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**Comparison of Two Freshwater  
Turtle Species as Monitors of  
Environmental Contamination**

L. Meyers-Schöne  
B. T. Walton

Environmental Sciences Division  
Publication No. 3454

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ENVIRONMENTAL SCIENCES DIVISION

COMPARISON OF TWO FRESHWATER TURTLE SPECIES AS MONITORS  
OF ENVIRONMENTAL CONTAMINATION'

L. **Meyers-Schöne**<sup>2</sup> and B. T. Walton

Environmental Sciences Division  
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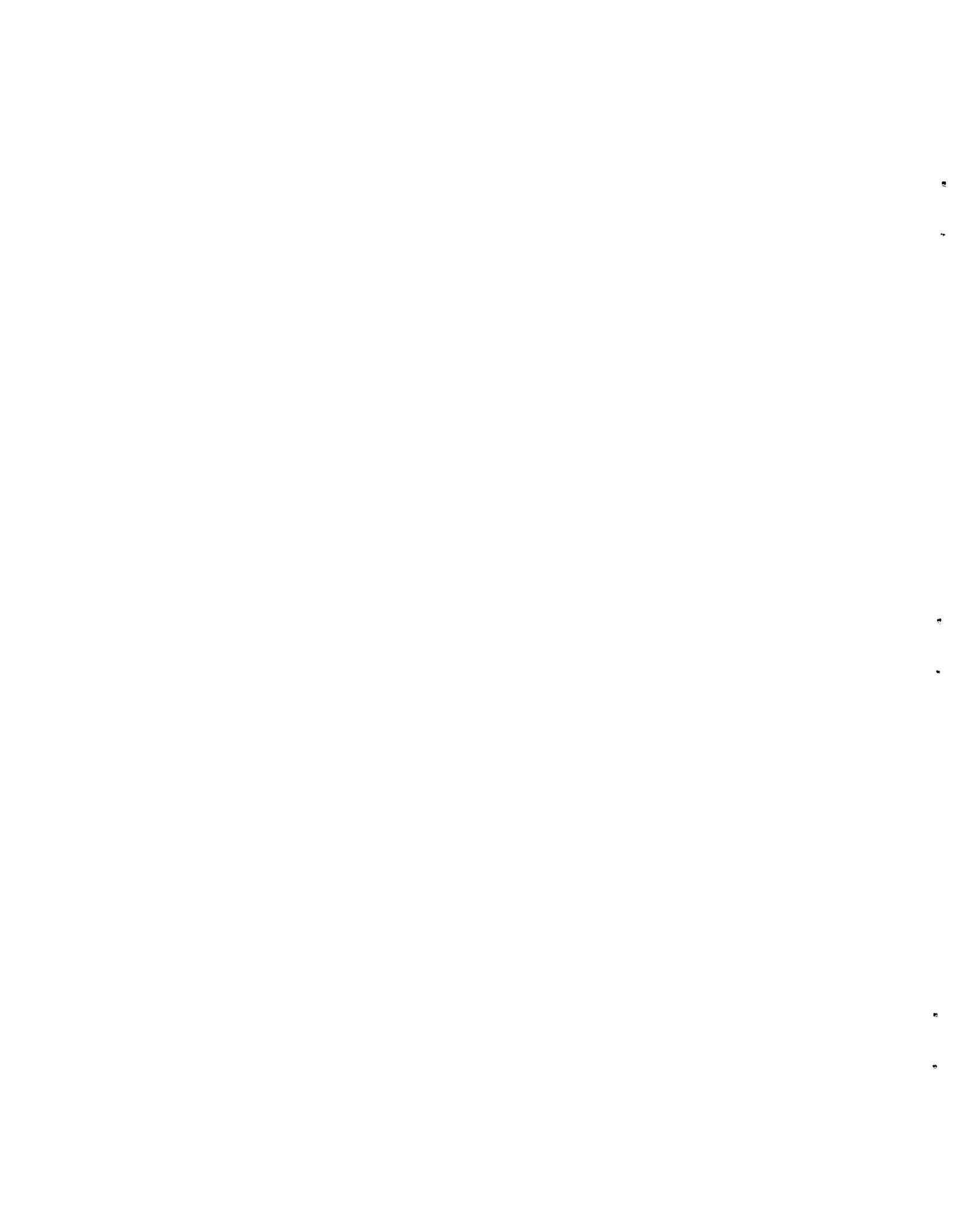
