AN *IN SITU* INVESTIGATION OF GENETIC DAMAGE IN WILD RODENTS INHABITING A SITE CONTAMINATED WITH AROCLOR 1254

BY

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PREFACE

This study is part of a larger effort working toward the development of mammalian *in situ* genetic biomonitors. It examines the frequency of structural chromosome aberrations in standard bone marrow metaphase preparations of three rodent species inhabiting a site contaminated with Aroclor 1254. These data are compared to those of conspecifics from uncontaminated reference areas. In the case of 2 species, comparisons are also made to conspecifics from an area contaminated with substances associated with the induction of structural chromosome aberrations and significant variation in whole cell DNA content.

It is my wish to express gratitude to my major advisor, Dr. Karen McBee, for her support throughout this endeavor and for sharing her knowledge and insight on the field of toxicology. Gratitude is also expressed to my master's committee members Dr. S.L. "Bud" Burks, for the inspiration and encouragement he provided and Dr. Bill Warde, for his guidance through the statistical analysis of the data.

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CHAPTER I

INTRODUCTION

Effects of hazardous wastes and monitoring of disposal sites have become important topics of investigation due to increasing public concern about risks associated with such materials and the efficiency of their containment. This document describes a project that involved *in situ* biomonitoring of a hazardous waste site using chromosome structural aberration frequency as an end point. *In situ* monitoring is the use of resident species to assess the potential hazard a waste site poses to humans and to other components of the environment.

The goals of this project were to conduct an *in situ* investigation of genetic damage in wild rodents from a site contaminated with the polychlorinated biphenyl (PCB) Aroclor 1254 and to compare these data to the amount of damage in conspecifics from uncontaminated reference sites and a site known to contain clastogens. Specific objectives were:

1. To collect live rodents from a waste site contaminated with the PCB Aroclor 1254 and from three uncontaminated reference areas.

2. To conduct standard chromosome aberration assays on animals from all sites.
3. To compare results from PCB site animals to conspecifics from uncontaminated reference areas and to animals from an area contaminated with known clastogens.

Aroclor 1254 has not been demonstrated to be mutagenic when tested alone in standard laboratory assays such as standard bone marrow chromosome aberration assays, micronucleus assays, sperm abnormality assays and Salmonella/microsome assays (Green et al., 1975a and 1975b; Dikshith et al., 1975; Heddle and Bruce, 1977; Schoeny et al, 1979; Schoeny, 1982). However, conflicting data exist concerning the mutagenicity of PCBs. Aroclor 1254 has been demonstrated to be mutagenic in kidney cells of three Cyprinid species (Al-Sabti, 1985). Significant genetic effects have been observed in Salmonella typhimurium exposed to the single congener 4-chlorobiphenyl (Wyndham et al., 1976). This same study determined that Aroclor 1254 was essentially inactive as a mutagen. One study (Sargent et al., 1989) suggested the possibility of synergistic genotoxic interaction of individual PCB congeners.

The hypothesis tested in this study was that the amount of genetic damage in rodents caught on the PCB spill site would not be significantly greater than the damage in conspecifics from uncontaminated reference sites. In

addition, the amount of damage in rodents from the site contaminated with PCBs would be significantly less than the amount in those from a site containing known clastogens. By concurring with a previous rodent standard bone marrow metaphase chromosome assay conducted under laboratory conditions (Green et al., 1975a), this study further strengthens the validity of the use of in situ indicator species for the assessment of genetic hazard in polluted environments. Should this hypothesis have been rejected, it would have suggested the presence of a previously undetected clastogen at the site. Alternatively, if the hypothesis was rejected and the absence of a biologically available confirmed clastogen was assured, it would imply that laboratory assays did not accurately assess synergistic interactions which may occur within PCB wastes or between PCBs and waste site environments. The observation of statistically significant damage in animals from the PCB site would have indicated that further research was necessary to more realistically assess the potential hazard PCB wastes pose in waste site environments. Had these alternative events taken place, they would have strengthened the validity of the use of mammalian in situ genetic biomonitors by indicating actual effects which were not predicted by laboratory assays.

CHAPTER II

LITERATURE REVIEW

Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a group of halogenated aromatic compounds first created in 1881 (Eisler, 1986). Their commercial use began in the 1930's and continued into the late 1970's. PCBs were used as flame retardants, plasticizers, concrete sealants, hydraulic fluids, pesticide solvents, paint additives and dielectric fluids for transformers and capacitors (Hansen, 1987; Eisler, 1986; WHO, 1976). The major PCB producer in the United States, Montsanto, sold commercial mixtures under the trade name Aroclor. Foreign mixtures include Kanechlor (Japan), Phenoclor (France), Clophen (West Germany) and others (Eisler, 1986). PCBs are generally inert but when ignited or exposed to strong sunlight may form other toxic substances such as phenolics and dibenzofurans (Anon., 1983).

Due to their long persistence, widespread use and improper handling and disposal, PCB residues are now a ubiquitous contaminant found even in remote areas of the earth (WHO, 1976). The persistence of PCBs, coupled with

their toxic properties, led to the worldwide restriction of their use. In 1972, Japan banned production after a mass poisoning incident involving PCB contaminated rice bran oil (Anon., 1986; WHO 1976). In 1979 the United States Environmental Protection Agency restricted production, distribution and use of PCBs to closed systems presumed not to release the chemicals into the environment. Although not produced in the United States since 1973, older capacitors and other closed systems still contain PCBs. Therefore they are still in use to a limited extent. The USEPA has determined that PCB transfomers near commercial and public buildings must be replaced or reclassified by October 1st, 1990 (Motter, 1989).

As their name suggests, polychlorinated biphenyls are biphenyl structures upon which two or more hydrogen atoms have been substituted by chlorine atoms. There are 10 possible locations for chlorine substitution making 209 possible congeners. Chlorination of biphenyls is a random event affected by the method used to create them. Chlorination is not uniform during production, therefore several congeners are created at one time. Accordingly, commercial mixtures are composed of several congeners. Aroclors are named for their percent chlorine content, for instance Aroclor 1254 contains 54% chlorine and 1262 has 62% chlorine. Lower chlorinated biphenyl molecules are generally more water soluble. Solubility is also effected

by the degree of chlorine substitution in the ortho positions. Ortho substitutions affect the planarity of a PCB molecule and therefore influence vapor pressure and water solubility. Vapor pressures increase with increasing chlorination (Hansen, 1987).

Genetic Toxicology and PCBs

Hazardous materials which cause genetic damage are especially dangerous due to the potential for cancer induction, teratogenic effects and reduction of fertility. There has been much research on the effects of both natural and man made substances on DNA (Ames et al., 1975; Preston et al., 1981; Ray et al., 1987). Genetic damage in somatic cells has been linked to the development of cancer (Miller and Miller, 1981; Sasaki, 1982). Germ cell mutations may reduce fertility, cause birth defects, miscarriages and retardation in young (Hook, 1982; Hsu, 1983).

Conflicting data exist concerning the potential for PCBs to cause genetic damage. This is possibly due to differences in *in vitro* or *in vivo* methods, species or tissue selection and source and type of PCB mixture. Green and colleagues (1975a) determined that laboratory Osborne-Mendel rats orally dosed with subacute and acute doses of Aroclor 1254 or 1242 did not exibit statistically significant increases in the frequency of chromosomal abnormalities in bone marrow cells or spermatogonial cells.

Dominant lethal tests using Osborne-Mendel rats dosed with Aroclors 1242 and 1254 also indicated that these substances were not mutagenic (Green et al., 1975b).

Schoeny and collegues (1979) used the Ames Salmonella/mammalian microsome assay to evaluate the mutagenicity of several compounds, including PCBs. Among their findings was that the PCB mixture Aroclor 1254 was nonmutagenic in Salmonella/microsome assays. Schoeny (1982) later examined the mutagenicity of several of the individual PCB isomers present in Aroclor 1254 and of polychlorinated dibenzofurans (PCDFs) which are contaminants of commercial PCB mixtures. The results of this study indicated that, even in the presence of hepatic microsomes, plates exposed to PCBs and PCDFs did not exibit statistically significant increases in reversion frequency (Schoeny, 1982). As inducers of hepatic enzymes, PCBs and some PCDFs may modify the mutagenicity of known chemical carcinogens (Ames et al., 1975; Schoeny et al., 1979).

Salmonella/microsome assays conducted by Wyndham and collegues (1976) demonstrated that the isomer 4-tetrachlorobiphenyl was highly mutagenic, but Aroclors 1254 and 1268 were essentially inactive. They concluded that mutagenic activity decreased with increased chlorination.

Aroclor 1254 is the most commonly used inducer of hepatic microsomal enzymes (S9 fractions) for genotoxicity testing (Kirkland et al., 1989). S9 fractions are used to

mimic mammalian metabolism in a variety of in vitro tests. Researchers conducting standard mutagenicity testing observed higher than normal levels of chromosomal aberrations in Chinese hamster ovary cells (CHO) incubated with 11 different batches of Aroclor-1254 induced S9 mix. Human lymphocyte cultures exposed to aliquots from the same batches of S9 fraction did not exhibit abnormal levels of aberrations. Bacterial reversion assays, unscheduled DNA synthesis assays and mammalian cell hgprt mutation tests also exhibited normal mutation rates. The researchers suspected that the cytochrome P-450 enzymes present in the induced S9 fractions produced active oxygen species (AOS) which are known to be clastogenic. They further deducted that the AOS were inactivated by blood components in the human lymphocyte cultures but remained active in the CHO cultures. They suggested that the instability of CHO chromosomes was a contributing factor to AOS sensitivity (Kirkland et al., 1989).

Al-Sabti (1985) injected three species of fish with different dose levels of Aroclor 1254 and examined kidney cells for chromosomal aberrations. Significantly more chromosomal aberrations were found in the kidney cells of dosed fish in comparison to conspecifics injected with corn oil or distilled water. An increase in aberration frequency was observed with increased dosage.

