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ANNUAL TECHNICAL REPORT
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Submitted by

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1. Summary

All field work at a site contaminated with polychlorinated biphenyls (PCBs) in Pryor, Oklahoma has been completed. Standard chromosome aberration assays on Peromyscus leucopus (white-footed mouse), Sigmodon hispidus (cotton rat), and Reithrodontomys fulvescens (fulvous harvest mouse) from the Pryor site and from three matched reference sites has been completed. All samples of spleen tissues for flow cytometric analyses (FCM) have been prepared. Trial runs for FCM analysis have been carried out and all FCM analyses should be completed by the end of the year. PCB tissue content analysis has been completed on seven animals randomly chosen from the Pryor sites and shows significant increases over background levels. Field analyses at a second site in Payne County, Oklahoma contaminated with a mixture of radioactive and chemical wastes has been underway for one year. Data collection has involved monthly sampling for several demographic variables and tri-monthly sampling for cytogenetic and tissue residue variables. Field work for this year has been completed and slides are currently being analyzed. Laboratory validation experiments involving exposure to known levels of known clastogens have been initiated. Both the white-footed mouse and the cotton rat have been examined for genetic effects at no exposure and at four increasing levels of exposure to the known clastogen TEM (triethylenemelamine) for either a subchronic period of five days or an acute exposure period of 24 hours. Chromosome aberration data are currently being analyzed and tissues for FCM analysis have been preserved.

2. Research Objectives

One of the most hazardous aspects of the alteration of the environment by human activity is the pollution of air, soil, and water with clastogenic chemicals (chemicals which alter chromosome structure). There are numerous techniques for detection of clastogenic activity in suspect compounds but these techniques generally are restricted to controlled laboratory situations which may not realistically indicate the effects of the complex interactions among pollutants and the biological, geological, and physical components of the environment. The research proposed in this project aims at the continued development and validation of in situ monitors for the evaluation of cytogenetic interactions among environmental pollutants and exposed organisms.

The project centers on the identification of cytogenetic effects of environmental mutagens on populations of two common, wild rodent species, Peromyscus leucopus (white-footed mouse) and Sigmodon hispidus (cotton rat) inhabiting two chemically contaminated sites and matched, pristine, control sites. Two genetic assays (Standard metaphase chromosome analysis {CA} and flow cytometric analysis of DNA content variation {FCM}) will be used. The battery will allow determination of the relative usefulness of each method. Each assay system also could provide types of information not readily available from the others. In addition, tissue samples from these mice will be analyzed for content of specific chemical pollutants.

Specific objectives for the continued development of in situ biomonitors are: 1) Identification of cytogenetic effects of environmental clastogens in P. leucopus and S. hispidus using two independent assays; 2) Comparison of the usefulness of each of the assays; 3) Comparison of genetic responses in animals inhabiting environments contaminated with two different complex mixtures of pollutant chemicals; 4) Documentation of the presence of clastogenic environmental toxins in tissues of animals used in the cytogenetic assays.

A second part of this project will aim to document that these species provide similar results in classical laboratory clastogen assays and therefore are valid genotoxin monitors. Animals will be dosed with two powerful toxins and appropriate negative controls. Specific objectives for validation include: 1) Identification of cytogenetic effects on laboratory reared P. leucopus and S. hispidus exposed to PCB (Aroclor 1254) and triethylenemelamine (TEM); 2) Comparison of effects of exposure to a powerful clastogen (TEM) and a powerful toxin not shown to be a clastogen (Aroclor 1254); 3) Comparison of responses in P. leucopus and S. hispidus used in laboratory assays to levels of response seen in environmentally exposed animals; 4) Comparison of responses in P. leucopus and S. hispidus to historical data bases available for commonly used laboratory models for cytogenetic assays (eg. Sprague-Dawley rats and several strains of Mus musculus).

3. Status of the Research Effort

3.1 Pryor PCB-Contaminated Site

Effects of hazardous wastes have become important topics of investigation due to increasing public concern about risks associated with such materials. A number of techniques have been developed in response to this concern. Several of these methods involve in situ analyses to determine the effects of hazardous compounds on exposed organisms. Most of these in situ methods are still in the essential process of testing and validation, however. The purpose of this portion of the project was 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 detected in conspecifics from uncontaminated reference sites.

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 (1). In situ biomonitoring can serve as a sentinel of environmental quality indicating if the nature of toxicity of resident wastes has changed, or if leakage or unreported disposal of additional waste has occurred (2, 3). Interactions may occur between different compounds in a waste site, and, although harmless by themselves, certain chemicals can increase the toxicity of others (2).

Aroclor 1254 has not been demonstrated to be mutagenic when tested alone in established laboratory assays such as standard bone marrow chromosome aberration assays, micronucleus assays, sperm head abnormality assays, and Salmonella/mammalian microsome assays (4, 5, 6, 7, 8, 9). However, conflicting data exist concerning the mutagenicity of PCBs. Aroclor 1254 has been demonstrated to be mutagenic in kidney cells of three cyprinid fish species (10). Significant genetic effects have been observed in S. typhimurium exposed to the single congener, 4-chlorobiphenyl but the mixture Aroclor 1254 was essentially inactive as a mutagen in the same test system (11). The possibility of synergistic genotoxic interaction among individual PCB congeners has also been suggested (12).

