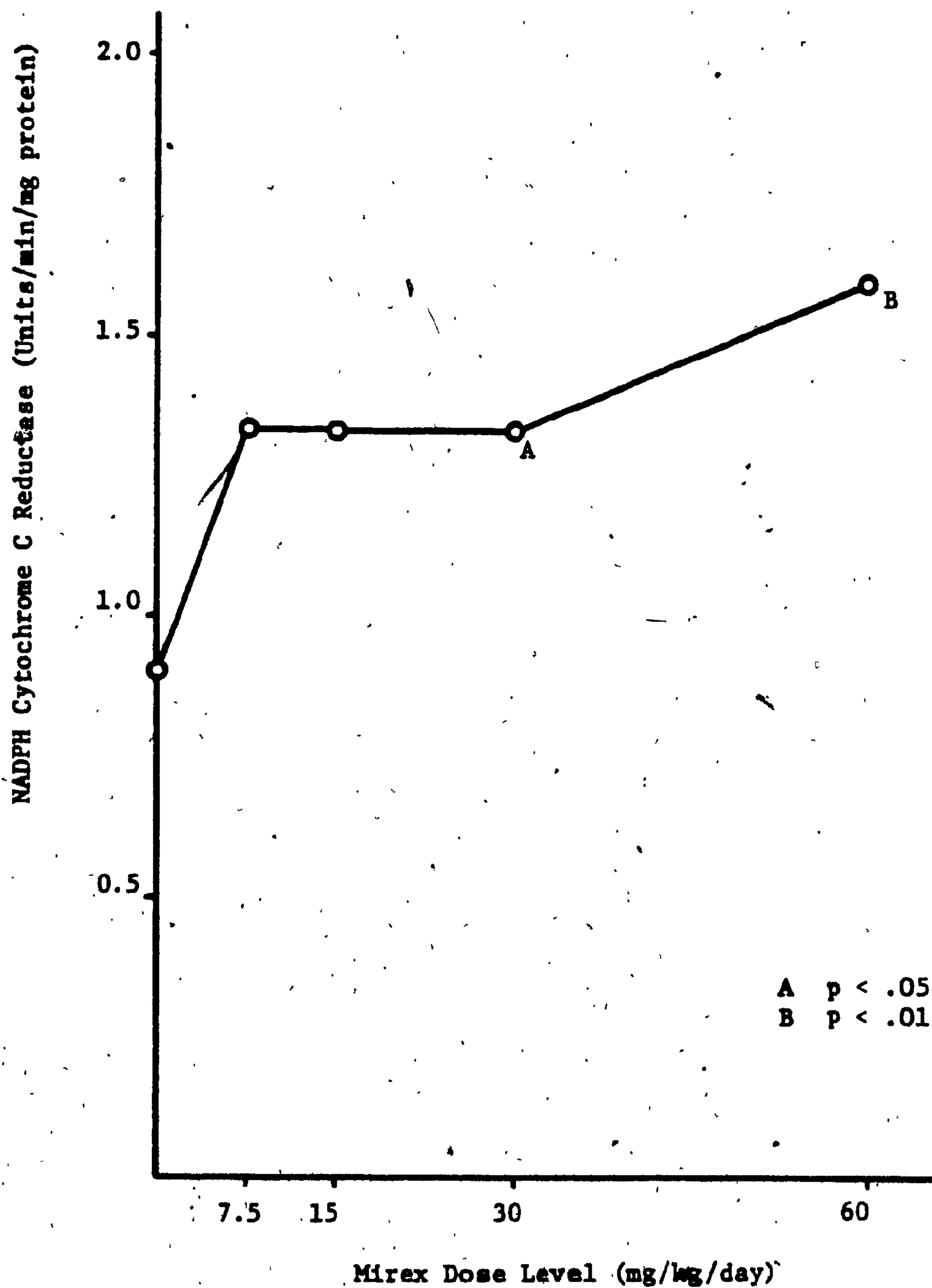


FIGURE 8 EFFECT OF INCREASING MIREX DOSAGE ON NADH CYTOCHROME C REDUCTASE ACTIVITY

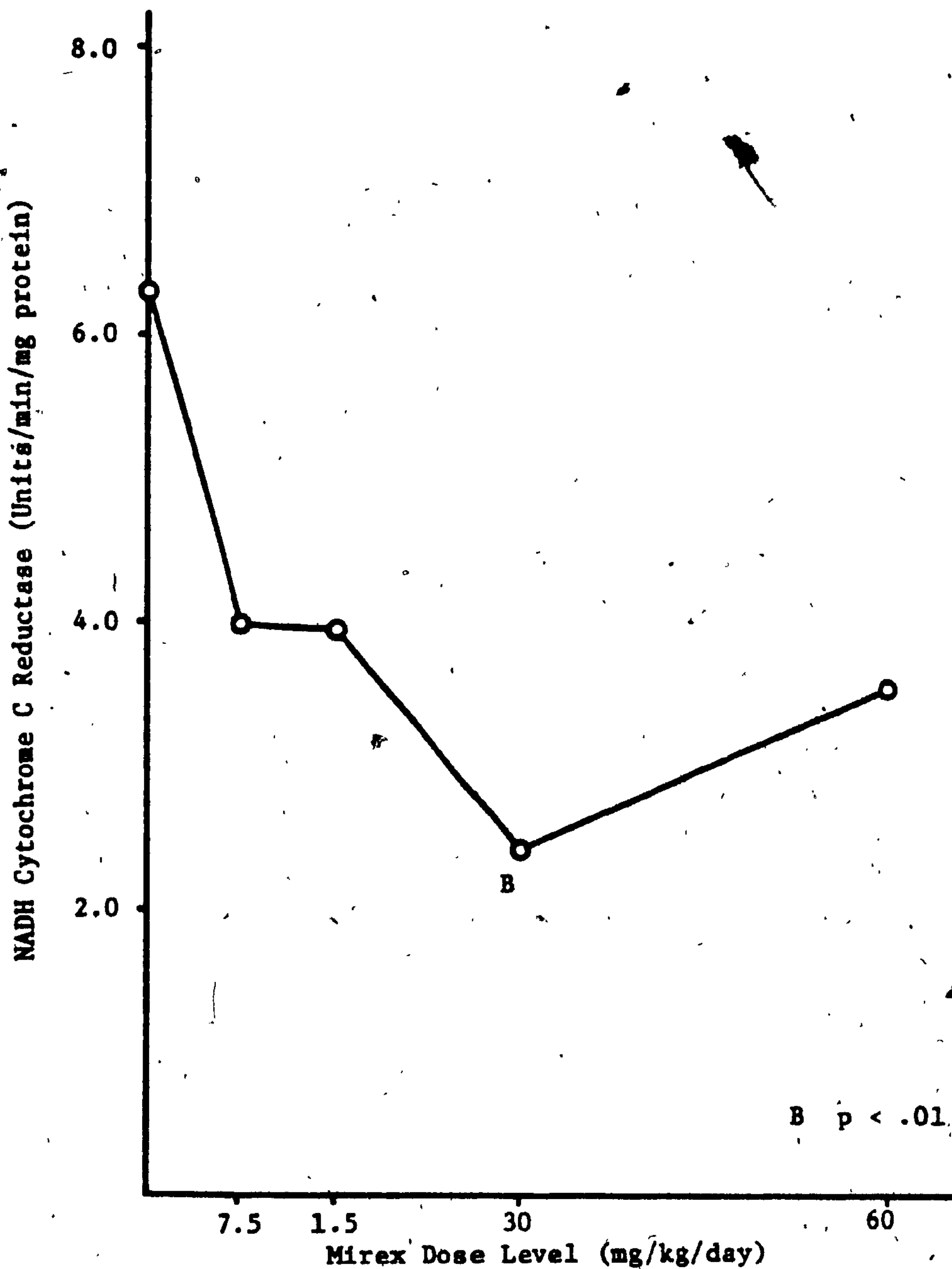
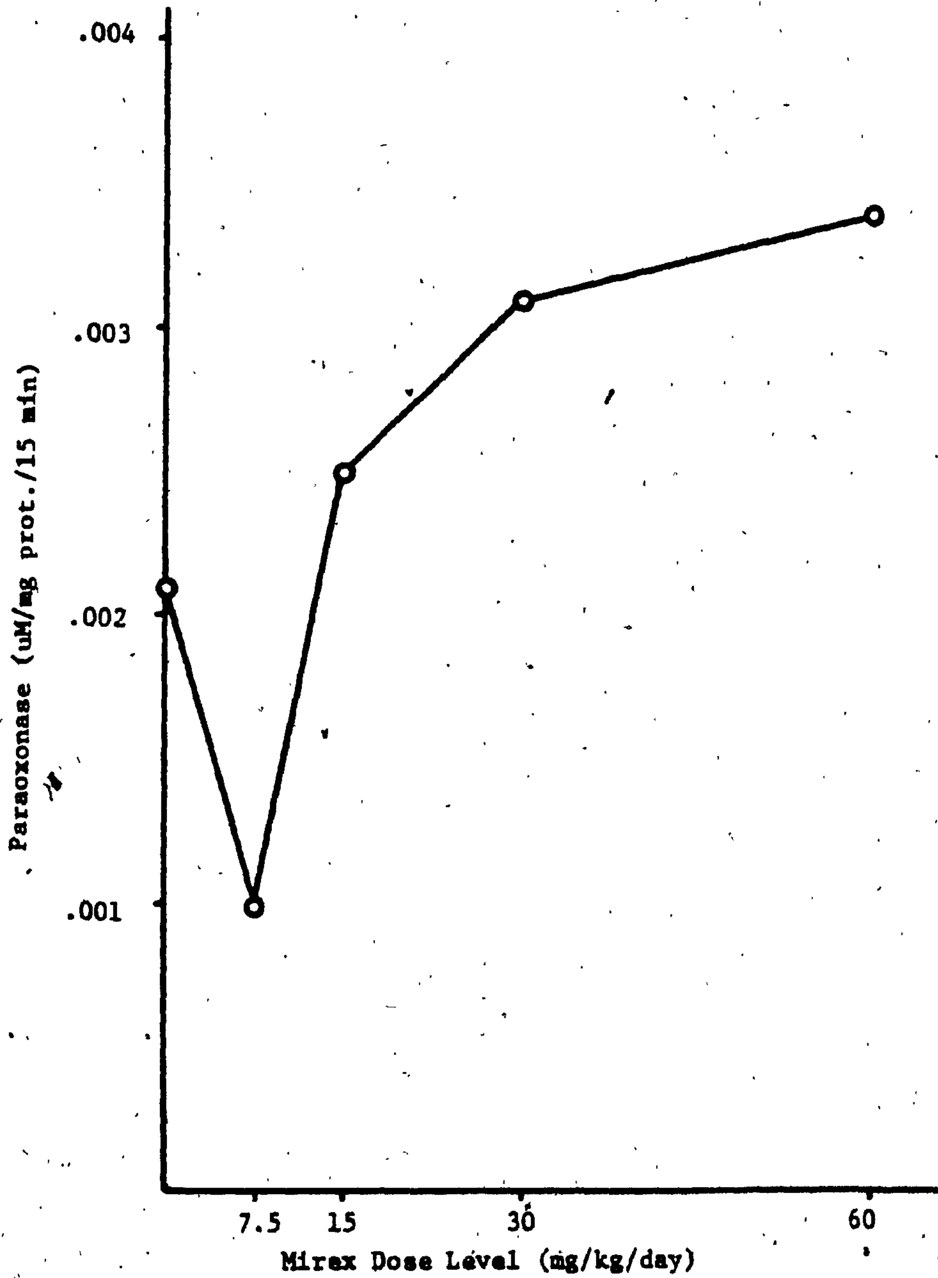


