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Title: The Effects of Mirex on Biliary Excretion of Bile Salts and Phenolphthalein Glucuronide in Male Rats

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Rats exposed to one or two doses of 60 mg mirex/kg by gavage exhibited hypertrophied livers and various degrees of hepatobiliary dysfunction. Although single dosed animals demonstrated elevated whole liver bile flow after 96 hours, the single dose was insufficient to significantly inhibit whole liver hepatobiliary performance as measured by the biliary excretion of exogenously supplied phenolphthalein glucuronide (PG) and endogenous bile salts. Two doses of mirex on consecutive days lead to significantly greater liver hypertrophy than a single dose, reduced excretion of bile salts and PG in the first 15 minutes following infusion on both a per gram and whole liver basis, and depressed bile salt excretion on a gram liver basis. Maximal disruption of hepatobiliary excretory performance was apparent 96 hours after the second mirex dose.

The Effects of Mirex on Biliary Excretion of Bile Salts and Phenolphthalein Glucuronide in Male Rats

by

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Max Deinzer permitted me to work in his laboratory after hours, enabling me to generate mirex residue data.

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The support of my friends kept me going, I will not forget the love I received or the love I failed to reciprocate while under the graduate school burden.

Last and most, I am indebted to Larry Curtis for his help, support, and guidance over nearly five years, but most of all, for his patience.

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INTRODUCTION

The liver performs a multitude of functions, one of the more important of which is the excretion of endogenous and exogenous compounds in the bile. For many of these compounds, the hepatobiliary system is their only mode of removal and so the performance of this system is critical. These compounds are generally of high molecular weight (450+ daltons), may be cationic, anionic, or neutral, and include such physiologic and natural chemicals as bile acids, insulin, bilirubin, steroids, heavy metals, alkaloids, cardioactive glycosides, and aflatoxins. In addition, the liver is responsible for the removal of many drugs including some antibiotics as well as other xenobiotics (Smith, 1971; Klaassen and Watkins, 1984). These compounds may or may not be metabolized prior to excretion.

Solutes enter hepatocytes across sinusoidal and lateral cell membranes which composes approximately 87% of the total cell surface. The remainder of the liver plasma membrane borders the bile canaliculus, the site of

bile secretion. Bile canaliculi are formed between hepatocytes which are otherwise separated from each other by small, approximately 1 um, paracellular spaces. These apical regions are bordered by junctional complexes which serve as barriers to isolate the bile canaliculi from the blood which otherwise perfuses the entire hepatocyte.

Whereas the kidney operates predominantly via a pressure/filtration process, bile is believed to be formed by an osmotic mechanism. The liver excretes many organic anions (the bile salts) into the bile canaliculus and water follows down an osmotic gradient (Boyer, 1980). A sodium gradient is generated at the sinusoidal membrane surfaces by the Na+/K+ ATPase which concentrates Na+ outside the cell. Bile acids are transported from the sinusoid and into the hepatocyte via a Na+ coupled carrier. This carrier is sodium specific but can transport other anions besides bile acids into the cell. Once inside the cell, bile acids are rapidly bound by soluble proteins which act to decrease their intracellular activity. They may be transported through the cell in membrane confined structures. Bile acids can then diffuse down a concentration gradient across canalicululer membranes. Should the concentration of bile acids reach a critical concentration in the canalicular lumen, they will form micelles. Other anions

driven into the bile by the electrochemical gradient (-30 to -40 mV in the rat (Claret and Bazet, 1972)) can also aggregate in the micelles. By doing so, a concentration gradient is maintained where bile acids continue to diffuse into the canaliculus and in so doing, maintain the driving force of bile formation.

There are also mechanisms of bile formation independent of bile acid concentration. The paracellular shunt pathway refers to the contributions to bile made through the junctional complexes. These complexes have been shown to have characteristics of both leaky and tight junctions and have limited permeability (Friend and Gilula, 1972). The degree of permeabilty is related to the number of microfilaments present in the complexes. Inorganic ions enter bile predominantly through these junctions, as well as some small, uncharged molecules. The permeability of these junctions increases with the degree of the osmotic gradient created by the presence of bile acids in the lumen of the canaliculi. If additional bile acids are administered to an animal, the osmotic gradient will increase, leading to an increase in the permeability of the junctional complexes. An infusion of water and electrolytes (Layden et al., 1978) will result. The bile may also be affected by resorption of salts and water in the biliary tree. Ultimately, the bile will be isoosmotic with respect to the serum (Wheeler, 1968).

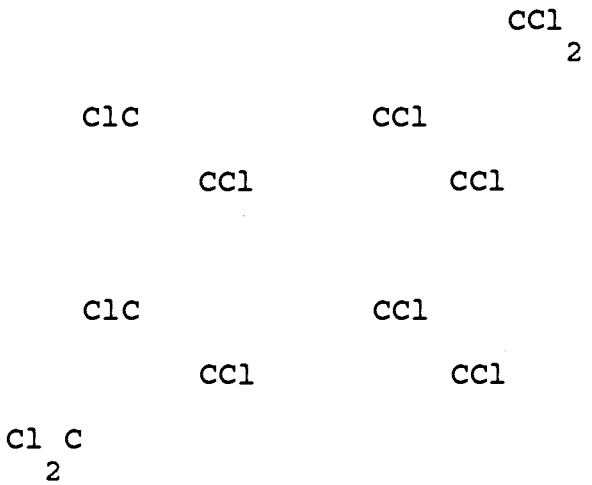
Nonpolar molecules have been shown to partition into cell membranes (Colley and Metcalfe, 1972; Kenega, 1975; White, 1976). This tendency can be predicted to a large extent by the lipophilic nature of the molecule, often indicated by the octanol-water partition coefficient (Kow). However, as cell membranes are also composed of as much as 60% or more protein (Lehninger, 1975), charge interactions and steric effects can also affect the ability of molecules to diffuse into and through the hydrophobic region of a biological bilayer.

As a result of xenobiotics entering cell membranes, the ability of the membrane to function properly may be affected. Roth (1979) and Pritchard (1979) describe mechanisms by which membrane function may be altered. The incorporation of xenobiotics into a membrane may result in disordering of the membrane structure, both by changing the fluidity of the membrane and by disturbing the microenvironment of the enzymes present. In addition, membrane proteins may undergo conformational changes following exposure of hydrophobic regions of the proteins to xenobiotics, leading to swelling of the membranes. A well-documented example is the sensitivity of Na+/K+ ATPases to changes in membrane fluidity resulting from interactions of lipid soluble molecules with hyrophobic regions of the membranes (Goldstein,

1983). Such perturbations of the membrane bilayer can lead to disruption of cell processes which may result in mild intoxication, sickness, and even death.