Human lymphocyte cultures were used to test mutagenicity of Aroclor 1254 as well as that of planar (3,4,3',4' tetrachlorobiphenyl) and nonplanar (2,5,2',5' tetrachlorobiphenyl) congeners which are found in Aroclor 1254 (Sargent et al., 1989). At low PCB concentrations a significantly increased frequency of chromosomal aberrations and mitotic index supression were observed. A synergistic effect was also observed when compounds were tested together. Their conclusions suggested that the overall biological effect of Aroclor 1254 is not due to the additive effect of each constituent but the synergistic interaction of a few components present in low concentrations (Sargent et al., 1989).

Exposure and Uptake of PCBs

Exposure of the young through breast milk has been documented in rodents by Anderson et al. (1986), Sager et al. (1987), and Linzey (1988) and in humans by Mes and Davies (1979). A study comparing PCB content in the milk and milk fat of Inuit mothers to that of non Inuit mothers demonstrated the importance of fish and marine mammal consumption as a route of exposure. Inuit mothers consumed more fish and marine mammals and had higher concentrations of PCB residues in their milk and milk fat (Dewailly et al., 1989). Milk intended for human consumption has also been implicated as an exposure source (Frank and Braun, 1989).

The most common exposure route is through the consumption of contaminated foods, especially fish (Anon., 1986; Fiore et al., 1989). The first human epidemic of PCB poisoning occurred through the mass consumption of contaminated rice oil by Japanese in 1968. The victims of this poisoning are called Yusho patients. Yusho symptoms differ from those of PCB poisoning due to the presence of polychlorinated dibenzofuran (PCDF) contamination in the toxic rice bran oil (Kuratsune et al., 1987; Kashimoto and Miyata, 1987).

Due to the nonpolar, lipophilic nature of PCB molecules, the principal mechanism of uptake is passive lipid diffusion. PCBs may be absorbed from the atmosphere through skin or the walls of the lung. In aquatic organisms, uptake via the gills is the most significant route, although some residues may enter through the epidermis. Absorption into the gut from ingested contaminated food is the most common route of uptake in both terrestrial and aquatic species (Shaw and Connell, 1986).

Distribution and Storage of PCBs

The highly lipophilic nature of PCBs allows them to enter the biota easily and their resistance to metabolism enables them to remain stored in the organism, rather than be excreted. Factors affecting PCB accumulation include physicochemical properties of specific components, ambient

concentration, duration of exposure, temperature, species, age, weight, diet and lipid content (Hamdy and Gooch, 1986). Seasonal patterns may also affect uptake and excretion of PCBs. Such variables include seasonal availability of certain food sources, elimination post breeding by females to young through the placenta and breast milk and periods of fasting during spawning, migration and rut (Strout, pers. comm.). Organisms occupying the top trophic levels aquire most of their PCB burden from biomagnification (Shaw and Connell, 1986). Biomagnification is rapid and follows pathways similar to that of DDT placing aquatic life, predators and birds at highest risk (Anon., 1983).

PCBs are removed from the blood rapidly, are initially deposited in the liver and muscles, and then translocated to adipose tissue and skin (USEPA, 1980). Tissue concentrations generally increase with the age of an individual (Phillips, 1986).

Metabolism and Excretion

Biotransformation by hepatic cytochrome P-450(s) of specific congeners is the key event in determining rate of metabolism and elimination. Metabolism and excretion of PCBs in the liver reduces it's burden and ultimately causes the redistribution of residues within the organism (Sipes and Schnellman, 1987). Metabolism of PCBs is dependant on chlorine content and location of substituted chlorines, with

the more highly chlorinated congeners being more resistant to metabolism. The capacity to metabolize PCBs declines from mammals to birds to fish. Major metabolic products are phenolic derivatives or dihydrodiols formed either by direct hydroxylation or through formation of arene oxide intermediates (USEPA, 1980). Arene oxide intermediates have been implicated in some of the toxic effects of PCBs (Sipes and Schnellman, 1987).

Excretion of PCBs is closely coupled with metabolism. Primary routes are through the urine and bile. In females residues may leave the body by being passed to offspring through the placenta and mother's milk (WHO, 1976; USEPA, 1980). Residues may also be found in the eggs of avian species (Zicus et al., 1987; Barrett et al., 1985). Lower chlorinated biphenyls are metabolized and excreted more easily than highly chlorinated congeners. Biliary excretion is more significant than urinary elimination, especially for highly chlorinated congeners. Excretion through urine occurs for several days after exposure while biliary excretion occurs over a longer time span. Accordingly, duration of excretion is positively correlated with increasing chlorine content (WHO, 1976; USEPA, 1980).

Organismal Response to PCB Poisoning

Alterations in physiological function induced by PCBs are related to the biochemical events of enzyme induction or

alteration and the alteration of metabolic processes. Induction of hepatic microsomal enzymes is the earliest biochemical change observed in response to PCBs (Hansen, 1987). P-450 microsomal enzymes catalyze the metabolism of lipophilic xenobiotics into excretable metabolites. The metabolites formed are not necessarily less toxic than the parent compound (Parkinson and Safe, 1987).

The interaction of PCBs with hydrophobic domains of enzymes enables these compounds to affect enzyme activity (Gamble, 1986). PCB exposure alters enzymes of glycolysis, gluconeogenesis, drug metabolism, oxidative phosphorylation and alters phospholipid, triglyceride and sterol metabolism. Alteration of enzymes involved in glucose metabolism will lead to metabolic trauma if uncompensated. Inhibition (at low doses) and stimulation (at high doses) of oxidative phosphorylation will, in turn, alter cellular respiration. PCBs also alter the concentration, metabolism and mobilization of lipids. The general trend is an increase in generation of triglycerides. Phospholipid synthesis is inhibited by prolonged exposure and stimulated by an acute dose. Cholesterol metabolism is generally inhibited by PCBs. This has been attributed to interference with lipoprotein metabolism and transport (Gamble, 1986). PCBs have also been demonstrated to alter brain catecholamine levels. Catecholamine is important in stimulating thermogenesis in brown adipose tissue (Hansen, 1987).

PCBs are acutely toxic at high doses, acute exposure is not likely to occur under natural conditions. Symptoms of acute poisoning are collectively referred to as 'wasting syndrome'. Wasting syndrome is characterized by weight loss, diarrhea, terminal ataxia, unusual stance and gait and loss of pain stimuli response. These symptoms are attributed to deterioration of the central nervous system and dehydration (Bruckner et al., 1973; USEPA, 1980).

Histopathological changes attributed to PCBs are usually associated with the liver. These include the formation of neoplasia, adenofibrosis, organ enlargement, vacuolization and fatty deposits (Bruckner et al., 1973; WHO, 1976; Fuller and Hobson, 1986). Initiation of tumors in the lung has also been demonstrated in one study (Anderson et al., 1986). Cellular changes include the presence of atypical mitochondria and increased volumes of liposomes, smooth and rough endoplasmic reticulum and circumnuclear cytoplasm (Gamble, 1986; Fuller and Hobson, 1987). The occlusion of uteri in pinniped species has also been observed (Olsson, 1987).

Kuratsune et al. (1987) found that significantly more male yusho victims died of cancer, especially cancer of the liver. This study provided correlative data on carcinogenesis, but a proponderance of supporting epidemiological studies will be necessary before PCBs can be confirmed as human carcinogens by the United States

Environmental Protection Agency (USEPA, 1980). PCBs have been demonstrated to be potent tumor inducers in rodents; however, rodents appear to be extremely susceptible to this response. For this reason, confidence in extrapolation to humans is low. PCBs can not be generalized as liver tumor promoters because they inhibit tumor initiation by known carcinogenic xenobiotics under natural exposure conditions (Hayes, 1987).

While the liver is considered to be the primary target organ, the reproductive and immune systems are also affected by PCBs (Fuller and Hobson, 1986). Atrophy of lymphoid tissue has been suggested as the origin of immunosupressive effects of PCBs (WHO, 1976). This atrophy is accompanied by a reduction in circulating lymphocytes and leukocytes, suppressed antibody responses, enhanced virus susceptibility and suppression of natural killer cells (Hansen, 1987). Tryphonas et al. (1989) studied the immunotoxic effects of PCBs in rhesus monkeys and found a dose related response where increased PCB levels reduced the ability of subjects to respond to a T-dependant antigen (sheep red blood cells). Other detrimental effects on the immune system were observed but the exact mechanism of immunotoxicity is not yet fully understood (Tryphonas et al., 1989). Dewailly et al., (1989) noted that the infection rate of Inuit children is 10 to 15 fold higher than children in societies not as dependant of marine mammals and fish as a food source.

Reproductive sensitivity to sublethal doses increases in higher mammalian orders. A suggested cause of reproductive effects is the alteration of steroid patterns and estrogenic activity of lower chlorinated biphenyls. The result of PCB effects on reproduction is a reduction in an organism's ability to produce offspring (Fuller and Hobson, 1986).

The effects of early postnatal exposure of male rats to PCBs through nursing was evaluated by Sager et al., (1987). Weight gain was reduced during the period of exposure, but returned to normal after weaning. When these males were mated to unexposed females, significant reductions in implantation and number of embryos were observed.

Merson and Kirkpatrick (1976) studied the effects of PCBs on reproduction in captive white footed mice (Peromyscus leucopus). Their results indicated that a diet containing 200 ppm or more Aroclor 1254 will inhibit reproduction in this species. Dosed individuals had significantly fewer litters and fewer pups per litter than control animals. Offspring of PCB dosed parents did not survive beyond 21 days.

Linzey (1987, 1988) exposed members of the species P. leucopus to 10 ppm chronic dietary Aroclor 1254. Mice exposed as adults had significantly fewer offspring at weaning than control animals. Those exposed at 12 weeks of age had smaller litters, longer periods between births and fewer offspring at weaning. This study (Linzey, 1987)

suggested that the reproductive effects of PCBs are related to age at exposure. Second generation offspring exhibited significant reductions in reproductive success when compared to the parental generation and controls (Linzey, 1988). These animals were also smaller at 4, 8 and 12 weeks of age. At 8 and 12 weeks the uteri and ovaries of females were smaller than those of their unexposed couterparts and dosed males had smaller accessory glands. Testicular weights of dosed and control males were not significantly different. These results confirmed that the effects of PCBs are accumulated through generations.