'Submitted as a dissertation by L. **Meyers-Schöne** to the Graduate Council of the University of Tennessee in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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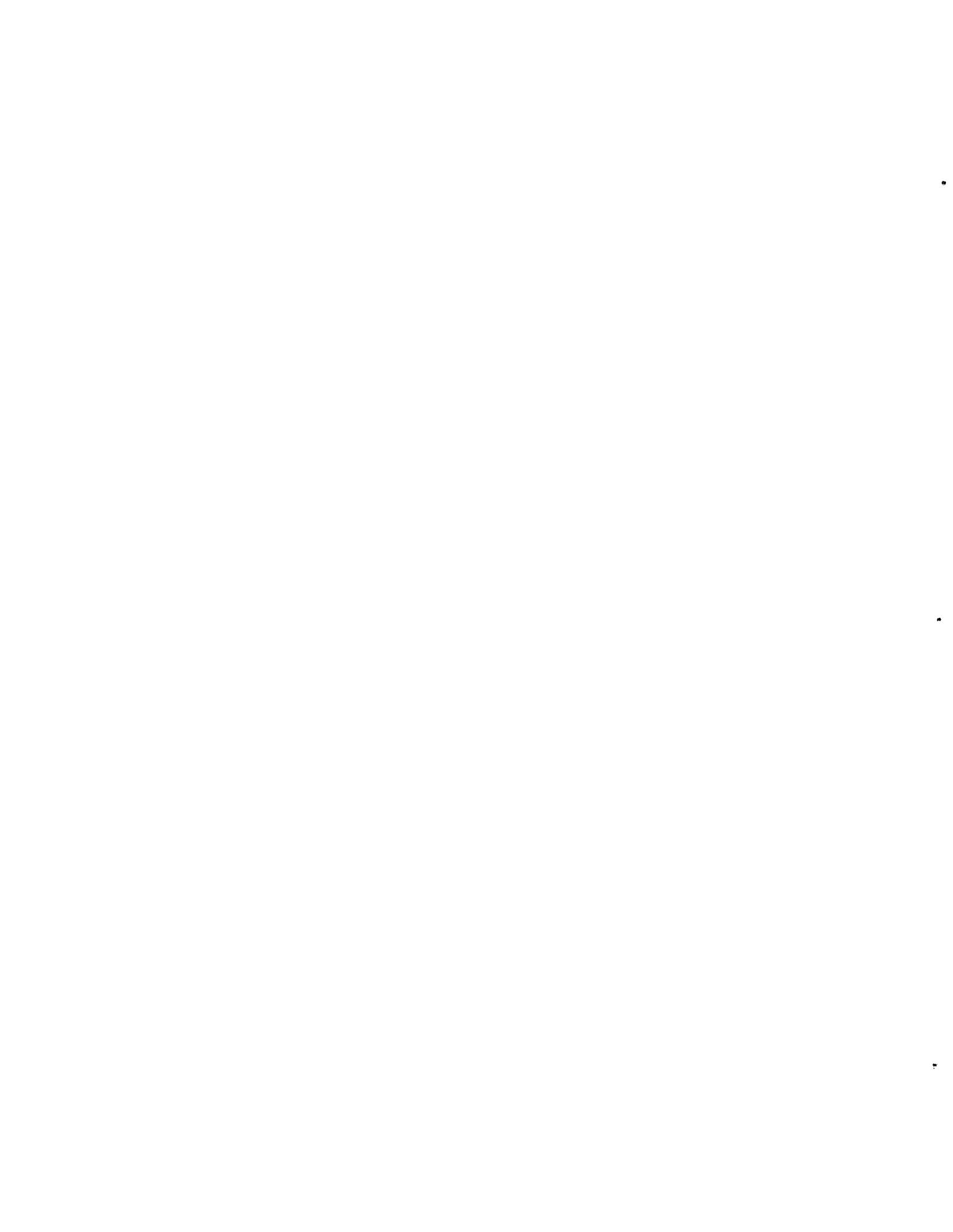
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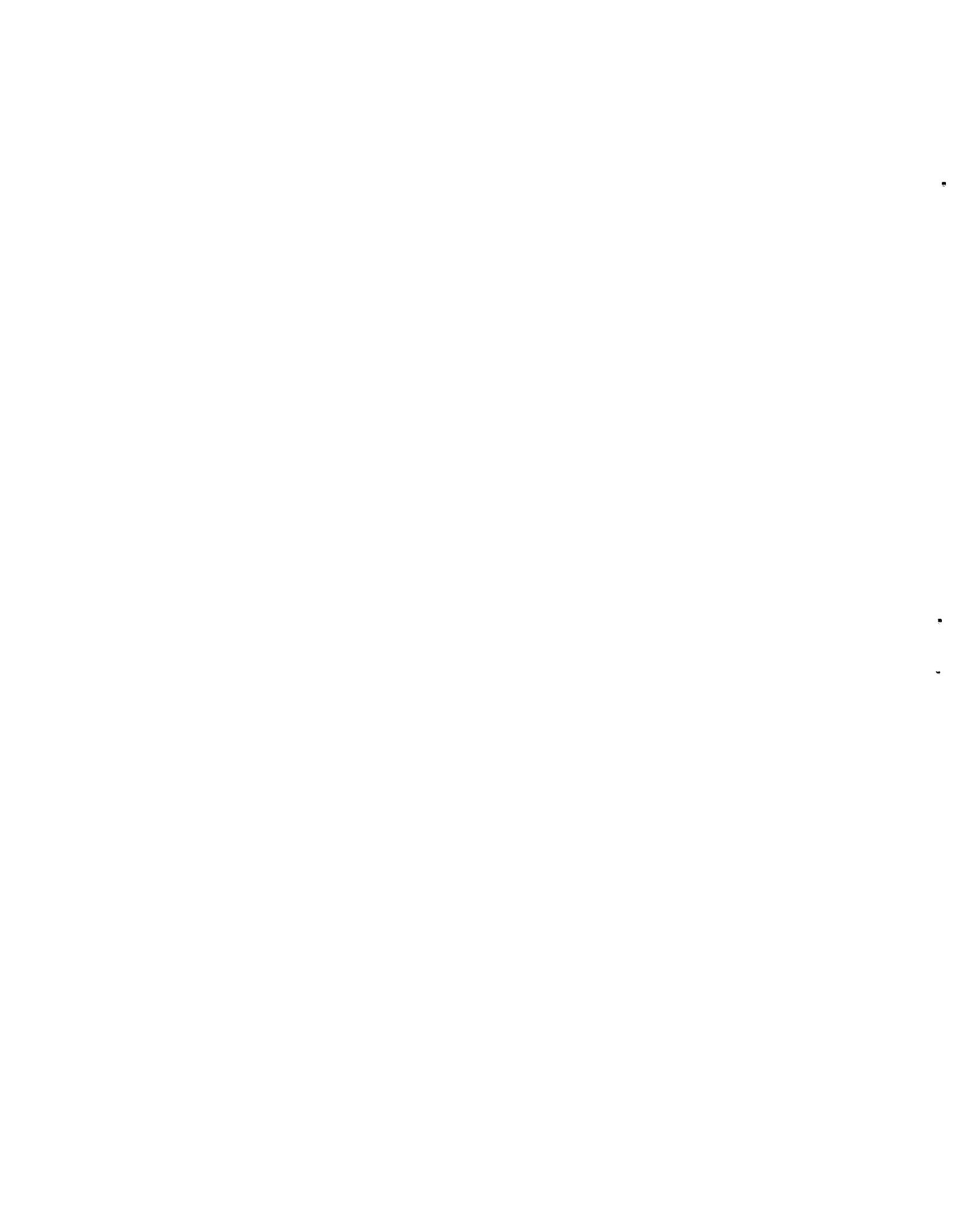


