DEVELOPMENT OF AN ENVIRONMENTAL BIOMONITOR USING COTTON RATS (SIGMODON HISPIDUS)

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PREFACE

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

Table Page

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER V

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

One of the most critical environmental issues today is the pollution of soil and water with toxic chemicals. In recent years, the concern for potential toxicity from the exposure to multiple toxic chemicals has been amplified by various environmental problems, including those associated with hazardous waste sites. However, there are no accepted methods to determine the additive, antagonistic, or synergistic effects of such chemical mixtures in the environment, nor are there well-defined assay systems whereby the potential risk to animals and humans environmentally exposed to these pollutants can be reliably assessed.

Because of possible adverse biological effects to human and animals resulting from toxic chemicals, a major priority has been the development of early warning sentinels which will identify and define areas of contamination. Simple quantitation of toxic substances in water and soil with comparison to levels of toxins known to cause disease in laboratory animals is not an acceptable means of determining the potential hazard of toxins in the environment (Clark et al., 1982; Rowley et al., 1983). Additionally, analytical assays for specific toxins typically recognize only those toxins for which specific procedures were performed. Toxicity in the natural environment is usually the result of exposure to complex mixtures of toxins, and their metabolites or degradation products, where additive insults of low levels of multiple toxins may cause deleterious effects (Rowley et al., 1983). Furthermore, there is

limited scope of investigation in this area because of the complexity of experiments required for a chemical mixture that has more than two components. There have been some recent studies dealing specifically with toxicity of complex mixtures such as a dump site samples (Plotkin and Ram, 1984; Silkworth et al., 1984), combustion products from tobacco smoke (Heckman and Dalbey, 1982; Bassi et al., 1984; Rylander, 1984), diesel fuel and gasoline (Dalbey and Lock, 1982; MacFarland, 1984), contaminated water samples (Bull, 1984; Kool et al., 1985), contaminated fish (Villeneuve et al., 1981; Chu et al., 1984), by-products from synfuel operations and coal combustion (Cunningham et al., 1985; Springer et al., 1986), and chemical mixtures of environmental concern (Yang and Rauckman, 1987). Toxins which have been "aged" in the environment may be more toxic than corresponding quantities of technical mixtures (out of a bottle) which are used in virtually all laboratory experiments. This effect has been demonstrated with polychlorinated biphenyls (Hornshaw et al., 1983).

Resident wildlife species have been used successfully to monitor a variety of toxic effects from environmental contaminants (Dixon, 1982; Hutton, 1982; Smith and Rongstad, 1982; Ellenton et al., 1983; Helwig and Hora, 1983; Rowley et al., 1983; Thalken and Young, 1983). Biological monitoring of exposure to various chemicals has advantages over routine analyses of soil and water concentrations of toxins. It is difficult to relate these routine analyses to actual bioavailability of chemicals to resident wildlife, which is the real determinant of the toxic risk in the exposed subjects.

The cotton rat (Sigmodon hispidus) is a small, robust rodent with a scaly, sparsely haired tail that is shorter than the combined length of head and body. Total length, 200-275 mm; tail, 90-110 mm; hind foot, 30-35 mm; ear, 15-20 mm; weight, 50-150 g (Schwartz and Schwartz, 1981). Cotton rat may serve as a bioindicator of environmental pollutants due to its abundant distribution throughout much of the

United States (Lyane, 1974; Grant et al., 1982), its ease of capture, and its sufficient size to allow multiple assays on single animals. Furthermore, its short life expectancy (less than one year), its confined area of movement (less than one hectare) and its close association with the soil have advantages for a toxicologic study of a specific area (Fleharty and Mares, 1973; Lyane, 1974).

Population Dynamics

Population dynamics of resident wildlife species can potentially change in response to exposure from toxic environmental contaminants. This has been demonstrated with wild voles in the Love Canal Emergency Declaration Area, which was considered safe for man and animals (Rowley et al., 1983). Population studies on voles from this area indicated a significant decline in the population density because of the loss of mature females and increased in utero mortality. Subsequent evaluation of individuals living in this area suggested toxicity in humans, as well (Rowley et al., 1983). Similarly, Pomeroy and Barrett (1975) have studied the effects of pesticides on population density in cotton rats. Pesticides were found to inhibit reproduction which resulted in a lower peak population density.

Population studies tend to be tedious, time consuming, and must be rigidly controlled (Rowley et al., 1983). Even when coupled with toxicologic and pathologic evaluation of tissues, potential long-term effects, especially carcinogenesis, may be difficult to assess from population studies. Development of alternate tests, that could indicate possible deleterious long-term effects, are merited.

Mixed Function Oxidase Enzyme System

The mixed function oxidase (MFO) enzyme system is of considerable interest; importance of this enzyme resides not only in its ability to detoxify certain foreign chemicals, but also its ability to produce carcinogens or reactive toxic metabo-

lites. The MFO enzyme system is induced by exposure to a wide variety of toxins such as dioxin, polyhalogenated biphenyls (PCBs and polybrominated biphenyls), alkanes, olefins, aromatic carbon systems (monocyclic and polycyclic hydrocarbons and halobenzenes), nitrogen-based systems (nitrosamines, aromatic amines, azo dyes), sulfur-based systems, and metal-based systems (Conney, 1982; Guengerich and Liebler, 1985; Payne et al., 1987). Thus, evaluation of induction of the MFO system in wildlife is a means of indicating exposure and toxicity of a variety of chemicals in our environment (Knight and Walker, 1982). The level of MFO induction is evaluated by assaying for cytochrome P-450 (Omura and Sato, 1964). Rodents are a sensitive model due to their typically high P-450 levels, their marked P-450 inducibility, and their abundance in nature (Dent et al., 1976; Kahl et al., 1980; Cresteil et al., 1986). Cytochrome P-450 activity in fish has been documented as a criterion for determining water pollution. However, the use of P-450 levels has not been consistently successful due to low inherent levels of cytochrome P-450 and marked variation in inducibility in fish (Lindstrom-Seppa et al., 1985). Cotton rats may be a more suitable model due to their high cytochrome P-450 concentrations compared to laboratory rat, their marked inducibility (Elangbam et al., 1989), and ubiquitous distribution in the southeastern United States (Lyane, 1974; Grant et al., 1982).

The specificity of the mixed-function oxygenase system depends upon cytochrome P-450 (terminal electron acceptor), of which there are at least 10 different isoenzymes in laboratory rat liver (Ryan et al., 1984; Astrom and DePierre, 1985). A single isoenzyme appears to be the product of a discrete structural gene (Nebert and Negishi, 1982). Many of these isoenzymes can be selectively induced by various xenobiotics. In laboratory rats, phenobarbital causes at most a 1-fold increase in total cytochrome P-450 activity; however specific phenobarbital isoenzymes P-450b and P-450e increase 40-fold. 3-Methylcholanthrene shows as much as a 70-fold

increase in P-450c (Thomas et al., 1987). Evaluation of isoenzymes induced by different environmental contaminants is a more sensitive indicator than total cytochrome P-450. However, typical methods for evaluation of cytochrome P-450 isoenzymes are difficult. They require either complex immunologic or recombinant DNA methods (Burke et al., 1985; Shires et al., 1987). Commercial reagents are not available, thus requiring isolation of the specific isoenzyme by numerous separations, production of monoclonal antibodies, and development of assays for quantitation that have not been standardized and have technical problems in quantitation. Problems in developing these assays include the low solubility of cytochrome P-450 and its tendency for aggregation (Rothwell et al., 1985; Seidel et al., 1984; Waxman, 1986; Thomas et al., 1987). In addition, total reliance on immunologic and recombinant-DNA methods is suspect in view of evidence that microsomes contain variable proportions of enzymatically inactive yet antigenically recognizable cytochrome P-450 apoproteins (Guengerich et al., 1982; Seidel et al., 1984).

The isoenzymes of cytochrome P-450 have unique but overlapping substrate specificities (Guengerich, 1977). As a consequence, same substrate may be metabolized by two or more isoenzymes at different rates. Therefore, many of the "model" substrates which have been used do not appear to differentiate adequately between individual isoenzymes or between induced and constitutive isoenzymes (Guengerich et al., 1982; Kaminsky et al., 1983). In Sprague-Dawley rats, while phenobarbital causes a 40- to 60-fold increase in immunologically measured isoenzyme(s), it only increases microsomal activity of aminopyrine N-demethylation and aldrin epoxidation (reactions considered to be isoenzyme specific) 3- to 6-fold (Guengerich et al., 1982; Ryan et al., 1982). The O-dealkylation of phenoxazone ethers is much more specific. Several investigators are using metabolism of specific phenoxazone ethers (ethoxy-, pentoxy-, and benzyloxy-), which are highly specific for the major isoenzymes, as an indicator of cytochrome P-450 isoenzyme induction in laboratory rats

(Burke et al., 1985; Lubert et al., 1985; Rettie et al., 1986).

The specificity of O-dealkylation of phenoxazone ethers and degree of increased metabolism by specific cytochrome P-450 isoenzymes has been demonstrated in laboratory rats. When compared to uninduced microsomes, phenobarbital-induced microsomes showed a 283-fold increase in O-dealkylation of pentoxyphenoxazone, while a 9-fold increase was observed with 3-methylcholanthrene-induced microsomes. In the same group of experiments, O-dealkylation of ethoxyphenoxazone was increased 51-fold by 3-methylcholanthrene and only 6-fold by phenobarbital (Burke et al., 1985). Similar findings have been demonstrated with other cytochrome P-450 isoenzyme inducers (Burke and Mayer, 1983; Burke et al., 1985; Lubert et al., 1985; Rettie et al., 1986).

Light and Ultrastructural Hepatic Pathology

Induction of microsomal enzymes has several consequences for the organism. Increased activity of microsomal enzymes enhances the rate of biotransformation of the inducer itself and exogenous and endogenous substances that are metabolized by this system. As a consequence, induction can cause accelerated catabolism of drugs, increased rate of inactivation of physiological hormones and vitamins, and modification of biotransformation of other exogenous substances. Activation of some parent compounds can result in formation of reactive metabolites that initiate toxic and carcinogenic events. Increased hepatic microsomal activity is associated with hepatomegaly and centrilobular hepatocellular swelling. Ultrastructurally, the outstanding effect is the proliferation of smooth endoplasmic reticulum in centrilobular hepatocytes. There is correlation between alteration in the structure and content of organelles and biochemical changes observed in the subcellular fractions (Fouts and Rogers, 1960; Staubli et al., 1969; Weibel et al., 1969; Toftgard et al., 1986; Woods and Fowler, 1986). Ultrastructural hepatic alterations not only corre-

late with biochemical events of detoxification or activation but also with toxic effects of a parent compound or its metabolites to specific organelles.

Hematology and Serum chemistry

Serum enzyme concentrations are now routine diagnostic aids for assessing toxic exposure. Enzymes are useful in part because of their specific activity and tissue-specific production. Determination of the activity of hepatic enzymes released into circulation from damaged liver has become one of the most useful tools in the study of hepatotoxicity. Pollutants such as halogenated hydrocarbons have been shown to increase liver weights, and concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in mice (Munson et al., 1982).

Chemicals toxic to bone marrow can decrease the circulating numbers of all three major groups of formed cells (pancytopenia). Agents regularly associated with pancytopenia provided exposure is sufficiently intense include benzene, arsenic, lindane, chlordane, mustards, chloramphenicol, trinibotoluene, gold, hydantoin derivatives, and phenylbutazone (Smith, 1986).

Conclusion

Environmental pollution has become a critical issue in recent years due to urban industrialization. However, there are no well defined assays or tests to determine the potential toxic hazards in man and animals. The cytochrome P-450-mediated hepatic mixed-function oxygenase system is involved in the metabolism of endogenous and exogenous compounds including, variety of environmental pollutants. The role of biotransformation is well characterized. Induction of the cytochrome P-450 by phenobarbital and 3-methylcholanthrene-type compounds has been demonstrated in a variety of mammals, lower vertebrates and invertebrates, likely to be exposed to environmental pollutants. This sensitive induction response

may be useful in the development of a biological monitoring system.

This study was designed to develop a reliable, quick and sensitive method to monitor environmental toxicity through the evaluation of hepatic cytochrome P-450 levels in wild cotton rats (Sigmodon hispidus). Population characteristics, organ pathology, hematology and serum biochemistries and ultrastructural hepatic pathology (selected cotton rats) were included for complete toxicological evaluation.

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CHAPTER II

INDUCTION OF HEPATIC CYTOCHROME P-450 ACTIVITY IN WILD COTTON RATS (SIGMODON HISPIDUS) BY PHENOBARBITAL AND 3-METHYLCHOLANTHRENE

Introduction

Cytochrome P-450 enzymes are a family of hemoproteins which are important in the metabolism of drugs, carcinogens, steroid hormones, fatty acids, and endogenous and exogenous toxins (Conney, 1982; Guengerich and Liebler, 1985; Payne et al., 1987). The bioactivation (enzyme) index of cytochrome P-450 has been used in fish as a criterion for monitoring environmental pollution (Payne et al., 1984).

For toxicological evaluation, small rodents are superior to fish and other aquatic species because they are phylogenetically closer to man and live in close proximity to man. Wild cotton rats (Sigmodon hispidus) are ubiquitous throughout the Southeast quadrant of the United States, easy to capture, have a generation interval of less than one year and a limited range of movement (less than one hectare). Adult cotton rats range in size from 110 to 225 g and 100 to 200 g for males and females respectively (Chipman, 1965). This enables the collection of sufficient body fluids and tissues for pathological and toxicological assays on individual animals rather than having to pool fluids and tissues from several animals. Furthermore, this species may prove to be an excellent model for monitoring environmental contamination.