A population of white footed-mice in an area contaminated with polychlorinated biphenyls and heavy metals was examined by Batty et al. (1990). Liver, kidney, spleen and adrenal weights of animals from the contaminated site were significantly higher in the winter. Significant decreases in whole body weight of contaminated site animals were observed in the summer while liver weights were still significantly higher. In addition, testis weights of PCB site males were significantly lower during the summer months. Fewer juveniles and subadults were captured at the contaminated site during the breeding season, implying that the population was either not reproducing effectively or was subject to increased predation pressure.

While PCBs are considered to be reproductive poisons causing retarded fetal growth and spontaneous abortion,

structural malformations in mammals are rare (USEPA, 1980). Malformations that have been observed include cleft lip, cleft palate, brachydactyly and syndactyly (Fuller and Hobson, 1986). Teratogenic effects of Aroclor 1254 observed in birds include leg, toe and neck deformities. Effects were less severe than those observed in Aroclors 1242 and 1248 (Tumasonis et al., 1973). Injection of PCBs into eggs has been demonstrated to cause beak abnormalities in chicks (McLaughlin, et al., 1963). PCB induced reproductive failure in birds includes reduced hatchability (Peakall, 1986).

Use of In Situ Biomonitors

The importance of conducting in situ studies lies in the fact that the toxicity of a pollutant is affected by the physical and chemical characteristics of the environment in which it exists. Factors which may alter the toxicity of a pollutant include pH, organic and mineral content of the soil, exposure to sunlight, osmotic pressure, ion composition and exchange capacity, buffering capacity, moisture and temperature (Babich and Stotzky, 1983).

In situ biomonitoring can serve as a sentinal of environmental quality indicating if the nature of the toxicity of resident wastes has changed, or if leakage or unreported disposal of additional waste has occurred (Sandhu and Lower, 1987; McBee and Bickham, 1990). Interactions may

occur between different compounds in a waste site, and, although harmless by themselves, certain chemicals can increase the toxicity of others (Sandhu and Lower, 1987).

Previous work using rodents as in situ indicators used several species. Rowley et al. (1983) observed survivorship and liver, adrenal and seminal vesicle weights in Microtus pennsylvanicus caught at Love Canal, Niagara Falls, New York. These animals were exposed to a complex mixture of lindane, chlorobenzenes, benzylchlorides and trichlorophenol contaminated with dioxin. Mean life expectancy, reproductive success and organ weights were lowest in animals from the contaminated area when compared to both the control site and an intermediate site. These factors were also lower for animals occupying the intermediate site when compared to those from the control, implying the occurrence of chemical waste migration.

Tice et al. (1987) observed frequencies of chromosomal aberrations, sister chromatid exchanges, occurrence of micronuclei, sperm head abnormalities and reduction of mitotic index to assess genetic damage in Peromyscus leucopus from a hazardous waste site in New Jersey. The results of this study suggest seasonal variability in sensitivity and supports the use of white-footed mice to assess genetic hazard in waste site environments.

Work done by McBee et al. (1987) and McBee and Bickham (1988) also supports the use of P. leucopus as an in situ

indicator species and introduces the use of another species, the hispid cotton rat (Sigmodon hispidus). Through the analysis of standard bone marrow karyotypes (McBee et al.,1987) and flow cytometric histograms and CVs (McBee and Bickham, 1988), significantly higher levels of genetic damage were observed in individuals of both species caught from a hazardous waste site when compared to conspecifics from pristine areas.

Thompson et al. (1988) observed chromosome aberration and aneuploidy frequencies in standard bone marrow karyotypes of S. hispidus from two superfund waste sites. Again, comparisons were made to wild conspecifics from an uncontaminated site and similar results were obtained to that of McBee et al. (1987).

Blood samples from deer mice (Peromyscus maniculatus) trapped near two metal smelters in Manitoba, Canada were analyzed for increased hemoglobin and hematocrit values. According to Kucera, (1988) increased hemogram values are a normal physiological response to respiratory distress due to sulfur dioxide inhalation. The data from this study were plotted against distance from the smelters and the identification of an impact area was suggested (Kucera, 1988).

Hepatic cytochrome P-450 induction in S. hispidus was successfully used as an indicator of environmental contamination by Elangbam et al. (1989). Cytochrome P-450

is important in the metabolism of xenobiotics, detoxifying some compounds and activating others to form reactive metabolites (Elangbam et al., 1989).

The application of in situ biomonitoring to the ecological assessment of hazardous waste sites is a rapidly developing field of study. In many instances it is more time and cost effective than conventional laboratory bioassays and substrate analyses. Further development and standardization of in situ methods can provide alternative methods which will allow more efficient allocation of resources and effort in waste site management.

CHAPTER III

DESCRIPTION OF STUDY AREAS

Pryor PCB Site

The PCB site was located near the city of Pryor in Mayes County, Oklahoma on federal property managed by the U. S. Army Corps of Engineers (Fig. 1). The area contaminated with Aroclor 1254 covered approximately 1.5 ha. Black jack (Quercus marilandica), post oak(Q. stellata), native grasses and Smilax sp. were the most common plant species. An intermittent stream flowed through the area into Pryor Creek. The site was fenced off to prevent disturbance by cattle grazing on a nearby field west of the site. Being protected from grazing pressure, the area was densely vegetated in comparison to the surrounding land.

PCB contamination was limited to the upper 9-12 inches of the soil and ranged in concentration from 0.3 to 863.0 ug/g. The distribution of PCB concentrations in the surface soil is displayed in figure 2. Contamination was due to intentional illegal dumping dating back to the 1950's. PCB contaminated oil used in liquid oxygen pumps was reportedly discharged across Army Corps property into Pryor Creek from waste lagoons belonging to the neighboring fertilizer plant.

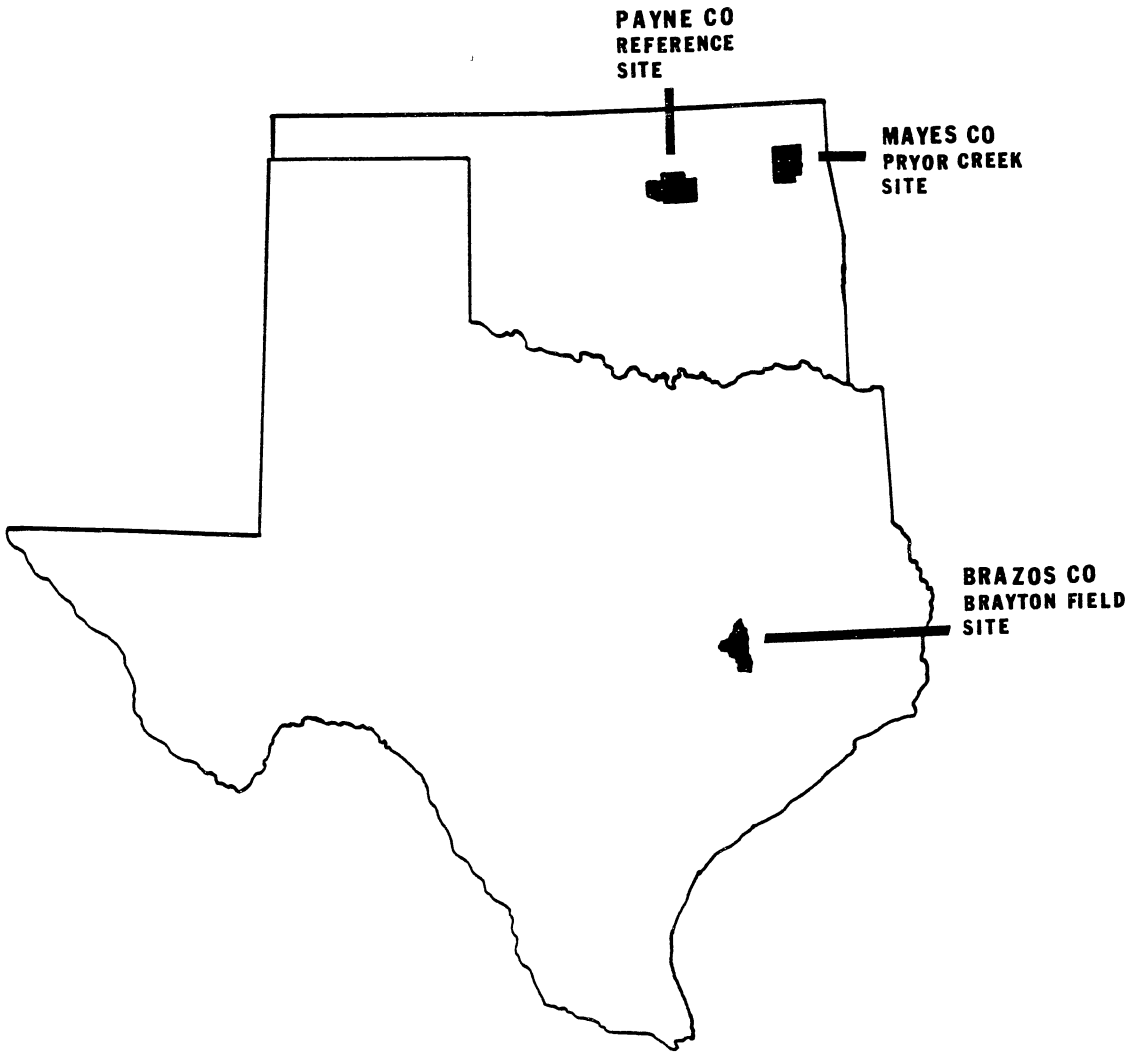
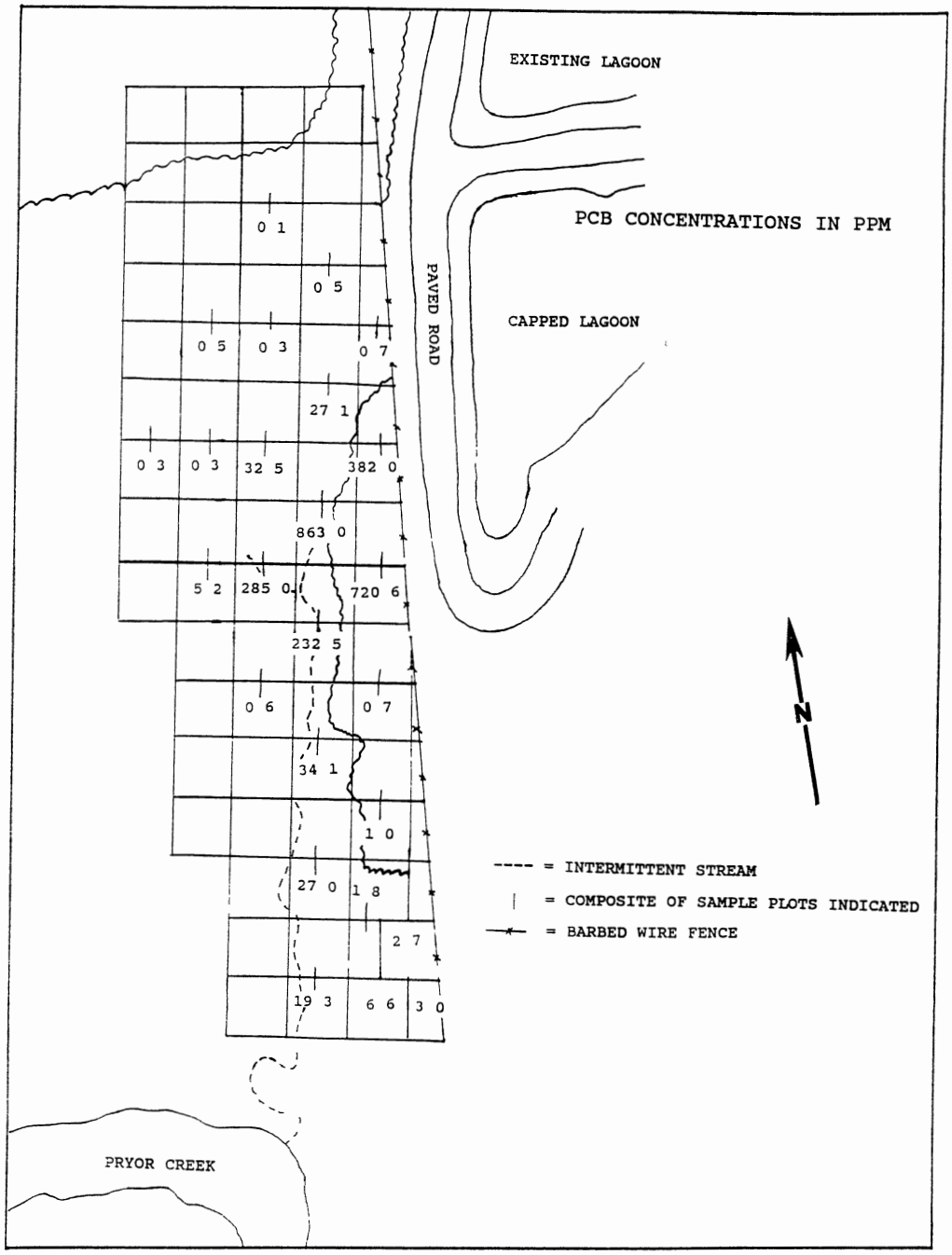