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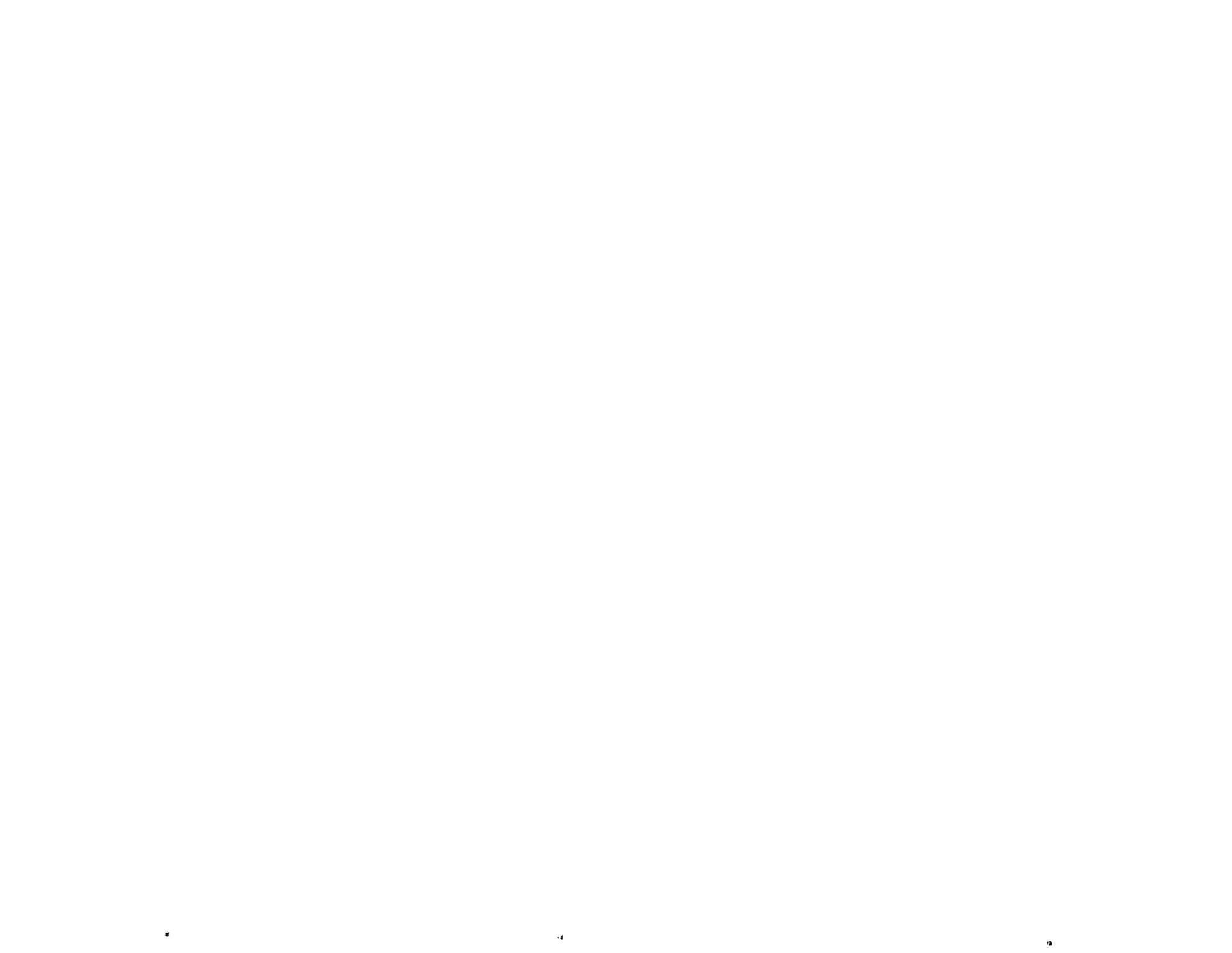
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ABSTRACT