Much work has been done to determine the effects of organochlorine exposure on hepatobiliary function, particuarly in terms of the excretion of organic anions. This work has been useful both for helping to elucidate the fundamental mechanisms of the hepatobiliary system, and also to understand how the system handles stresses such as organochlorine exposure. The voluminous information generated by such investigations has been reviewed and succinctly summarized by Mehendale (1979).

Figure 1. Structural Model of Mirex



Mirex (dodecachlorooctahydro-1,3,4-methano-2H cyclobuta[c,d]pentalene, CAS Registry Number 2385-85-5) was introduced for fire ant control in the southeastern United States in 1959. It acts principally as a stomach poison and has little contact insecticidal activity. After a latent period of 3 days (in insects) death occurs as a result of damage to the central nervous system. It is highly resistant to chemical and biological degradation and only slowly degraded by sunlight. Its principal photodegradation product is 8-monohydromirex (Alley, et al., 1973 and 1974) which is similarly toxic and persistent in the environment. Both are insoluble in water and strongly lipophilic. As a result, mirex tends to bioaccumulate where there is contamination of primary trophic levels (Waters et al., 1977).

Exposure of rats to mirex in the feed (>80 ppm) typically leads to liver hypertrophy (Mehendale et al., 1972; Gibson et al., 1972; Kendall, 1974; Mehendale, 1981 Yarbrough et al., 1984), with vacuolation and swelling evident at the cell level (Larson et al., 1979). With rats fed 365 ppm in the food, Kendall (1974) observed hepatocyte enlargement, glycogen depletion, and periportal liposis.

Rats given a single oral dose of mirex were able to excrete 18-59% of the total dose within 7 days, virtually all in the feces (Mehendale et al., 1972; Water et al., 1977). The bulk of that was excreted within 48 hours. Rats with an existing body burden were less efficient in absorbing mirex in food and passed 25% of it in the feces. Body burdens were unchanged over a 28 day period. Once absorbed into lipid, metabolic turnover is extremely slow (Gibson et al., 1972; Mehendale et al., 1972). An initial half life of 39 hours and a second half life of 100 days was estimated by Mehendale et al. (1972).

In addition to liver hypertrophy, mirex has been shown to cause a proliferation of endoplasmic reticulum associated with an induction in mixed-function oxidases (Baker et al., 1972; Mehendale et al., 1973; Kendall, 1974; Febacher and Hodgson, 1976; Peppriell, 1981).

It is well established that the liver is a major site of xenobiotic metabolism and elimination. Mehendale (1977a, 1977b, and 1981) and Curtis and Mehendale (1978, 1980, and 1981) have demonstrated that rats exposed to the organochlorine analogs mirex and kepone exhibited hepatobiliary dysfunction. Excretion of xenobiotic metabolites in the bile was inhibited by preexposure to these chemicals. The decreased ability of animals to

excrete the chemicals was correlated with the inhibition of bile canaliculi enriched fraction (BCEF) ATPase activities. The disordering of the membranes by the organochlorines could have resulted in decreased ATPase activities because of resulting perturbations in enzyme microenvironments. Curtis and Hoyt (1984) reported that preexposure to mirex and kepone resulted in increased permeability of the biliary tree, leading to a decrease in hepatobiliary concentrative performance.

This study was performed to further characterize the effects of mirex exposure on the hepatobiliary system. Much of the work was similar to that previously performed. However, the role of endogenous bile salts was also investigated to obtain new information on mirex induced hepatic dysfunction. Phenolphthalein glucuronide excretion was also measured to assure that the dose studied corresponded with previously reported hepatobiliary dysfunction.

MATERIALS AND METHODS

Animals and Treatment

Adult (300-400 g) male Sprague-Dawley rats (Simonson Breeding Laboratories, Gilory, CA) were maintained in an animal facility on a 12-hour dark/light cycle. The rats were provided with water and Purina rat chow ad libitum until use. Animals were housed 2 to 4 in a plastic cage which contained a layer of chipped cedar wood bedding.

Test animals were gavaged with mirex in corn oil (60 mg/kg) at hour zero. Some groups were given an additional dose 24 hours later. Controls received pure corn oil only. The time period for each group was the time expired from the last mirex treatment. Seven test groups were used: 48 hour (48-1X), 96 hour (96-1X), and 14-day single dose (14-1X); 48 hour (48-2X), 96 hour (96-2X), and 14-day double dose (14-2X), and controls (C) which were matched in time after gavage with each mirex dose group.

Surgical Procedure

Animals were anesthetized with sodium pentabarbital (50 mg/kg ip), and light anesthesia was maintained with additional pentabarbital as needed throughout the experiment. Body temperature was monitored with a rectal thermoprobe and maintained at 37'C by means of themostatically controlled heating pads. Following anesthesia, the femoral vein was cannulated with PE-50 tubing. An abdominal incision was made, the renal pedicles ligated, and the common bile duct of all rats was cannulated with PE-10 tubing. At the termination of the experiments, the rats were sacrificed with an overdose of pentabarbital, their livers excised and stored at -20' C until use.

Mirex Residues

Mirex residues in rat liver were measured using a modification of the method of Hallett, et al. (1976). A 0.4 to 1.0 g portion of each frozen liver was ground with 5 g of Florisil (80-200 mesh) in a mortar and pestle until the resulting powder was uniformly pink and free flowing. The Florisil-liver mixture was dry packed

between 1 cm plugs of anhydrous sodium sulfate in a 22 mm i.d. glass column and extracted with 100 ml of acetonitrile. The eluate was transferred to a 250 ml separatory funnel and shaken with 100 ml hexane and 25 ml of water, allowed to clarify, and the aqueous phase discarded. The hexane fraction was reduced to approximately 5 ml on a rotary-evaporator and applied to a 12 mm i.d. column wet packed with 5.0 g of fully activated Florisil (baked at 450' C overnight and stored at 130' C). The mirex was eluted from the column with 30 ml of hexane. The extract was diluted or concentrated so that the resulting mirex concentration was about 15 ng/ml.