Figure 1. Study site locations



adapted from (U.S. Army Corps of Engineers, 1987)

Figure 2. Distribution of surface soil PCB residues

Groundwater samples were determined to be free from PCBs, but were contaminated with low levels of trichloroethene, tetrachloroethene, barium, chromium and lead. A scan of soil samples for priority pollutants revealed only low levels of chromium, barium and cadmium. It was determined by Corps investigators that Aroclor 1254 was the only contaminant present in sufficient quantity to be of regulatory concern (U.S. Army Corps of Engineers, 1987).

The site contaminated with known clastogens was Brayton Field Fireman's Training School in Brazos County, Texas (Fig. 1). This site was contaminated with partially combusted hydrocarbons, PCBs and heavy metals.

Reference Areas

The original reference site in Mayes County was located 300 meters north of the contaminated site. Trap success on this site was poor so it was relocated 200 meters west of the original location for the trapping session in May 1989. Despite improved ground cover at the new location, trap success was not improved. The field in which both reference sites were located was continuously grazed which probably contributed to poor trapping success. Vegetation of the original reference site mainly consisted of native grasses. A single Peromyscus leucopus was captured at this site, unfortunately it's bone marrow preparation was not scorable. Because poor trapping success on the Mayes county reference

sites had been anticipated, two additional reference sites had been established in Payne County (Fig. 1). The two Payne County reference areas were on property belonging to Oklahoma State University. Both are formerly cultivated land undergoing early succession. The Wildlife Research Annex (Annex) is on the western outskirts of Stillwater and has a mixture of native grasses, honey locust (Gladitsia triacanthos), sumac (Rhus sp.) and oaks (Quercus sp.). The area is fenced and gated. A storage building and a trailor are located near the gate and human activity on the property is primarily limited to this area. A barbed wire fence separates the buildings from the old field where study specimens were obtained.

The other Payne County site was approximately 8.9 miles west of Stillwater near Lake Carl Blackwell (Lake Carl). It was also a grassy area, with vegetation similar to that of the Annex but had a greater quantity of sumac (Rhus sp.). Collection of animals from the Wildlife Research Annex began in the summer of 1988 and collection at Lake Carl Blackwell began in the winter of 1989.

CHAPTER IV

DESCRIPTION OF SPECIES EXAMINED

Three rodent species, the white-footed mouse (Peromyscus leucopus), the hispid cotton rat (Sigmodon hispidus), and the fulvous harvest mouse (Reithrodontomys fulvescens) were examined in this study. These species were chosen on the basis of their relative abundance on the waste site. Other rodent species were present, but not used due to infrequent capture. These included members of the genera Mus and Microtus. The use of the three selected species permits the observation of effects in slightly different niches because each species varies in soil association, feeding habits and home range size.

Peromyscus leucopus

The white-footed mouse (P. leucopus) is a member of a genus that is widely distributed throughout North America. Of the more than 40 species in the genus, information about the white-footed mouse and it's close relative the deer mouse (P. maniculatus) accounts for the majority of what is known about the genus (Hooper, 1968).

P. leucopus is a medium sized member of the genus with an average total body length of 153.5 mm and is

characterized by small ears and an indistinctly bicolored tail which is shorter than the head and body. This species prefers the cover of wooded, brushy habitat and does not use runways. The diet includes seeds, nuts, plant material, fungi and invertebrates. Breeding can occur year round, but most young are produced in the late fall and early winter. Females produce 1-6 altricial young which become sexually mature at ten to eleven weeks of age. Nests are made in any place that provides adequate shelter (Schmidly, 1983). Reported home ranges for P. leucopus range from 0.03 to 1.7 acres (0.01-0.69 ha) (Stickel, 1968).

Members of the species P. leucopus may have great potential as genetic biomonitors because the species is abundant and easily captured, they generally limit their activity to small areas and the relatively low baseline frequency of genetic events allow for the detection of small increases in genetic damage (Boucher, 1985). Members of the species have been used as a genetic biomonitor in two studies (McBee et al., 1987; Tice et al., 1987), both of which indicated that their use for in situ monitoring was feasable.

Peromyscus leucopus has been the subject of much cytogenetic research (Stangl and Baker, 1984; Greenbaum and Baker, 1978). They have a diploid number of 48 and a fundamental number of 70 (Fig. 3). Their sex chromosomes are a large submetacentric X and a small metacentric