MEYERS-SCHONE, L. and B. T. WALTON. 1990. Comparison of two freshwater turtle species as monitors of environmental contamination. **ORNL/TM-11460**. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 181 pp.

Two species of turtles that occupy different ecological niches were compared for their usefulness as monitors of contamination in freshwater ecosystems. Trachemys scripta (Agassiz) (yellow-bellied slider) and Chelydra serpentina (Linnaeus) (common snapping turtle) were selected for comparison based on species abundance and differences in food habits and sediment contact. A review of the literature on contaminants in turtles and results of preliminary surveys conducted at the field sites, which are included in this study, were used to direct and focus this research project.

White Oak Lake, a settling basin for low-level radioactive and nonradioactive contaminants, and **Bearden** Creek Embayment, an uncontaminated reference site upriver, were used as study sites in the investigation of turtles as indicators of chemical contamination. Turtles were analyzed for concentrations of strontium-90, cesium-137, cobalt 60, and mercury in specific target tissues, and for **single-**stranded DNA breaks, a non-specific indicator of possible exposure to genotoxic agents in the environment. Significantly higher concentrations of <sup>90</sup>Sr, <sup>137</sup>Cs, <sup>60</sup>Co, and mercury were detected in turtles from White Oak Lake than in turtles from the reference site. In addition, turtles from White Oak Lake contained a significantly greater amount of DNA damage than those from the reference site. Although this suggests greater exposure of White Oak Lake turtles to genotoxic agents, further studies are needed to establish the cause of the enhanced amount of single-stranded breaks.

Interspecific comparisons of the turtles from White Oak Lake indicated that diet may play a significant role in the exposure of turtles to certain contaminants. No difference was detected between the concentrations of <sup>90</sup>Sr, <sup>137</sup>Cs, and <sup>60</sup>Co between the two species.

Mercury concentrations in C. sernentina were significantly higher than in T. scrinta. No difference was detected in the amount of DNA damage between T. scrinta and C. sernentina. Although both species were effective monitors of contaminants in White Oak Lake, C. sernentina (the carnivorous species) may be of greater value in the detection of contaminants with high biomagnification potential such as methylmercury. In addition, less variability detected in the C. sernentina data than in the T. scrinta data suggests the former species as having greater uniformity of exposure to the contaminants in White Oak Lake, thereby favoring the use of C. sernentina as a bioindicator species.

## 1. INTRODUCTION

Biological monitoring, defined here as the use of biota for the detection of chemical contaminants in the environment, can provide information useful to the control and regulation of toxicants in the environment. Such information is used to determine the degree of contamination at a site and can indicate the bioavailability of chemical contaminants. In addition, the monitoring of contaminants in biota can be used to estimate the impact of a contaminant on an indicator species and on the ecosystem as a whole. Such information can also be used to evaluate potential risks to humans (e.g., Suter 1989). Essential to such biological monitoring efforts are the selection of an appropriate indicator species (National Research Council 1986) and the use of suitable analytical techniques.

Several **taxa** have been used to monitor contaminants in aquatic ecosystems, included among them are fish (e.g., Schmitt et al. **1985**), aquatic plants (e.g., Ohlendorph et al. 1986) and invertebrates (e.g., Huckabee et al. **1979**), and turtles (e.g., Albers et al. 1986). This study focuses on the use of turtles as biological monitors. Freshwater turtles have several favorable characteristics that make them useful for biological monitoring of contaminants. They are often common in ponds, lakes, and some river systems and several species often occur at the same site. They are long-lived, and may reach an age of 10 to 50 years in the wild depending on the species and environmental conditions (Gibbons 1987). This allows for long-term exposure to contaminants. Turtles are mobile within ecosystems. Many species may travel distances ranging from a few hundred meters to several kilometers during a year. The fact that turtles are both long-lived and mobile allows **for the** integration of exposure over time and space. These attributes are very important for biomonitoring. In addition, liver, fat, kidney, muscle and bone, key tissues useful in residue and biochemical **analyses**, are available in large quantities. Important ecological differences also exist among species. When species feed at different-trophic levels, useful information may be obtained on the bioavailability and biomagnification of contaminants. Similarly, the extent of contact with sediments, which is a sink for non-volatile contaminants in slow moving waters, is highly species specific. A sedentary species would have

greater contact with the sediment than a pelagic species and, therefore, would have greater exposure to contaminants that accumulate in sediment. A variety of chemical contaminants have been reported in turtles (e.g., Albers et al. 1986, Olafsson et al. 1983, Scott et al. 1986); however, the importance of turtle species selection in the monitoring of specific contaminants has not been addressed. The purpose of this study is threefold: (1) to compare concentrations of selected contaminants in two turtle species, (2) to compare the response of each species to a nonspecific biochemical indicator of contaminant exposure and (3) to evaluate the influence of food and sediment contact on contaminant exposure.