FIGURE 9 EFFECT OF INCREASING MIREX DOSAGE ON PARAOXONASE ACTIVITY



median diameter of 1.9 microns with a geometric standard deviation of 1.73 (Figure 10). This size distribution analysis also demonstrated that greater than 84% of the aerosol mass was smaller than 3.3 microns in diameter and therefore capable of penetrating to the secondary bronchi and lower levels of the lungs (89).

Mortality following parathion exposure generally increased with dose level and occurred within 2 days of exposure (Table 4). Two mortalities were observed in mirex-treated groups at 4 and 5 days after treatment. These latter deaths were considered a delayed effect of mirex treatment rather than due to parathion exposure because signs of cholinesterase inhibition in these rats had disappeared by day 2, and symptoms of acute mirex toxicity (i.e., muscular weakness, anemia and bloody mucus in the feces) were observed immediately before death. Consequently, these latter deaths were excluded from the LC50 calculation. Clinical signs observed in the majority of animals were typical of cholinesterase inhibition and included salivation, ataxia and muscle tremors. Animals surviving the parathion exposure had generally returned to normal by the second day after treatment. Parathion dose-mortality curves were constructed for the corn oil control and mirex-treated groups (Figures 11 and 12). The LC50 values and their 95% confidence intervals for the control and mirex-treated groups were calculated to be 0.128 mg/L (0.097, 0.168 mg/L) and 0.213 mg/L (0.201, 0.225 mg/L), respectively. The two dose mortality curves were found to be parallel (95% confidence) and the relative toxicity of parathion in mirex- treated animals compared to corn oil controls was found to be 0.60 (95% confidence).