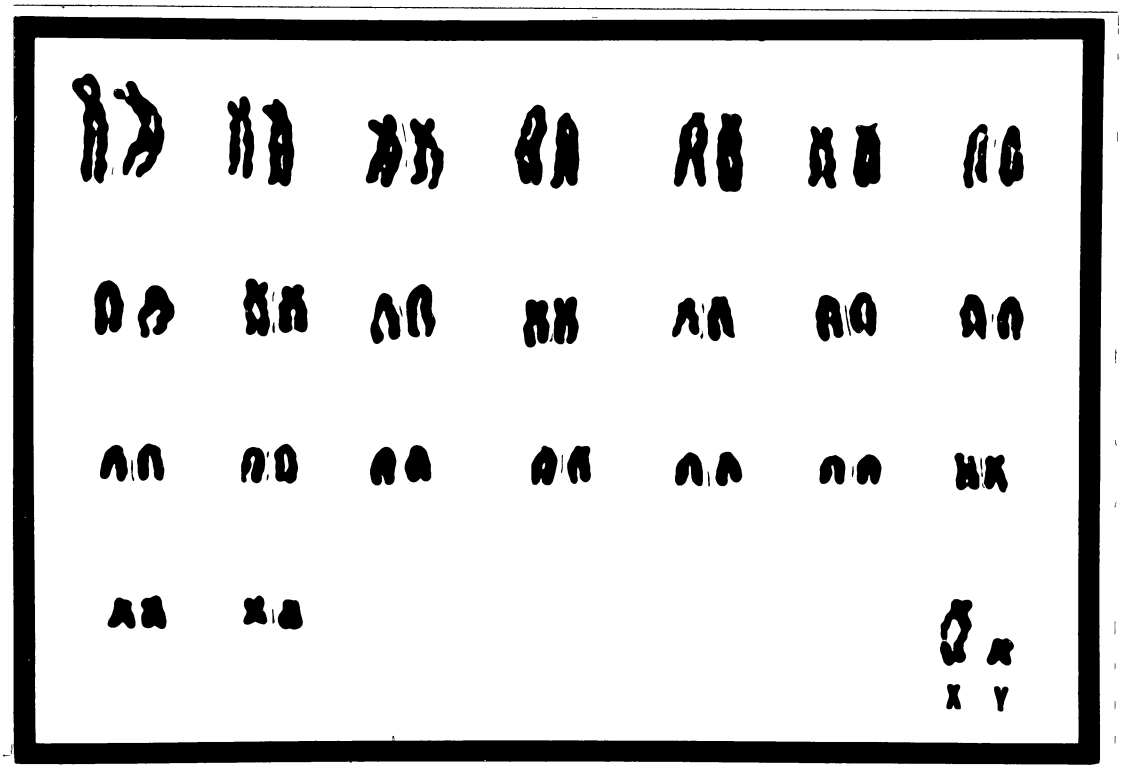


Figure 3. Standard karyotype of Peromyscus leucopus OK00103

Y. Autosomes include 12 submeta- or subtelocentric and 13 acrocentric chromosomes (Robbins and Baker, 1981).

Sigmodon hispidus

The hispid cotton rat (S. hispidus) is a coarse-haired, medium-sized rat with an average total length of 257 mm. This species inhabits dense grassy areas in which surface runways are made, but may also be found in habitats with adequate overhanging cover such as brush tangles and dense forbs (Schmidly, 1983). They eat mainly green vegetation, with monocots composing a major part of their diet (Inglis, 1955). Nests are lined with woven grass and are either located in a burrow or above ground in a shallow depression (Schmidly, 1983). They breed throughout the year, but exhibit seasonal peaks in reproduction. Two to 7 young are born after a gestation of 27 d. Newborns are sparsely haired, very active and open their eyes within 36 h after birth. They become sexually mature at 2 months, but do not reach maximum body size until they are 6 months old (Cameron, 1977; Waggoner, 1975). A study of S. hispidus in Oklahoma (Goertz, 1964) indicated that in periods of high population density, males had a home range from 0.31 to 0.72 acres (0.12-0.29 ha) and females ranged 0.30 to 1.20 acres (0.12-0.48 ha). When population densities were low home range varied from 0.55 to 9.90 acres (0.22-4.01 ha) for males and 0.80 to 3.30 acres (0.32-1.34 ha) for females

(Goertz, 1964). A review of different studies in different parts of the range has indicated that no general trend in home range size exists for this species (Layne, 1974).

The species has been used as a genetic biomonitor in two different studies (McBee et al., 1987; Thompson et al., 1988). Both studies supported their use for this purpose. However, it was noted in McBee et al. (1987) that they possessed a significantly lower baseline frequency of chromosomal lesions than Peromyscus leucopus.

S. hispidus has a diploid number of 52 and a fundamental number of 52 (Fig. 4). Their karyotype consists of a large telocentric X chromosome, a metacentric Y chromosome, 1 pair of biarmed and 24 pairs of acrocentric autosomes (Elder, 1980).

Reithrodontomys fulvescens

The fulvous harvest mouse (R. fulvescens) has an average total length of 156 mm and is the smallest of the three species examined in this study. Individuals typically inhabit fence rows and grassy areas with scattered brush. They will feed on insects, seeds and herbaceous vegetation. Nests may be on the ground or 0.3-1.0 meters above ground in bushes or trees (Schmidly, 1983). They have a diestrous cycle in which two to four young are born after a gestation of about twenty-one days. Young begin to grow hair within three to four days and open their eyes nine to twelve days

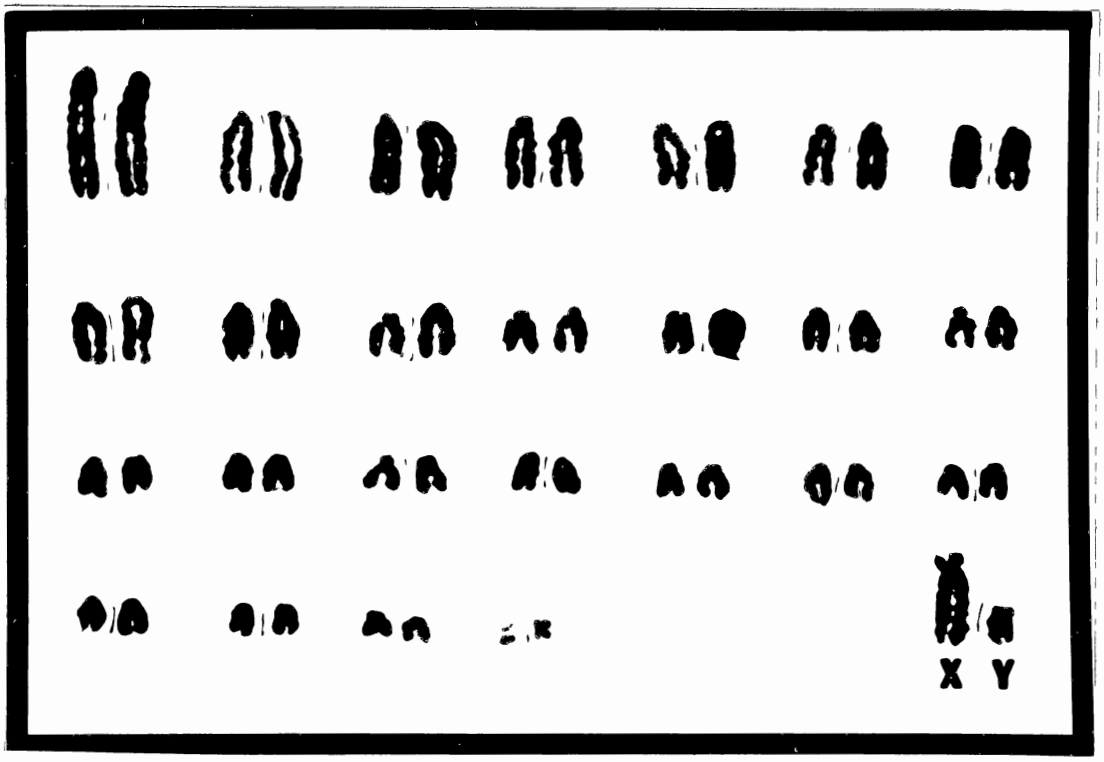


Figure 4. Standard karyotype of Sigmodon hispidus OK00053

after birth (Cameron, 1977; Packard, 1968). Home range was observed to be slighly larger for females (0.24 ha) than males (0.19 ha) (Packard, 1968). This species has not been previously used as an *in situ* genetic biomonitor.

The standard karyotype of the fulvous harvest mouse (*R. fulvescens*) has a diploid number of 50 and fundamental number of 48-49 (Fig. 5). Their sex chromosomes are a large submetacentric X and a small acrocentric Y. All 24 pairs of autosomes are acrocentric (Carleton and Myers, 1979).

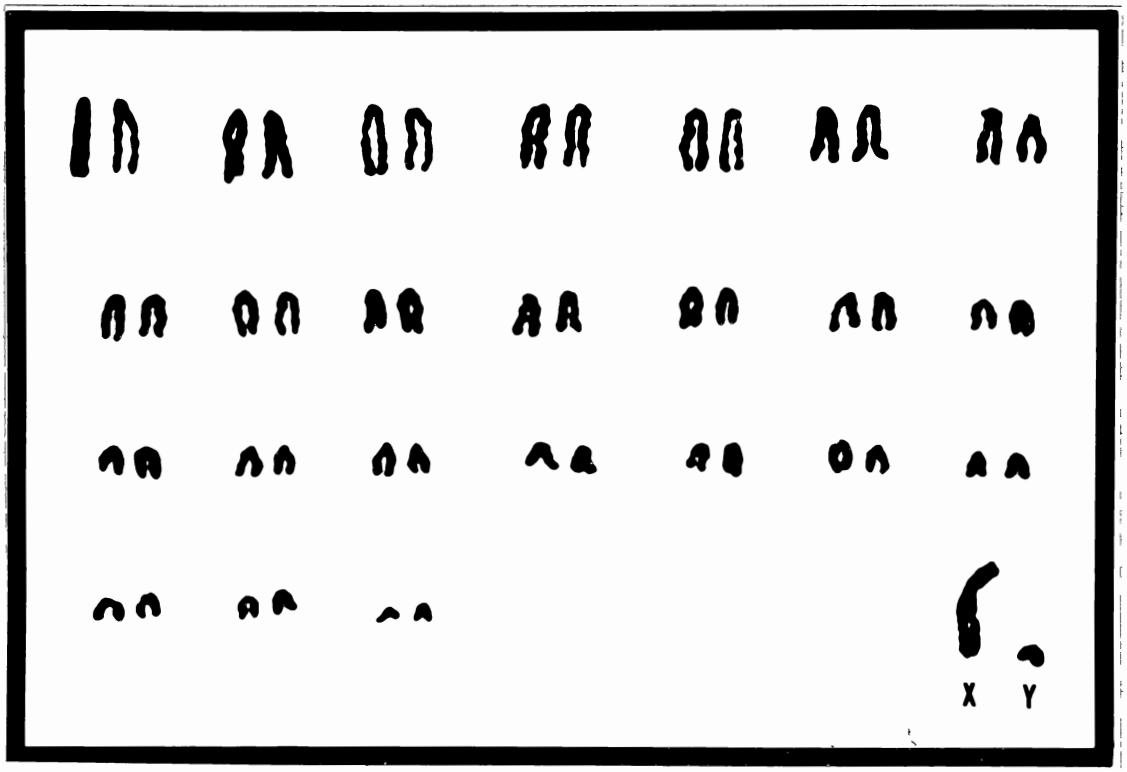


Figure 5. Standard karyotype of Reithrodontomys fulvescens OK00109

CHAPTER V

METHODS

Field Methods

Live capture of specimens was achieved using standard aluminum Sherman live traps baited with scratch grain and rolled oats. Juveniles and nontarget species captured were released. Trap grids had originally been established on both the contaminated site and a reference site 300 m north of it. The reference site was relocated 200 m west in May of 1989 due to poor trapping success. Traps were arranged 5 meters apart in 3 rows of 22 traps to match the size and dimensions of the contaminated site.