Traditionally, cytochrome P-450 inducing agents are grouped into two classes. One, represented by phenobarbital, induces P-450b and P-450e; the other, represented by 3-methylcholanthrene, induces P-450c and P-450d isoenzymes. The types and amounts of cytochrome P-450 vary among species, organs, health status, sex, and stress of the animal (Astrom et al., 1986; Sipes and Gandolfi, 1986, Thomford and Dziuk, 1986). If the levels of cytochrome P-450 of wild cotton rats are to be used in monitoring environmental pollution, it is necessary to characterize the inducibility and concentration of cytochrome P-450 in this species.

This study was designed to determine the concentration and inducibility of cytochrome P-450 in the livers of cotton rats after intraperitoneal (ip) administration of phenobarbital and 3-methylcholanthrene.

Materials and Methods

Pregnant female cotton rats were collected from uncontaminated areas with Sherman live traps. They were housed in individual polycarbonate cages with wire tops containing wood shavings as bedding. They were maintained using 12-hour photoperiod, were fed rat chow (Rodent laboratory chow 5001, Purina Mills, Inc.), and received water free choice. Their young were weaned at 3 weeks and housed 2 animals of the same sex to a cage until they weighed a minimum of 85 grams. Before initiating experiments, each rat was weighed and placed in an individual cage. Induction studies were conducted using phenobarbital, 70 mg/kg in 0.09 % saline ip for 4 days, and 3-methylcholanthrene, 25 mg/kg in corn oil ip for 3 days. Four males and four females received each agent. Two groups of four males and four females each, one group receiving saline and one receiving corn oil by ip injection were served as controls. On the day after the last injection the animals were fasted overnight then killed by cervical dislocation. Animals were exsanguinated by severing the aorta. Livers from both control and treated rats were weighed and total cytochrome P-450

levels were assayed (Omura and Sato, 1964). Microsomal protein was determined on solubilized microsome samples (Smith et al., 1985). Representative liver samples were fixed in 10% buffered formalin and processed for histopathologic examination. Results were analyzed using a Student's t-test (Steel and Torrie, 1980).

Results and Discussion

The liver weight to body weight ratio was significantly ($P < 0.05$) increased 139% (males) and 165% (females) of the control value in rats receiving phenobarbital and 156% (males) and 158% (females) of the control value in rats receiving 3-methylcholanthrene (Table 1). Microscopically, the hepatic lobules of treated livers were enlarged due to hypertrophy of hepatocytes particularly in centrilobular areas (Fig. 1,2). The total hepatic cytochrome P-450 concentrations were higher in animals receiving phenobarbital and 3-methylcholanthrene than in control animals (Table 1). The concentration of cytochrome P-450 was similar between male and female animals. Furthermore, the concentration of hepatic cytochrome P-450 in treated rats was more than that reported for laboratory rats (Sumner and Lodola, 1987): 1.3 $\pm$ 0.1 nmoles/mg protein and 1.0 $\pm$ 0.14 nmoles/mg protein for phenobarbital and 3-methylcholanthrene respectively. The cytochrome P-450 inducibility in laboratory rats is reported to be approximately 50 to 100% of the control value (Kahl et al., 1980). In contrast, the value in wild cotton rats was 146% to 209% of the control value, indicating greater cytochrome P-450 response. Previous induction studies on cotton rats dealt only with 3-methylcholanthrene, an inducer of P-450c and P-450d. This response leading to higher P-450 concentration may be related to species differences in regulatory genes. It has been reported that P-450 induction is regulated by a cluster of genes (Kahl et al., 1980). Consequently, genetic differences in capacity for the induction of cytochrome P-450 can cause large differences in xenobiotic metabolism, thereby leading to significant differences in concentration and induction

of cytochrome P-450 among different species.

Biotransformation enzyme activity in fish has been documented as a criterion for determining water pollution (Payne et al., 1984). However, the use of cytochrome P-450 levels in fish has not been consistently successful due to low inherent levels of cytochrome P-450, and marked variation in inducibility (Lindstrom-Seppa et al., 1985). Cotton rats are likely to be more useful model due to their typically high cytochrome P-450, their marked inducibility and ubiquitous distribution in Southeast United States. Our initial studies at a contaminated toxic waste disposal site suggest that hepatic cytochrome P-450 in the cotton rats may be a useful indicator of environmental contamination (Elangbam et al., 1987).

Summary

The concentration and inducibility of hepatic cytochrome P-450 in wild cotton rats (Sigmodon hispidus) using phenobarbital and 3-methylcholanthrene are presented. After treatment, the total cytochrome P-450 concentration was significantly elevated ($P < 0.05$) to 146 to 209% of the control value. There was centrilobular hypertrophy of hepatocytes in the livers of treated rats and a corresponding increase in liver to body weight ratios. Cotton rats are a likely model for hepatic cytochrome P-450 induction studies and as a biomonitor of environmental contamination.

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Table 1. Liver weights and levels of hepatic cytochrome P-450 from control and treated animals

Treatment and sex	Control males (n=4)	Treated males (n=4)	Control females (n=4)	Treated females (n=4)
a. Phenobarbital:				
Liver weight (g)	2.61 ±0.13	4.36 ±0.35	2.84 ±0.42	3.57 ±0.18
Liver_weight ---------- x 100 Body weight	2.75 ±0.11	3.82* ±0.10 (139%)	2.41 ±0.08	3.97* ±0.13 (165%)
P-450 nmoles/mg of protein	2.11 ±0.13	4.36* ±0.42 (206%)	1.74 ±0.31	3.64* ±0.14 (209%)
b. 3-Methylcholanthrene:				
Liver weight (g)	2.90 ±0.17	4.63 ±0.18	2.71 ±0.25	3.40 ±0.17
Liver_weight ---------- X 100 Body weight	2.86 ±0.15	4.47* ±0.13 (156%)	2.50 ±0.09	3.95* ±0.07 (158%)
P-450 nmoles/mg of protein	2.10 ±0.14	3.07* ±0.21 (146%)	1.84 ±0.10	3.51* ±0.26 (191%)

(n) = Number of rats
Data expressed as Mean±SE
* = Statistically different from control rats ($P < 0.05$)
Number in parenthesis indicates the percentage of the control value

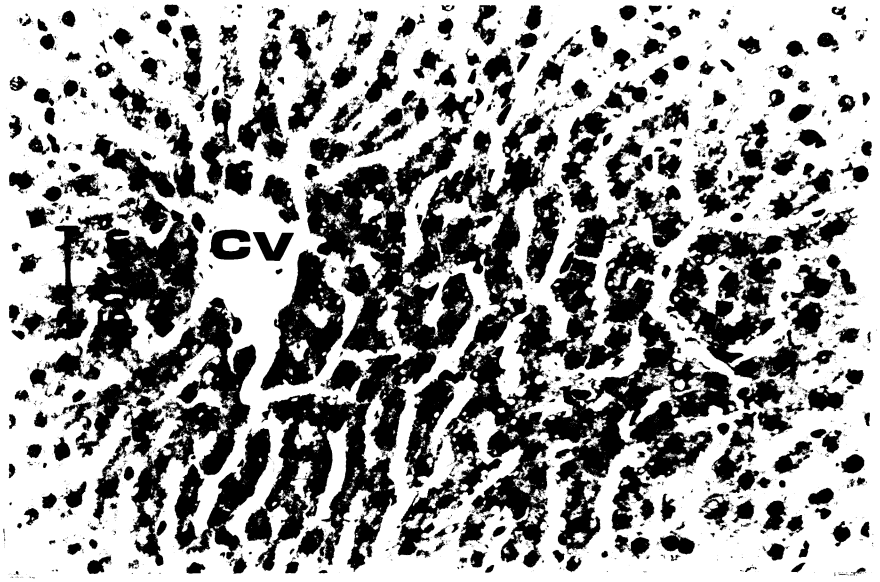

Fig. 1. Liver from control animal illustrating normal size and appearance of hepatocytes. Central vein (CV).

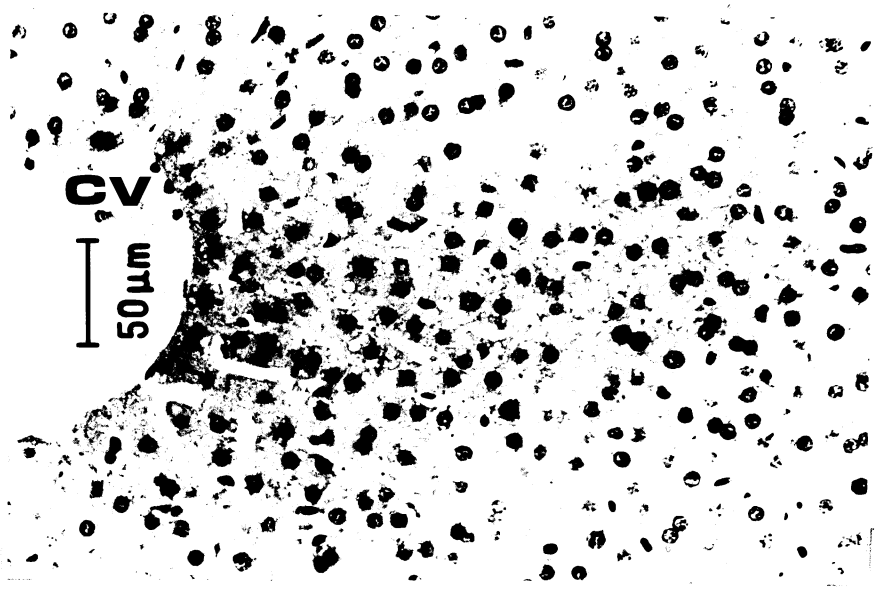

Fig. 2. Liver from animal treated with phenobarbital illustrating the enlargement of centrilobular hepatocytes and nuclei (arrow). Note increased cytoplasmic vacuoles. Central vein (CV).

CHAPTER III

DEVELOPMENT OF THE COTTON RAT (SIGMODON HISPIDUS) AS A BIOMONITOR OF ENVIRONMENTAL CONTAMINATION WITH EMPHASIS ON HEPATIC CYTOCHROME P-450 INDUCTION AND POPULATION CHARACTERISTICS

Introduction

Biological monitoring of exposure to various chemical pollutants has been employed for environmental toxic evaluation (Christian, 1983; Rowley et al., 1983). The impact of pollutants can be predicted by biochemical responses of exposed wild animals. Responses linked to biochemical detoxification processes would be especially meaningful because of their direct link to detoxification function. They are generally quite sensitive and precede the onset of more serious pathology at cellular and tissue levels.

The mammalian liver and its cytochrome P-450 is central to xenobiotic metabolism. Cytochrome P-450, a family of hemoproteins with distinct activity profiles catalyzes metabolism of an almost limitless number of xenobiotics as well as certain endogenous compounds. This enzyme system serves as a route of detoxification as well as a route of metabolic activation of parent compounds to reactive metabolites that initiate toxic and carcinogenic events (Guengerich and Liebler, 1985). The induction of cytochrome P-450 enzyme in fish has been extensively studied and validated as a criterion for monitoring water pollution (Payne et al., 1984).

Wild cotton rats (Sigmodon hispidus) are potentially superior to fish for

toxicological studies because they are phylogenetically closer to man and live in close proximity to man. They are ubiquitous throughout the Southeastern United States, easy to capture, have a generation interval of less than one year, and limited range of movement (less than one hectare). Additionally, fish are aquatic and unsuited for evaluation of terrestrial pollution.

The present study was designed to develop a reliable, quick, and sensitive method to determine environmental toxicity hazards in man and animals through the evaluation of hepatic cytochrome P-450 levels in wild cotton rats. Population characteristics and organ pathology were included for complete toxicological evaluation.

Materials and Methods

Royal Hardage (RH) toxic waste disposal site, a declared superfund site, was selected for the field study. This site is located near the town of Criner in McClain County, Oklahoma and covers approximately 25 hectares. According to the information provided by the Oklahoma State Department of Health (1986 report), solid wastes as well as drummed liquids were placed in areas known as the main pit, barrel mound and sledge mound during 1972 to 1980. The main pit and barrel mound together cover a 6 meters deep area of approximately 18,580 square meters. The locations of the main pit, barrel mound, sludge mound, evaporation ponds and north pit are shown in Figure 1. The study site encompassed the main pit and barrel mound areas which were dominated by tall grass cover with the exception of a few areas of stained, bare soil. An uncontaminated control (CS) site with ecologically similar habitat to RH was selected approximately 2.5 km Southwest of RH.

Cotton rats were live-trapped for a 3-day population study using an 8 x 8 trapping grid with 10 meters spacing between trap stations and 10.2 x 10.2 x 22.9 cm Sherman aluminum traps baited with rolled oats. Individual trap stations were iden-

tified with labeled flags. Cotton rats were weighed, sexed, and marked by toe clipping prior to their release. The third day's captives were returned to the laboratory for further evaluations.