FIGURE 10 PARATHION AEROSOL PARTICLE SIZE DISTRIBUTION

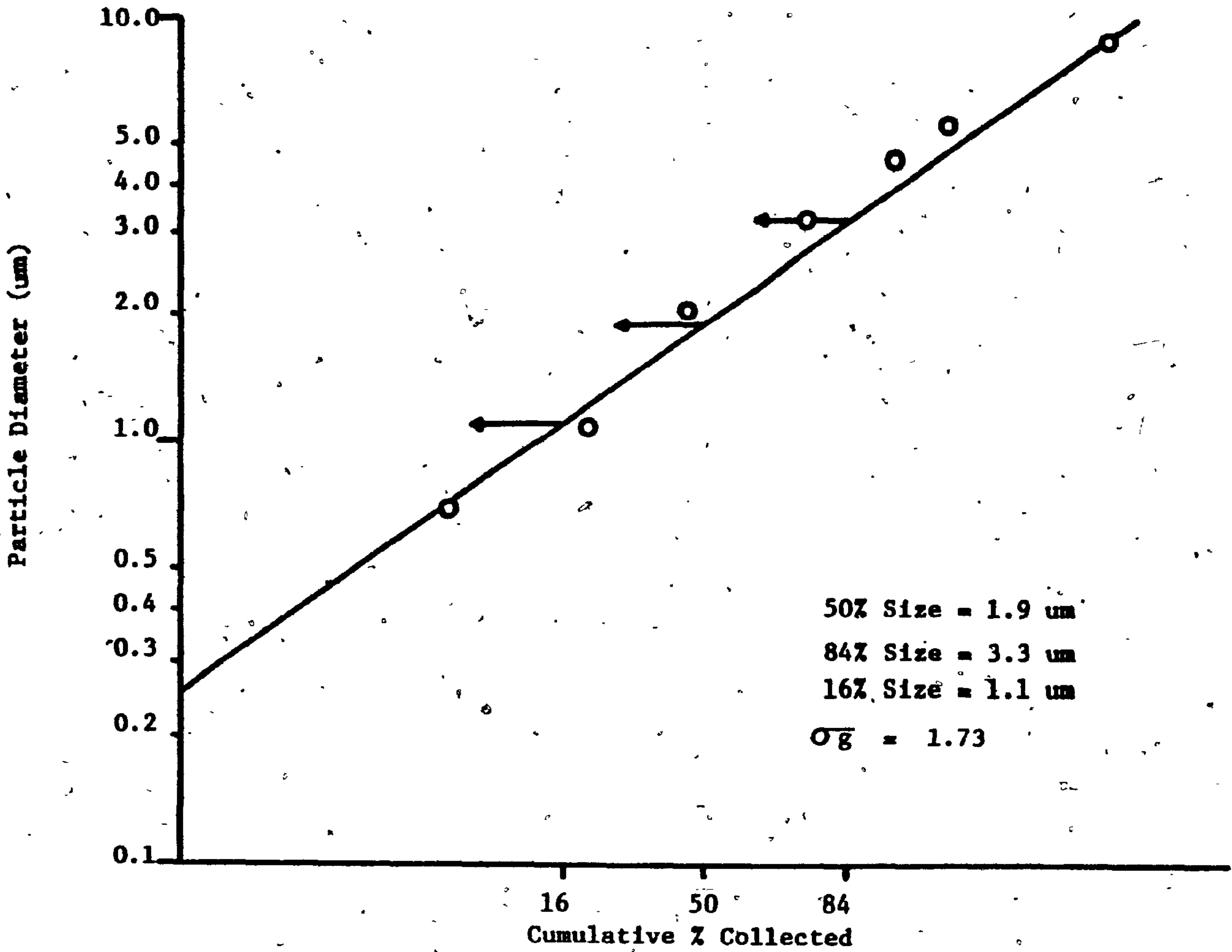


TABLE 4

MORTALITY IN CORN OIL CONTROL AND MIREX-TREATED GROUPS FOLLOWING PARATHION EXPOSURE

Group No.	Gravimetric Chamber Parathion Concentration (mg/L)	Days Following Exposure							Mortality** (%)
		1	2	3	4	5	6	7	
Corn Oil Control									
7	0 (Air Control)								0
2	0.055								0
5	0.100	1							10
4	0.105	3							30
3	0.122	6	1						70
6	0.159	6							60
Mirex (15 mg/kg/day)									
70	0 (Air Control)								0
20	0.055								0
30	0.122				1*				0
50	0.182		1			1*			10
60	0.214		6						60
40	0.225		6						60

* Mortality considered a delayed effect of mirex treatment.
** Cumulative mortality (%) 3 days following parathion exposure.

FIGURE 11 PARATHION DOSE-MORTALITY CURVES FOR CONTROL AND MIREX TREATED RATS

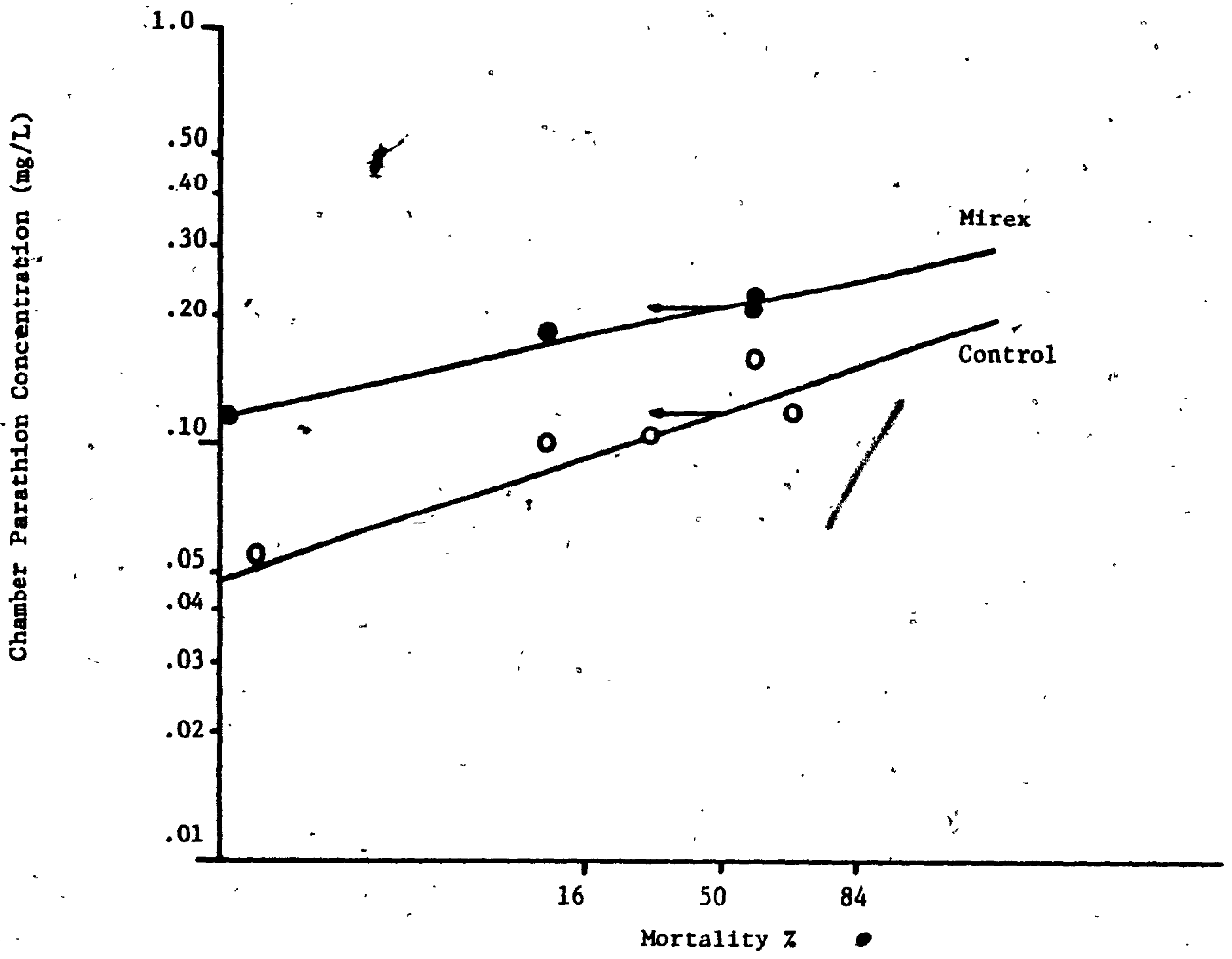
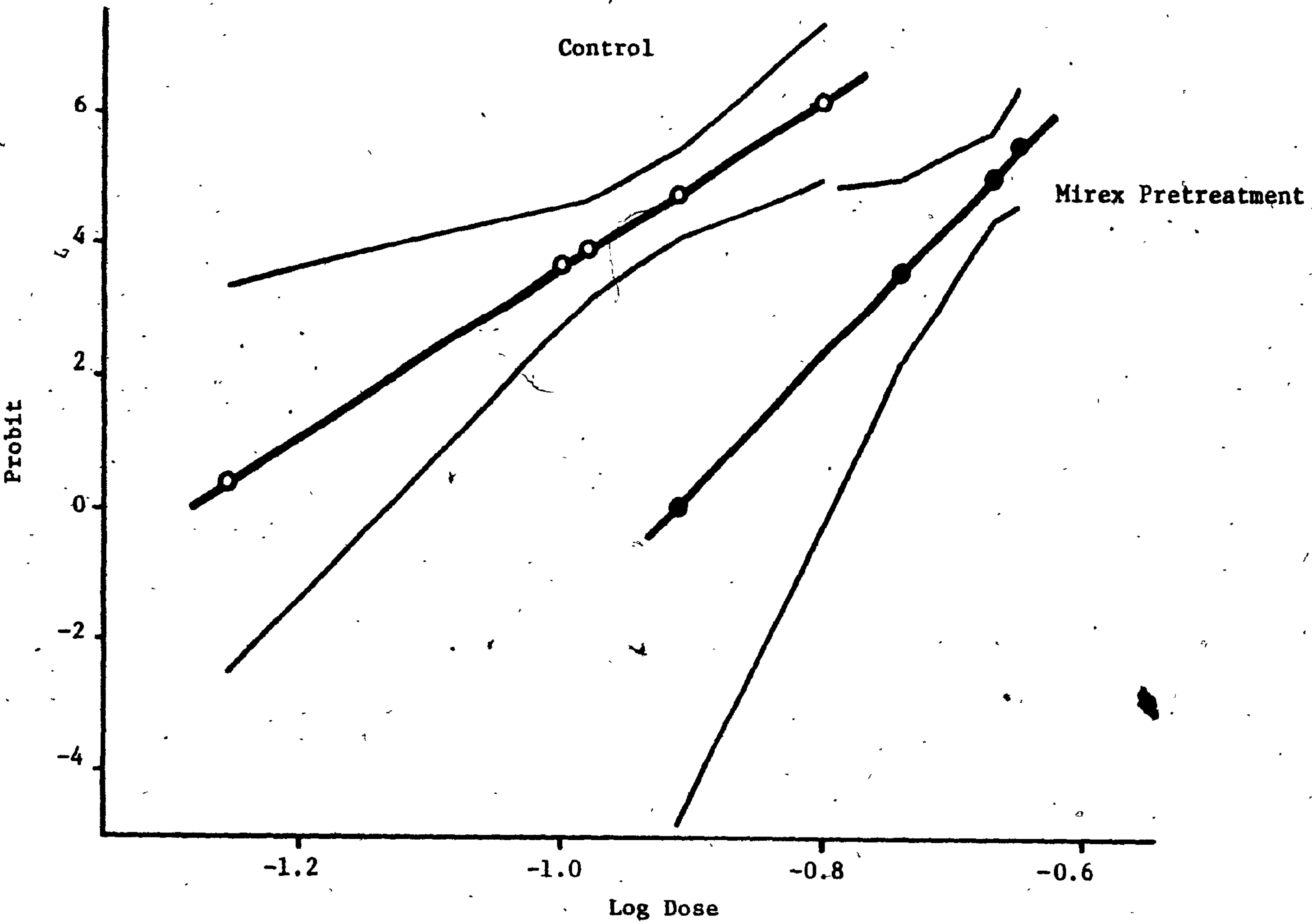