The Pryor sites were trapped for three periods during this study. There were three trap nights in August of 1988, four in January of 1989 and two in May of 1989. A fourth trapping period scheduled for early November of 1989 was cancelled because on-site incineration to destroy the soil's PCB burden had been initiated. The process of incineration not only eliminated the contaminant from the site but all vegetation and vertebrate species as well.

Poor trap success at the Pryor reference sites had been anticipated; therefore, two additional sites were

established in Payne county. One reference site was located near Lake Carl Blackwell and the other was at the Oklahoma State University Wildlife Research Annex. The first trapping on the Lake Carl Blackwell reference site replicated the grids used in Mayes County. Thereafter, lines of 100 traps were used on both Payne county sites. Traps at these sites were selectively placed in suitable habitat for the target species. Lake Carl Blackwell was trapped for a total of four nights and the Annex was trapped for 9 nights.

Standard Bone Marrow Metaphase Chromosome Preparation

Most captured animals were returned to Oklahoma State University's CYTOSU laboratory within 48 h of capture and processed for standard bone marrow metaphase preparations. Several were maintained in captivity beyond 48 h until it was possible to process them at CYTOSU and two R. fulvescens were processed in the field. No difference in the quality of preparations was evident with respect to these different processing schedules. Because mitotic index (number of cells undergoing division or MI) of animals retained in the lab may decrease, those not processed within 48 h were injected with a solution of 1:1:7 bakers yeast, sugar and warm water 24 h prior to sacrifice. Lee and Elder (1980) determined that this increased the MI without increasing

chromosome breakage or variations in chromosome number.

The protocol used was a modification of methods described by Baker et al. (1982) and Patton (1967). Marrow was flushed from each specimen's femurs and tibias with warm (37^{o}C) 0.075 M KCl. Tissue was aspirated to produce a single cell suspension and incubated for 27-30 minutes at 37^{o}C. After incubation, the suspension was centrifuged at 600 rpm for 90 seconds to pellet the marrow cells. The resulting supernatant was gently decanted leaving about 0.5 ml of solution above the pellet. Modified Carnoy's fixative (3:1 methanol/glacial acetic acid) was added to the tube and the pellet was gently resuspended and again centrifuged for 90 seconds at 600 rpm. After the initial wash in fixative, all of the supernatant was removed and replaced with fresh Carnoy's. The pellet was again resuspended and centrifuged. This last step was repeated twice to insure saturation of cells with fixative and eliminate cytosol components from the final slides. Before preparing slides, all supernatant was removed, replaced with a smaller volume of fixative and the pellet was resuspended. A few drops of this suspension were then dropped onto clean, dry, labeled slides and ignited with a flame to enhance spreading of chromatids. Slides were allowed to air dry before staining in a 2% Giemsa-phosphate buffer for 5 minutes. Stained slides were thoroughly rinsed with distilled water and air dried.

Prepared slides for each animal were number coded and

examined in random order to insure that the origin of the specimen was unknown while being scanned (Brusick, 1980). One hundred metaphase spreads were scored for each animal and any aberrations found were identified and indicated on that specimen's score sheet. A copy of the score sheets used is provided in Appendix A. The mean number of aberrant cells per individual and lesions per cell were calculated for use in statistical analysis. Aberrations of interest included chromatid breaks (cB), chromosome breaks (CB), dicentric (D) and ring (R) chromosomes, acentric fragments (ACF) and translocation figures (TR). Only metaphase spreads with a complete complement (2n) or only differing by one member (2n +/- 1) of it's diploid number were scored. Each aberrant cell and standard spreads for each specimen are documented in photomicrographs located at the Oklahoma State University CYTOSU laboratory. Specimens examined are listed in Appendix B. The voucher specimens are housed in the zoology research collection, Department of Zoology, Oklahoma State University.

Statistical Analysis

Previous in situ cytogenetic studies used Students t-tests to compare waste site and reference site populations (McBee, 1985; McBee et al., 1987; Tice et al., 1987; Thompson et al., 1988). TOXSTAT, a statistical program designed for the analysis of assays for NPDES permitting,

was selected to analyze this data set (Gulley, et al., 1989). No statistical transformations were made due to the presence of zero in the data set. The chi-square test of normality and Bartlett's test for homogeneity of variance were used to determine if it was necessary to analyze the data with nonparametric statistics. Chi-square test of normality was chosen over Shapiro-Wilk's because of greater familiarity with the former test.

If the assumptions of normality and homogeneity of variance were confirmed, Bonferroni's t-test was selected to analyze the data. Alternative nonparametric tests used were Wilcoxon's rank sum test with Bonferroni adjustment and Kruskal-Wallis test. Wilcoxon's rank sum test was the preferred method of analysis, Kruskal-Wallis test was only used when TOXSTAT's Wilcoxon procedure would not proceed due to the absence of tabled values for the degrees of freedom required. The flow chart used to determine which statistical test was most appropriate is provided in appendix C.

Comparisons were made between conspecifics from the PCB waste site and the Mayes and Payne county reference sites, between the Brazos county waste site, the PCB site and all references and between all reference groups.

CHAPTER VI

RESULTS AND DISCUSSION

Summary statistics for Peromyscus leucopus, Sigmodon hispidus and Reithrodontomys fulvescens are provided in Table I. The S. hispidus data sets for both proportion of lesions and number of aberrant cells per individual failed chi-square tests of normality, making it necessary to analyze the data sets with Wilcoxon's rank sum test. This analysis indicated no significant increase in levels of genetic damage in PCB site animals when compared to reference animals with respect to the proportion of lesions (rank sum = 34.00, alpha = 0.05) or number of aberrant cells per individual (rank sum = 34, alpha = 0.05).

Because TOXSTAT used average sample size to compute Bartlett's X^2 values, the statistic was hand calculated for the P. leucopus and R. fulvescens data sets with correction factors to compensate for unequal sample sizes. The P. leucopus data set failed Bartlett's test of homogeneity of variance with respect to the proportion of lesions per individual, but passed when the number of aberrant cells per individual was tested. Kruskal-Wallis analysis of the lesion data set indicated no significant increase in PCB site animals when compared to reference conspecifics (rank

sum = 91.50, P = 0.05). Bartlett's t-tests also did not indicate significantly higher numbers of aberrant cells per individual in PCB site animals (t = -0.017, P = 0.05).

The R. fulvescens data sets passed the tests of homogeneity of variance. Bartlett's t-tests did not indicate a significantly higher amount of chromosome damage in PCB site animals for the proportion of lesions (t = -0.333, P = 0.05) or the number of aberrant cells per individual (t = -0.351, P = 0.05).

Although the expected results were obtained for all three species, this does not imply that they are all equally sensitive genetic biomonitors. The average baseline lesion and aberrant cell frequency for reference P. leucopus in this study was greater than that for S. hispidus and R. fulvescens. The lowest aberration frequency was observed in S. hispidus with means of 0.333 aberrant cells per individual and 0.003 lesions per 100 cells scored. Previous evaluation of members of the species P. leucopus for use as genetic biomonitors noted the importance of low baseline aberration frequency for detecting low level genetic effects (Boucher, 1985). Differences between species in baseline aberration frequency is not necessarily due to differential stability of chromosomes. Efficiency of repair mechanisms and xenobiotic metabolism along with ecological condition of a species may affect intensity of exposure to genotoxins and lesion duration (McBee, 1985; Woodruff and Thompson, 1982;

TABLE I

SAMPLE SIZES, MEANS AND VARIANCES FOR THE NUMBER OF ABERRANT CELLS AND LESIONS PER 100 CELLS SCORED FROM ANIMALS CAUGHT AT PRYOR PCB SITE AND THREE REFERENCE SITES

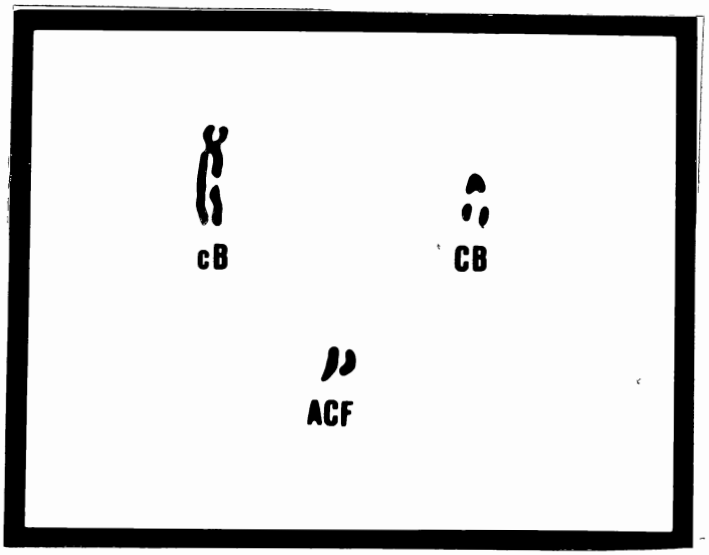
	P. leucopus			*S. hispidus*			*R. fulvescens*	
	Aberrant Cells Per 100 Scored							
	N	Mean	Var	N	Mean	Var	N	Mean	Var
Annex	22	1.727	3.732	6	0.333	0.267	3	0.667	0.333
Lake Carl	2	0.500	0.500	0	---	---	7	0.857	1.143
Pryor Ref	0	---	---	3	0.333	0.333	0	---	---
Pryor PCB	6	1.833	4.967	5	0.600	0.300	9	0.889	0.861
	Lesions Per 100 Cells Scored							
	N	Mean	Var	N	Mean	Var	N	Mean	Var
Annex	22	0.018	<0.001	6	0.003	<0.001	3	0.007	<0.001
Lake Carl	2	0.005	<0.001	0	-----	-----	7	0.010	<0.001
Pryor Ref	0	-----	-----	3	0.003	<0.001	0	-----	-----
Pryor PCB	6	0.018	0.001	5	0.006	<0.001	9	0.009	<0.001

Hsu, 1983).