Cotton rats returned to the laboratory were placed in individual polycarbonate cages with wire tops containing wood-chips as bedding. Rats were fasted overnight with water provided ad libitum, immobilized by cervical dislocation, and exsanguinated by severing the aorta. No laboratory food was provided to these rats during holding period. Cotton rats were necropsied and livers quickly removed, weighed and processed for cytochrome P-450 assay. Total cytochrome P-450 contents were measured with a recording spectrophotometer (Shimadzu MPS200). The spectrophotometer and recorder were set to scan from 400 to 500 nm (1 cm/nm). Cytochrome P-450 was quantitated on the basis of the difference spectrum between the carbon monoxide reduced cytochrome complex and the reduced cytochrome itself according to Omura and Sato (1964). Liver microsomal protein was determined on solubilized samples (Smith et al. 1985). Fresh weights of testes, adrenal glands, kidneys and spleen were recorded. Liver, kidney, adrenal, pancreas, representative samples of intestinal areas, reproductive organs and brain were fixed in 10% buffered formalin for histopathology.

Population characteristics including density (Grant et al., 1982), biomass (Grant and French, 1980), effective trapping area (Grant et al., 1982), sex ratios, trappability (Krebs and Boonstra, 1984) and age structure (Stafford and Stout, 1983) were determined. Body weights were used as an index of age: 0-59.9 g = juvenile, 60-99.9 g = subadult, 100+ g = adult (Stafford and Stout, 1983).

Statistical significance of organ weights and Cytochrome P-450 values was analyzed using Student's t-test (Steel and Torrie, 1980). The accepted level of significance was $P < 0.05$.

Results and Discussion

Over-all, male cotton rats and to a lesser extent females from RH showed an induction of hepatic cytochrome P-450 (Table 1) with means of 122% and 119% of control (CS) values in males and females, respectively. The mean concentration of cytochrome P-450 of 1.05 nmoles per mg of protein found in RH males was significantly higher ($P < 0.5$) than the CS males (0.80 nmoles per mg of protein). While, cytochrome P-450 levels in RH females were higher than CS but no statistical significant difference was observed. The liver to body weight ratio in both sexes was consistent within RH and CS groups.

The Mixed Function Oxidase (MFO) enzyme system is of considerable interest; the importance of this enzyme system resides not only in its ability to detoxify certain foreign chemicals, but also its ability to produce carcinogens or reactive toxic metabolites. Several environmental pollutants have been shown to induce the MFO enzyme system (Guengerich and Liebler, 1985). The level of MFO induction is evaluated by assaying cytochrome P-450. The hepatic cytochrome P-450 induction (122% and 119% of the control value in males and females respectively) demonstrated in this study is an appealing candidate as a new bioindicator for monitoring chemical exposure. Simple quantitation of toxic substances in water and soil with comparison to the levels of toxins known to cause disease in the laboratory may not be an acceptable means of determining the potential hazard of toxins in the environment (Clark et al., 1982; Rowley et al., 1983). Analytical assays for specific toxins typically recognize only those toxins for which specific procedures were performed. Toxicity in the natural environment is usually the result of exposure to a complex mixture of toxins and their metabolites or degradation products (Rowley et al., 1983). Additive insults of low levels of multiple toxins may cause deleterious effects. Toxins which have been "aged" in the environment may be more toxic than corre-

sponding quantities of technical mixtures (out of bottle) which are used in virtually all laboratory experiments. This effect has been demonstrated with polychlorinated hydrocarbons (Hornshaw et al., 1983). Biochemical detoxification response therefore, is especially meaningful and often precedes the onset of more serious cellular changes.

Cytochrome P-450 activity in fish has been documented as a criterion for determining water pollution. However, the use of P-450 levels has not been consistently successful due to low inherent levels of cytochrome P-450 and marked variation in inducibility in fish (Lindstrom-Seppa et al., 1985). Cotton rats are an excellent prospective model due to their high cytochrome P-450 concentrations compared to laboratory rat and their marked inducibility.

Characteristics of cotton rat populations for the two study sites (RH and CS) are shown in Table 2. Population density at the CS site (68 cotton rats per hectare) greatly exceeded the density at RH site (21 cotton rats per hectare). Maximum trappability did not differ appreciably between sites. Biomass density showed a similar trend with 5331 g/ha at CS which was 290% of the RH biomass density. The age structure indicated a higher percentage of juveniles in CS site (20%) compared to the RH site (11%).

Results of this study are in agreement with population studies on wild voles in Love Canal, New York (Rowley et al., 1983) where thousands of organic and inorganic chemicals were buried. Population difference between CS and RH due to immigration from surrounding areas was considered an unlikely possibility. The low population density at the RH site might be attributed to decreased recruitment or increased mortality from environmental pollutants. The impact of pollutants is further elucidated by decline in juvenile percentage and biomass density at RH site. The lower percentage of juveniles at the RH site could have been due to increased susceptibility of juveniles to pollutants, decreased conception, or increased fetal

mortality.

Mean testes, kidney, spleen, thymus and adrenal weights relative to body weights did not differ significantly between treatment groups for male and females. A variety of parasites (nematodes and cestodes) infected the liver and intestine of cotton rats from both RH and CS sites. The parasitic lesions in liver consisted of nodular cystic masses (cysticercus) of approximately 4 mm in diameter, located beneath the capsule, and varied in number from 1 to 7. Except for the presence of adult worms (nematodes and cestodes) in the lumen, no macroscopic changes were seen in the gastrointestinal tract. The other organs and tissues were essentially normal. Histopathologic examination of the organs and tissues of each rat did not reveal toxin-associated lesions. 50 percent of rats from each site (RH and CS) had cut sections of strongyloides (larval and adult forms) within the intestinal crypts with minimal inflammatory response. Absence of light microscopic lesions in the various organs and tissues in RH rats indicates the limitations of histopathologic evaluation. Tissue alterations are dependent on type of toxins, time and frequency of exposure and several other factors. Standard histopathologic evaluation may not be feasible in detecting low level exposure to toxins. More sophisticated pathologic tissue evaluation such as morphometry of liver at the light microscopic level to determine hepatocyte and lobule size and at the electron microscopic level to determine cellular alteration and especially hypertrophy of smooth endoplasmic reticulum (SER), the site of cytochrome P-450 (Guengerich and Liebler, 1985) may be fruitful. These studies are promising and warrant further testing to determine their validity.

Summary

A three day population study of wild cotton rats (Sigmodon hispidus) from Royal Hardage (RH) waste disposal site, a declared superfund site and similar rats from an uncontaminated (CS) habitat was conducted using Sherman live traps. The

third day's captives were returned to the laboratory for evaluation of total hepatic cytochrome P-450 and complete necropsy including organ weights. The level of mean hepatic cytochrome P-450 in the RH rats was 122% of the control value in males whereas in female, the value was 119%. Additionally, there was an appreciable difference in population density (21 cotton rats/ha for RH versus 68 for CS). The biomass density and juvenile percentage were also remarkably different. These results suggest that biotransformation response (hepatic cytochrome P-450 induction) along with population studies in cotton rats would be especially meaningful in assessing environments contaminated with hazardous chemicals.

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Table 1. Body weights, liver weights and mean hepatic cytochrome P-450 levels from control (CS) and Royal Hardage exposed (RH) cotton rats

Sites and sex	Terminal body weight (g)	Relative liver weight (%)	Cytochrome P-450 nmoles/mg of protein
CS males (n=5)	101.19±12.23	4.10±0.21	0.80±0.03
RH males (n=8)	103.22±14.12	4.09±0.17	1.05±0.03* (122%)
CS females (n=5)	98.59±9.29	3.71±0.41	0.92±0.08
RH females (n=4)	78.95±15.56	4.58±0.34	1.09±0.04 (119%)

All data are expressed as X±SE
Number in parenthesis indicates the percentage of the control value
* = Statistically different from control ($P < 0.05$; Student t-test)
n = No. of rats

Table 2. Population characteristics

Sites	Population density (rats/ha)	Biomass density (g/ha)	Maximum trappability (%)	Juvenile (%)	Sub-adult (%)	Adult (%)
CS	68	5331 (290%)	90.90	20	54	26
RH	21	1903	86.80	10.50	63.20	26.30

Number in parenthesis indicates the percentage of the CS value
CS = Control site; RH = Royal hardage toxic waste disposal site

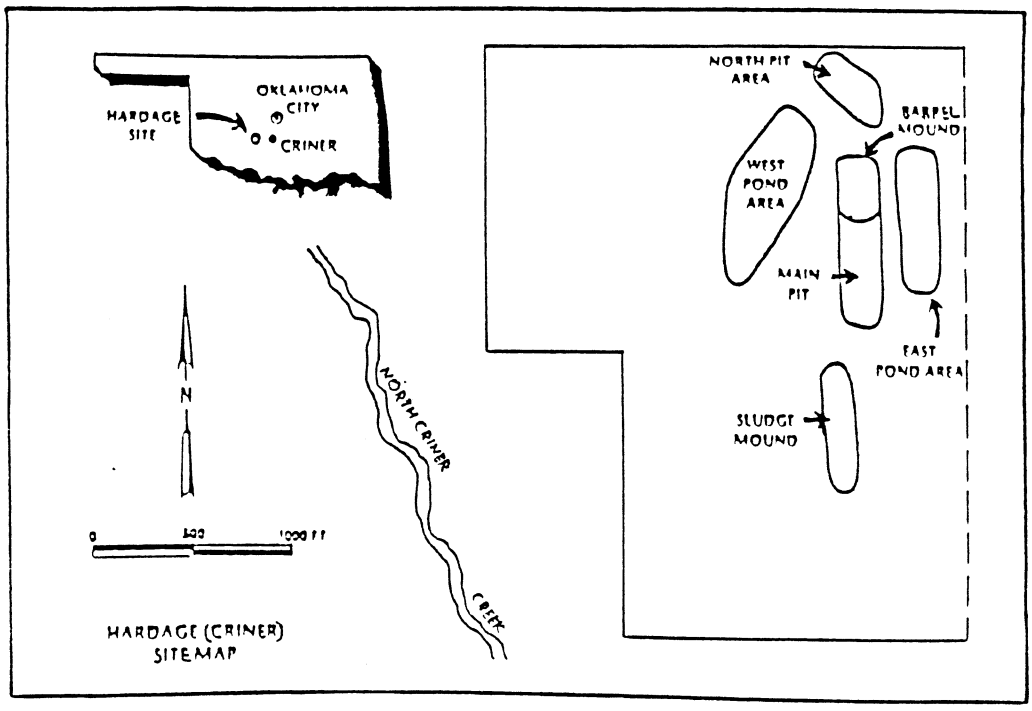

Figure. 1. Map of the Hardage (Criner) site showing locations of main pit, barrel mound, evaporation ponds (west and east ponds) and north pit.

CHAPTER IV

EVALUATION OF ULTRASTRUCTURAL HEPATIC RESPONSE TO ENVIRONMENTAL TOXICANTS IN WILD COTTON RATS (SIGMODON HISPIDUS)

Introduction

The cytochrome P-450-mediated microsome monooxygenase is involved in the metabolism of endogenous substrates, such as steroid hormones, as well as xenobiotics, including many drugs and carcinogens (Conney, 1982; Payne et al., 1987). The induction of cytochrome P-450 enzymes to certain chemicals plays a significant role in the rate of metabolism of these compounds and could lead to the development of a biological monitoring system of sufficient sensitivity to discriminate environmental quality differences for a wide variety of pollution conditions (Payne et al., 1987). There is a correlation between the alteration in structure and content of organelles and biochemical changes observed in the subcellular fractions (Fouts and Rogers, 1960; Weibel et al., 1969; Staubli et al., 1969; Toftgard et al., 1986; Woods and Fowler, 1986).

Hepatic lobules are composed of hepatocytes organized in three microcirculatory zones (periportal, midzonal, and centrilobular). The hepatocytes in each of these zones contain enzymes which are involved in various biochemical reactions. The predominant location of the mixed-function oxidation system in the liver lobule is the centrilobular zone (Farber and Fisher, 1979). Ultrastructural changes in the hepatocytes not only correlate with biochemical events of detoxification but also

with toxic effects of a parent compound or its metabolites.

The objective of this study was to characterize the ultrastructural alterations in the liver of wild cotton rats (Sigmodon hispidus) following exposure to polychlorinated biphenyls (PCB) contaminated habitat.

Materials and Methods

Cotton rats used for breeding stock, were collected from uncontaminated areas (Blackwell, Stillwater) with Sherman live traps. They were housed in the laboratory animal facility in individual polycarbonate cages with wire tops and wood shavings as bedding. They were maintained using 12-hour photoperiod and received commercial rat chow and water ad libitum. After a 2-week acclimatization, a male was paired with a female for 1 wk to breed. The offspring were weaned at 3 weeks and housed 2 animals of the same sex to a cage until they weighed at least 90 grams. Prior to the experiments, rats were placed in individual cages. Induction studies included the use of phenobarbital in saline (an inducer of P-450b and P-450e) 70mg/kg IP for 4 days. Five female and 5 male cotton rats were administered phenobarbital. Five male and 5 female cotton rats received intraperitoneal (IP) injections of saline and served as controls.

An industrial site near Pryor, Oklahoma (PO), a known PCB-contaminated area was selected for the field study. This site is located in Mayes County, Oklahoma. According to information provided by the United States Corps of Engineers (1987), the site has Aroclor 1254 (PCB mixture) concentration in the soil greater than 800 PPM. The contaminated site was dominated by tall-grass cover. An uncontaminated control site with an ecologically similar habitat to PO was selected approximately 0.50 km Southwest of PO.

Cotton rats were live-trapped for 3 consecutive days, using an 3 x 22 trapping grid with 5 m spacing between trap stations and 10.2 x 10.2 x 22.9 cm Sherman

aluminum traps baited with rolled oats. Individual trap stations were identified with labeled flags. The third day's captives were returned to the laboratory for further evaluation.