The mirex residue was injected into a Varian 3740 gas chromatograph equipped with a Supelco SPB-5 0.75 mm X 30 m Widebore capillary column (Supelco Inc., Bellefonte, PA) and an electron capture detector. The oven was maintained at 245'C, the injection port at 260'C, and the detector at 320'C. Quantitation was accomplished by using the paired sample:standard technique and measuring peak heights. At least 3 pairs of injections were used in quantitating each residue. Each liver was tested twice. A third sample was taken from one liver as the first two results differed by more than 15%. In each sample set, a control liver was spiked at approximately

0.5 ppm in the extraction column and carried through the entire procedure. Recoveries averaged 98% (n=11) and ranged from 82-114%.

Endogenous bile acid excretion

Bile acids are critical in bile formation (see above). However, little work has been done to determine the effects of cholestatic compounds on endogenous bile acid excretion. Basal endogenous bile acid excretion was determined by collecting bile for 7.5 minutes and determining the concentration of total bile acids present by the method of Berthelot, et al., 1970.

Biliary Excretion of Phenolphthalein Glucuronide

Phenolphthalein Glucuronide (PG) was used as a model anionic compound for assessing the modification of the biliary excretory system as it has been previously shown to be a sensitive indicator of hepatobiliary dysfunction (Curtis and Mehendale, 1979). Phenolphthalein Glucuronide (PG) (Sigma Chemical Company, St. Louis, MO) was administered intravenously (10 umol/kg) as a bolus into the femoral cannula and flushed with the use of a syringe containing heparinized saline. Bile was

collected over four fifteen minute periods following the PG injection and the volumes recorded. The concentration of PG in the bile was determined by the method of Gustafson and Benet (1974), and the rate of PG excretion and the percent total dose excreted calculated.

Statistics

Statistics were generated using the Number Cruncher Statistical System program, version 4.1 (Dr. Jerry L. Hintze, Kaysville, Utah). Comparisons between groups means were made via one-way analysis of variance with the significance level set at $p < 0.05$.

Test results for control groups were combined as statistical analysis indicated that the six groups were similar in all respects.

RESULTS

Mirex Concentrations in Liver and Liver Burdens

Within 48 hours of the last mirex treatment, test animals exhibited their highest mirex liver concentrations and liver burdens (mirex liver concentration x liver weight). However, both mirex concentrations and burdens dropped rapidly over the next 48 hours. By day 14, hepatic mirex levels and burdens appeared to be stabilizing.

All test groups had mirex liver concentrations and burdens significantly higher than control rats (Fig. 2, panels A and B, respectively). Double dose groups had significantly higher (approximately double) mirex concentrations and burdens than corresponding single dose groups. The concentrations and total liver burdens of 48-hour groups were about twice that of corresponding 96-hour groups, while those of 14-day and 96-hour groups were similar.

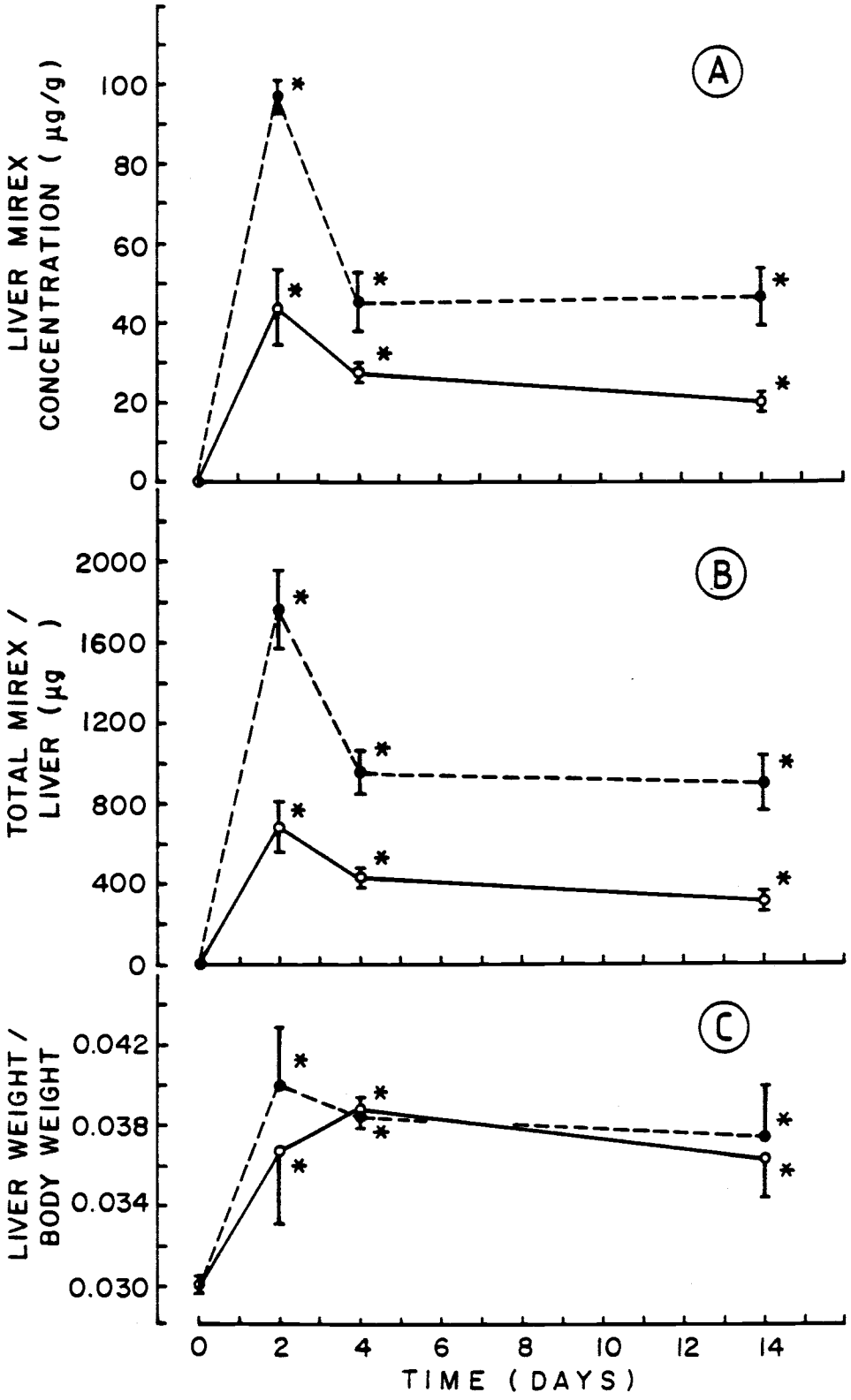
Figure 2. A: Concentration of mirex in the liver in ppm. Sample sizes were, by group; Controls:15, 48-1X:3, 96-1X:7, 14-1X:5, 48-2X:3, 96-2X:5, 14-2X:3.