Chromatid breaks, chromosome breaks and acentric fragments were the most common aberrations observed (Fig. 6). The relative frequencies of each type are provided in Table II, this information is also illustrated for pooled reference animals in Figure 7. The majority of aberrant cells posessed only a single lesion. Chromatid breaks were observed more frequently than chromosome breaks and acentric fragments. A single triradial was observed in a reference site P. leucopus, but no ring or dicentric chromosomes were observed in this study.

In other studies involving known clastogens (Al-Sabti, 1985; McBee, 1985; McBee et al., 1987; Thompson et al., 1988), a greater variety of structural aberrations was observed. However, in these studies, chromatid breaks were the most common aberrations in control and exposed organisms.

Table III provides the sample sizes, means and variances of the Brazos County data. The P. leucopus data sets passed tests of normality and homogeneity of variance. Bartlett's t-tests did not indicate a significant increase in the proportion of lesions ($t = -7.675$, $P = 0.05$) or number of aberrant cells per individual ($t = -10.127$, $P = 0.05$) in Fireman's Training School waste site animals when compared to PCB site and reference animals. The failure to detect a significant difference could be attributed to the



cB = chromatid break
CB = chromosome break and
ACF = acentric fragment

Figure 6. Examples of the three most commonly observed types of chromosome aberrations

TABLE II

ABERRATION TYPE AND PERCENT OCCURENCE
IN PRYOR PCB SITE AND
REFERENCE ANIMALS

Species	# Cells Observed	# Lesions	% cB	% CB	% ACF
P. leucopus					
Reference*	2400	49	67	6	25
Pryor PCB	600	12	83	8	8
S. hispidus					
Reference	900	2	100	---	---
Pryor PCB	500	3	100	---	---
R. fulvescens					
Reference	1000	9	89	10	---
Pryor PCB	900	8	75	12	12

*HIGH PERCENTAGE OF ACENTRIC FRAGMENTS DUE TO A SINGLE CELL POSSESSING 7 ACF's

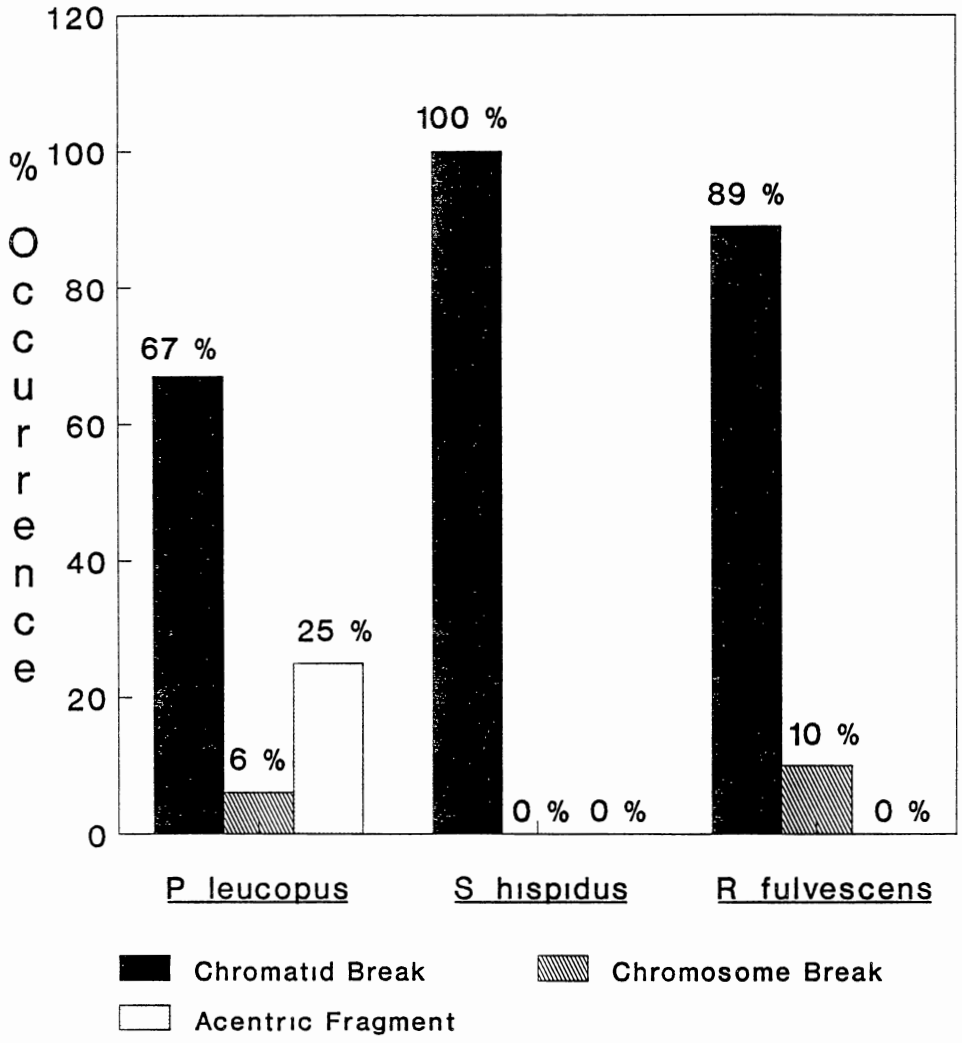


Figure 7. Distribution of aberration types observed in reference animals from this study

TABLE III

SAMPLE SIZES, MEANS AND VARIANCES FOR THE NUMBER OF ABERRANT CELLS AND LESIONS PER 50 CELLS FOR ANIMALS CAUGHT AT FIREMAN'S TRAINING SCHOOL (FS) AND 2 REFERENCE SITES (CS1 AND CS2)

	P. leucopus			*S. hispidus*		
	Aberrant Cells Per 50 Scored					
	N	Mean	Var	N	Mean	Var
CS1	12	1.42	1.90	11	1.091	1.091
CS2	14	1.79	1.10	0	----	----
FS	32	5.34	5.52	10	3.900	1.878
	Lesions Per 50 Cells Scored					
	N	Mean	Var	N	Mean	Var
CS1	12	0.038	0.002	11	0.025	0.001
CS2	14	0.041	0.001	0	----	----
FS	32	0.156	0.008	10	0.110	0.003

from McBee, 1985

high variances encountered in data sets from wild caught animals. No significant differences were found between reference P. leucopus populations for the proportion of lesions (t statistic range of t = 0.759 - -1.92, P = 0.05) or the number of aberrant cells per individual (t statistic range of t = 0.798 - -2.293, P = 0.05).

Comparison of the S. hispidus data sets from the two studies required Kruskal-Wallis analysis. A significantly higher frequency of genetic damage in the animals from the Fireman's Training School was indicated for both proportion of lesions (rank sum = 300.50, P = 0.05) and number of aberrant cells per individual (rank sum = 302.00, P = 0.05). Comparison of reference populations with Bartlett's t-tests indicated no significant differences with respect to the proportion of lesions (t statistic range t = <0.001 - -2.179, P = 0.05) or the number of aberrant cells per individual (t statistic range t = <0.001 - -2.103, P = 0.05).

PCB residues in the carcasses of three reference site and four contaminated site S. hispidus were analyzed by the U.S. Fish and Wildlife Service (1989). The results of this analysis are provided in Table IV. The analysis is broken into different components by degree of chlorination (6Cl = ug/g PCB congeners with 6 chlorines per molecule). Values for PCB site animals are clearly elevated above background levels in reference animals. Total PCB content was very

TABLE IV

CARCASS* PCB CONTENT (ug/g) OF SIGMODON HISPIDUS SPECIMENS FROM PRYOR PCB AND REFERENCE SITES

	2-4Cl	5Cl	6Cl	7CL	8CL	9CL	TOTAL
Pryor PCB							
OK00002	<0.05	0.10	0.23	0.06	<0.05	<0.05	<0.50
% total	0	----	----	----	0	0	
OK00003	<0.05	2.69	8.55	0.98	0.14	<0.05	12.36
% total	0	22	69	8	1	0	
OK00004	<0.05	1.41	2.55	0.38	0.15	<0.05	4.39
% total	0	32	58	8	3	0	
OK00082	<0.05	0.31	0.94	0.17	<0.05	<0.05	1.42
% total	0	22	66	12	0	0	
Pryor Ref							
OK00005	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.50
OK00006	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.50
OK00007	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.50

(U.S. Fish and Wildlife Service, 1989)

* LIVER, SPLEEN, FEMUR AND SMALL SAMPLES OF INTESTINE AND MUSCLE TISSUE WERE REMOVED FROM EACH SPECIMEN

NOTE: 2-4Cl = 2-4 chlorines per molecule, 5 = 5 chlorines per molecule etc.

high in female OK0003 (12.36 ug/g). This individual did not have any aberrant cells. Comparable values for other mammals in the literature include the livers of male river otters (Lutra canadensis) at a mean of 9.3 ug/g (Henny et al., 1981), carcasses of adult female little brown bats (Myotis lucifugus) at mean 11.4 ug/g (Clark and Krynitsky, 1976) and blubber, sperm oil and kidneys of male sperm whale (Physeter macrocephalus) 9.9, 10.5 and 9.4 ug/g respectively and the blubber and kidneys of females, 15.5 and 9.2 ug/g (Aguilar, 1983).