All rats were fasted overnight with water provided ad libitum, immobilized by cervical dislocation, and exsanguinated by severing the aorta. The cotton rats were necropsied, and their livers quickly removed, weighed and volumes determined (Elias and Hyde, 1980). Each carcass was labeled and submitted to the United States Corps of Engineers for PCB analysis. Total hepatic cytochrome P-450 levels were assayed by the method of Omura and Sato (1964). Microsomal protein was determined on solubilized microsome samples (Smith et al., 1985). Liver, kidney, adrenal, pancreas, representative samples of intestinal areas, reproductive organs, and brain were fixed in 10 % neutral buffered formalin for histopathology. Liver tissue for transmission electron microscopic examination was minced to approximately 1-mm cubes and fixed in Karnovsky's fixative. Tissue was rinsed in three changes of cacodylate-H2O-sucrose buffer for 30 min (10 min each rinse), post-fixed in a 1:1 solution of osmium tetroxide and 0.27 M cacodylate buffer for one hr. Tissue was rinsed in three changes of cacodylate-H2O-sucrose buffer for 30 min (10 min each rinse). Dehydration consisted of consecutive 10 min rinses each of 50%, 70% , 90% reagent grade ethyl alcohol followed by three 10 min rinses in 100% ethyl alcohol and 10 min three rinses in propylene oxide. Tissue was then allowed to remain for 24 hrs in 1:1 propylene oxide and DER resin. The tissue was embedded in fresh 100 % DER and polymerized at 60^{o}C with 5-8 Hg vacuum for 48 hrs. Thick (55 nm) sections were cut with glass knives on a Sorvall MT 6000 Ultracut ultramicrotome, mounted on glass microscope slides, and stained with Mallory's stain. Centrilobular hepatocytes were selected for thin sectioning. Thin (0.55nm) sections were stained with uranyl acetate and lead citrate and examined with a Jeol 100C XII transmission electron microscope (Jeol Ltd, Tokyo, Japan).

Results

Pryor contaminated rats :

The population of the cotton rats in the Pryor site including the uncontaminated was relatively low. Three collections in January 1988, June 1988 and January 1989 with 3-day trappings each time, resulted in a total collection of nine cotton rats (6 rats on first collection followed by 1 in June 1988 and 2 in January 1989). Nine cotton rats (6 contaminated and 3 uncontaminated) were included in the study.

At necropsy, major gross lesions in the contaminated rats were confined to the liver. The livers were friable and enlarged with accentuated lobular patterns. Additionally, one rat had multiple irregular white foci of 1-3 mm in diameter in the central hepatic lobe. These hepatic gross changes were not observed in the uncontaminated rats. The other organs from both contaminated and uncontaminated rats were essentially normal.

Liver weight, liver volume, liver-weight to body-weight ratio and liver volume to body-weight ratio are presented in the Table 1. Both liver-weight to body-weight and liver volume to body weight were significantly ($P < 0.05$) increased in the contaminated rats. This increase was associated with increased activity of total hepatic cytochrome P-450. The total hepatic cytochrome P-450 in the contaminated rat was 144 % that for the uncontaminated cotton rat. Of the nine cotton rats, seven (excluding the last collection on January, 1989) was included for whole body PCB analysis. Except for one cotton rat (PCB concentration < 0.5 ppm), whole body PCB concentration of PO rats (1.42 to 12.36 ppm) greatly exceeded the concentrations in CO rats (< 0.5 ppm). There were substantial light microscopic changes in the livers of contaminated rats. These alterations included mild to moderate enlargement of centrilobular and midzonal hepatocytes including enlarged nuclei and increased

cytoplasmic lipid droplets. Within a single hepatocyte, these lipid droplets ranged from numerous fine droplets to one or more larger droplets (Fig. 1). The periportal hepatocytes were minimally affected. The liver from one rat from the contaminated site had multiple, locally extensive foci of coagulation necrosis. The livers of the uncontaminated rats were essentially normal (Fig. 2). Incidental extrahepatic lesions seen in both contaminated and uncontaminated rats included mild interstitial nephritis, intestinal strongyloidiasis, and cestodiasis and focal interstitial mononuclear cell infiltrations in the pulmonary interalveolar septa.

Ultrastructurally, the most striking feature of centrilobular hepatocytes of the contaminated rats was the proliferation of smooth endoplasmic reticulum (SER). The cytoplasm was filled with SER, which displaced the mitochondria (Fig. 3). The proliferation of SER consisted of both vesicular and tubular membranes. The rough endoplasmic reticulum (RER) was disorganized with long profiles woven between mitochondria and in one case encircling the nucleus. The rough endoplasmic reticulum were dilated and continuous with the proliferated SER. Additionally, RER had fewer than normal attached ribosomes. Membrane-bound lipid droplets of various sizes were in the cytosol, and most were enclosed by SER. The presence of lipid droplets varied among rats; two rats had substantially more lipid droplets compared to cells from other rats.

Compared with contaminated rats, the centrilobular hepatocytes of uncontaminated rats had less SER, uniformly distributed mitochondria (Fig. 4), and fewer membrane-bound lipid droplets. However, one rat had minimal mitochondrial degeneration (one or two mitochondria involved) characterized by microconcretions.

Phenobarbital treated rats:

The light microscopic hepatic changes of phenobarbital treated rats were

comparable to contaminated rats from Pryor. The ultrastructural changes of centrilobular hepatocytes were an extensive proliferation of the SER similar for that seen in the contaminated rats (Fig. 5). The lipid droplets, which were bounded by smooth double membranes, varied greatly in size and appeared to coalesce to form large droplets. The presence of lipid droplets in the cytosol was more prominent in phenobarbital treated rats than in contaminated rats. Hepatocytes with severe lipid vacuolation appeared to have less RER than hepatocytes with mild lipid changes. Saline-treated rats had comparable ultrastructural changes to uncontaminated rats (Fig. 6). However, lipid droplet accumulation was more pronounced in saline treated rats than in uncontaminated rats.

Discussion

The phenomenon of increased liver weight associated with induction of microsomal enzymes has been observed in rabbits with dermal application of Aroclor (PCB mixture). Similarly, hepatomegaly, liver weight increases and increased friability have been reported in rats fed polybrominated biphenyls (Sleight and Sanger, 1976; Raber et al.,1986). This increase in the liver weight was related to hypertrophy of the hepatocytes with increased organelle (SER) content. These findings are in agreement with significantly increased liver weight and volume of the contaminated rats in the present study.

The proliferation of SER is a reversible response to a variety of exogenous substances including PCB and polybrominated biphenyls (PBB) and is a morphological reflection of enhanced enzyme activity (Sleight and Sanger, 1976; Farber and Fisher, 1979; Render et al., 1982). Increased SER following PCB treatment, has been reported in the livers of mice, monkeys, guinea pigs, and rats (Nishizumi, 1970; Kimbrough et al., 1972; Vos and Notenboom-ram, 1972). Biotransformation enzymes of the mixed-function oxidation system are associated with electron transport

chains on the SER (Farber and Fisher, 1979); therefore, proliferation of the SER results in increased activity of the MFO enzymes. Since detoxification of PCB and PBB is dependent on the MFO enzymes, the logical result of PCB-induced hepatotoxicity would be proliferation of SER in hepatocytes containing substantial mixed-function oxidation capability. Thus, the zonal distribution of the PCB-induced increase in SER, is greater in centrilobular hepatocytes (Farber and Fisher, 1979).

Increased SER originates from continuous growth and budding from preexisting cisternae of RER (Orrenius and Ericsson, 1966). Vos and Notenboom-Ram (1972) have reported numerous ultrastructural changes in rabbit hepatocytes due to PCB treatment. Included in their observations were degranulation of RER in hepatocytes from animals after dermal application of PCB for 28 days. In the present study, the ribosomes in many hepatocytes from contaminated cotton rats were affected similarly. According to Farber and Fisher (1979), the dispersal of ordered aggregates of ribosomes and detachment of ribosomes from RER can occur under various conditions, including anoxia, ischemia, and hepatotoxcosis. This phenomenon has been associated with inhibition of protein synthesis during liver injury induced by carbon tetrachloride and ethionine. Ethionine-induced ultrastructural and biochemical changes can be reversed by the administration of a metabolic antagonist of ethionine such as methionine or adenine.

Intracytoplasmic membrane-bound lipid droplets were found throughout the cytosol and varied in number and size. In PCB-treated rats, fatty livers were produced as a result of the decreased availability of non-esterified fatty acids for hepatic mitochondrial oxidation (Mehlman et al., 1974).

Polychlorinated biphenyls have been identified as widespread, persistent environmental contaminants. They have been detected in fish (Brunn and Manz, 1982; Zabik et al., 1982), birds (Barbehenn and Reichel, 1981), and humans (Safe, 1984). The PO contaminated cotton rats were exposed to known PCB polluted site

for an unknown period. Their whole body PCB content confirmed the bioavilability of the toxicant. How PCB entered or enters the body is still unknown. Most likely, the mode of exposure would be through ingestion. However, inhalation through dust filled air and percutaneous absorption are also possible. Additionally, transfer of PCB from maternal to fetal and suckling rats has been reported (Takagi et al., 1986) whereby the highest concentration of PCB was in the fetal placenta followed by the fetal liver, heart, skin, muscle, blood, lung and brain and the dam's milk.

In conclusion, the ultrastructural evaluation of liver together with hepatic microsomal assay, hepatic light microscopy, liver weight and volume from wild cotton rats provide a meaningful method of biomonitoring for environmental contamination.

Summary

A total of six cotton rats (Sigmodon hispidus) were collected from a known polychlorinated biphenyls contaminated site. Three cotton rats, serving as control, were collected from a nearby uncontaminated site. There were significant increases in the liver weight and volume of the contaminated rats compared with uncontaminated rats. Associated with increased liver weight and volume, the total hepatic cytochrome P-450 activity was greatly increased (144 % of the control value). Light microscopic hepatic changes in contaminated rats included moderate enlargement of centrilobular and midzonal hepatocytes with vacuolated cytoplasm. Ultrastructural alterations of centrilobular hepatocytes of the contaminated rats consisted of abundant smooth endoplasmic reticulum, disorganized rough endoplasmic reticulum, lipid droplets, and detached ribosomes. These changes were similar to hepatic ultrastructural alterations produced in cotton rats by three-day phenobarbital treatment. Lipid droplet formation in the cytosol was a more prominent feature in the phenobarbital-treated rats.

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Table 1. Body weights, liver weights, Liver volume and total hepatic cytochrome P-450 levels from contaminated and uncontaminated cotton rats

Measurement	Uncontaminated site (n=3)	PCB-Contaminated site (n=6)
Body weight (g)	93.06±3.97	74.73±7.84
Liver weight (g)	2.99±0.24	4.52±0.53
Liver volume (cubic cm)	2.78±0.25	4.15±0.50
Liver weight / Body weight X 100	3.25±0.41	6.22±0.64 *
Liver volume / Body weight X 100	3.02±0.41	5.71±0.61 *
P-450 (nmoles/mg protein)	1.70±0.21	2.45±0.26 (144%)

All data are expressed as Mean±SE
Number in parenthesis indicates the percentage of the control value
* = Statistically different from uncontaminated ($P < 0.05$)
n = Number of rats

Figure 1. Liver from a contaminated cotton rat. Swollen centrilobular hepatocytes with numerous cytoplasmic lipid droplets. Central vein (CV).

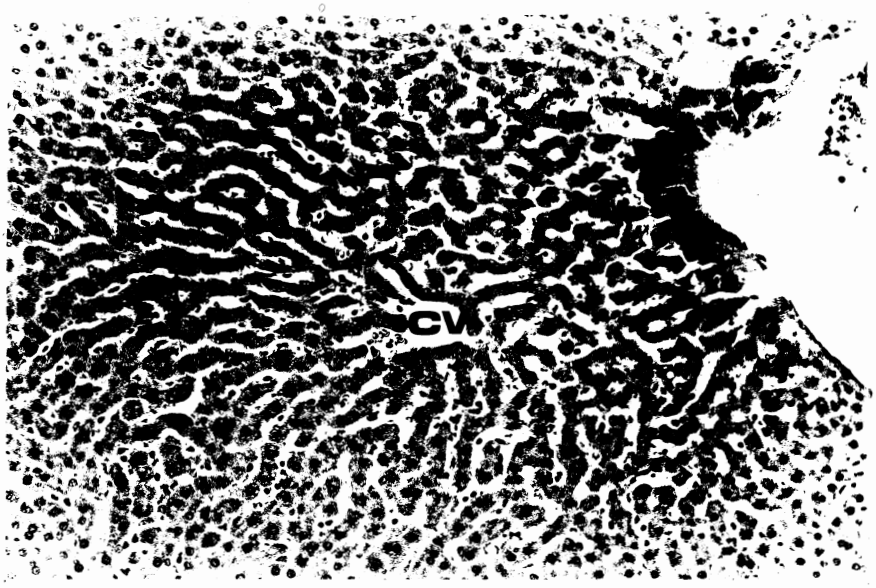

Figure 2. Liver from an uncontaminated cotton rat. Normal hepatocytes. Central vein (CV).

Figure 3. Centrilobular hepatocyte from a contaminated cotton rat. Note proliferation smooth endoplasmic reticulum.