Previous work has indicated that wild rodents can be effectively used as in situ genetic biomonitors. McBee et al. (13) and McBee and Bickham (14) found significantly higher levels of genetic damage as indicated by increased frequencies of chromosomal lesions and increased variation in nuclear DNA content in individuals of P. leucopus and S. hispidus trapped at a hazardous waste site when compared to conspecifics from pristine sites. Tice et al. (15) observed increased frequencies of chromosomal aberrations, sister chromatid exchanges, micronuclei, sperm head abnormalities, and reduction of mitotic index as evidence of genetic damage in P. leucopus from a hazardous waste site in New Jersey. Increased frequencies of chromosome aberrations and

aneuploidy were also observed in bone marrow cells of S. hispidus from two superfund sites in Texas compared to conspecifics from a pristine site (16).

If laboratory genetic toxicity assays accurately reflect the potential for genetic hazard from exposure to PCBs the rodents examined in this study should have chromosomal aberration frequencies not significantly different from animals collected at reference sites. If, however, rodents from this contaminated site show significantly elevated levels of chromosomal damage, alternative explanations must be sought.

MATERIALS AND METHODS

Species Descriptions

Three rodent species, Peromyscus leucopus (white-footed mouse), Sigmodon hispidus (hispid cotton rat), and Reithrodontomys fulvescens (fulvous harvest mouse) were examined. These species were chosen for analysis based on their abundance on the waste site. The use of these three species permits observation of effects in different niches because each differs in soil associations, feeding habits, and home range sizes. Other rodent species were present, including members of the genera Mus and Microtus, but were not used in the analysis due to infrequent capture. The genus Peromyscus is widely distributed throughout North America. Of the more than 40 species in the genus, information about P. leucopus and it's close relative, P. maniculatus (deer mouse) accounts for the majority of what is known about the genus (17). Peromyscus leucopus has been the subject of extensive cytogenetic research (18, 19). The species has a diploid number (2n) of 48 and a fundamental number (FN) of 70. Sex chromosomes are a large submetacentric X and a small metacentric Y. Autosomes include 12 submeta- or subtelocentric and 13 acrocentric chromosomes (20). Members of this species may have great potential as genetic biomonitors because the species is abundant, generally limits activity to small areas and has a relatively low baseline frequency of genetic events which should allow for the detection of small increases in genetic damage (21).

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. It may also be found in habitats with adequate overhanging cover such as brush tangles and dense forbs (22). Sigmodon hispidus has 2n = 52 and FN = 52. The karyotypes consists of a large telocentric X chromosome, a metacentric Y chromosome, 1 pair of biarmed autosomes and 24 pairs of acrocentric autosomes (23).

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. The karyotype for R. fulvescens has 2n = 50 and FN = 48-49.

Sex chromosomes are a large submetacentric X and a small acrocentric Y. All 24 pairs of autosomes are acrocentric (24).

Site Descriptions

Pryor PCB-The site contaminated with PCBs was located near the city of Pryor, Mayes County, Oklahoma on federal property managed by the U.S. Army Corps of Engineers. The area contaminated with Aroclor 1254 covered approximately 1.5 ha. Oak and native grasses were the most common plant species. An intermittent stream flowed through the area into Pryor Creek. The site was fenced to prevent cattle grazing on adjacent fields from gaining access. Therefore, the area was densely vegetated compared to surrounding land. PCB contamination was limited to the upper 9-12 inches of soil. Concentrations ranged from 0.3 to 8.63.o μg/g. 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 and Aroclor 1254 has been considered the only contaminant present in sufficient quantity to be of regulatory concern (25). .

Pryor Ref-The original reference site in Mayes County was located 300 m north of the contaminated site. Trap success was poor during the first sampling period so the reference site was relocated 200 m west of the original location for the second sampling period. Despite improved ground cover at the new location, trap success was not improved. Therefore, two additional reference sites were established in Payne County.

Lake Ref and Annex Ref-The two Payne County reference area were on property belonging to Oklahoma State University. Both are formerly cultivated land undergoing early succession. Annex Ref was located on the outskirts of Stillwater, Oklahoma and had native grasses, sumac, and oaks as the most common vegetation. Lake Ref was located 8.9 mi west of Stillwater near Lake Carl Blackwell and showed similar patterns of land use and vegetation.

Trapping Protocol

Specimens were live-captured using Sherman aluminum live traps baited with scratch grain and rolled oats. Juveniles and nontarget species were released. Trap grids were established in August 1988 on the Pryor PCB and Pryor Ref sites. Traps were arranged 5 m apart in three rows of 22 traps each. Dimensions were determined by the size of the area known to be contaminated by PCBs. Traps were opened and baited during the late afternoon and checked at sunrise during each of three trapping periods. The Pryor sites were trapped for three nights in August 1988, four in January 1989, and two in May 1989. The first trapping period at Lake Ref replicated the grid system used at the Pryor sites. Thereafter, lines of 100 traps were used at both Lake Ref and Annex Ref. Traps were selectively placed in optimal habitat for target species. Lake Ref was trapped for four nights and Annex Ref was trapped for nine nights.

Chromosome Preparation

Animals were returned to the laboratory within 48 h of capture and processed for standard bone marrow metaphase chromosome preparations. Because mitotic index (number of cells undergoing division) may decrease in animals retained in captivity, animals maintained in the laboratory for more than 48 h were injected with a solution of 1:7:7 bakers' yeast, sugar, and warm (37°) water 24 h prior to processing to stimulated bone marrow mitoses. Lee and Elder (26) found this process had no effect on chromosome breakage or variation in chromosome number.

