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Development of the Cotton Rat (*Sigmodon hispidus*) as a Biomonitor of Environmental Contamination with Emphasis on Hepatic Cytochrome P-450 Induction and Population Characteristics

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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 the 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 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.

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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 identified 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.

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 $\bar{x} \pm SE$
Number in parenthesis indicates the percentage of the control value
*Statistically different from control ($P < 0.05$; Student t-test)
n = No. of rats

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 corresponding 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 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, their marked inducibility and ubiquitous distribution in the Southeastern United States.

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%).

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

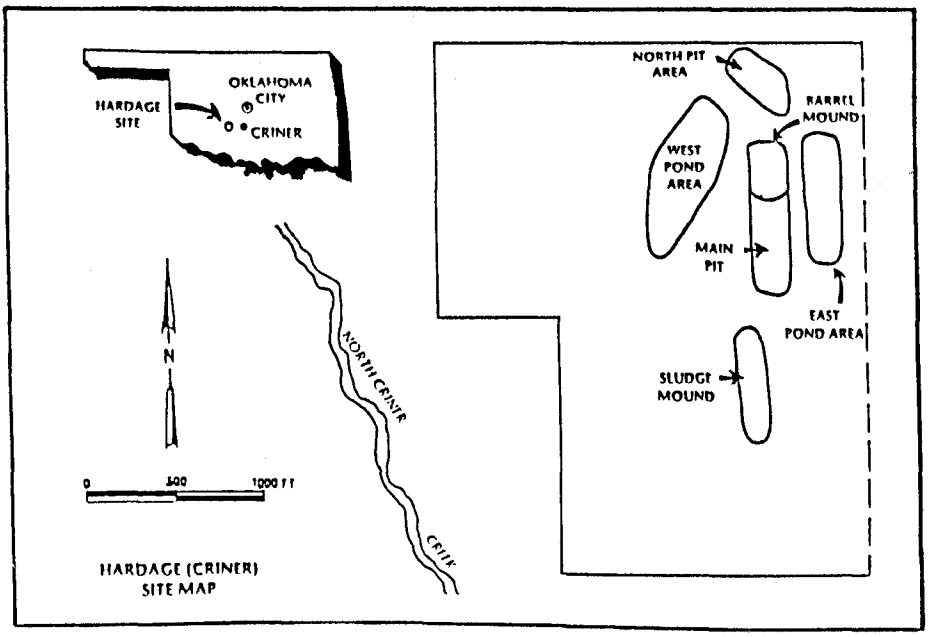

Figure. 1. Map of the Hardage (Criner) site showing locations of main pit, barrel mound, evaporation ponds (west and east ponds) and north pit.

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 may have been 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 essen-

tially 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 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.

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