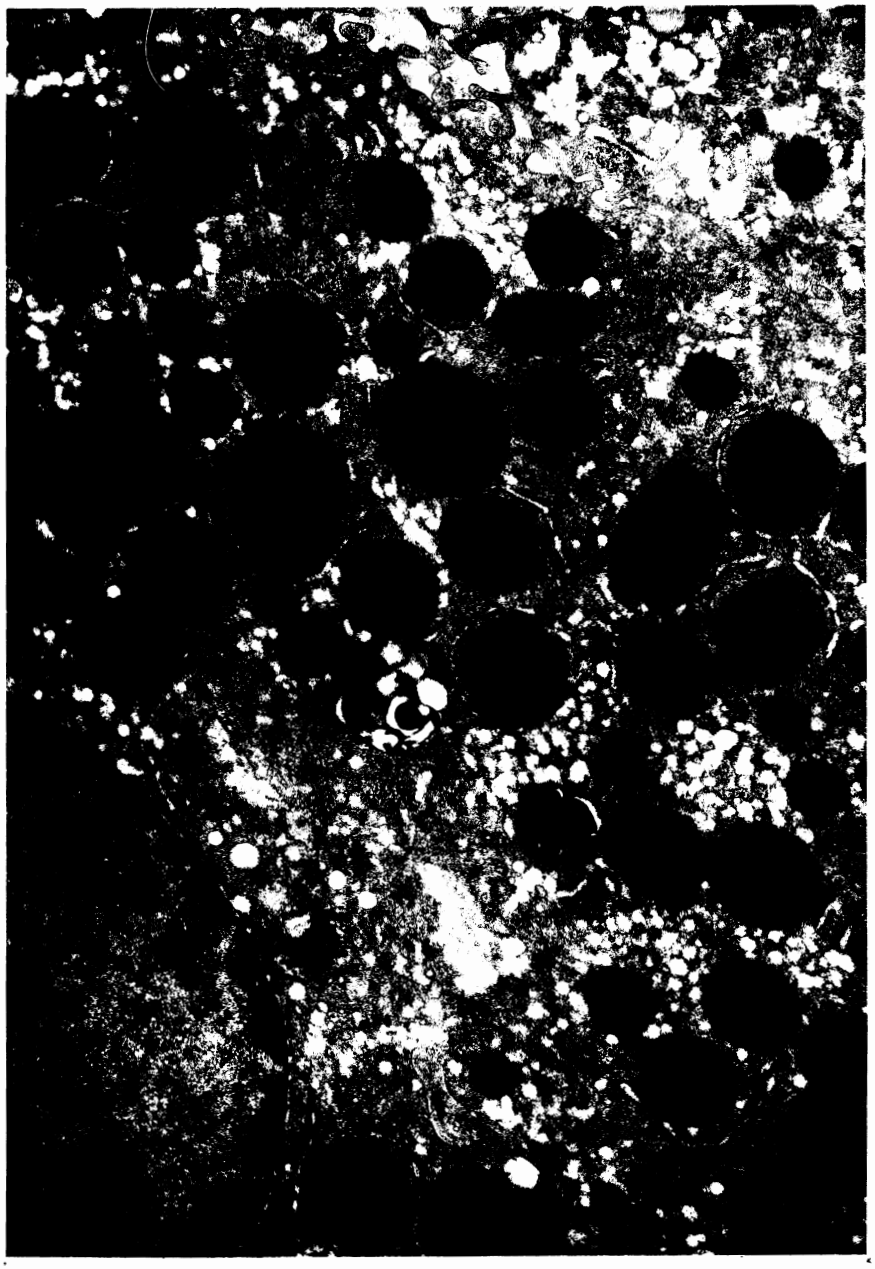

Figure 4. Electron micrograph of centrilobular hepatocyte from an uncontaminated cotton rat. Diffusely scattered mitochondria and network of endoplasmic reticula of rough and smooth types. Occasional mitochondrial concretions.

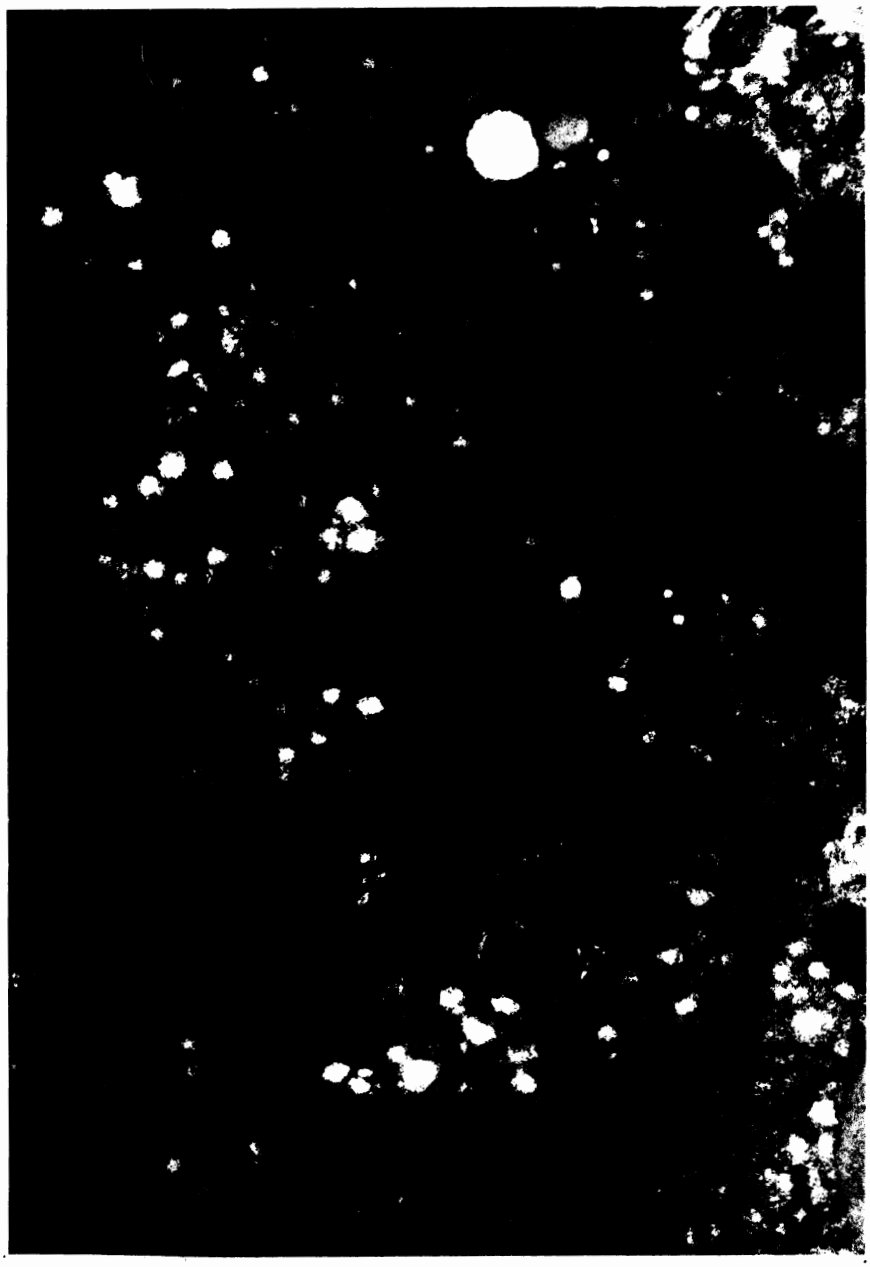

Figure 5. Electron micrograph of centrilobular hepatocyte from a phenobarbital treated cotton rat. Note proliferation of smooth endoplasmic reticulum and cytoplasmic membrane-bound lipid droplets.

Figure 6. Electron micrograph of centrilobular hepatocyte from a control cotton rat. Note diffusely scattered mitochondria and network of endoplasmic reticula of both rough and smooth types.

CHAPTER V

A STUDY OF O-DEALKYLATION OF RESORUFIN ETHERS AS AN INDICATOR OF HEPATIC CYTOCHROME P-450 ISOENZYME INDUCTION IN THE COTTON RAT (SIGMODON HISPIDUS) : A METHOD FOR MONITORING ENVIRONMENTAL CONTAMINATION

Introduction

Cytochrome P-450, the terminal oxygenase of the mixed-function oxidase system is widely distributed in many tissues but is present in the highest concentration in hepatic endoplasmic reticulum. This enzyme system plays an essential role in the metabolism of a broad range of xenobiotics, and endogenous and exogenous substrates (Guengerich and Liebler, 1985). Evaluation of hepatic cytochrome P-450 induction in wild animals has been suggested as a biological monitor to various environmental contaminants. Hepatic microsomes contain multiple cytochrome P-450 isoenzymes, each possessing broad and overlapping substrate selectivity (Ryan et al., 1982). The different isoenzymes function in both the activation and detoxification of foreign compounds and some forms of cytochrome P-450 have been implicated in the activation of a variety of chemical carcinogens (Guengerich, 1977; Prough et al., 1984; Wilson et al., 1984).

Many of the model substrates which have been widely used for measuring cytochrome P-450 induction do not differentiate adequately among individual isoenzymes, nor between induced and constitutive isoenzymes (Guengerich et al., 1982;

Kaminsky et al., 1983). In recent years, O-dealkylation of resorufin ethers has gained a considerable importance. This reaction, which is highly specific for the major isoenzymes, can be used as an indicator of cytochrome P-450 isoenzyme induction. Novak and Qualls (1990) have demonstrated that O-dealkylation methoxy-, ethoxy-, pentoxy- and bezyloxyresorufins in cotton rat (Sigmodon hispidus) was similar to Sprague-Dawley rats for 3-methylcholanthrene inducible forms of cytochrome P-450.

The objective of this study was to evaluate the induction of major hepatic cytochrome P-450 isoenzymes through O-dealkylation of various resorufin ethers in the cotton rat (Sigmodon hispidus) as an indicator of environmental contamination with toxic chemicals.

Materials and Methods

Chemicals:

Propoxy-, butoxy-, hexoxy-, octoxy - and decoxyresorufins were synthesized from resorufin by the method of Mayer et al. (1977) using appropriate alkyl iodides. Resorufin, I-iodopropane, 2-iodobutane, I-iodohexane, I-iodoctane and I-iododecane, were purchased from Aldrich Chemical Company (Milwaukee, WI 53201, USA). Methyl-, ethyl-, pentyl- and benzyloxyresorufins were obtained from Molecular Probes Inc. (Junction City, Oregon 97448, USA).

Animals:

Cotton rats collected from uncontaminated areas (Near Stillwater, Oklahoma) with Sherman live traps were used as breeding stock for a captive outbred colony. Animals were housed in our laboratory animal facility in individual polycarbonate cage with wire tops and wood shavings as bedding. Animals were maintained

under a 12 hour photoperiod and commercial rat chow was provided ad libitum. After 2 weeks of acclimatization a male was placed with a female for two weeks. Offspring were weaned at three weeks of age and housed in pairs (same sex) until they reach a desired body weight. Induction studies were performed using phenobarbital, 70 mg/kg in 0.09 saline IP for 4 days, and 3-methylcholanthrene (3MC), 25 mg/kg in corn oil IP for 3 days. Four males born to wild-caught females in our outbred colony received each agent. Two groups of four males each, one group receiving saline and one receiving corn oil by IP injection served as controls.

Two known contaminated sites near Criner and Pryor, Oklahoma) were selected for field trials. The Royal Hardage (RH) toxic waste disposal site, a declared superfund site, is located near the town of Criner in McClain County, Oklahoma. This site was used as a toxic waste dump during 1972 to 1980. An industrial site near Pryor, located in Mayes county, Oklahoma is an aroclor 1254 (polychlorinated biphenyl mixture) contaminated site. According to the United States Corps of Engineer (1987), the site has soil concentration of Aroclor 1254 greater than 800 ppm. Cotton rats were live-trapped from contaminated and nearby uncontaminated sites (CO for RH and CP for PO) with ecologically similar habitat. The distances between contaminated and uncontaminated sites for Criner and Pryor were approximately 2.5 and 0.5 km respectively.

Microsomal preparation:

Cotton rats returned to the laboratory from field studies and rats from laboratory studies receiving phenobarbital, 3MC and control cotton rats, were fasted overnight and killed by cervical dislocation. Liver microsomes were prepared using methods previously described (Omura and Sato, 1964). Microsomal protein was measured by the method of Smith et al. (1985).

O-dealkylation reactions:

The dealkylation of alkoxyresorufins (methoxy-, ethoxy-, propoxy-, butoxy-, pentoxy-, hexoxy-, benzyloxy-, octoxy- and decoxyresorufins) was measured at 37^{o}C using a SLM 48000 spectrofluorometer (SLM Instruments Inc. 810 West Anthony Drive, Urbana, IL 61801, USA). The fluorometer was set to an excitation wavelength of 570 nm, and an emission wavelength of 585 nm with slit widths of both monochrometers at 2 nm. The fluorescence increase as a function of time from resorufin formation measured by the spectrofluorometer was concurrently plotted on HP7475A Plotter (Hewlett-Packard Company, 16399 W. Bernardo Drive, San Diego, CA 92127-1899). Reaction mixtures consisted of microsomal protein (0.30 to 1.50 mg/ml), 1978 ul 1mM Na/K buffer (pH 7.6), 10 ul substrate (1mM in dimethyl sulfoxide). Reactions were initiated by addition of 50 mM nicotinamide adenine dinucleotide phosphate (NADPH) in 1mM Na/K buffer. The rate of formation of resorufin was calculated by comparing the rate of increase in relative fluorescence to the fluorescence of a known amount of resorufin.