Chromosome suspensions were prepared following a modification of the methods of Baker et al. (27) and Patton (28). Marrow was flushed from femurs and tibias with warm (37°) 0.075 M KCl. Tissue was aspirated to produce a single cell suspension and incubated for 27 to 30 min at 37°. After incubation, the suspension was centrifuged at 600 rpm for 90 sec. Resulting supernatant was gently decanted leaving about 0.5 ml of solution above the cell 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 sec at 600 rpm. After the initial wash in fixative, all of the supernatant was removed and replaced with fresh fixative. The pellet was again resuspended and centrifuged. This step was repeated twice to ensure saturation of cells with fixative and to eliminate cytosol components from the final slides. A few drops of the final suspension were dropped onto clean, dry, microscope slides and ignited to enhance spreading of chromatids. Slides were allowed to air dry before staining in a 2% Giemsa-phosphate buffer solution for 5 min. Stained slides were thoroughly rinsed with distilled water and air dried.

Scoring Protocol

Prepared slides for each animal were number coded and examined in random order to ensure that the origin of the specimen was unknown while being analyzed for damage. One hundred complete, metaphase chromosomal spreads were scored for each animal and all detected aberrations were logged on score sheets. Types of aberrations scored included chromatid breaks (cB), chromosome breaks (CB), dicentric (D) and ring (R) chromosomes, acentric fragments (ACF), and translocation figures (TR). Mean number of aberrant cells per individual and lesions per cell were calculated for use in statistical analyses.

Statistical Analyses

Previous in situ cytogenetic studies used Student's t-tests to compare waste site and reference site populations (13, 15, 16, 29). TOXSTAT, a statistical program designed for the analysis of assays for NPDES permitting, was used with this data set (30). No statistical transformations were made due to the presence of zeros 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 use nonparametric statistics. If the assumptions of normality and homogeneity of variance were confirmed, Bonferroni's t-test was selected. 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 with TOXSTAT's Wilcoxon procedure would not proceed due to the absence of tabled values for degrees of freedom required.

Voucher Specimens

Each aberrant cell and two standard normal cells for each specimen are documented in photomicrographs housed in the Department of Zoology, CYTOSU Laboratory. Voucher skeletal specimens are housed in the Department of Zoology Vertebrate Collections.

RESULTS AND DISCUSSION

Summary statistics for P. leucopus, S. hispidus, and R. fulvescens are provided in Table 1. The S. hispidus data sets for both lesions per cell and number of aberrant cells per individual failed chi-square tests of normality, making it necessary to use Wilcoxon's rank sum test. This analysis indicated no significant increase in levels of genetic damage in Pryor PCB site animals when compared to reference animals for either lesions per cell (rank sum = 34.00, α =0.005) or number of aberrant cells per individual (rank sum = 34, α = 0.05).

Because TOXSTAT used average sample size to compute Bartlett's X^2 values, the statistic was hand calculated for P. leucopus and R. fulvescens with correction factors to compensate for unequal sample sizes. The P. leucopus data set failed Bartlett's test of homogeneity of variance for lesions per cell but passed for number of aberrant cells per individual. Kruskal-Wallis analysis of data for lesions per cell indicated no significant increase in Pryor PCB 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 Pryor PCB site animals (t = -1.017, P = 0.05).

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

Although all three species showed similar results in this study, this does not imply that they are all equally sensitive genetic biomonitors. The average baseline lesions per cell and aberrant cells per individual for P. leucopus from reference sites was greater than that for either S. nispidus or 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 cell. Previous evaluation of P. leucopus for use as a genetic biomonitor noted the importance of low baseline aberration frequency for detecting low level genetic effects (23). 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 lesion duration and intensity of exposure to genotoxins (29, 31, 32).

Chromatid breaks, chromosome breaks, and acentric fragments were the most common aberrations observed (Fig. 2). Relative frequencies of each type of aberration are provided in Table 2. The majority of aberrant cells possessed only a single lesion. Chromatid breaks were observed more frequently than chromosome breaks or acentric fragments. A single triradial was observed in a reference site P. leucopus, but no ring or dicentric chromosomes were observed. In other studies involving known clastogens (12, 13, 16, 29) a greater variety of structural aberrations was observed; however, chromatid breaks were the most commonly observed aberrations in both reference and exposed animals.

PCB residues in the carcasses of three Pryor Ref and four Pryor PCB S. hispidus were analyzed by the U.S. Fish and Wildlife Service (33). These analyses are summarized in Table 3. The analysis is broken into different components by degree of chlorination (e.g. 6Cl = µg/g PCB congeners with 6 chlorines per molecule). Values for Pryor PCB site animals are elevated above levels of Pryor Ref animals. Total PCB content was exceptionally high in one female (OK 00003) measuring 12.36 µg/g, yet this animals had no observable aberrant cells. It is important to note that the 4-chlorobiphenyls (4Cl) concentrations were below the detection limit of 0.05 µg/g. This PCB congener is the one most often associated with mutagenicity, (13, 14, 33).

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 when compared to reference animals. This is in agreement with previous laboratory studies investigating the mutagenicity of Aroclor 1254 (6, 7, 10, 11, 13). 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 use of in situ genetic biomonitoring is a promising approach to waste site evaluation because it demonstrates an actual impact on an organisms's health. Establishing the presence of contaminants on a site does not necessarily mean that resident organisms are exposed to and/or affected by those contaminants. Demonstration of toxic effects 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.