FIGURE 12 PROBIT ANALYSIS WITH FIDUCIAL LIMITS (99%)



3.3 Hepatic microsomal metabolism of parathion and paraoxon

Hepatic microsomal metabolism of parathion and paraoxon *in vitro* was determined in control and mirex-treated animals as previously described (Section 2.6).

Parathion metabolism by microsomal protein (Table 5) was increased with NADH and to a greater extent with NADPH compared to metabolism without cofactors in both control and mirex-treated groups. Paraoxon formation was dependent on NADH and to a greater extent on NADPH in both control and treated animals. Mirex-treated animals displayed increased NADH and NADPH-dependent parathion metabolism compared to controls, with NADPH-dependent metabolism being elevated by 50% (significant at $p < .05$). Similarly, NADH and NADPH-dependent paraoxon production was elevated by 180% and 117%, respectively in mirex-treated animals (significant at $p < .01$). NADH-dependent conversion of parathion to paraoxon (calculated as uM paraoxon produced/uM parathion metabolized) increased from 11.3% in controls to 23.5% in mirex-treated animals and NADPH-dependent conversion increased from 18.5% to 26.7%.

Paraoxon metabolism by microsomal protein (Table 6) was independent of NADH or NADPH. Some increase in the rate of paraoxon metabolism was noted with mirex-treatment (6%, 11% and 22% with no cofactor, NADH and NADPH, respectively), however, no statistical significance was observed.

TABLE 5 PARATHION METABOLISM BY MICROSOMAL PROTEIN

Treatment	Animal No.	Cofactor: Parathion Remaining (uMoles) None	NADH	NADPH	Parathion Metabolized (uMoles/mg Protein/15 min) None	NADH	NADPH	Paraoxon Produced (uMoles/mg Protein/15 min) None	NADH	NADPH
Corn Oil	101	-	-	-	-	-	-	0	0.0017	0.0035
Control	102	-	-	-	-	-	-	0	0.0021	0.0051
	103	0.485	0.461	0.430	0.0120	0.0145	0.0177	0	0.0018	0.0037
	104	0.482	0.427	0.380	0.0123	0.0180	0.0229	0	0.0011	0.0026
	105	0.523	0.494	0.430	0.0133	0.0183	0.0293	0	0.0033	0.0064
	106	0.502	0.461	0.438	0.0129	0.0183	0.0213	0	0.0022	0.0046
	107	0.476	0.450	0.405	0.0132	0.0160	0.0207	0	0.0014	0.0033
	108	0.499	0.504	0.456	0.0107	0.0102	0.0153	0	0.0009	0.0027
	109	0.439	0.442	0.395	0.0168	0.0165	0.0214	0	0.0014	0.0029
	110	0.576	0.518	0.488	0.0024	0.0083	0.0113	0	0.0010	0.0024
Mean		0.498	0.470	0.428	0.0117	0.0150	0.0200	0	0.0017	0.0037
± S.D.		0.0398	0.0321	0.0347	0.00414	0.00382	0.00537	0	0.00072	0.00128
Mirex	201	-	-	-	-	-	-	0	0.0041	0.0062
15 mg/kg/day	202	-	-	-	-	-	-	0	0.0055	0.0099
	203	0.475	0.379	0.311	0.0118	0.0208	0.0273	0	0.0057	0.0088
	204	0.479	0.456	0.385	0.0136	0.0162	0.0242	0	0.0042	0.0056
	205	0.547	0.480	0.404	0.0110	0.0250	0.0408	0	0.0086	0.0151
	206	0.577	0.482	0.423	0.0052	0.0268	0.0402	0	0.0086	0.0124
	207	0.416	0.329	0.251	0.0192	0.0282	0.0364	0	0.0042	0.0084
	208	0.513	0.487	0.455	0.0112	0.0145	0.0187	0	0.0013	0.0020
	209	0.502	0.427	0.340	0.0126	0.0222	0.0333	0	0.0032	0.0067
	210	0.593	0.532	0.467	0.0010	0.0096	0.0187	0	0.0025	0.0056
Mean		0.513	0.447	0.380	0.0107	0.0204	0.0300[A]	0	0.0048[B]	0.0080[B]
± S.D.		0.0583	0.0656	0.0743	0.00548	0.00650	0.00901	0	0.00239	0.00375

A $P < .05$
B $P < .01$

TABLE 6 **PARAOXON METABOLISM BY MICROSOMAL PROTEIN**

Treatment	Animal No.	Cofactor: Paraoxon Remaining (uMoles) None	NADH	NADPH	Paraoxon Metabolized (uMoles/mg Protein/15 min) None	NADH	NADPH
Corn Oil Control	101	0.409	0.449	0.466	0.0222	0.0176	0.0156
	102	0.475	0.481	0.462	0.0171	0.0163	0.0189
	103	0.443	0.446	0.446	0.0164	0.0160	0.0160
	104	0.488	0.478	0.481	0.0117	0.0127	0.0124
	105	0.491	0.490	0.490	0.0189	0.0190	0.0190
	106	0.509	0.517	0.520	0.0119	0.0110	0.0106
	107	0.536	0.531	0.529	0.0068	0.0074	0.0076
	108	0.529	0.523	0.515	0.0075	0.0082	0.0090
	109	0.502	0.515	0.498	0.0103	0.0088	0.0106
	110	0.509	0.520	0.502	0.0092	0.0081	0.0099
Mean		0.489	0.495	0.491	0.0132	0.0125	0.0130
± S.D.		0.0387	0.0309	0.0271	0.00517	0.00441	0.00413
Mirex 15 mg/kg/day	201	0.462	0.461	0.462	0.0189	0.0190	0.0189
	202	0.477	0.487	0.446	0.0180	0.0166	0.0226
	203	0.479	0.479	0.459	0.0114	0.0114	0.0133
	204	0.476	0.455	0.479	0.0139	0.0163	0.0136
	205	0.523	0.533	0.503	0.0160	0.0140	0.0203
	206	0.514	0.510	0.495	0.0196	0.0205	0.0238
	207	0.489	0.488	0.482	0.0115	0.0116	0.0123
	208	0.523	0.530	0.517	0.0099	0.0089	0.0106
	209	0.498	0.503	0.490	0.0131	0.0124	0.0141
	210	0.543	0.540	0.524	0.0080	0.0084	0.0107
Mean		0.498	0.499	0.486	0.0140	0.0139	0.0159
± S.D.		0.0262	0.0298	0.0253	0.00397	0.00411	0.00500

3.4 The effect of mirex on other biological parameters with potential to influence parathion toxicity

3.4.1 Endogenous substrate competition with parathion and paraoxon for metabolic enzymes

Metabolism of parathion and paraoxon by crude liver homogenate was measured as described in Materials and Methods (Section 2.7). Parathion metabolism increased an average of 19.6% in mirex-treated animals compared to controls (significant at $p < .05$) and paraoxon production increased 123.8% (significant at $p < .01$) (Table 7). The efficiency of parathion conversion to paraoxon increased from 12.1% in controls to 22.7% in mirex-treated animals. Paraoxon detoxification by crude liver homogenate increased 32% with mirex treatment (significant at $p < .05$). These trends are consistent with the mirex-induced changes observed in the microsomal metabolism of parathion and paraoxon (Tables 5 and 6, respectively).