B: Total mirex liver burden in ug. Sample sizes were, by group; Controls:13, 48-1X:3, 96-1X:4, 14-1X:5, 48-2X:3, 96-2X:3, 14-2X:3.

C: Liver size as percent body weight. Sample sizes were, by group; Controls:37, 48-1X:3, 96-1X:20, 14-1X:9, 48-2X:7, 96-2X:19, 14-2X:6.

Vertical lines represent $\pm$ 1 se. Asterisks (*) represent significant difference from controls at $p < 0.05$. Solid lines (-----) and open circles represent single dosed groups, dashed lines (- - -) and solid circles represent double dosed groups.

Figure 2.


LIVER MIREX CONCENTRATION (µg/g)
100
80
60
40
20
0
A
TOTAL MIREX / LIVER (µg)
2000
1600
1200
800
400
0
B
LIVER WEIGHT / BODY WEIGHT
0.042
0.038
0.034
0.030
C
0
2
4
6
8
10
12
14
TIME (DAYS)

Liver Size

By 48 hours, the livers of treated rats had hypertrophied significantly in response to mirex exposure. Interestingly, single dosed animals exhibited greatest liver hypertrophy after 96 hours while double dosed animals showed the greatest response after only 48 hours (Fig. 2, panel C).

Bile Excretion

The volume of bile excreted on a per gram liver basis (Fig. 3, panel A) was not significantly different between controls and any of the other groups except 96-2X animals, which demonstrated decreased bile flow.

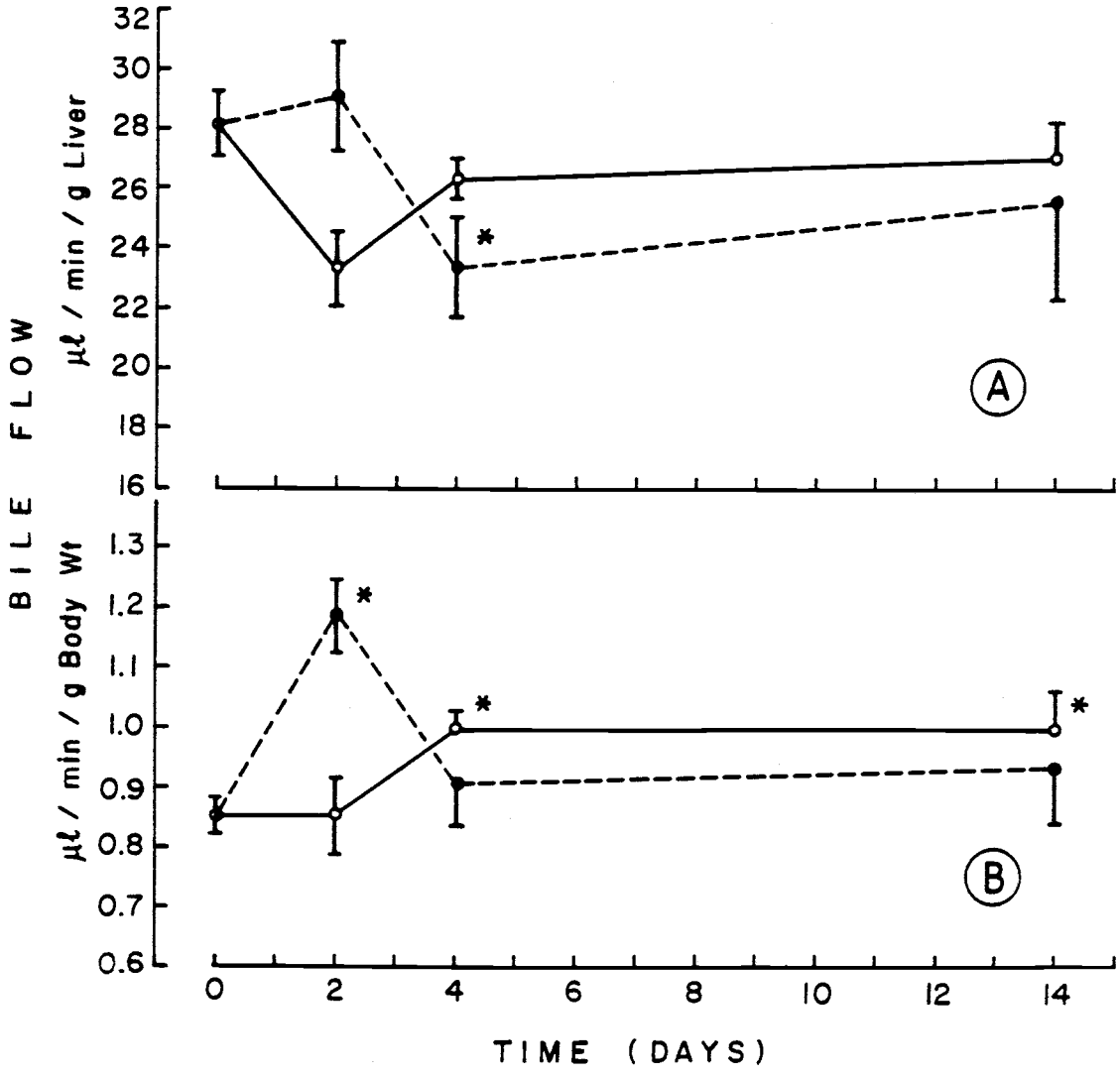
On a per gram body weight basis (Fig. 3, panel B), bile flow of groups 96-1X, 48-2X, and 14-1X were significantly higher than controls. Group 48-2X bile flow was significantly higher than all other groups.

Figure 3. A: Bile excretion in ul/minute/g liver. Sample sizes were, by group; Controls:29, 48-1X:3, 96-1X:14, 14-1X:8, 48-2X:7, 96-2X:10, 14-2X:6.