3.2 Payne County Radioactive Waste Disposal Site

A second portion of the proposed project was to conduct the same type of cytogenetic study at a second site contaminated with a different complex mixture of environmental pollutants. Original plans were to sample an old military explosives and chemical disposal site in Mayes County, Oklahoma. When approval to collect on the site was delayed, an alternate study site was selected in Payne County, Oklahoma. This site has similar vegetation and soil patterns but is contaminated with a complex mixture of radioactive materials, solvents, pesticides, and waste ammunition. It is on property owned by Oklahoma State University and served as the disposal facility for hazardous wastes generated in research, maintenance, and physical plant activities at the University from the early 1950s through 1980. In 1980 the site was closed as a disposal facility and is currently under review for remediation. Because the site includes a number of compounds known to be clastogenic (e.g. radioactive materials, benzene, phenol) it should provide provide a valuable comparison point for validation of in situ genetic biomonitoring. All types of data collection described for the Pryor PCB site and its reference sites have also been conducted at the Payne Co. site and a matched reference site.

In addition, several demographic variables have also been monitored for this site in order to address possible correlations between observed genetic damage and long-term population effects. The mutagenic properties of radiation are well documented (34) and dramatic demographic consequences (e.g. reduced survivorship, altered population densities, altered age distributions, altered reproductive potential) have been demonstrated in wild rodent populations exposed to radioactivity (35). Mutagenic properties of a variety of chemical pollutants are also well documented in both laboratory assay systems and in wild rodents populations and alterations in normal demography patterns also have been demonstrated in rodent populations exposed to chemical waste (36). However, coordinated studies of both genetic and demographic effects in populations exposed to chemical and radioactive wastes have not been conducted.

Materials and Methods

Species Descriptions

Peromyscus leucopus, S. hispidus, and R. fulvescens as described above have been monitored at the Payne Co. site. A fourth species, Neotoma floridana (Florida woodrat) has also been added to the analysis. This species is the largest of the four weighing up to 350 g. They may live in burrows at the base of trees or build large surface level nests and inhabit brushy or woody areas (37). They have 2n = 52 and FN = 58. There are two large submetacentric, six small submetacentric, and 42 acrocentric autosomes. The X chromosome is a large submetacentric and the Y is a large metacentric (38).

Trapping Protocol, Chromosomal Preparations, and Statistical Analyses

Trap grids were established on the site and at a matched reference site at the Annex Ref site described above. Trap stations were marked by stakes placed at 10 m intervals to form 100 m by 100 m grids. Sherman aluminum live traps were run at dawn and dusk for five day intervals once a month from September 1989 through May 1990. All animals captured were identified to species and sex, sex and reproductive condition determined, weighed, and examined for evidence of external parasites and external terata. Animals were then assigned a unique identifying number, marked, and released. Every third month a random sample of captured adults were returned to the laboratory for assessment of genetic damage using standard metaphase chromosome analysis and flow cytometry. Tissues were also preserved for tissue residue analysis and DNA strand-break analysis. Chromosome preparation procedures and scoring protocols are as described earlier. Statistical analyses for chromosomal data will be as described above. Analyses of demographic variables will follow methods outlined by Kapustka et al. (39).

Current Status

To date, demographic data for 9000 trap nights have been collected and 74 individuals have been processed for genetic analyses. Species and numbers marked are summarized in Table 4. Data analyses are currently in progress. Although demographic analyses are not yet complete, skewed sex ratios have been observed at the contaminated site compared to the reference site. Since, genetic analyses are also not yet complete, number codes randomly assigned to animals to ensure their location of capture is unknown at the time of analysis have not been decoded. However, 14 animals have already been scored for chromosome aberrations. The percent aberrant cells ranges from one percent (well within previously determined background levels [13]) to 15 % (close to highest levels observed in previous studies [13]). These aberrations include chromatid breaks and acentric fragments as well as more complex lesions characteristic of misrepair such as triradials, dicentrics, and multiple translocations. Species and numbers of animals processes for genetic endpoints are summarized in Table 5.

3.3. Triethylene Melamine Dosing Experiments

A third portion of the proposed project involved documentation of response to known clastogens in standardized laboratory assays of mutagenicity for two of these wild species of rodents. Challenge experiments for intraperitoneal exposure to triethylene melamine (TEM) have been conducted for both P. leucopus and S. hispidus. TEM is a potent clastogen that has been used in numerous laboratory assays with rodents including CFN laboratory rats and CD1 laboratory mice (40, 41). Information on responses of P. leucopus and S. hispidus in similar studies should provide valuable data for calibration of

laboratory derived data on the severity of genetic effects against effects actually observed in the field.

MATERIALS AND METHODS

Dosing Protocol

Twenty individuals of lab reared, adult P. leucopus were randomly assigned to one of five different groups, two males and two females per group. Animals were dosed intraperitoneally on each of five days with either 0.00 mg/kg, 0.25 mg/kg, 0.50 mg/kg, 0.75 mg/kg, or 1.00 mg/kg body weight for a subchronic exposure period of 120 h. On days four and five of the exposure period animals were also given subcutaneous yeast injections as described earlier. Twenty individuals of S. hispidus were randomly assigned to similar dose groups as for P. leucopus but received only a single dose of TEM for an acute exposure period of 24 h. On the first day after the end of the exposure period for each group animals were processed for genetic endpoints as described above. Spleen tissues for flow cytometric analysis and skeletal muscle and brain tissues for DNA strand-break analysis were also preserved.

Current Status

Slides from both studies are currently being scanned. Mitotic indices have been determined for the P. leucopus study (Fig. 3) and show evidence of increasing cytotoxicity as dose level increases. Analysis of chromosome aberration data for S. hispidus is approximately half completed and should be finished during December 1990. Analysis of chromosome aberration data for P. leucopus should be completed by April 1991.