3.4.2 Parathion uptake via the lung

Parathion uptake via the lung was determined indirectly by measuring respiratory rate, and by measuring plasma parathion levels in control and mirex-treated animals following inhalation exposure as per Sections 2.8 and 2.13, respectively.

3.4.2.1 Respiratory rate

Considerable variation was observed in the respiratory rates of control and mirex-treated groups, however, the mean rates and the range of values observed in the two groups were similar (Table 8).

TABLE 7 PARATHION AND PARAOXON METABOLISM BY CRUDE LIVER HOMOGENATE

Treatment		Animal No.	Parathion Metabolism			Paraoxon Metabolism	
			Parathion Remaining (uMoles)	Parathion Metabolized (uMoles/mL)	Paraoxon Produced (uMoles/mL)	Paraoxon Remaining (uMoles/mL)	Paraoxon Metabolized (uMoles/mL)
Corn Oil		101	0.263	0.337	0.045	0.419	0.181
Control		102	0.263	0.337	0.036	0.443	0.157
		103	0.234	0.366	0.045	0.431	0.169
	Mean		0.253	0.347	0.042	0.431	0.169
	± S.D.		0.0167	0.0167	0.0052	0.0120	0.0120
Mirex		1001	0.190	0.410	0.091	0.372	0.228
15 mg/kg/day		1002	0.205	0.395	0.091	0.355	0.245
		1003	0.161	0.439	0.100	0.404	0.196
	Mean		0.185[A]	0.415[A]	0.094[B]	0.377[A]	0.223[A]
	± S.D.		0.0224	0.0224	0.0052	0.0249	0.0249

A $P < .05$
B $P < .01$

TABLE 8 RESPIRATORY RATE IN CONTROL AND MIREX-TREATED ANIMALS

Treatment		Animal No.	Respiratory Rate (Breaths/min)
Corn Oil Control		101	142
		102	84
		103	318
		104	118
		105	112
		106	230
		107	226
		108	126
		109	252
		110	236
	Mean		184
	± S.D.		77.3
Mirex 15 mg/kg/day		1001	270
		1002	124
		1003	230
		1004	216
		1005	104
		1006	196
		1007	66
		1008	234
		1009	226
		1010	216
	Mean		188
	± S.D.		66.4

3.4.2.2 Parathion level in plasma following inhalation exposure

The parathion content of plasma following a 3-hour inhalation exposure was similar in control and mirex-treated animals and no significant difference in the group mean values was observed (Table 9).

3.4.3 Lung metabolism of parathion and paraoxon

Dearylation of parathion and paraoxon was measured in lung and liver microsomal and soluble protein fractions as described in Section 2.9.

Lung microsomal and soluble fractions did not catalyze dearylation of parathion or paraoxon (Table 10). Some dearylation was seen with liver soluble protein having NADH as cofactor, however, the highest rate of dearylation of both substrates was observed with liver microsomal protein.

3.4.4 Plasma metabolism of parathion and paraoxon

Metabolism of parathion and paraoxon by plasma was measured as described in Section 2.10.

Parathion was metabolized by plasma of control and mirex-treated animals and paraoxon was not one of the products formed. Parathion metabolism appeared to decrease in the mirex-treated group, but no statistical significance was observed (Table 11). Paraoxon metabolism by plasma was significantly decreased with mirex treatment ($p < .01$) (Table 12).

TABLE 9 PARATHION AND PARAOXON LEVELS IN PLASMA FOLLOWING INHALATION EXPOSURE

Treatment	Animal No.	Parathion Level (uM/mL Plasma)	Paraoxon Level (uM/mL Plasma)
Corn Oil Control	1	0.0287	0
	2	0.0227	0
	3	0.0313	0.0047
	4	0.0240	0
	5	0.0514	0
Mean		0.0316	0.0009
± S.D.		0.01159	0.00210
Mirex 15 mg/kg/day	6	0.0240	0
	7	0.0272	0
	8	0.0460	0
	9	0.0207	0
	10	0.0374	0
Mean		0.0311	0
± S.D.		0.01043	0

TABLE 10 PARATHION AND PARAOXON CONVERSION TO p-NITROPHENOL BY LIVER AND LUNG FRACTIONS (ug p-Nitrophenol/mg protein/15 minutes)

	Substrate			
	Parathion		Paraoxon	
Enzyme Source	NADH	NADPH	NADH	NADPH
Liver Microsomal	1.84	2.45	7.35	7.96
Liver Supernatant	0.34	0	0.67	0
Lung Microsomal	0	0	0	0
Lung Supernatant	0	0	0	0

TABLE 11 PARATHION METABOLISM BY PLASMA PROTEIN

Treatment		Animal No.	Parathion Metabolism (uM/0.5 mL/15 min)	Paraoxon Produced (uM/0.5 mL/15 min)
Corn Oil Control		1	0.0118	0
		2	0.0083	0
		3	0.0163	0
		4	0.0013	0
		5	0.0057	0
	Mean		0.0087	0
	± S.D.		0.00573	0
Mirex 15 mg/kg/day		6	0.0057	0
		7	0.0055	0
		8	0	0
		9	0.0085	0
		10	0	0
	Mean		0.0040	0
	± S.D.		0.00379	0

TABLE 12

PARAOXON METABOLISM BY PLASMA PROTEIN

Treatment	Animal No.	Paraoxon Remaining (uMoles)	Paraoxon Metabolized (uMoles/mg Protein/15 min)	pNitrophenol Produced (uMoles/mg Protein/15 min)
Corn Oil	101	0.449	0.0108	0.0080
Control	102	0.301	0.0099	0.0074
	103	0.314	0.0136	0.0104
	104	0.289	0.0131	0.0106
	105	0.301	0.0121	0.0080
	106	0.330	0.0114	0.0092
	107	0.274	0.0147	0.0074
	108	0.302	0.0130	0.0086
	109	0.336	0.0111	0.0095
	110	0.353	0.0101	0.0073
Mean		0.325	0.0120	0.0086
± S.D.		0.0494	0.00159	0.00123
Mirex 15 mg/kg/day	1001	0.465	0.0038	0.0057
	1002	0.327	0.0110	0.0088
	1003	0.478	0.0030	0.0054
	1004	0.338	0.0110	0.0085
	1005	0.367	0.0082	0.0055
	1006	0.379	0.0095	0.0028
	1007	0.358	0.0108	0.0045
	1008	0.383	0.0079	0.0034
	1009	0.421	0.0054	0.0045
	1010	0.432	0.0061	0.0052
Mean		0.395[B]	0.0077[B]	0.0054[B]
± S.D.		0.0519	0.00298	0.00193

[B] $P < .01$

3.4.5 Liver histopathology

Livers of control and mirex-treated animals were processed for microscopic examination as described in Section 2.11.

Histopathological findings in the livers of mirex-treated rats consisted of frequent mitotic figures, coarse lipid vacuolization and possibly some hepatocyte enlargement. These features were not present in control livers.

3.4.5.1 Hepatic blood flow

Hepatic blood flow was measured by BSP retention in the blood as described in Section 2.10.