The decision tree method was used for selecting the appropriate procedure for hypothesis testing (Gad and Weil, 1984). Data were first subjected to normality test using Univariate procedure (SAS Institute, 1985). Since the data were normal (normality test), the unpaired TTest procedure (SAS Institute, 1985) was used for further comparisons.

Results and Discussion

Effects of phenobarbital-induced and 3-methylcholanthrene-induced microsomes on O-dealkylation of alkoxyresorufins :

The rate of O-dealkylation of the homologous n-alkyl ethers of resorufin with increasing alkoxy chain lengths by cotton rat microsomes to a common metabolite, resorufin was different among ethers (methoxy-, ethoxy-, propoxy-, butoxy-, pentoxy-

, hexoxy, benzyloxy-, octoxy- and decoxy-). Methoxy ether was the most actively metabolized substrate by (both saline and corn oil treated controls) microsomes (Fig. 2), whereas phenobarbital-induced microsomes showed no specific preference for any ether. In comparison, 3-methylcholanthrene (3MC)-induced microsomes were selective for ethoxy ether (Fig. 1) with a decreasing order of selectivity (rate of metabolism) of ethoxy > benzyloxy > methoxy > propoxy > pentoxy > octoxy > butoxy > hexoxy > decoxy.

Control microsomes in Sprague-Dawley rats showed a selective preference for an ethyl ether (Burke et al., 1985). The best substrate for phenobarbital-induced microsomes has been reported to be pentyl ether in Sprague-Dawley rats (Burke and Mayer, 1983; Burke et al., 1985; Lubert et al., 1985). However, cotton rats had no substrate selectivity for phenobarbital-induced microsomes and this agrees with previously reported findings for the cotton rat (Novak and Qualls, 1990).

The rate of metabolism of methoxy-, ethoxy-, propoxy-, pentoxy- and benzyloxyresorufins by 3MC-induced microsomes differed significantly ($P < 0.05$) between control and treated cotton rats (Fig. 3). It is important to note that in the 3MC-induced microsomes, the substrate showing the highest rate of metabolism was not necessarily the substrate showing the greatest degree over controls. Ethoxyresorufin was the fastest substrate for 3MC-induced microsomes, but the induction of the metabolism of propoxyresorufin was greater (24-fold increase) than the induction of ethoxyresorufin. The study of Novak and Qualls's (1990) study on cotton rats unfortunately, did not include O-dealkylation of propoxyresorufin by 3MC-induced microsomes. Highest substrate selectivity for propoxy and butoxyresorufins by 3MC-induced microsomes has been reported in C57BL strain of mice (Lum et al., 1986).

Marked species difference exists in the toxic response of animals to various chemicals. Some of these differences reflect quantitative as well as qualitative differences in drug metabolism and induction of 3MC-induced microsomes (also desig-

nated as cytochrome P-448) by drugs and other chemicals. A chemical may act as an inducer in one species but have no effect or even cause inhibition in another (Hansen and Fouts, 1971; Hook et al., 1975). Iwasaki et al. (1986) reported the induction of P-448 activity by 2-aminoanthrecene in rat but not in hamster. Furthermore, in hamsters, 7-ethoxyresorufin-O-deethylase activity is induced by 3MC but not by phenobarbital (Blaich et al., 1988). The activity of pentoxyresorufin-O-deethylase, which is useful as a specific enzymatic assay for P-450b in the rat liver, cannot be used to probe phenobarbital-like inducers in the hamster liver (Blaich et al., 1988).

Effects of environmental pollutants on microsomal O-dealkylation of alkoxyresorufins :

Based on the results from the 3MC induction study, microsomal O-dealkylation of methoxy-, ethoxy-, propoxy-, pentoxy- and benzyloxyresorufins was selected for field studies.

Twenty two cotton rats (12 RH and 10 CO) were collected from the Criner study area. Significant differences in O-dealkylation of methoxy ($P < 0.0001$), ethoxy ($P < 0.0009$), propoxy ($P < 0.0012$), pentoxy ($P < 0.0005$) and benzyloxyresorufins ($P < 0.0023$) were observed between RH and CO in male cotton rats (Table 1). Females however, had an apparent increase (3 to 8-fold increase) in O-dealkylation rate for all of five ethers in RH rats but which was not statistically significant from CO rats.

Population densities of cotton rats on the Pryor study area were low in both PO and CP. Three collections in January 1988, June 1988 and January 1989 with three-day trapping resulted in a total collection of nine cotton rats (6 rats on first collection followed by 1 in June 1988 and 2 in January 1989). Because of the limited number of animals, no definitive conclusions can be drawn regarding the effect of

environmental contaminants on O-dealkylation of all ethers tested. Despite this cautionary note, there were significant differences on O-dealkylation of all ethers between PO and CP rats. Cotton rats from PO had significantly higher rate of O-dealkylation reactions ($P < 0.05$) than that of controls (Table 2). Aroclor 1254 (PCB mixture) has been categorized as a "mixed" type of inducer, because it possesses the properties of both phenobarbital-type and 3MC-type inducers (Ryan el at., 1979). Since cotton rats from Pryor were exposed to a known Aroclor 1254 contaminated area, increased rate of O-dealkylation observed in PO rats may be real and could be attributed to Aroclor 1254. However, further study with a large sample size (adequate number of both sexes) is warranted.

Cytochrome P-448, 3MC-induced microsomes (also designated as P-450c and P-450d in the rat) are induced by polycyclic aromatic hydrocarbons and other chemical carcinogens (Ioannides et al., 1984), while cytochrome P-450 (also designated as P-450b and P-450e in rats) is exemplified by drugs such as phenobarbital (Lu and West, 1980). Cytochrome P-450 tends towards deactivation while in contrast cytochrome P-448 tends toward metabolic generation of toxic intermediates (Ioannides et al., 1984; Phillipson et al., 1985). The induction of cytochrome P-448 has been associated with mutagenic and carcinogenic activity of the inducing agent (Ioannides et al., 1984) and with genetic potential for malignancy (Nebert and Jensen, 1979). Study of cytochrome P-448 induction in wild cotton rats, therefore, can be used an a reliable bioindicator of environmental toxic exposure.

In laboratory rats, phenobarbital causes a 40 to 60-fold increase in specific phenobarbital isoenzymes (P-450 b and P-450 e) compared with 1-fold increase in total cytochrome P-450. Three methylcholanthrene causes as much as a 70-fold increase in P-450c (Thomas et al., 1987). However, evaluation of cytochrome P-450 isoenzymes is difficult and requires complex immunologic and recombinant-DNA methods (Burke et al., 1985; Shires et al., 1987). Furthermore, total reliance on

immunological and recombinant-DNA methods is moreover, of questionable validity because of variable proportions of enzymatically inactive yet antigenic cytochrome P-450 apoproteins (Guengerich et al., 1982; Seidel et al., 1984).

The specificity of O-dealkylation of resorufin ethers and degree of increased metabolism by specific cytochrome P-450 isoenzymes has been demonstrated in laboratory rats. When compared to uninduced microsomes, phenobarbital-induced microsomes showed a 283-fold increase in O-dealkylation of pentoxyresorufin, while a 9-fold increase was observed with 3MC-induced microsomes. In the same group of experiments, O-dealkylation of ethoxyresorufin was increased 51-fold by 3MC and only 6-fold by phenobarbital. O-dealkylation of ethoxyresorufin, therefore, constitutes a highly specific and sensitive method for the determination of major isoenzymes induced by 3MC in a variety of species (Guengerich et al., 1982). Pentoxyresorufin has been reported as specific for drugs such as phenobarbital, SKF-525A whereas the dealkylation of benzyloxyresorufin is preferentially induced by isosafrole in Sprague-Dawley rats (Burke et al., 1985). Significant differences in dealkylation of methoxy, ethoxy, propoxy, pentoxy and benzyloxyresorufin between RH and CO male cotton rats indicate the induction of major isoenzymes by environmental toxicants.

Compared with laboratory induction studies, the rate of O-dealkylation was considerably lower in RH cotton rats. These differences may be related to several factors such as nutrition, type of toxic agent, nature of exposure, duration of exposure and most importantly, dose of the toxicant (low or high). Maximal induction of cytochrome P-448 activity by 2-acetylaminofluorene occurs at about 2 mg/kg and at higher doses the extent of induction is drastically reduced, possibly because of the hepatotoxicity of the inducing agent (Iwasaki et al., 1986). Likewise, at very low doses, induction of cytochrome P-448 may be insignificant.

From the evaluation of alkoxyresorufin O-dealkylation by environmental

contaminant-induced microsomes it was apparent that there were individual differences particularly among females. This diminishes the value of using female cotton rats to assess the toxic effects of environmental contaminants. Sex differences occur in the O-dealkylation of alkoxyresorufins in cotton rats, the females exhibiting a higher O-dealkylation rate (Novak and Qualls, 1990). Although the mean rate of alkoxyresorufin O-dealkylation in females was higher than males, this does not conclusively prove that increased metabolic activity was the only factor for individual metabolic differences in females. Variation may also be linked to hormonal (steroid and thyroxine) status (Gustaffson et al., 1984).

Studies have indicated that fetal and newborn animals of most mammalian species including man have limited ability to metabolize xenobiotics because of the low levels of hepatic mixed-function oxidases and other enzymes involved in detoxification that are present at birth. However, postnatal development is rapid, adult levels of activity being reached within a few weeks (Kato et al., 1964; Short and Davies, 1970). Finally, marked fall in the maximal activity of phenobarbital-inducible and 3MC-inducible forms of microsomes occurs in senescent animals whether expressed in terms of microsomal protein or unit of cytochrome P-450. However, there are no age-related changes in enzyme (ethoxyresorufin O-deethylase and aldrin epoxidation) activities (Wynne et al., 1987). Age-related effects in alkoxyresorufins O-dealkylation are unlikely in the present study. Since enzyme activities are not affected by increased age, the evaluation of enzyme activity (alkoxyresorufin O-dealkylation) should be more advantageous than total cytochrome P-450 assays which are subjected to age related quantitative change.

Summary

The O-dealkylation of homologous series of n-alkyl ethers (methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, benzyloxy, octoxy, decoxy) was studied in hepatic

microsomes from control, phenobarbital and 3-MC pretreated cotton rats. Wild cotton rats collected from two known contaminated sites (Royal Hardage, Criner and Pryor creek, Pryor) with adjacent uncontaminated sites were examined for O-dealkylation of five ethers (methoxy, ethoxy, propoxy, pentoxy and benzyloxyresorufins) by hepatic microsomes. The rate of resorufin formation by hepatic microsomes in control cotton rats was highest for methoxyresorufin. In 3MC-induced microsomes, ethoxyresorufin was the best substrate. Compared with controls, 3MC-induced microsomes had the highest substrate selectivity to propoxyresorufin (24-fold increase). The Criner study indicated a significant increase in the rate of resorufin formation by hepatic microsomes in contaminated male rats. There was apparent increase in O-dealkylation rate for contaminated females by hepatic microsomes, however, this increase was not statistically significant. Similar significant increase in O-dealkylation rate by hepatic microsomes was seen in Pryor contaminated rats. It is concluded that O-dealkylation of alkoxyresorufins in wild cotton rats by hepatic microsomes may provide a reliable method for monitoring environmental contamination.

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Table 1. Criner study : Effect of environmental contaminants on microsomal O-dealkylation of resorufin ethers in cotton rats

Animals and Statistics	Resorufin ethers: Methoxy	Ethoxy	Propoxy	Pentoxy	Benzyloxy
Males:					
RH (n=8)	[a] 0.364 ±0.035	[a] 0.311 ±0.050	[a] 0.204 ±0.035	[a] 0.027 ±0.003	[a] 0.336 ±0.048
CO (n=5)	[a] 0.103 ±0.019	[a] 0.045 ±0.015	[a] 0.027 ±0.016	[a] 0.009 ±0.002	[a] 0.117 ±0.025
Fold induction	4-fold	8-fold	8-fold	3-fold	3-fold
Significance	0.0001	0.0009	0.0012	0.0005	0.0023
Females:					
RH (n=4)	[a] 0.626 ±0.297	[a] 0.954 ±0.554	[a] 0.540 ±0.253	[a] 0.038 ±0.010	[a] 0.525 ±0.204
CO (n=5)	[a] 0.152 ±0.020	[a] 0.268 ±0.045	[a] 0.180 ±0.029	[a] 0.014 ±0.004	[a] 0.069 ±0.017
Fold induction	4-fold	4-fold	3-fold	3-fold	8-fold
Significance	NS	NS	NS	NS	NS