B: Bile excretion in ul/minute/g body. Sample sizes were, by group; Controls:29, 48-1X:3, 96-1X:14, 14-1X:8, 48-2X:7, 96-2X:10, 14-2X:6.

Vertical lines represent $\pm$ 1 se. Asterisks (*) represent significant difference from controls at $p < 0.05$. Solid lines (-----) and open circles represent single dosed groups, dashed lines (- - -) and solid circles represent double dosed groups.

Figure 3.


BILE FLOW
µℓ / min / g Liver
µℓ / min / g Body Wt
32
30
28
26
24
22
20
18
16
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
A
B
*
0
2
4
6
8
10
12
14
TIME (DAYS)

Bile Salt Excretion

Although the concentration of excreted bile salts (Fig. 4, panel A) and the rate of bile salt excretion on a per gram liver basis (Fig 4., panel B) was lower in mirex exposed rats than controls, only double dosed groups were significantly lower. On a per gram body weight basis, there were no significant differences in bile salt excretion between any of the groups (Fig. 4, panel C), although there was an apparent decrease in bile salt excretory ability in groups 96-2X and 14-2X.

Phenolphthalein Glucuronide Excretion

Only group 96-2X excreted statistically less of the PG dose than controls in an hours time (Fig. 5, panel A). Remarkably, 10 days later, group 14-2X excreted significantly more PG than controls (Fig. 5, panel A).

During the first 15 minutes following PG administration, control rats had a higher average rate of PG excretion on a liver basis than all other groups (i.e., significantly higher than 96-1X, 14-1X, 48-2X, 96-2X groups). While the other groups with depressed PG excretion rates had similar excretion rates compared to

Figure 4. Total bile salt excretion.

A: Biliary concentration of bile salts (mM). Sample sizes were, by group; Controls:25, 96-1X:13, 14-1X:6, 48-2X:8, 96-2X:11, 14-2X:5.

B: Bile salt excretion in nmol/min/g liver. Sample sizes were, by group; Controls:25, 96-1X:13, 14-1X:6, 48-2X:8, 96-2X:11, 14-2X:5.

C: Bile salt excretion in nmol/min/g body. Sample sizes were, by group; Controls:25, 96-1X:13, 14-1X:6, 48-2X:8, 96-2X:11, 14-2X:5.

Vertical lines represent $\pm$ 1 se. Asterisks (*) represent significant difference from controls at $p < 0.05$. Solid lines (-----) and open circles represent single dosed groups, dashed lines (- - -) and solid circles represent double dosed groups.

Figure 4.

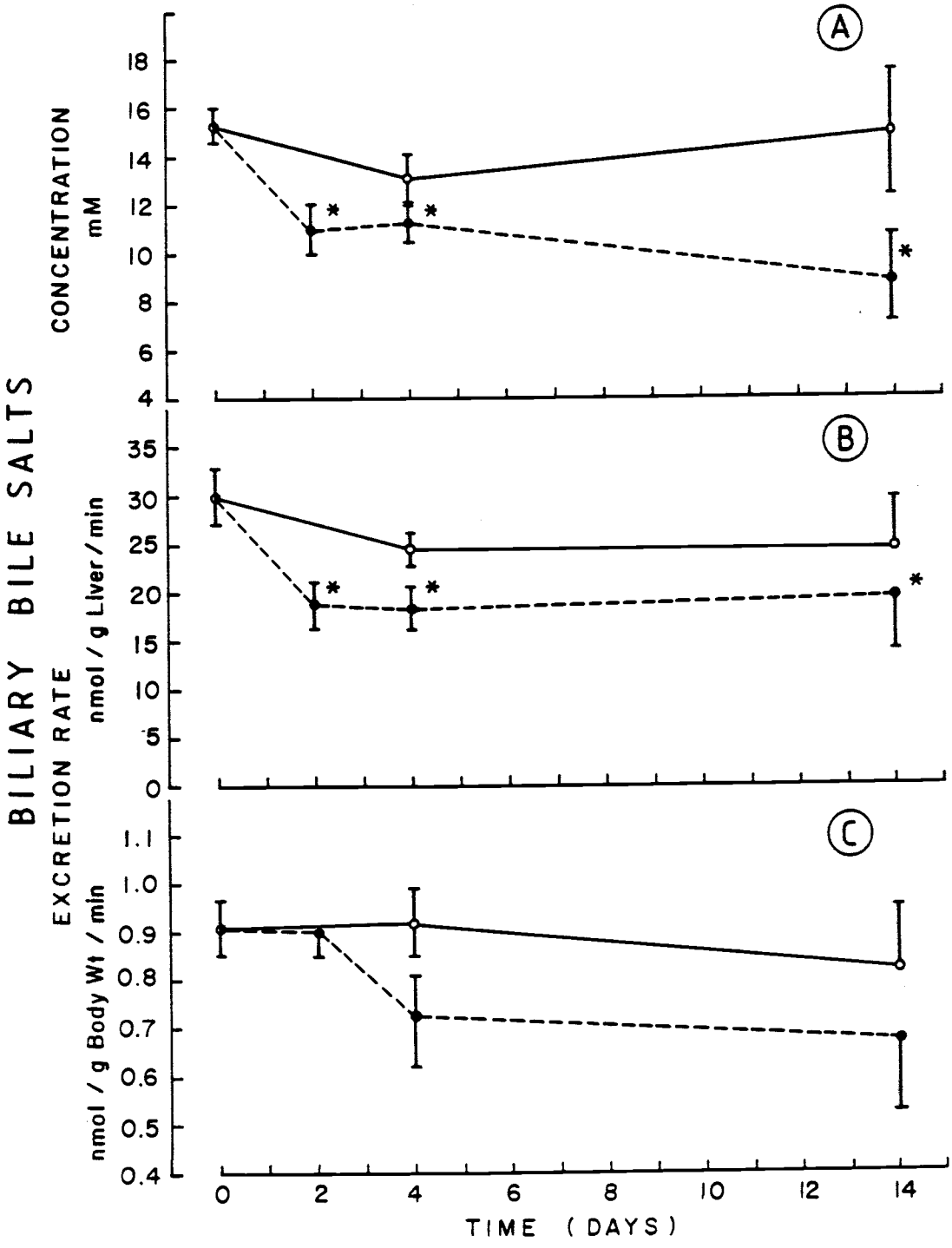

BILIARY BILE SALTS
CONCENTRATION
mM
EXCRETION RATE
nmol / g Liver / min
nmol / g Body Wt / min
A
B
C
TIME (DAYS)
0
2
4
6
8
10
12
14

Figure 5. Phenolphthalein glucuronide excretion.