Selection of the two species was based on several criteria. The species were to occupy different ecological niches, that is, occupy different **trophic** levels and differ in the degree of contact with sediments. The availability of information in the literature on the accumulation of contaminants by these species was a criterion for selection. Finally, the species selected had to be abundant in the study sites and readily trappable.

Studies on the uptake of pesticides, **PCBs**, metals and radionuclides by turtles are presented in the literature review (Section 2) as are investigations on biochemical and histopathological responses of turtles to chemical and physical stressors. The use of turtle growth rates as a possible indicator of exposure to contaminants in the environment is also evaluated. The information from this review indicated that Trachemys scripta (Agassiz) (yellow-bellied slider turtle, Remydidae) and Chelydra serpentina (Linnaeus) (common snapping turtle, Chelydradae) are species frequently used to monitor chemical contaminants in freshwater wetlands.

Site reconnaissance data from a contaminated and a reference site on the Oak Ridge Reservation are presented in Sections 3 and 4. Preliminary sampling of turtles from the two study sites revealed **T. scripta** and **C. serpentina** as the most frequently trapped species from both sites. Section 4 also contains data on the distribution and concentrations of specific contaminants in turtles from the contaminated site. These data were used to define contaminants and key tissues to be

used for residue analysis. Data on the food habits of three species of turtles were collected (Section 4) and used to focus the study on two species that occupy different **trophic** levels. Information both from the literature review (Section 2) and from the preliminary surveys (Section 4) showed **T. scriptiona** and **C. serpentina** as the two species most appropriate for the purposes of this study. Natural history background on these species are presented in part A of Section 5.

Based on results from preliminary studies, **T. scriptiona**, a pond turtle that feeds primarily on vegetation, was compared with **C. serpentina**, a carnivorous species more closely associated with sediment, to determine their usefulness as indicators of contaminants in a freshwater ecosystem. These species were collected from a site containing radioactive and non-radioactive contaminants and **from a** , reference site. (Site descriptions are in Section 3.) Turtles were analyzed for tissue residues of **strontium-90**, cesium-137, cobalt-60, and mercury, and for DNA damage, a non-specific indicator of exposure to genotoxic agents (Section 5). **In** addition, gut analyses were performed on both species to establish dietary sources of contamination (Section 5). Comparisons were **made between** sites and between species in order to evaluate the importance of species selection **when turtles are** used to monitor chemical contaminants **in freshwater** environments.

## 2. REVIEW OF TURTLES AS MONITORS OF ENVIRONMENTAL CONTAMINANTS

The utilization of turtles as indicators of environmental contamination is not a novel idea. Turtles have been used increasingly as bioindicators during the last ten years, yet they have not been used as extensively as fish in aquatic environments or small mammals in terrestrial environments. This trend coincides with increased public and governmental concern for the health of the environment. Although several studies report chemical contaminants in turtles, the majority of these investigations are surveillance reports on chemical contaminants within a few individual turtles of various species with no reference made to contaminant concentrations in the surrounding environment. Collectively, these studies demonstrate that turtles can accumulate chlorinated organic compounds, metals, and radionuclides from the environment. Turtles have proven to be especially useful as biomonitors of polychlorinated biphenyls (**PCBs**) (e.g., Stone et al. 1980, Watson et al. 1985) and strontium-90 (e.g., Holcomb et al. 1971). In general, affinities of the chemical contaminants for specific tissues in turtles (e.g., Meeks 1968, Stone et al. 1980) were similar to those reported in birds and mammals (e.g., Matsumura 1975, Eisler 1986b). There is also some indication that organochlorine pesticides and **PCBs** may be transferred to eggs in utero (e.g., Flickinger and King 1972, Stone et al. 1980). Also, the detection of chemical contaminants in turtles collected kilometers away from the nearest point source provides evidence for the movement of such contaminants through the ecosystem and, in some instances, indicates the ability of turtles to concentrate trace amounts of contaminants from the environment (Holcomb et al. 1971). Finally, a few studies have focused on the use of biochemical parameters and growth rates in turtles as indicators of exposure to contaminants in the environment (e.g., Bickham et al. 1988). These studies, however, often present inconclusive results.