3.4. Current Status for Flow Cytometry Analyses

A Partec Pas II Flow cytometer has been purchased with Air Force matching funds and is now in place and functioning. Tissue preparation methods have been developed and training trials have been conducted (Fig. 4). Spleen tissue from 63 individuals from the Pryor PCB study has been prepared as single cell suspensions and fixed. DNA content analysis will be completed in December. Spleens from the 74 individuals from the Payne Co. radioactive site have also been prepared but analysis will not begin until the PCB site analyses are completed. Spleen tissues from the TEM challenge experiments will be analyzed after the radioactive site samples. All flow cytometric analyses should be completed by December 1991.

3.5. Current Status for DNA Strand-Break Analysis

Skeletal muscle and brain tissue from the 177 individuals analyzed in the three portions of this project have been stored in liquid nitrogen for extraction of DNA for the strand-break assay. All equipment for DNA extraction and analysis requested in the Request for No Cost Extension

except the rotor for the ultracentrifuge has been purchased and is in place and functioning. DNA extraction on these sample will begin in December 1990 but analyses will not be completed until 1992.

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Table 1. Sample sizes, means, and variances for aberrant cells per individual and lesions per cell for animals collected from Pryor PCB and three reference sites.

One hundred cells were scored for each individual.

ABERRANT CELLS PER INDIVIDUAL

	P. leucopus			S. hispidus			R. fulvescens		
Location	N	Mean	Var	N	Mean	Var	N	Mean	Var
Annex Ref	22	1.73	3.73	6	0.33	0.27	3	0.67	0.33
Lake Ref	2	0.50	0.50	0	--	--	7	0.86	1.14
Pryor Ref	0	--	--	3	0.33	0.33	0	--	--
Pryor PCB	6	1.83	4.97	5	0.60	0.30	9	0.89	0.86

LESIONS PER CELL

	P. leucopus			S. hispidus			R. fulvescens		
Location	N	Mean	Var	N	Mean	Var	N	Mean	Var
Annex Ref	22	0.018	<0.001	6	0.003	<0.001	3	0.007	<0.001
Lake Ref	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

Table 2. Aberration types and frequency of occurence in Pryor PCB and pooled reference site animals.

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

Table 3. Carcass* PCB content (µg/g ww) for selected Sigmodon hispidus from Pryor PCB and Pryor Ref sites (from U.S. Fish and Wildlife Service,33).

			Pryor PCB				
ID Number	2-4Cl	5Cl	6Cl	7Cl	8Cl	9Cl**	Total
OK00002	<0.05	0.10	0.23	0.06	<0.05	<0.05	0.39
%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	0.7	<0.05	<0.05	1.42
%Total	0	22	66	12	0	0	
			Pryor Ref				
ID Number	2-4Cl	5Cl	6Cl	7Cl	8Cl	9Cl**	Total
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

*Liver, spleen, femur, and partial intestine and skeletal muscle removed.

**Number of chlorine atoms per molecule.

Table 4. Species and numbers of rodents marked for demographic analyses at a radioactive waste disposal site and a matched reference site in Payne County, Oklahoma.

Species	Radioactive Site	Reference Site
Neotoma floridana	4 females, 7 males	none
Peromyscus leucopus	11 females, 17 males	5 females, 16 males
Reithrodontomys fulvescens	10 females, 15 males	11 females, 13 males
Sigmodon hispidus	14 females, 27 males	23 females, 29 males

Table 5. Species and numbers of rodents processed for genetic endpoints from a radioactive waste disposal site and a matched reference site in Payne County, Oklahoma.

Species	Number
Neotoma floridana	none
Peromyscus leucopus	9 males, 10 females
Reithrodontomys fulvescens	12 males, 11 females
Sigmodon hispidus	23 males, 9 females