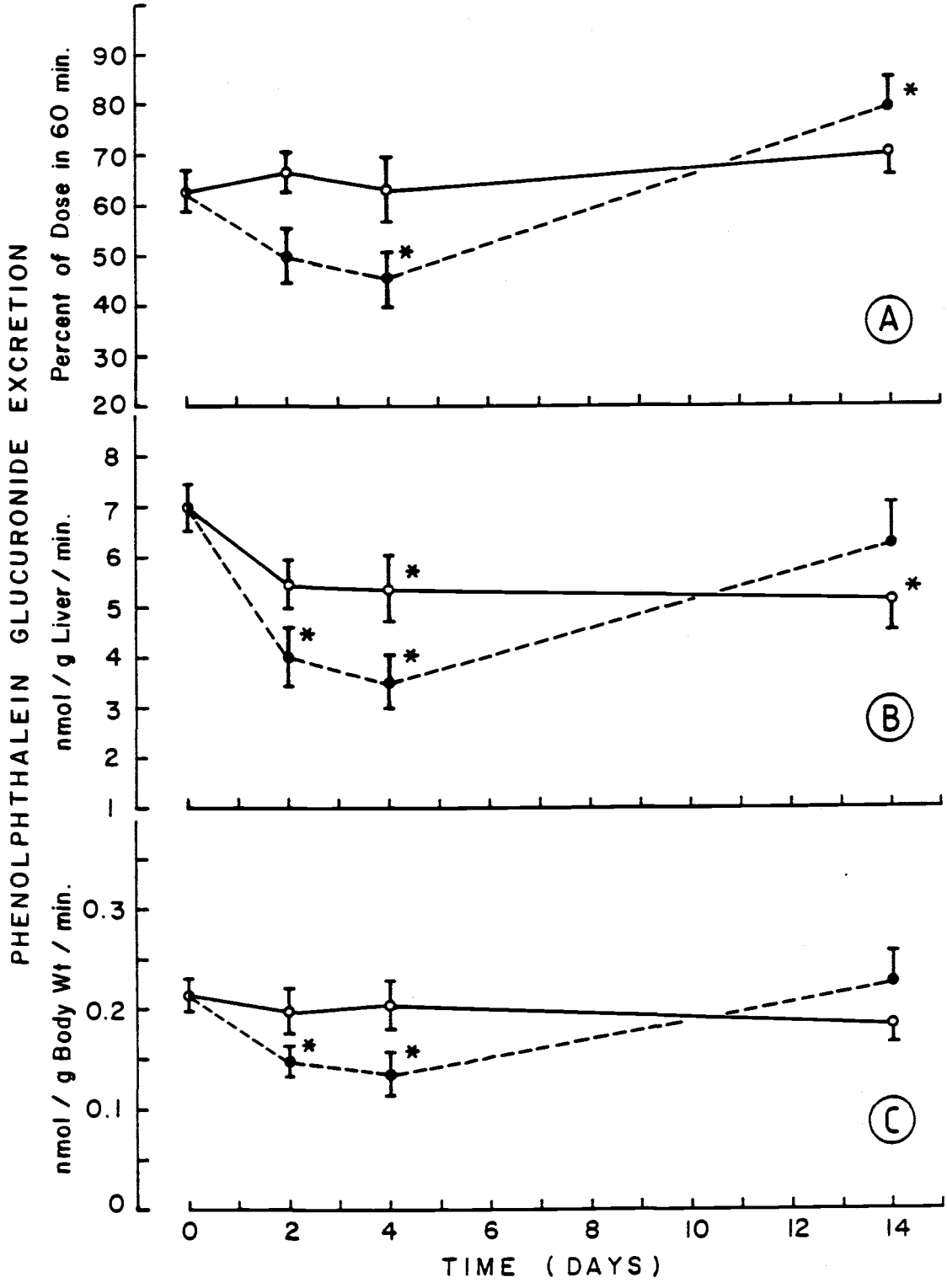
A: Percent dose excreted in 60 minutes.
Sample sizes were, by group; Controls:28, 48-1X:3, 96-1X:14, 14-1X:8, 48-2X:6, 96-2X:10, 14-2X:6.

B: PG excreted (nmol/min/g liver) in first 15 minutes following injection.
Sample sizes were, by group; Controls:28, 48-1X:3, 96-1X:14, 14-1X:6, 48-2X:8, 96-2X:10, 14-2X:6.

C: PG excreted (nmol/min/g body) in first 15 minutes following injection.
Sample sizes were, by group; Controls:28, 48-1X:3, 96-1X:14, 14-1X:6, 48-2X:8, 96-2X:10, 14-2X:6.

Vertical lines represent $\pm$ 1 se.
Asterisks (*) represent significant difference from controls at $p < 0.05$.
Solid lines (-----) and open circles represent single dosed groups, dashed lines (- - -) and solid circles represent double dosed groups.

Figure 5.


PHENOLPHTHALEIN GLUCURONIDE EXCRETION
Percent of Dose in 60 min.
nmol / g Liver / min.
nmol / g Body Wt / min.
A
B
C
TIME (DAYS)
0
2
4
6
8
10
12
14

controls in the next 15 minute time interval, group 96-2X had a significantly lower PG excretion rate for the next three 15 minute intervals (data not shown).

On a body weight basis (Fig. 5, panel C), control rats had a higher average rate of PG excretion in the first 15 minutes than all other groups except 14-2X, but only significantly higher than 48-2X and 96-2X. Again, group 96-2X demonstrated the greatest impairment of PG excretory ability of the test groups, and group 14-2X animals showed the greatest recovery. Over the next three 15 minute time intervals, group 14-2X excreted significantly more PG than controls. For the next 30 minutes, group 96-2X continued to excrete less than controls (data not shown).