It is important to note that the 4-chlorobiphenyls (4Cl) concentrations were below the detection limit of 0.05 ug/g. This PCB is the one most often associated with mutagenicity, (Sargent, et al., 1989; USEPA, 1980; Wyndam et al., 1976). Lesser chlorinated biphenyls are less persistent and are metabolized or degraded more easily than PCB molecules with 5 or more chlorine atoms (USEPA, 1980). It is possible that the disparity in studies examining the mutagenicity of Aroclor 1254 is due to contamination with other chemicals, changes in constituent components or even metabolic efficiency of exposed organisms.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Summary

The purpose of this study was to provide information relevant to the development of mammalian in situ genetic biomonitors. The results strengthen the validity of this approach by demonstrating that three wild rodent species chronically exposed to Aroclor 1254 did not exhibit significantly elevated chromosome aberration frequencies over controls. This is in agreement with previous studies investigating the mutagenicity of Aroclor 1254 (Green et al., 1975a, 1975b; Wyndam et al., 1976; Schoeny et al., 1979; Schoeny, 1982).

Conclusions

By agreeing with established laboratory assays this study confirms that in situ genetic evaluations of waste sites are comparable and their application to waste site assessment is a valid approach.

The presence of low levels of known clastogens on the PCB site could have been of biological significance even though the levels were not high enough to be of

regulatory concern. Had significantly higher levels of chromosomal aberrations been observed in waste site animals, they could have been attributed to these contaminants, an interaction between the PCBs at the site and these contaminants, or to environmental interaction with the PCBs.

The use of in situ genetic biomonitoring is a promising approach to waste site evaluation. This method does not simply establish the presence of and exposure to waste site contaminants, it demonstrates an actual impact on an organism's health. The occurrence of potentially cytolethal lesions in the chromosomes of bone marrow cells can be detrimental if they occur in high frequencies. This will, in turn, affect the survivorship of an individual and thus the population.

However, biomonitoring need not be limited to genetic endpoints. Other endpoints used should be of biological significance and not merely indicate exposure. The consequences of alterations in selected endpoints should be well understood so that the ultimate effect on environmental quality may be extrapolated with greater confidence.

Lesions occurring in bone marrow suggests that lesions may also occur in other parts of the body, including germ cells. The environmental significance of germ cell damage is greater than that of somatic cell damage due to the impairment of the ability of an individual to produce viable offspring thereby impacting the population structure.

Establishing the presence of harmful contaminants on a site does not necessarily mean that resident organisms are exposed to and/or affected by it's presence. The demonstration of cytotoxicity in resident species not only establishes bioavailability of contaminants, it indicates a decrease in environmental quality through detrimental effects on the health of components of that environment.

In the development of new techniques it is important to recognize which elements of a study design require highest priority. In this endeavor, site access and the lack of a suitable proximal reference site were topics of concern. Ideally 10 individuals of each species would have been examined from each site. Unfortunately access to the site was limited and trapping sessions could not be planned as often as desired. Even with numerous trapping sessions, capture of desired species in sufficient numbers is not guaranteed. The ability to apply an assay to several species will permit investigators to take full advantage of which species are present in sufficient numbers.

Had significant differences been observed for the parameters examined, they could have been attributed to the distance between the waste site and reference sites rather than to waste site contaminants. The lack of significant differences imply that, while distant references are not preferred, they are an alternative. This alternative should not be used when a suitable reference site is available near

the waste site being examined. A proximal reference site would have permitted concurrent sampling. Concurrent sampling allows for more efficient use of time and strengthens results by eliminating concerns about geological and climatic differences as well as temporal effects.

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APPENDIXES

APPENDIX A

SCORE SHEETS USED TO RECORD OBSERVED CELLS

Scorer _____

OK _____ Species _____ Sex · _____
Locality/Treatment _____

No/sl	Coordinates	2N	cB	CB	R	D	TRF	ACF	Remarks
1									
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50									

OK _____ PAGE 2

No/sl	Coordinates	2N	cB	CB	R	D	TR	ACF	Remarks
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	TOTAL								

TOTAL ABERRANT CELLS _____

APPENDIX B

SPECIMENS EXAMINED IN THIS STUDY

PRYOR PCB SITE, MAYES COUNTY, OKLAHOMA

OK #	Species	Gender (M or F)
OK00084	Peromyscus leucopus	M
OK00085	"	F
OK00086	"	M
OK00087	"	M
OK00088	"	F
OK00142	"	M
OK00002	Sigmodon hispidus	M
OK00003	"	F
OK00004	"	F
OK00082	"	M
OK00083	"	F
OK00089	Reithrodontomys fulvescens	M
OK00090	"	F
OK00091	"	F
OK00092	"	F
OK00095	"	F
OK00097	"	M
OK00099	"	F
OK00100	"	M
OK00141	"	M

PRYOR REFERENCE SITE, MAYES COUNTY, OKLAHOMA

OK #	Species	Gender (M or F)
OK00005	Sigmodon hispidus	M
0K00006	"	F
OK00007	"	F

OSU WILDLIFE RESEARCH ANNEX, PAYNE COUNTY, OKLAHOMA

OK #	Species	Gender (M or F)
OK00008	Peromyscus leucopus	F
OK00009	"	F
OK00010	"	M
OK00011	"	M
OK00012	"	M
OK00013	"	F
OK00014	"	F
OK00015	"	M
OK00017	"	F
OK00018	"	F
OK00020	"	F
OK00021	"	F
OK00022	"	F
OK00023	"	F
OK00028	"	F
OK00029	"	F
OK00031	"	M
OK00032	"	F
OK00045	"	F

OSU WILDLIFE RESEARCH ANNEX, PAYNE COUNTY, OKLAHOMA

OK #	Species	Gender (M or F)
OK00047	Peromyscus leucopus	M
OK00052	"	M
OK00108	"	F
OK00046	Sigmodon hispidus	F
OK00048	"	F
OK00053	"	M
OK00054	"	F
OK00114	"	F
OK00121	"	M
OK00117	Reithrodontomys fulvescens	M
OK00219	"	M
OK00220	"	F

LAKE CARL BLACKWELL, PAYNE COUNTY, OKLAHOMA

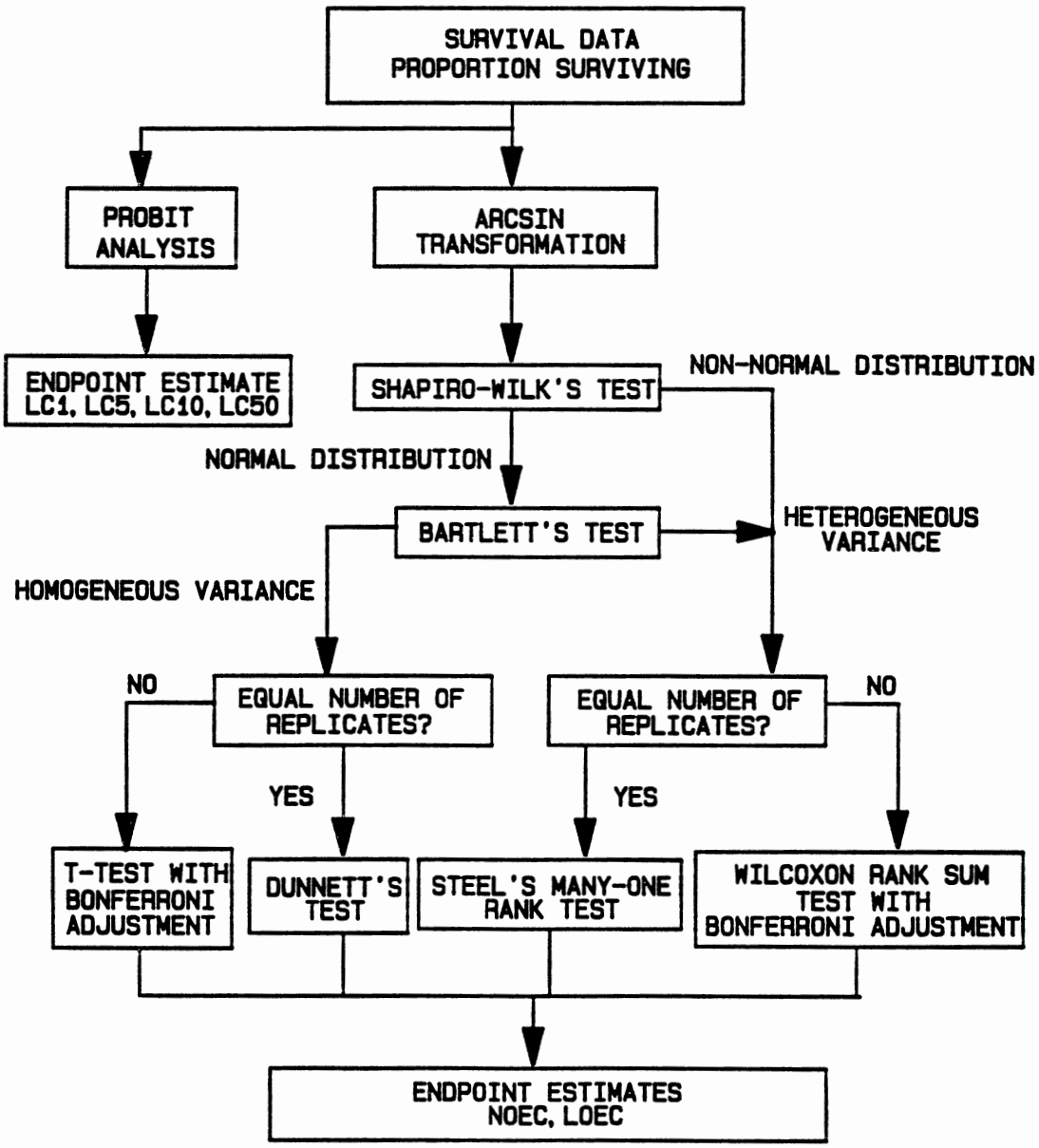
OK #	Species	Gender (M or F)
OK00102	Peromyscus leucopus	F
OK00103	"	M
OK00104	Reithrodontomys fulvescens	F
OK00105	"	M
OK00109	"	M
OK00110	"	M
OK00112	"	F
OK00115	"	F
OK00116	"	M

APPENDIX C

FLOW CHART USED TO ASSIST IN SELECTION OF STATISTICAL TESTS

STATISTICAL ANALYSIS OF FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

SURVIVAL



(USEPA, 1989)

VITA

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