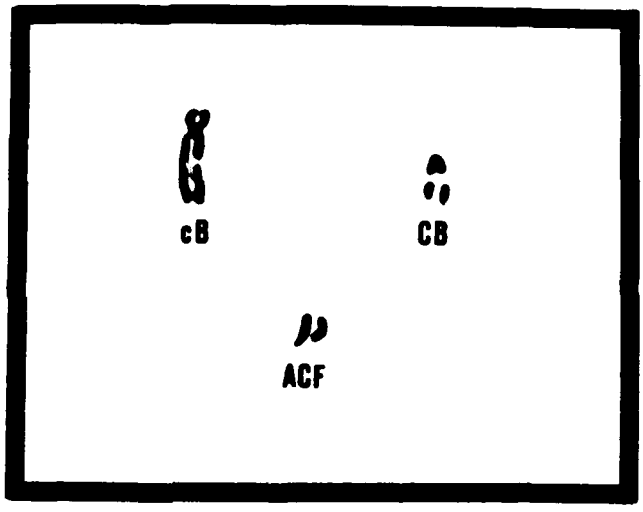
FIGURE LEGENDS

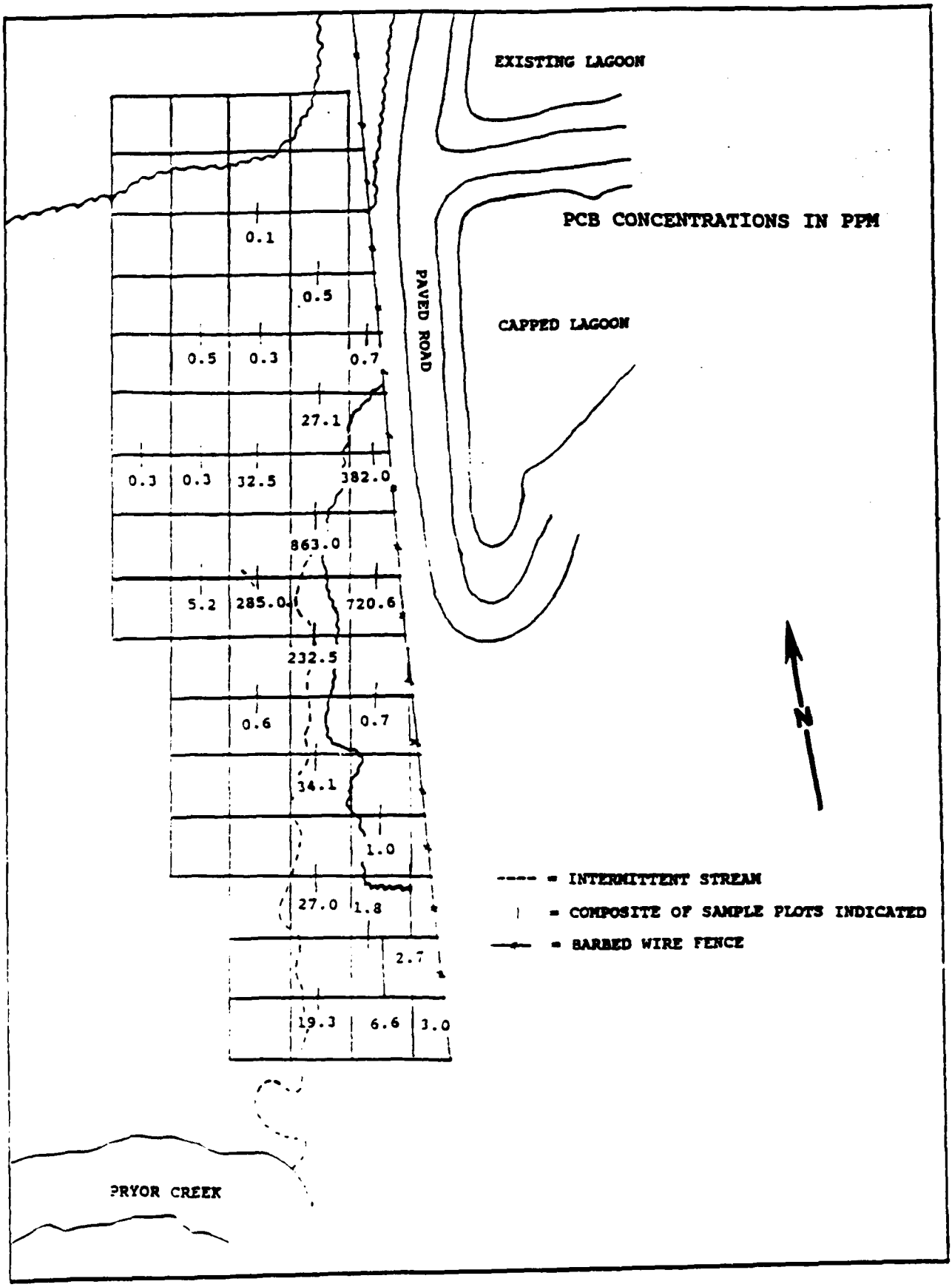
Figure 1. Distribution of surface soil PCB residues, values are concentrations in ppm. Adapted from U.S. Army Corps of Engineers (25).

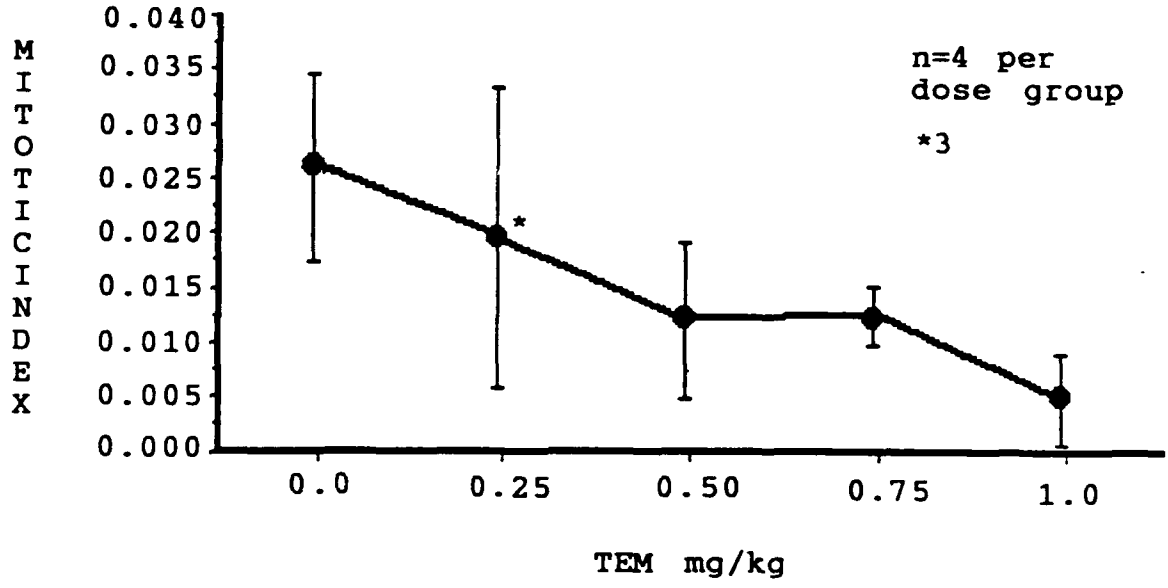
Figure 2. Representative chromosomal aberrations, cB = chromatid break, CB = chromosome break, ACF = acentric fragment.