DISCUSSION

Exposure of rats to the organochlorines chlordecone and mirex inhibits the excretion of PG and polar metabolites of imipramine despite stimulated bile flow (Mehendale, 1976; and Curtis and Mehendale, 1979). In addition, Curtis and Mehendale (1979) demonstrated a possible link between decreased mitochondrial ATPase activities and the decreased ability of rats to excrete these compounds after exposure to chlordecone in the feed. Using isolated, bile canalicular enriched fractions (BCEF) of mirex-exposed rat livers, Curtis and Mehendale (1981) demonstrated decreased activities of 5'nucleotidase, glucose-6-phosphatase, Na+/K+ ATPase, and both oligomycin sensitive (mitochondrial) and insensitive Mg2+ ATPases. As these enzymes are membrane bound and are found in different organelles, it seems likely that the mirex becomes incorporated into the cellular membranes and acts as a general membrane perturbant by changing membrane fluidity. It was shown that the decreased enzyme activities was not due to competitive inhibition (Mehendale, 1981; Curtis and Mehendale, 1981).

Work with organic anions such as imipramine and sulfobromophthalein in isolated, perfused rat livers has shown that metabolism of these compounds to readily

excretable polar metabolites is not affected by mirex (Mehendale, 1977a) or kepone (Mehendale, 1977b) pretreatment. As polar metabolites of these compounds accumulated in the perfusate, it is clear that hepatocyte uptake and metabolism was not greatly affected. In addition, when mirex exposed rats are supplied with PG (Mehendale, 1981) or taurocholate (Curtis and Hoyt, 1984), both readily excreted in the bile, excretion of these compounds is impaired. As impaired excretion of polar metabolites of monochlorobiphenyl has also been shown in isolated, perfused rat livers following mirex exposure (Mehendale, 1979), it can be concluded that mirex inhibition of hepatobiliary excretion of organic anions is not substrate specific and does not affect the metabolism of these compounds. It appears that the transfer of the organic anion metabolites from the hepatocyte into the bile canaliculi is the process impaired. It is unclear whether the underlying mechanism is related to cellular energy production or inhibition of the transport systems (Mehendale, 1979).

Mehendale (1981) was able to demonstrate a linear relationship between mirex liver residues and hepatic dysfunction as measured by the ability of exposed rats to excrete exogenously supplied PG over 60 minutes. He also showed that hepatic dysfuction was not due to competitive inhibition of the transport process.

Mirex exposure apparently inhibits hepatobiliary performance by interfering with the transfer of compounds through the hepatocyte and into the canalicular lumen. For some compounds, active transport is required for them to pass through this membrane. Others may penetrate freely, drawn by an osmotic and electrochemical gradient. Mirex has been shown to interfere with the functioning of mitochondrial (oligomycin sensitive) ATPases, which could disrupt the energy supply of the hepatocyte. Under such conditions, it is conceivable that the cell would budget the limited energy available to functions most critical for the cell's survival. As a consequence, it is possible that processes requiring energy involved with biliary excretion could be compromised (Curtis and Mehendale, 1979).

The paracellular junction has been shown to isolate the bile from liver perfusate, but allows some molecules to be drawn into the canalicular lumen by the osmotic and electrochemical gradient. Water and nonpolar molecules such as erythritol and some sugars have been shown to be among those molecules. Many researchers (Forker, 1969; Dubin et al., 1980; Elias et al., 1980; Bajwa and Fujimoto, 1983; Jaeschke et al., 1983) have done work that indicates that the cholestatic agents colchicine, phalladoin, and alpha-napthylisothiolynate affect the

structure of the junctional complexes leading to an increase in the permeability of the of the biliary tree. As a result, the efflux of biliary solutes and water into the intercellular spaces can occur, leading to a decrease in bile flow (Curtis and Hoyt, 1984).

Curtis and Hoyt (1984) found that mirex exposed rats had an increase in permeability of the paracellular pathway by using radiolabeled erythritol and sucrose and measuring their bile:plasma ratios. A decrease in the ability to excrete exogenously supplied xenobiotic metabolites was partially explained by the increased permeability of the biliary tree.

It has been previously shown that after acute mirex exposure, an initial high liver burden rapidly declines as the compound redistributes and reaches equilibrium in the tissues with the bulk of it in adipose tissue (Gibson et al., 1972). This was evident whether mirex liver concentration or total liver burden was examined. Liver hypertrophy, a characteristic of mirex exposure, was significant for all doses and time points.

Organochlorine exposure has been shown to cause an increase in bile flow on a whole liver basis although on a per gram basis the flow is often similar to or less than controls. It is of interest that although group 48-2X had the highest bile flow of all the groups on a whole

liver basis, the other double dosed groups had flows similar to controls. Data of PG excretion indicate that hepatobiliary dysfunction was maximal in group 96-2X. Excretion of PG was maximally depressed at 96 hours for both doses on a per gram and whole liver basis, and bile salt excretion on a per gram liver and concentration basis were also depressed. It was expected that bile excretion of group 96-2X on a gram liver basis would be depressed but that whole liver flow would be increased due to the effects of liver hypertrophy. Single dosed animals demonstrated increased whole liver bile flows at this time, as is common for organochlorine poisoned rats. The decreased whole liver bile flow could be a manifestation of the toxic effects of mirex (Curtis, pers. comm.). It is the only group which demonstrated significantly decreased bile flow on a per unit liver basis. As bile flow on a whole liver basis was not elevated, it is possible that the liver's integrity was so highly compromised that the anticipated increase in whole liver bile flow was prevented by efflux of bile through junctional complexes, inhibition of sinusoidal N+/K+ ATPases, or a combination of these and other factors. By 14 days, double dosed rats had fully recovered the ability to excrete PG in the first 15 minutes following dosing, and significantly outperformed controls in PG excretion over an hours time.

Bile salt excretion is of primary importance in bile formation. Endogenous bile salt excretion was significantly reduced in all double dosed groups on a gram liver basis, as well as biliary concentration. It seems that double dosed animals received a sufficient mirex dose to yield a cholestatic effect while single dosed animals did not. As it is the concentration of bile salts in the bile that are responsible for establishing the osmotic gradients critical to bile formation, the fact that bile salt concentrations in mirex exposed animals (double dosed) were lower than controls is of importance. Double dosed groups demonstrated a significantly reduced ability to concentrate bile acids in the bile. This could be due to an increased flux of fluid into or out of the bile, decreased activities of Na+/K+ ATPase (the enzyme which generates the driving force of bile formation), a decreased ability of the hepatocyte to transport the bile acids into the canalicular lumen, decreased hepatic bile acid concentrations, or a combination of the above factors.