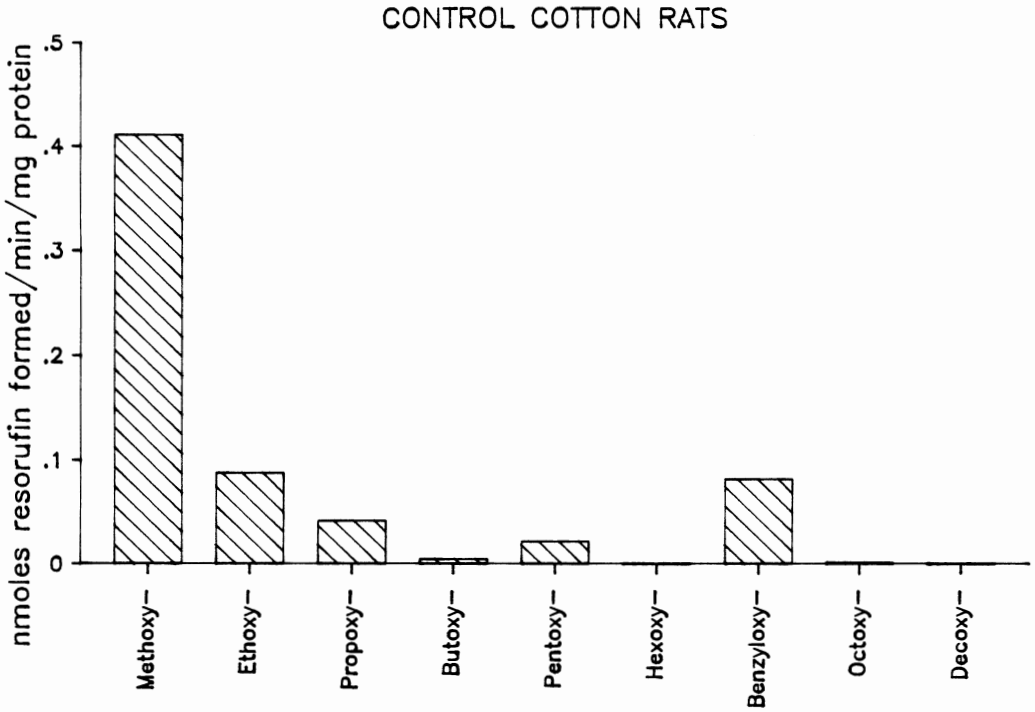
a = nmoles of resorufin formed / min / mg microsomal protein (expressed as Mean ± Standard error)

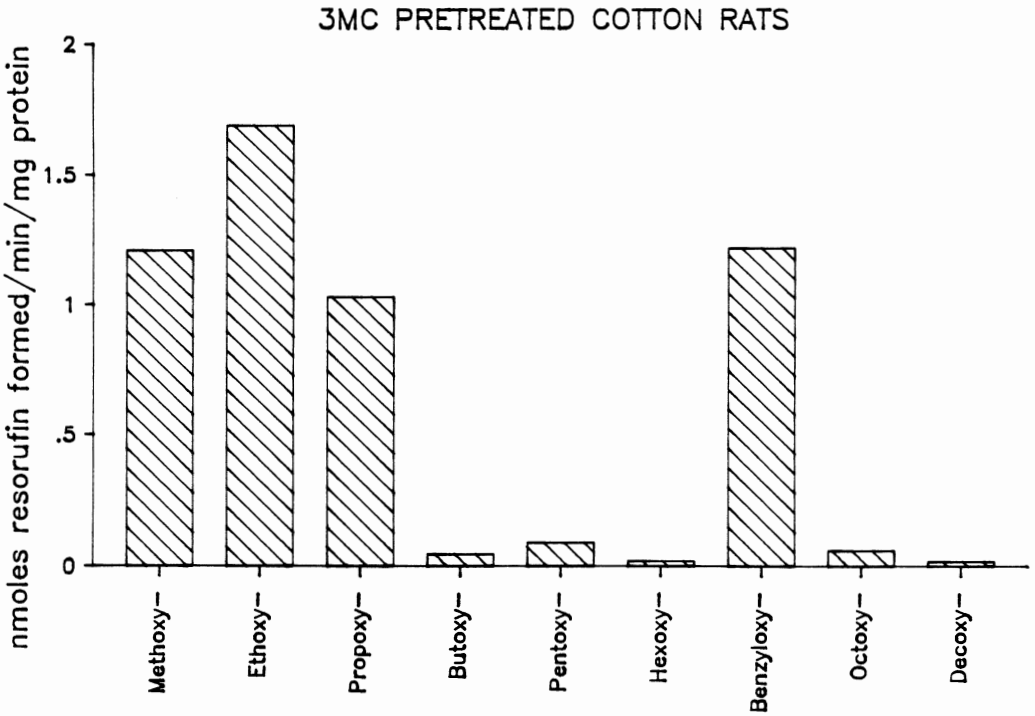
Table 2. Pryor study : Effect of environmental contaminants on microsomal O-dealkylation of resorufin ethers in cotton rats

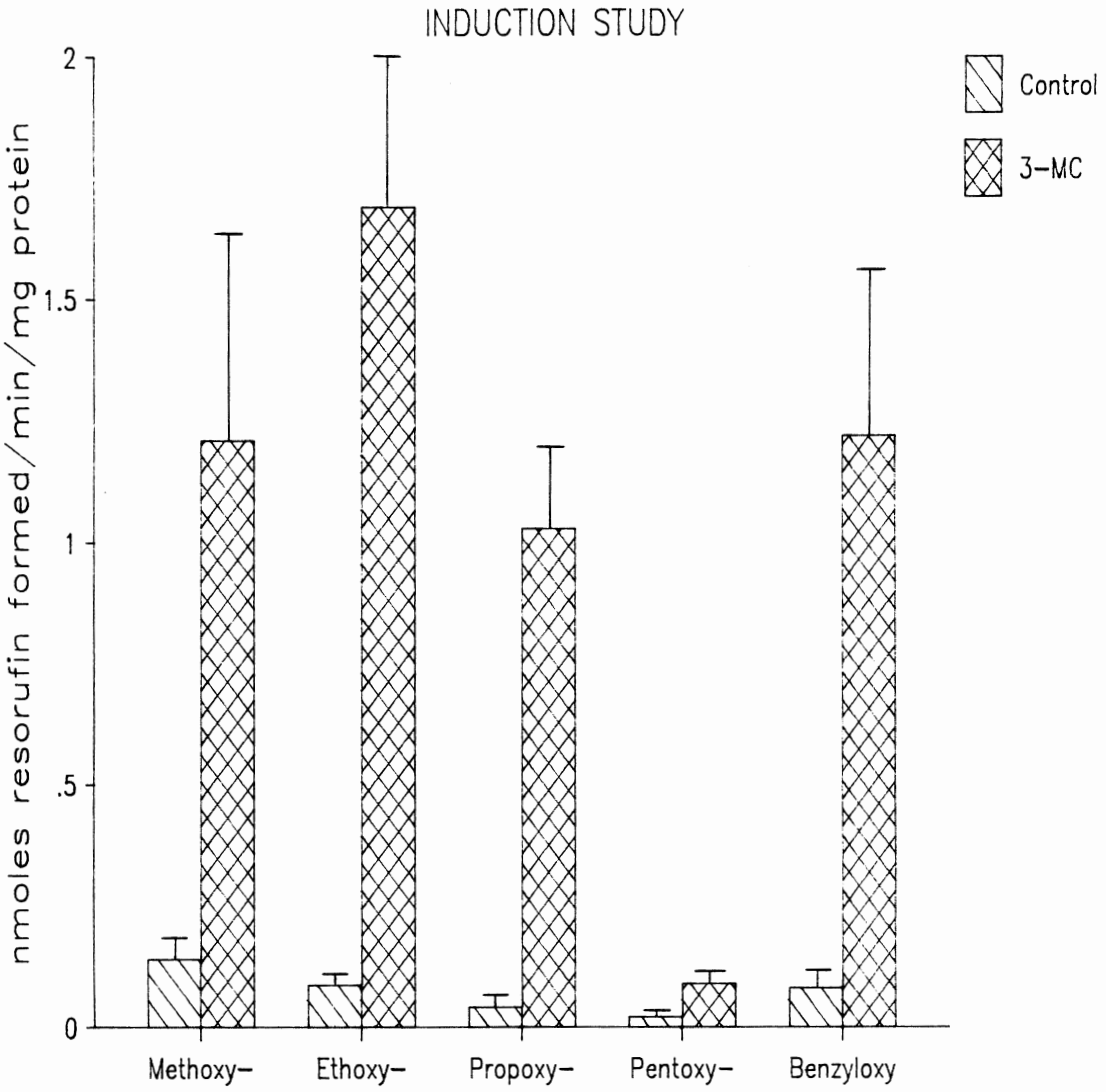
Animals and Statistics	Resorufin ethers Methoxy	Ethoxy	Propoxy	Pentoxy	Benzyloxy
PO (n=6)	1.417[a]P ±0.203	1.330[a] ±0.182	1.266[a] ±0.264	0.070[a] ±0.013	0.444[a] ±0.114
PC (n=3)	0.248[a] ±0.026	0.302[a] ±0.039	0.178[a] ±0.047	0.021[a] ±0.009	0.074[a] ±0.006
Fold induction	6-fold	4-fold	7-fold	3-fold	6-fold
Significance	0.0022	0.0021	0.0088	0.0166	0.0224

a = nmoles of resorufin formed / min / mg microsomal protein (expressed as Mean ± Standard error)

Figure 1. Relationship between reaction rate and alkyl ether chain length by liver microsomes of control and 3-methylcholanthrene (3MC) pretreated cotton rats.


CONTROL COTTON RATS
nmoles resorufin formed/min/mg protein
0
.1
.2
.3
.4
.5
Methoxy–
Ethoxy–
Propoxy–
Butoxy–
Pentoxy–
Hexoxy–
Benzyloxy–
Octoxy–
Decoxy–


3MC PRETREATED COTTON RATS
nmoles resorufin formed/min/mg protein
0
.5
1
1.5
2
Methoxy–
Ethoxy–
Propoxy–
Butoxy–
Pentoxy–
Hexoxy–
Benzyloxy–
Octoxy–
Decoxy–


INDUCTION STUDY
Control
3-MC
nmoles resorufin formed/min/mg protein
2
1.5
1
.5
0
Methoxy-
Ethoxy-
Propoxy-
Pentoxy-
Benzyloxy

CHAPTER VI

HEMATOLOGIC AND BLOOD CHEMICAL CHARACTERISTICS OF WILD AND LABORATORY RAISED COTTON RATS (SIGMODON HISPIDUS)

Introduction

The hispid cotton rat (Sigmodon hispidus) is ubiquitous in its distribution in the southeastern United States, comprising an important component of small mammal communities in grass and brush dominated rangelands (Fleharty and Mares, 1973). Cotton rats are increasingly used as animal models of both human and animal diseases. Their susceptibility to a variety of pathogens has been used in the "choice species" study of respiratory syncytial virus (Prince et al., 1978), parainfluenza virus (Murphy et al., 1981), mycoplasma pneumoniae (Eaton et al., 1942) and filarial parasites (Court and Story, 1981). Because of their species specific susceptibility, cotton rats also are used as in vivo infection stock for Echinococcus multilocularis (Lubinsky, 1960). Recently, increased emphasis has been placed on the use of wild cotton rats as biomonitors to evaluate the effects of pollutants in the environment (Elangbam et al., 1989).

Despite their importance in the study of many diseases, there is a general lack of baseline physiological information for the species which hindered our efforts in assessing the health and general physiological status of both captive and wild caught cotton rats. Only selected hematologic values have been reported in this species (Dotson et al., 1987). Our primary objective was to compare selected

hematologic and serum chemical characteristics of wild caught cotton rats with those raised in the laboratory to establish baseline reference values. We also examined the effects of xenobiotic exposure on several blood parameters of wild cotton rats that were collected from a contaminated waste disposal site.

Materials and Methods

A total of 52 cotton rats (body weight > 60 g) was collected from three locations in central Oklahoma near the towns of Blackwell, Criner, and Stillwater from June to September 1987. Twenty-nine of these cotton rats were collected from Criner, Oklahoma which included 16 animals obtained from the Royal Hardage toxic waste disposal site and 13 from an adjacent uncontaminated control site. We collected cotton rats from the Royal Hardage site to examine the sensitivity of selected blood parameters to xenobiotic exposure. All three collection locations were from ecologically similar habitats, representing typical ungrazed tall-grass prairie. Cotton rats used in this study were live-trapped using Sherman aluminum live-traps baited with rolled oats.

Cotton rats collected from uncontaminated areas in Blackwell and Stillwater, Oklahoma were used as breeding stock for a captive outbred colony. Animals were housed in our laboratory animal facility at Oklahoma State University in individual polycarbonate cages with wire tops and wood shavings as bedding. Animals were maintained under a 12 hr photoperiod and commercial rat chow was provided ad libitum. After a 2-week acclimatization, each female was paired for two weeks with an adult male for breeding. Offspring born to captive females were weaned at three weeks of age and housed in pairs (same sex) until they reached a desired body weight (> 60 g) for blood sampling.

Wild-caught cotton rats were always returned to the laboratory prior to blood sampling. Both wild-caught and laboratory-raised cotton rats (captives), were fasted

overnight, immobilized by cervical dislocation, and bled by periorbital sinus puncture. A blood sample was collected in Microtainer brand tubes containing EDTA (Becton Dickson and Company, USA) as an anticoagulant for hematologies. A whole blood sample for serum was collected in plain glass tubes, allowed to clot for 2-3 hours, and centrifuged for 30 minutes at 3000 rpm (IEC CENTRA-R, International Equipment Company, Needham, Massachusetts 02194, USA). Cotton rats were necropsied for any gross abnormalities.

Hematologies were performed on a Coulter Counter Model-S (Coulter Electronics, Hialeah, Florida 33014, USA). Hematological parameters assayed in this study included white blood cell counts, total red blood cell counts, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Differential counts were conducted on Wright-stained blood smears by classifying a minimum of 100 cells.