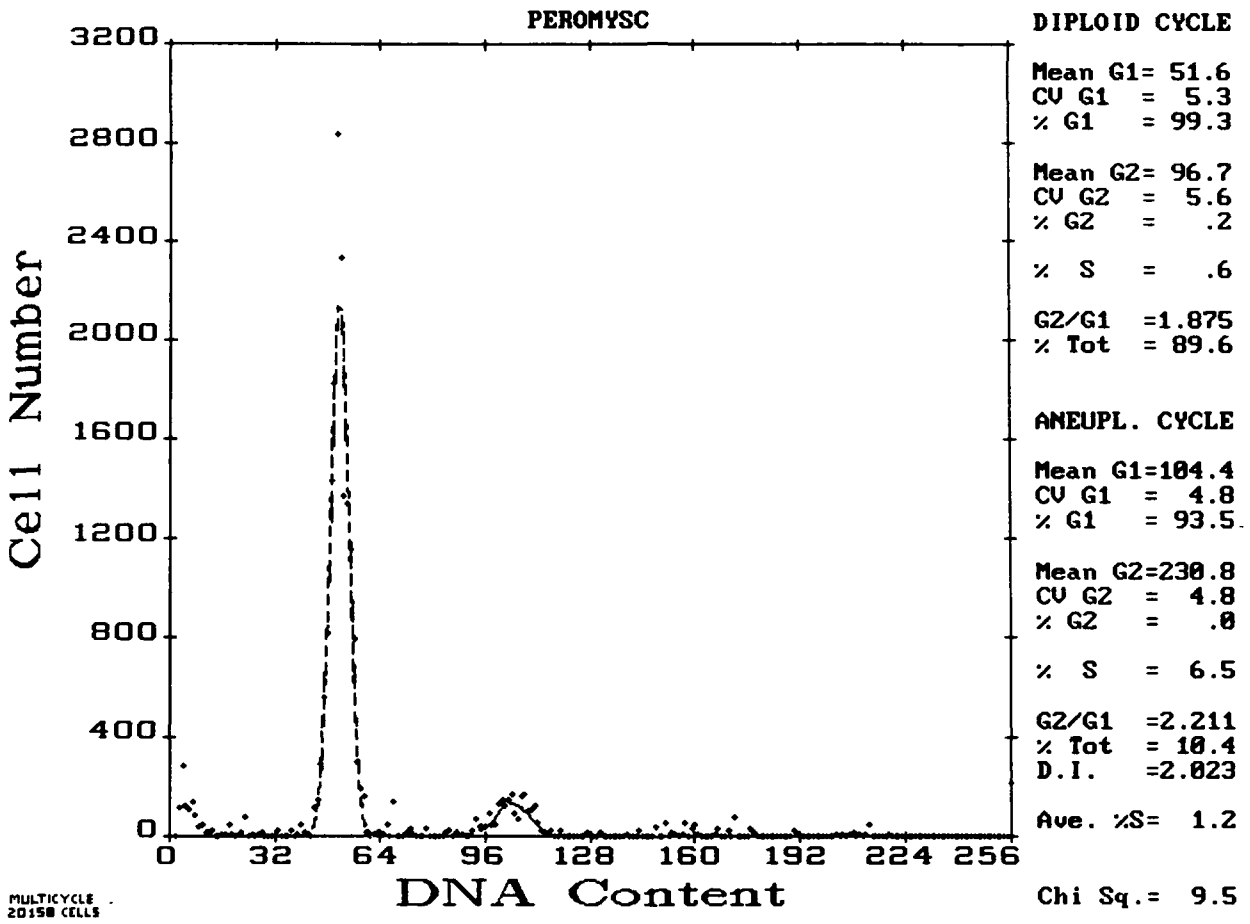
Figure 3. Mitotic index (MI) values for P. leucopus dosed with TEM. MI values represent the proportion of 1000 cells that are in mitosis.

Figure 4. DNA content histogram generated from spleen cells of a P. leucopus from Annex Ref.


cB
CB
ACF


EXISTING LAGOON
PCB CONCENTRATIONS IN PPM
PAVED ROAD
CAPPED LAGOON
0.1
0.5
0.5
0.3
0.7
27.1
0.3
0.3
32.5
382.0
863.0
5.2
285.0
720.6
232.5
0.6
0.7
34.1
1.0
27.0
1.8
2.7
19.3
6.6
3.0
N
---- = INTERMITTENT STREAM
| = COMPOSITE OF SAMPLE PLOTS INDICATED
—— = BARBED WIRE FENCE
PRYOR CREEK


MITOTICINDEX
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
n=4 per
dose group
*3
*
0.0
0.25
0.50
0.75
1.0
TEM mg/kg


PEROMYSC
3200
2800
2400
2000
1600
1200
800
400
0
Cell Number
0
32
64
96
128
160
192
224
256
DNA Content
MULTICYCLE
20150 CELLS
DIPLOID CYCLE
Mean G1= 51.6
CV G1 = 5.3
% G1 = 99.3
Mean G2= 96.7
CV G2 = 5.6
% G2 = .2
% S = .6
G2/G1 =1.875
% Tot = 89.6
ANEUPL. CYCLE
Mean G1=104.4
CV G1 = 4.8
% G1 = 93.5
Mean G2=230.8
CV G2 = 4.8
% G2 = .0
% S = 6.5
G2/G1 =2.211
% Tot = 10.4
D.I. =2.023
Ave. %S= 1.2
Chi Sq.= 9.5

4. Publications in Technical Journals

Owen, R. D., and **K. McBee**. Analysis of asymmetry and morphometric variation in natural populations of chromosome-damaged mice. Texas Journal of Science. IN PRESS. Preprint appended. Expected publication date November 1990.