Previous studies (Curtis and Mehendale, 1979; Curtis, 1979; Mehendale, et al., 1979) with chlorocarbon challenged, intact animals supplied with readily excretable anionic model compounds demonstrated a reduced

excretory ability that was dose related and not attributable to decreased bile formation (on a gram liver basis). Under similar conditions, Curtis and Mehendale (1981) had shown that oligomycin insensitive Mg2+ ATPase, the principal ATPase component of BCEF, was inhibited. They suggested that the results could be explained by impairment at the site of anion transport across the bile canalicular. My data would support the contention that such transfer sites could have been inhibited by the presence of mirex, especially at higher liver concentrations where rats were less able to concentrate bile salts in the bile.

The excretion of PG is an excellent indicator of the ability of the liver to excrete organic anions. Cumulative PG excretion (over 60 minutes) was inhibited for the 96-2X group, the time and dosage at which mirex had exerted its maximal cholestatic effect. By 14 days, the high dosage animals had fully recovered. Single dose animals were not significantly affected.

A sensitive indication of impaired organic anion excretion is the rate of excretion during the first 15 minutes as the bulk of the anion will be excreted in this time by a healthy animal. Even in single dosed animals, a significant decrease in excretory performance was apparent at 96 hours when examined on a per gram liver

basis. Hepatobiliary dysfunction was obvious as soon as 48 hours in high dose animals, with maximal effects apparent at 96 hours. Most interesting is the fact that by 14 days, high dosage groups had recovered whereas low dosage groups had not. It is possible that liver burdens in high dosage animals were sufficient to fully mobilize compensatory mechanisms whereas the low mirex dose did not stimulate such mechanisms to the same extent.

On a whole liver basis, it is clear that low dosage groups had adequately compensated for poor PG excretory performance by liver hypertrophy. By 14 days, high dosage groups had also demonstrated recovery but were still deficient in PG excretion at earlier time points, despite liver hypertrophy. Apparently, these groups had overcompensated for the cholestatic effects of mirex because during later time intervals, they significantly outperformed controls in excretion of remaining PG.

If the transfer of organic anions from the perfusate to the bile were truly non-substrate specific, results of bile salt and PG excretion tests should have been very similar. On a per gram liver basis they are close at 48 and 96 hours. At 14 days, low dose groups were still deficient in PG but not bile salt excretion. On a whole liver basis, the ability of high dosage rats to excrete PG was significantly compromised at the first two time

points while bile salt excretion was virtually identical at 48 hours and somewhat depressed at 96 hours. After 14 days, PG excretion for high dosage rats was slightly higher than controls while bile salt excretion was lower. If bile salts and PG were excreted by the same mechanisms, there should be competitive inhibition of one by the other. This is unlikely as sinusoidal membrane proteins have been identified that are fairly specific for bile acids and other proteins have been associated with the uptake of other organic anions (Klaassen and Watkins, 1984). The discrepencies in the PG and bile salt data cannot be explained on the basis of bile flow, nor can they be explained by any of the other factors studied as these factors were the same for each test. It is possible that bile salts were metabolized back to cholesterol esters and incorporated into membranes to reduce hyperfluidity that was likely caused by mirex.

Kakis and Yousef (1978) noted rapid and progressive changes in bile canaliculi membrane composition following the infusion of lithocholic and taurocholic acids. The former agent also induced a significant and progressive decrease in Na+/K+ and Mg+ ATPase activity of the BCEF membranes and was incorporated into the BCEF membranes, in contrast to taurocholic acid. In only 30 minutes, lithocholic acid treated animals demonstrated a 6-7 fold

increase in cholesterol in the BCEF while taurocholic acid treated animals demonstrated a 2-fold increase. Perhaps the depressed 14 day bile salt levels of high-dose animals could be partially explained by a shift in cell priorities in their use.

Curtis and Mehendale (1981) showed decreases in BCEF ATPase activity in chlorocarbon exposed rats. Direct membrane effects were proposed as the mode of action as the chemical could be "washed out" in vitro and enzyme activity restored (Desaiah et al., 1977).

It has been previously shown that mirex exposure leads to decreased per gram liver bile flow but increased whole liver bile flow due to hypertrophy in chronically exposed rats. In my study, bile flow averages on a liver weight basis were decreased for all groups except 48-2X, but only significantly decreased in group 96-2X. Group 48-1X demonstrated similarly decreased bile flow compared to 96-2X animals, but statistical significance was precluded by a small sample size.

All treated group bile excretion averages were higher than the control average on a body weight basis. Although other double dosed groups exhibited bile flow rates in the control range, group 48-2X demonstrated the highest whole liver bile flow of all the groups. This is especially interesting as single dosed animals

demonstrated bile flow rates identical to controls at 48 hours while at later time points single dosed animals had significantly elevated bile flow rates. Again, this discrepency could be explained by greater induction of compensatory mechanisms by double dosed animals.

Biliary bile salt concentrations of treated animals were lower than controls, the double dosed animals being significantly affected at all time points. This could indicate a dose dependent response.

When bile salt excretion is looked at in terms of per gram liver, it is clear that bile salt excretion is depressed. Under chlorocarbon stress it is common for hepatic organic anion excretion efficiency to decrease, due to depressed ability of the animal to excrete bile salts into the canalicular lumen, increased flux of water into the lumen, and/or fluid contributions at some point(s) in the ductular network.

There were no statistically significant differences between groups when bile salt excretion is examined on a body weight basis. However, bile salt excretion appeared to be inhibited at 96 hours and 14 days for double dosed animals. Large variability in individual animal performance seems to have prevented the difference from having statistical significance. As bile salt excretion is critical to bile formation, this apparent excretory

deficiency is extremely important.

It can be concluded that although a single mirex dose of 60 mg/kg resulted a hypertrophied liver and stimulated whole liver bile flow, it was insufficient to significantly inhibit whole liver performance as measured by the excretion of exogenously supplied PG and endogenous bile salts. A double dose of mirex lead to significantly greater hypertrophy than single dose animals, reduced excretion of bile salts and PG in the first 15 minutes following infusion on both a per gram and whole liver basis, and depressed bile salt excretion on a liver basis. Maximal disruption of hepatobiliary excretory performance was evident 96 hours following the second mirex dose.

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