Serum samples were analyzed on a SMAC-II (Technicon Instruments Corporation, Tarrytown, New York 10591, USA) according to procedures specified by the manufacturer. Serum chemistries included aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, serum urea nitrogen (SUN), creatinine, cholesterol, alkaline phosphatase (AP), phosphorus, calcium, total protein, albumin, globulin, sodium, potassium, chloride, CO_2, creatine phosphokinase (CPK), gamma glutamyl transferase (GGT), uric acid and lactate dehydrogenase (LDH).

Differences in hematological and serum chemical parameters between cotton rats from the Royal Hardage contaminated site and those from an adjacent uncontaminated control site were compared by one-way analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS Institute, Cary North Carolina, 27511-8000, USA). Hematological and serum biochemical

data for 52 cotton rats, including rats from Royal Hardage contaminated site (excluding the parameters of MCHC, total protein, albumin, and chloride), were combined to derive baseline values for wild-caught cotton rats. Differences in mean hematological and serum chemical parameters between wild-caught and captive cotton rats were also analyzed by one-way analysis of variance.

Results and Discussion

Effect of Xenobiotic Exposure :

No major differences were observed in hematological and serum biochemical values between the control and Royal Hardage collection sites in Criner, Oklahoma. The only exceptions were with MCHC, total protein, albumin, and chloride (Table 1). Mean corpuscular hemoglobin concentration was significantly lower in cotton rats from the Royal Hardage site compared to those from the control. Decreased MCHC, an indication of hypochromic anemia, in contaminated cotton rats was probably due in part to the effects of environmental pollutants. Lead, a ubiquitous environmental pollutant, is known to cause hypochromic anemia as a result of altered erythrocyte life spans and impairment of heme synthesis (Goyer, 1986). Although lead exposure was possible at the Royal Hardage waste disposal site, we did not measure blood lead levels in collected cotton rats. Iron and copper deficiencies which also cause similar hypochromic anemia, are possible but considered unlikely.

Concentrations of total protein, albumin and chloride in cotton rats from the contaminated site were significantly higher than those from the uncontaminated site. These differences were probably related to dehydration. An increase in albumin does not occur except during dehydration (Duncan and Prasse, 1986). Whether dehydration was due to environmental pollutants or not requires further investiga-

tion.

Monitoring concentrations of serum enzymes of hepatic origin are useful tools in the study of hepatotoxicity (Cornish, 1971; Plaa and Hewitt, 1984). Exposure to water and soil pollutants, particularly halogenated hydrocarbons, can increase liver weights and elevate concentrations of AST and ALT in mice (Munson et al., 1982). However, we did not detect significant differences in concentrations of serum enzymes (AST, ALT, CPK, AP, LDH) between contaminated and uncontaminated collection sites.

The effects of exposure to environmental contaminants on hematological and serum biochemical parameters in wild cotton rats was inconclusive. Changes in hematological and serum biochemical parameters in response to contaminant exposure depends on the type, amount, and duration of exposure, which are unknown variables for the Royal Hardage site. More detailed studies will be required to evaluate the usefulness of these blood parameters in biomonitoring of environmental contamination.

Differences between Captive and Wild cotton rats :

Hematology

The MCV, MCH and counts of neutrophils, lymphocytes and basophils differed significantly between wild and captive cotton rats (Table 2). Mean corpuscular volume, MCH and neutrophil counts of captive cotton rats were higher than those from the wild. Conversely, lymphocyte and basophil counts were higher in wild than captive cotton rats. Dietary deficiencies of protein and iron in wild cotton rats could have caused lower MCV and MCH (Duncan and Prasse, 1986) compared to captives. The high plane of nutrition offered in the laboratory consistently resulted in large increases in body weight gain compared to wild cotton rats on a natural herbi-

vorous diet. Differences in leucocyte parameters between captive and wild cotton rats were probably a reflection of differences in the type and degree of parasitism. Wild cotton rats are undoubtedly exposed to a wider variety of antigens than those in captivity.

No significant differences between wild and captive cotton rats were seen in white cell, red cell, eosinophil and monocyte counts, Hgb, Hct, and MCHC (Table 2). With the exception of MCHC, hematological values (red and white cell counts, Hgb, Hct, MCV, MCH) observed in this study were generally outside the range reported by Dotson et al. (1987) for captive cotton rats. Discrepancies between these two studies could be due in part to differences in Coulter counter threshold settings, as well as, the conditions of sampling. Unlike Dotson et al. (1987), our experimental animals were fasted overnight prior to blood sampling. There are no previously reported information on differential counts for the cotton rat. However, comparisons with published values for laboratory rats and meadow voles (Microtus pennsylvanicus) indicated that cotton rats have considerably more variation in neutrophil, lymphocyte, basophil, eosinophil and monocyte counts among individuals (Clark, 1984; Kohn and Barthold, 1984). Despite this fact, mean values for leucocyte parameters in the cotton rat are not remarkably different from the laboratory rat (Clark, 1984).

Serum chemistry

Serum concentrations of creatinine, cholesterol, glucose, calcium, phosphorus, total protein, globulin, sodium, potassium and uric acids differed significantly between wild and captive cotton rats (Table 3). Cotton rats collected from the wild had higher concentrations of cholesterol, calcium, phosphorus, total protein, globulin, sodium, potassium, and uric acid than captives. Conversely, captive cotton rats had higher concentrations of glucose and creatinine than those from the wild. Most

of these differences between captive and wild cotton rats may be associated with differences in nutritional status. Additionally, higher concentrations of gammaglobulins in wild cotton rats could be a reflection of their antigenic-environment which might have contributed to elevated total protein levels.

Significant differences between captive and wild cotton rats were not noted in AST, ALT, total bilirubin, direct bilirubin, SUN, AP, albumin, chloride, CO_2, CPK, GGT and LDH levels. Concentrations of AST, ALT, AP, CPK and LDH were considerably greater for the cotton rat than values reported for the laboratory rat (Streett and Highman, 1971; Loegering, 1974). This was not completely unexpected since elevated serum enzyme levels are extremely common among wild animals, primarily because they are unaccustomed to handling by investigators. Serum concentrations of calcium, phosphorus, sodium, potassium and uric acids were within the normal ranges reported for laboratory rats (Burns et al., 1971).

We attempted to standardize procedures as much as possible in order to lower the handling effects on blood values. Interpretation of the results must still include the probable effects of collection and handling procedures upon measured blood parameters.

Summary

Blood values of 68 (52 wild and 16 captive) cotton rats (Sigmodon hispidus) from Oklahoma were examined. Twenty-nine of 52 wild cotton rats were collected from Criner, Oklahoma which included 16 animals obtained from Royal Hardage toxic waste disposal site and 13 from an adjacent uncontaminated site. Contaminated cotton rats had significantly higher serum concentrations of total protein, albumin and chloride levels and lower MCHC values than uncontaminated cotton rats.

Hematologic and serum chemistry values differed significantly between wild and captive cotton rats. Captive cotton rats had higher MCV, MCH and neutrophil

counts and lower lymphocyte and basophil counts than wild cotton rats. Serum concentrations cholesterol, calcium, phosphorus, total protein, globulin, sodium, potassium and uric acids in wild cotton rats were higher than captive cotton rats. Serum creatinine and glucose levels in captive cotton rats were higher than those from the wild. Compared with laboratory rats, cotton rats had appreciably higher levels of serum enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase and lactate dehydrogenase) than laboratory rats.

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Table 1. Statistical significant hematologic and serum biochemistry data of wild cotton rats (Sigmodon hispidus) from Royal Hardage contaminated and uncontaminated sites.

Determinations	Sites	Number of rats in sample	Range	Mean	SD
MCHC (%)	RH	16	29.7-32.5	31.32	0.94
	CO	13	31.9-34.9	32.67	0.74
Total protein (g/dl)	RH	16	6.5-8.6	7.44	0.53
	CO	13	5.2-8.3	6.65	0.79
Albumin (g/dl)	RH	16	3.3-4.2	3.71	0.25
	CO	13	2.6-4.1	3.38	0.40
Chloride (meq/l)	RH	16	103-134	113.69	8.01
	CO	13	105-122	108.08	5.42

RH : Royal Hardage contaminated cotton rats
CO : uncontaminated cotton rats

Table 2. Hematology of wild (W; n=52) and captive (L; n=16) cotton rats

Hematology	Animal source	Range	Mean	SD
White cells	W	4.0-77.90	11.43	9.96
($X\ 10^3$)	L	4.3-16.3	9.78	3.02
Neutrophils **	W	1.0-53.0	19.7	11.00
(%)	L	3.0-52.0	30.06	13.69
Lymphocytes *	W	30.0-91.0	69.04	13.12
(%)	L	40.0-94.0	60.69	10.078
Monocytes	W	0-23	4.0	4.0
(%)	L	1-25	3.88	3.37
Eosinophils	W	1-25	6.02	4.67
(%)	L	0-15	5.44	4.33
Basophils **	W	0-5	0.96	1.31
(%)	L	0-3	0.25	0.72
Red cells	W	5.16-8.32	7.16	0.70
($X\ 10^6$)	L	5.8-8.66	6.91	0.70
Hemoglobin	W	11.20-17.30	15.02	1.45
(g/dl)	L	12.8-18.8	15.01	1.42
Hematocrit	W	34.30-56.2	47.22	4.59
(%)	L	38.5-55.8	47.96	4.68

* = Statistically significant ($P < 0.05$) between wild and captive cotton rats

** = Statistically significant ($P < 0.01$) between wild and captive cotton rats

Table 2. Hematology of wild (W; n=52) and captive (L; n=16) cotton rats (contd)

Hematology	Animal source	Range	Mean	SD
Mean corpuscular volume (u^3) **	W	60-72	66.78	2.74
	L	60-81	72.06	6.83
Mean corpuscular hemoglobin (uug) **	W	19.10-25.0	21.21	1.24
	L	20.6-24.6	22.14	1.07
Mean corpuscular hemoglobin concentration * (%)	W	29.7-34.9	31.61	1.12
	L	28.6-33.2	31.18	1.76

* = Statistically significant ($P < 0.05$) between wild and captive cotton rats

** = Statistically significant ($P < 0.01$) between wild and captive cotton rats

Table 3. Serum chemistry of wild (W; n=52) and captive (L; n=16) cotton rats

Serum chemistry	Animal source	Range	Mean	SD
Aspartate aminotransferase (IU/L)	W	37-980	396.40	168.10
	L	4-2106	361.31	466.17
Alanine aminotransferase (IU/L)	W	65-411	225.50	80.38
	L	56-700	187.06	168.83
Total bilirubin (mg/dl)	W	0-0.3	0.90	0.07
	L	0.1-0.2	0.11	0.02
Direct bilirubin (mg/dl)	W	0-0.1	0.02	0.04
	L	0	0	0
Serum urea nitrogen (mg/dl)	W	18-52	27.50	6.61
	L	13-70	27.25	12.69
Creatinine* (mg/dl)	W	0.4-1.70	0.79	0.26
	L	0.7-1.30	0.93	0.20
Cholesterol** (mg/dl)	W	39-194	104.10	36.41
	L	62-113	81.25	15.51
Alkaline phosphatase (IU/L)	W	12-498	201	74.29
	L	103-359	204.13	74.87

* = Statistically significant ($P < 0.05$) between wild and captive cotton rats

** = Statistically significant ($P < 0.01$) between wild and captive cotton rats

Table 3. Serum chemistry of wild (W; n=52) and captive (L; n=16) cotton rats

Serum chemistry	Animal source	Range	Mean	SD
Glucose **	W	53-206	100.40	29.56
(mg/dl)	L	78-247	145.06	42.29
Phosphorus **	W	5.50-15	9.24	2.16
(mg/dl)	L	3.30-16.50	6.35	3.03
Calcium **	W	10-14.30	12.74	0.76
(mg/dl)	L	9.40-13.40	11.31	1.12
Total protein **	W	5.20-8.70	7.19	0.72
(g/dl)	L	3.80-7.70	6.06	0.94
Albumin	W	2.60-4.20	3.53	0.33
(mg/dl)	L	2.20-4.80	3.56	0.62
Globulin **	W	2.60-5.20	3.67	0.50
(mg/dl)	L	1.60-3.50	2.50	0.42
Sodium **	W	147-184	155.50	6.09
(meq/l)	L	129-183	138.25	14.75
Potassium **	W	4.10-9.90	7.84	1.06
(meq/l)	L	3.90-6.30	4.47	0.64
Chloride	W	99-134	110.26	6.38
(meq/l)	L	101-126	107.06	7.74
CO_2	W	4-25	14.80	4.93
(meq/l)	L	1-24	15.94	6.03

* = Statistically significant ($P < 0.05$) between wild and captive cotton rats
** = Statistically significant ($P < 0.01$) between wild and captive cotton rats

Table 3. Serum chemistry of wild (W; n=52) and captive (L; n=16) cotton rats (contd)

Serum chemistry	Animal source	Range	Mean	SD
Creatine phosphokinase (IU/L)	W	131-2240	677.43	484.81
	L	90-2500	674.25	775.69
Gamma glutamyl transferase (IU/L)	W	0-6	1.17	1.46
	L	1	1	
Uric acid ** (mg/dl)	W	0-6.50	1.97	0.88
	L	0.50-4.20	1.42	0.84
Lactic dehydrogenase (IU/L)	W	178-901	418.62	153.35
	L	181-7662	811.44	1775.46

* = Statistically significant ($P < 0.05$) between wild and captive cotton rats
** = Statistically significant ($P < 0.01$) between wild and captive cotton rats

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