No significant difference in BSP retention was observed between the control and mirex-treated groups (Table 13).

3.4.5.2 Storage of parathion and paraoxon in the liver

Parathion and paraoxon content of the liver following inhalation exposure to parathion were measured as described in Section 2.13.

Levels of parathion and paraoxon in liver were extremely low and appeared not to be altered by mirex treatment (Table 14).

3.4.6 Plasma and brain cholinesterase activities

Plasma and brain cholinesterase activities were measured as described in Section 2.14.

Cholinesterase activities in plasma and brain were not significantly altered by treatment with mirex (Tables 15 and 16).

TABLE 13 BSP RETENTION IN CONTROL AND MIREX-TREATED ANIMALS

Treatment		Animal No.	BSP Retention* (%)
Corn Oil Control		19	1.07
		20	0.72
		21	1.07
		22	3.29
		23	5.15
		24	0.86
	Mean		2.03
	± S.D.		1.802
Mirex 15 mg/kg/day		13	2.93
		14	1.43
		15	0.86
		16	0.93
		17	2.57
		18	1.86
	Mean		1.76
	± S.D.		0.855

*Retention presented as % of initial plasma BSP remaining 10 minutes after injection.

TABLE 14 PARATHION AND PARAOXON LEVELS IN THE LIVER

Treatment		Animal No.	Parathion Level (uM/g)	Paraoxon Level (uM/g)
Corn Oil Control		1	0	0
		2	0.0184	0
		3	0.0472	0
		4	0.0184	0
		5	0.0216	0
	Mean		0.0211	0
	± S.D.		0.01689	
Mirex 60 mg/kg		6	0	0
		7	0.0576	0.0088
		8	0.0184	0
		9	0	0
		10	0	0
	Mean		0.0152	0.0018
	± S.D.		0.02501	0.00394

TABLE 15 PLASMA CHOLINESTERASE ACTIVITY IN CONTROL AND MIREX-TREATED ANIMALS

Treatment		Animal No.	uM/mg Protein/min
Corn Oil Control		101	1.06
		102	0.74
		103	0.87
		104	0.62
		105	0.55
		106	0.65
		107	0.69
		108	1.16
		109	0.92
		110	0.74
	Mean		0.80
	± S.D.		0.198
Mirex 15 mg/kg/day		1001	0.69
		1002	0.71
		1003	0.64
		1004	0.83
		1005	0.98
		1006	0.82
		1007	0.77
		1008	0.49
		1009	0.74
		1010	0.67
	Mean		0.73
	± S.D.		0.131

TABLE 16 BRAIN CHOLINESTERASE ACTIVITY IN CONTROL AND MIREX-TREATED ANIMALS

Treatment		Animal No.	uM substrate/min/g
Corn Oil Control		1	81.0
		2	79.2
		3	96.0
		4	93.6
		5	98.4
		11	67.2
	Mean		85.9
	± S.D.		12.13
Mirex 15 mg/kg/day		6	88.8
		7	103.2
		8	88.8
		9	96.0
		10	55.2
		12	96.0
	Mean		88.0
	± S.D.		16.95

4.0 DISCUSSION

4.1 Liver weight and MFO alterations with mirex treatment

Liver weight and cytochrome P-450 activity have been reported to increase in the rat following a single dose or repeated administration of mirex (11). This present study confirms these changes and demonstrates that subacute administration of mirex also significantly increases hepatic microsomal NADPH cytochrome c reductase activity which has been implicated in oxidative metabolism of parathion (3), but does not increase paraoxon detoxification, via dearylation, at tolerable mirex dose levels. In relation to parathion metabolism, these *in vitro* findings point to an increase in hepatic paraoxon production with no change in paraoxon detoxification and suggest that mirex pretreatment will potentiate the toxicity of parathion.

4.2 Parathion LC50 evaluation in control and mirex-treated animals

Subacute mirex pretreatment decreases the acute inhalation toxicity of parathion by a factor of 1.7. The protection provided by mirex is consistent with that reported following treatment with other chlorinated hydrocarbon compounds including DDT, DDE, aldrin, chlorcyclizine, and several halogenated benzenes (13, 19, 46, 51, 52). However, the decrease in parathion toxicity is inconsistent with the predicted toxicity change based upon mirex-induced alterations in *in vitro* microsomal enzyme activities involved in parathion metabolism (Section 4.1).

4.3 Hepatic microsomal metabolism of parathion and paraoxon

Hepatic microsomal metabolism of parathion and paraoxon was examined in control and mirex-treated animals in an attempt to identify the mechanism of mirex protection against parathion toxicity.

Mirex treatment doubled the rate of parathion conversion to paraoxon without significantly altering the rate of paraoxon detoxification. These findings are in agreement with observed changes in microsomal enzyme activities involved in parathion metabolism (Section 4.1), but contradict the observed change in parathion toxicity.

A lack of correlation between toxicity change and alterations in one or more of the pathways influencing paraoxon levels has been reported following treatment with various enzyme inducers (13, 51, 52). These discrepencies have led to examination of factors other than hepatic metabolism (including extra-hepatic metabolism and plasma binding) as being responsible for the protection observed (15, 48, 51, 53, 54). It has also been suggested that *in vitro* measurement may not provide an accurate representation of parathion metabolism *in vivo* due to heterogeneity of the hepatocyte population being disrupted in liver preparations used for *in vitro* assay (65). This latter suggestion is supported by the finding that SER proliferation induced by phenobarbital is not uniform throughout the liver, but occurs first at centrilobular cells and progresses to other cells following prolonged treatment (83).

4.4 Other biological factors with potential to influence the inhalation toxicity of parathion

In light of the lack of correlation between changes in parathion metabolism and toxicity following mirex treatment, other biological factors with potential to influence parathion toxicity were examined in an attempt to explain the protection afforded by mirex.

4.4.1 Endogenous substrate competition with parathion and paraoxon for metabolic enzymes

Endogenous substrate competition with parathion and paraoxon was examined as a potential mechanism of mirex-induced protection to parathion. The metabolic studies reported in Section 3.3 utilized a microsomal fraction free of contamination with endogenous substrates likely to compete *in vivo* for metabolic enzymes. Consequently, the parathion and paraoxon metabolic studies were repeated using a more crude liver preparation containing the endogenous water-soluble components of liver tissue.

The trends observed in parathion and paraoxon metabolism with crude liver homogenate (Section 3.4.1) are consistent with the mirex-induced changes observed in microsomal metabolism (Section 3.3). This finding suggests that mirex does not alter the quantity of hepatic water soluble substrates to levels which compete with parathion or paraoxon for microsomal enzymes.

It has been reported that lipophilic materials are the major substrates of microsomal enzymes (75). Two endogenous lipophiles, triglycerides and lipoproteins, are reliant upon hepatic endoplasmic reticulum for their synthesis and intracellular transport (75, 78),

and the surface of lipid vacuoles in hepatocytes is intimately associated with SER (78). Mirex has been observed to induce lipid accumulation in hepatocytes (Section 3.4.5) and it is possible that competition of these lipophiles with parathion for microsomal enzymes may be a factor in parathion metabolism _in vivo_. This competition would likely not be observed _in vitro_ due to disruption of the association between the lipid vacuoles and endoplasmic reticulum.

4.4.2 Parathion uptake via the lung

Alteration in uptake of toxicants, either from the gut following oral administration or via the lung following inhalation administration, has not been examined as a potential modulator of toxicity resulting from inducer treatment. Therefore, the effect of mirex treatment on uptake of parathion via the lung was examined, by measuring respiration rate and plasma levels of parathion following inhalation exposure. At least one report indicates that treatment with an organochlorine alters the metabolic rate of the rat (62), which may impact on respiration rate and uptake via the lung.

4.4.2.1 Respiration rate

Uptake of any aerosol via the lung is dependent primarily on aerosol concentration, lung deposition or retention and ventilation rate of the individual (89). Deposition in the lung is governed primarily by the particle size distribution of the aerosol (89) and was considered to be constant in the LC50 exposures performed. The third factor influencing aerosol uptake, ventilation rate, was measured in control and mirex-treated rats.