Adkins, R. M., **K. McBee**, C. A. Porter, and R. J. Baker. Hybrid breakdown in Peromyscus leucopus and examination of the recombinational-breakdown model. Journal of Mammalogy. IN PRESS.

McBee, K. Chromosomal aberrations in native small mammals (Peromyscus leucopus) at a waste disposal site: II. Inherited aberrations and fragile sites indicated by G-band analysis. Environmental Toxicology and Chemistry. ACCEPTED FOR PUBLICATION, in revision. Draft copy appended

Shaw-Allen, P. L., and **K. McBee**. Chromosome damage in wild rodents inhabiting a site contaminated with Aroclor 1254. Environmental Toxicology and Chemistry. IN PREPARATION, Draft copy appended, expected submission date December 1990.

Tull, S. A., and **K. McBee**. Heavy metal bioaccumulation in wild rodents from a hazardous waste site. Environmental Toxicology and Chemistry. IN PREPARATION, expected submission date January 1991.

McBee, K., S. A. Tull, and A. Head. Triethylene melamine clastogenesis in Peromyscus leucopus. Environmental and Molecular Mutagenesis. IN PREPARATION, expected submission date June 1991.

5. Professional Personnel

Dr. Karen McBee, Principal Investigator
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Ms Kathleen Thies, Research Technician, June 1990 - present
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Department of Zoology, Oklahoma State University

Advanced Degrees Awarded

August 1990 - Patricia L. Shaw-Allen, Master of Science, Thesis Title: An In Situ Investigation of Genetic Damage in Wild Rodents Inhabiting a Site Contaminated with Aroclor 1254.

6. Interactions (Coupling Activities)

6.1. Papers presented at meetings, conferences, seminars.

McBee, K. In situ biomonitoring of environmental clastogens using wild rodents. Annual Meetings of the Society of Environmental Toxicology and Chemistry, Toronto, Canada, 28 Oct - 2 Nov 1989.

Thies, M. and K. McBee. Demographic and cytogenetic examination of a hazardous waste dump. Annual Meetings of the Oklahoma Academy of Sciences, Central State University, Edmond, OK, 10 Nov 1989.

McBee, K. In Situ analysis of genetic damage in wild rodents. Seminar at Dept. Biology, University of Central Arkansas, Conway, AK, 21 Feb 1990.

Thies, M., and K. McBee. Demographic and cytogenetic examination of rodents from a hazardous waste site. Annual Meetings of the Southwestern Association of Naturalists, University of North Texas, Denton, TX, 12-14 Apr 1990.

Tull, S. A., K. McBee, and S. Kimball. Heavy metal bioaccumulation in wild rodents from a hazardous waste site. Annual Meetings of the Southwestern Association of Naturalists, University of North Texas, Denton, TX, 12-14 Apr 1990.

McBee, K., P. L. Shaw-Allen, and S. A. Tull, Development of mammalian in situ genetic biomonitors. Oklahoma Center for the Advancement of Science and Technology Conference, Oklahoma City, OK 16 Apr 1990.

Thies, M., and K. McBee. Demographic and cytogenetic examination of rodents inhabiting a hazardous waste site. Annual Meetings of the Southcentral Chapter of the Society of Environmental Toxicology and Chemistry, Oklahoma State University, Stillwater, OK, 18 May 1990.

Tull, S. A., K. McBee, and S. Kimball. Heavy metal bioaccumulation in wild rodents from a hazardous waste site. Annual Meetings of the Southcentral Chapter of the Society of Environmental Toxicology and Chemistry, Oklahoma State University, Stillwater, OK, 18 May 1990.

Thies, M., and K. McBee. Demographic and cytogenetic examination of rodents inhabiting a hazardous waste site. Annual Meetings of the American Society of Mammalogists, Frostburg State University, Frostburg, MD 9-13 June 1990.

Tull, S. A., K. McBee, and S. Kimball. Heavy metal bioaccumulation in wild rodents from a hazardous waste site. Annual Meetings of the American Society of Mammalogists, Frostburg State University, Frostburg, MD 9-13 June 1990.

McBee, K. Mammals as bioindicators of environmental toxicity. Co-Chairman of symposium. American Society of Mammalogists, Frostburg State University, Frostburg, MD 9-13 June 1990.

McBee, K. Adaptation of mutagenicity and clastogenicity assays for avian species. Conference on Population Ecology and Wildlife Toxicology of Agricultural Pesticide Use: A Modeling Initiative for Avian Species. Kiawah Island, SC, 25 July 1990.

*McBee, K., and P. L. Shaw-Allen. Chromosome damage in wild rodents inhabiting a site contaminated with Aroclor 1254. Annual Meetings of the Society of Environmental Toxicology and Chemistry, Arlington, VA 11-15 Nov 1990.

*Abstract accepted for presentation.

6.2. Interactions with Other Researchers

Dr. Robert Owen, a biometrician at the University of Missouri, Kansas City, is collaborating with this laboratory on the development of methods to assess morphological and developmental abnormalities associated with induced genetic damage in bioindicator species. This collaboration has resulted in one publication thus far, and Dr. Owen has agreed to continue morphological analyses of all the animals taken from the PCB site and the radioactive site in this project.