Studies of contaminant concentrations in terrestrial, freshwater, and marine turtles are included in this review. All concentrations reported in the open literature since 1950 are summarized in APPENDIX A and are expressed on a wet weight basis unless otherwise noted. Dry

weight tissue concentrations reported by authors were converted to wet weight concentrations using conversion factors presented by Meeks (1968). Dry weight concentrations in egg yolks of Caretta caretta were converted to wet weight concentrations using a conversion factor obtained from a summary of weights in Ewert (1979). Concentrations reported in whole body and egg do not include shell unless otherwise noted. Furthermore, all concentrations are presented in standard international units (i.e.,  $\mu\text{g/g}$  and  $\text{Bq/g}$ ).

## 2.1 PESTICIDES

The only group of pesticides that have been measured in field collected turtles is the organochlorine pesticides. A review of pesticides in reptiles was published by Hall in 1980. The present review, however, is restricted to data published on organochlorine pesticide concentrations in turtles from 1950 to 1989 as they pertain to biological monitoring. The pesticides that have been **detected** in turtles are dichlorodiphenyltrichloroethane (DDT), aldrin (**1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene**), dieldrin (**1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene**), endrin (**1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene**), heptachlor (**1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane**), mirex (**dodecachlorooctahydro-1,3,3-metheno-2H-cyclobuta(cd)pentane**), chlordane (**1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene**), nonachlor (**1,2,3,4,5,6,7,8,8-nonachlor-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene**), and toxaphene (a mixture of various **chlorinated camphenes**).

The most frequently monitored pesticide in turtles has been DDT and its metabolites (Meeks 1968, Flickenger and King 1972, Hillestad et al. 1974, Reeves et al. 1977, **Punzo** et al. 1979, Stone et al. 1980, Clark and Krynitsky 1980, Clark and Krynitsky 1985, Albers et al. 1986). As in birds and mammals (**Matsumura 1975**), DDT in turtles is metabolized to dichlorodiphenyldichloroethylene (DDE) and **dichlorodiphenyl-dichloroethane (TDE)**, with **p,p'-DDE** as the major metabolite

(Owen and Wells 1976). The presence of DDT in the freshwater turtles (Reeves et al. 1977, Punzo et al. 1979, Stone et al. 1980, Albers et al. **1986**), sea turtles (**McKim** and Johnson et al. **1983**), and in the eggs of marine turtles (Hillestad et al. 1974, Clark and Krynitsky 1980, Clark and Krynitsky 1985) collected since the 1972 ban on the use of DDT in the United States reveals the persistence and wide-spread distribution of the pesticide in the environment.

In turtles, as with other vertebrate **taxa**, organo-chlorine pesticides have great affinity for tissues high in lipid content (Meeks 1968, Stone et al. 1980, Pearson et al. 1973). The partitioning and subsequent storage of pesticides in adipose tissues can protect a turtle against the toxic chlorinated organic compounds. The utilization of fat reserves results in the redistribution of the pesticide and or its metabolites. These compounds are eventually eliminated into the urine and feces (Matsumura 1975) or are transferred to eggs in utero (Flickinger and King 1972, Holcomb and Parker 1979, Punzo et al. 1979, Stone et al. 1980). Liver and kidney in turtles tend to contain concentrations of these pesticides that are lower than that detected in fat, but higher than that detected in other soft tissues (Meeks 1968, Pearson et al. 1973). High concentrations are expected in liver because it is the major site of detoxication for xenobiotics that enter the body. Organic chemicals that enter the body may be eliminated into urine in either the original form or as a metabolite of the parent compound, thus, high concentrations may be found in kidney as well. Concentrations of DDT and its metabolites in reproductive organs are usually within the range of concentrations detected in liver and kidney tissues (Meeks 1968). Both DDT and dieldrin have been detected in the brains of turtles exposed to these pesticides (Meeks 1968, Pearson et al. 1973).