Respiratory rate was not altered by mirex treatment (Section 3.4.2.1) suggesting that parathion uptake via the lung is not different in control and treated rats.

4.4.2.2 Plasma parathion levels following inhalation exposure

Parathion levels in plasma immediately following inhalation exposure were similar in control and treated groups (Section 3.4.2.2). This finding suggests that mirex protection against parathion is not a result of reduced parathion uptake via the lung and is in agreement with the conclusion of the respiratory rate evaluation.

4.4.3 Lung metabolism of parathion and paraoxon

Microsomal metabolism of parathion to toxic and nontoxic products in rat lung has been reported to occur at about 20% of the rate observed in rat liver, calculated on the basis of similar protein levels in the reaction media (34). Considering the relative amount of total microsomal protein in these two organs, the total lung metabolism of parathion is probably considerably less than 20% of total liver metabolism. In spite of the expected low level of parathion detoxification in lung compared to liver, the rate of detoxification was investigated because the lung is the first site of insult and potential metabolism following inhalation exposure.

Parathion and paraoxon dearylation by lung microsomal and soluble protein was not observed in control animals (Section 3.4.3). Paraoxon formation from parathion was not measured, however, it is likely to be similar to the dearylation rate since it is catalyzed by NADPH-dependent microsomal enzymes which catalyze parathion and

paraoxon dearylation (7, 31). The effect of mirex on lung metabolism of parathion and paraoxon was not investigated since it has been reported that microsomal enzymes of lung are not induced as are liver enzymes (34). Furthermore, no evidence of enzyme induction, in the form of increased lung size or weight, was observed in mirex-treated animals.

Therefore, it is unlikely that mirex protection against parathion is provided through altered parathion or paraoxon metabolism in the lung.

4.4.4 Plasma metabolism of parathion and paraoxon

Theoretically, inducer protection against parathion could be provided by increasing plasma detoxification or protein binding of parathion before it reaches the liver and is toxified. Similarly, increased paraoxon detoxification or protein binding in plasma en route from the liver to nerve synapses could explain inducer protection against parathion. Several investigators have suggested this latter mechanism to explain protection against parathion and paraoxon following treatment with some inducers (46, 48, 51, 54).

Parathion detoxification by plasma was slightly less in mirex-treated animals than controls (Section 3.4.4). This detoxification may consist of either metabolism to nontoxic products or protein binding and the HPLC analysis utilized did not permit the two to be distinguished from one another. However, the essential action of both mechanisms is detoxification, and it is this parameter which is being examined in relation to parathion toxicity. The observed decrease in

parathion detoxification with mirex would likely result in increased parathion being available for hepatic metabolism, which does not correlate with the observed change in parathion toxicity. Similarly, decreased paraoxon detoxification by plasma following mirex treatment (Section 3.4.4) would likely increase "free" paraoxon available to inactivate cholinesterase, which does not correlate with the parathion toxicity change observed.

In light of these findings, it is unlikely that mirex protection against parathion is provided by increased plasma detoxification of parathion or paraoxon.

4.4.5 Liver histopathology

Hepatocyte hyperplasia and lipid vacuolation induced by mirex are consistent with changes observed in the rat following treatment with a variety of chlorinated hydrocarbons (64, 67, 69, 70). These anatomical changes have potential to influence parathion toxicity by altering the flow rate of parathion-containing blood through the liver, or by providing for increased hepatic storage in lipophilic depots.

4.4.5.1 Hepatic blood flow

Reported alterations in hepatic blood flow following chlorinated hydrocarbon administration in the rat have varied from no change (62) to increased (84) and decreased (69) flow rates, however, no attempt to correlate this change with a change in parathion toxicity has been made. The effect of mirex on liver blood flow was investigated to test the hypothesis that induced hepatocyte hyperplasia and hypertrophy could cause a reduction in blood flow through the liver by constricting or occluding blood vessels and sinusoids. BSP was used for this evaluation

because it is a dye which is removed from plasma predominantly by the liver and its elimination is dependent primarily on blood flow through the liver (72, 82).

No significant change in BSP retention in plasma was observed following mirex treatment (Section 3.4.5.1), suggesting that blood flow through the liver and parathion delivery to hepatic enzymes is unaltered.

In order to attribute the observed 1.7-fold decrease in parathion toxicity following mirex to reduced parathion supply to hepatic toxification enzymes, a 1.7-fold reduction in hepatic blood flow would be necessary. This reduction in blood flow would increase the half-life of substances removed by the liver, including BSP, by a similar order of magnitude (86). Thus, the half-life of BSP would increase from 1.6 minutes in the control rat (Figure 13) to 2.7 minutes in the mirex-treated rat. The number of BSP half-lives elapsed in the 10 minutes allowed for BSP removal would decrease from 6.3 in the control rat to 3.7 in the treated rat, and the quantity of BSP remaining in the plasma would increase from 1.5% to 9% (Figure 14). Differences of this order of magnitude between control and treated rats were not observed. It is therefore concluded that mirex-induced protection against parathion was not provided by reduced blood flow through the liver.

4.4.5.2 Storage of parathion and paraoxon in the liver

Insecticide storage in fat depots of the body can effectively reduce the concentration of free insecticide in the blood and thus affect concentration-dependent processes such as their metabolism (28, 75). Parathion and paraoxon are lipid soluble molecules, and as such

FIGURE 13 BSP HALF-LIFE IN THE RAT

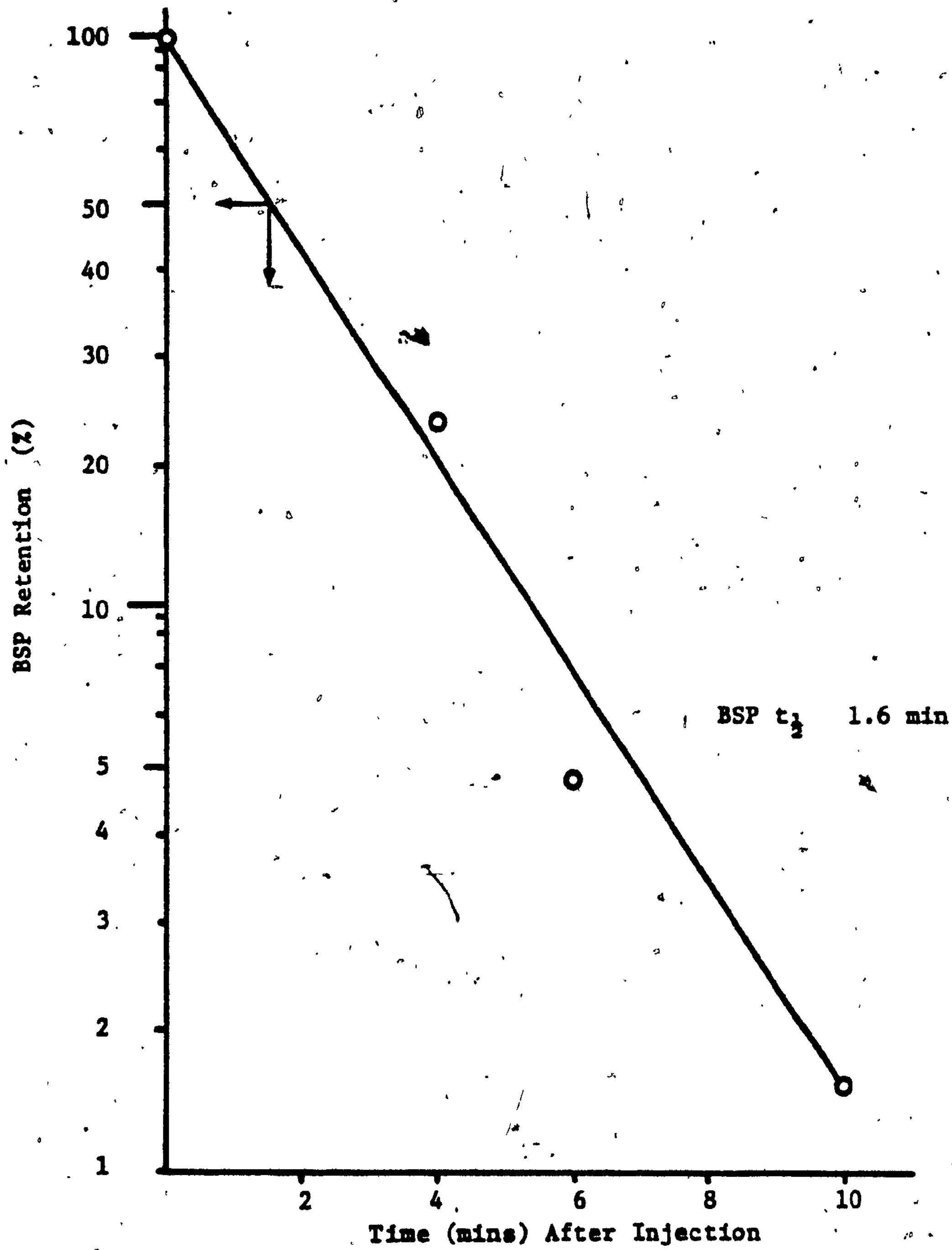
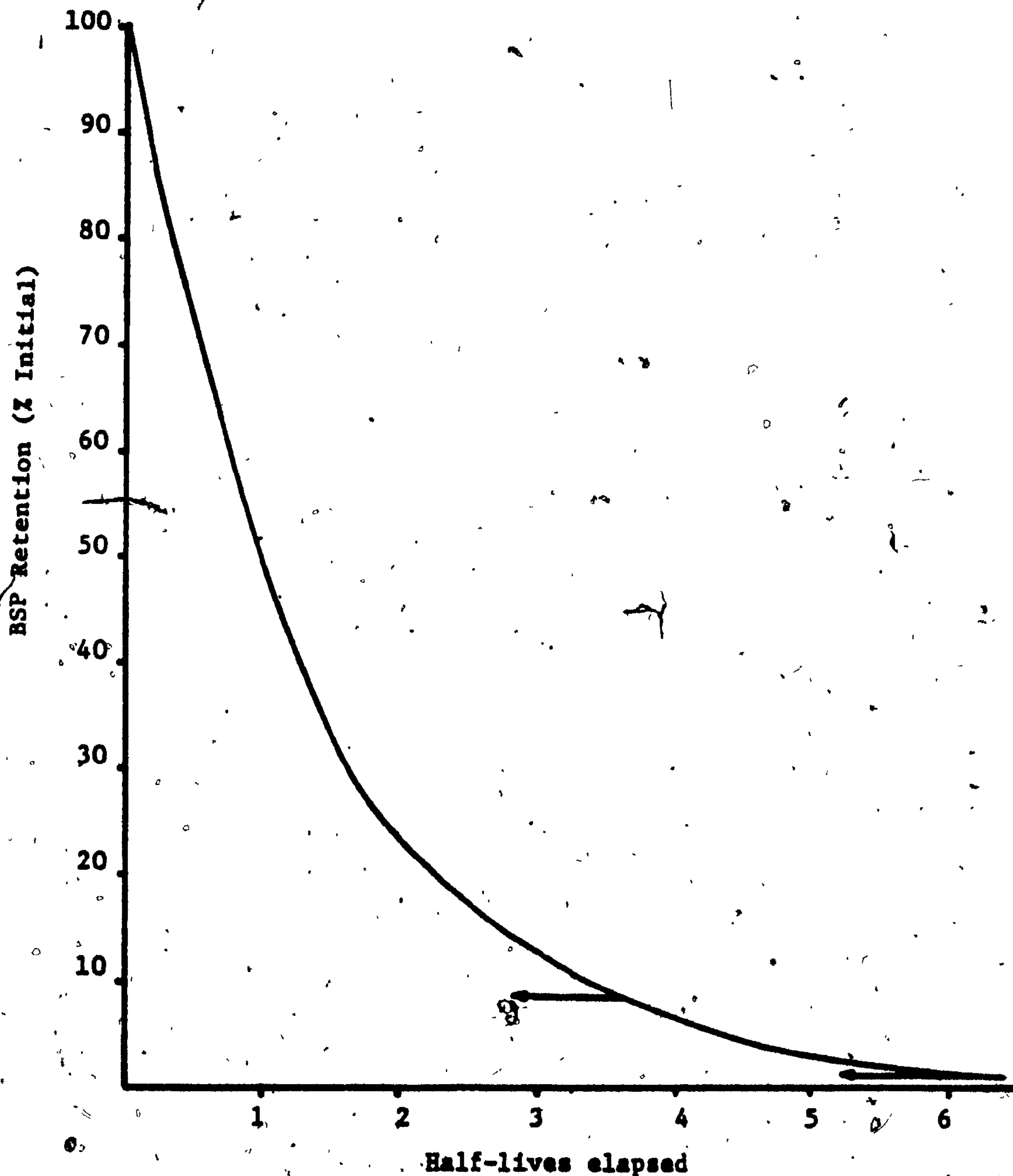


FIGURE 14 HALF-LIFE CURVE OF BSP ELIMINATION FROM THE BLOOD



may partition into fat depots. The fatty liver of mirex-treated animals may be an effective storage depot since the site of parathion and paraoxon metabolism, the ER, is closely associated with lipid storage vesicles (78).

Parathion and paraoxon levels in the liver following inhalation exposure were extremely low and not significantly altered by mirex treatment (Section 3.4.5.2). These findings suggest that the liver is not a major storage depot of parathion and paraoxon and that mirex protection is not afforded by storage of parathion before it can be activated to paraoxon or by storage of paraoxon before it can be released into the blood to be transported to its site of toxic action.

4.4.6 Plasma and brain cholinesterase activities

The toxic action of parathion results from binding of its oxygen analog, paraoxon, to cholinesterase enzymes at cholinergic nerve endings. The cholinesterase is inactivated by this binding and neurotransmitters in the nerve endings accumulate, resulting in overstimulation of the central and peripheral nervous systems (75). Increased levels of cholinesterases could provide some protection against parathion by binding more paraoxon, thereby increasing the amount of paraoxon production needed to cause mortality.

Plasma and brain cholinesterase activities were not altered by mirex pretreatment. This suggests that the protective effect of mirex against parathion is not mediated through increased levels of target enzymes of anticholinesterases.

4.5 Final comments

Mirex, like many chlorinated hydrocarbon insecticides, provides some protection to mammals against the toxicity of parathion and possibly other anticholinesterases whose metabolism is similar. The mechanism of protection appears not to be extra-hepatic in nature and may involve altered hepatic metabolism of parathion. Future studies should attempt to correlate changes in *in vivo* metabolism and toxicity since *in vitro* metabolism and toxicity generally do not correlate and because *in vitro* metabolism may not accurately represent what occurs *in vivo*. This protection may represent the single positive consequence of environmental contamination with persistant organochlorine compounds.

SUMMARY

The rat was afforded some protection against the inhalation toxicity of parathion by pretreatment with mirex for 4 days at 15 mg/kg/day. Hepatic enzymes involved in parathion activation increased following mirex treatment and the rate of parathion conversion to paraoxon by microsomes doubled without a change in the rate of paraoxon detoxification. Other biological parameters with potential to influence parathion toxicity were examined in an attempt to explain the protective effect of mirex. Parameters unaffected by mirex consisted of parathion uptake and metabolism by the lung, competition of endogenous substrates with parathion and paraoxon for metabolic enzymes, blood flow through the liver, toxicant storage in the liver, and activity of paraoxon target enzymes (cholinesterases). Plasma detoxification of parathion and paraoxon was reduced by mirex, however, this change was in the wrong direction to explain the observed protection against parathion.

These studies suggest that mirex protection against parathion is not provided by the extra-hepatic mechanisms examined. It is possible that the protection is afforded through altered hepatic metabolism of parathion, and that _in vitro_ parathion metabolism does not accurately reflect _in vivo_ metabolism.

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