Differences in the concentration of an organochlorine pesticide among species of turtles collected from a common site have been attributed to differences in food habits. Holcolmb and Parker (1979) monitored concentrations of mirex in Chrysemys scripta (**- Trachemys scripta**) and Terrapene Carolina (Eastern box turtle) collected from an

area that received four applications of the pesticide (5% mirex solution) during a period of eight years. Higher concentrations were detected in the more carnivorous T. Carolina than in C. scrinta, suggesting the biomagnification of mirex. Differences were also reported in the concentrations of DDE in the eggs of marine turtles (Clark and Krynitsky 1980). The eggs of Caretta caretta (loggerhead sea turtle) contained higher concentrations of **DDE than** those of Chelonia mydas (green sea turtle) collected from the same seashores. If the DDE in the eggs is assumed to have been transferred as **a metabolite** of DDT from gravid females, then it is possible that differences **in DDE** concentrations are attributed to differences in the diets of the adult turtles. Caretta caretta feeds primarily on fish, clams, sponges, and jellyfish, whereas, C. mydas feeds primarily on large seaweeds (Carr 1952). The biomagnification of DDT and its metabolites has also been reported in other vertebrate **taxa** (e.g., **Woodwell** et al. 1967, Mulhern et al. 1970, **Belisle** et al. 1972) therefore, the correlation between **carnivory** and elevated DDT concentrations in C. caretta is plausible. Flickinger and King (1972) also attributed a species difference in aldrin and dieldrin concentrations to the **food habits of the** turtles **examined**. **Higher** concentrations of these pesticides in T. scrinta than in Kinosternon flavescens (yellow mud turtle) were attributed to the consumption of aldrin-treated rice seed by the herbivorous T. scrinta. In this instance, the carnivore did not receive the highest exposure to the pesticide, indicating that direct ingestion of the contaminant may also serve as a critical route of exposure to turtles. These studies provide a limited amount of **evidence** that., dietary preferences may explain differences in contaminant concentration between turtles.

Interspecific differences have also been noted in the sensitivities of turtles to organochlorine pesticides. Phillips and Wells (1974) found species differences in the response of adenosine triphosphatase (ATPase) to DDT. Of the five species studied, C. serpentina and Trionyx spinifer (**- Trionyx spiniferus**, Eastern spiny soft-shell turtle) appeared to be the **most sensitive species** displaying the greatest inhibition of total ATPase activity at the highest

concentration of DDT used in the in vitro study (53  $\mu\text{M}$  DDT in the reaction mixture that included tissue homogenate, **NaCl, KCl, Na<sub>2</sub>ATP, MgCl<sub>2</sub>** and 1 **mM** DDT). Similar effects were observed among the species at lower concentration (5.3 and 0.53  $\mu\text{M}$  DDT) and were stimulatory at the lowest concentration in the organs of some species. A similar study was conducted by Wells et al. (1974) where the effect of dieldrin and aldrin on ATPase activity in tissues of *Graptemys geographica* (map turtle) was measured. The degree of inhibition, however, was not as great as that observed using DDT in the same species (Phillips and Wells 1974), especially for kidney and liver tissue. Organochlorine pesticides are generally considered central nervous system (CNS) toxicants. Neither of these studies, however, measured the effects of the pesticides on brain tissues. The **LD<sub>50</sub>** for technical grade DDT in rats is approximately 4.7 times greater than that for dieldrin (Murphy 1980), indicating dieldrin as the more toxic compound in rats. If, as in rats, dieldrin is more toxic than DDT in turtles, then the inhibition of ATPase activity (which can result in a decline in the active transport of compounds across cellular membranes) may not play as critical a role as the effect of organochlorines on the central nervous system. Additional research is needed before the physiological and biochemical responses of turtles to pesticides is understood.

Freshwater turtles are as effective as most vertebrate fauna in their ability to accumulate organochlorine pesticides, specifically **DDT**, from a contaminated wetland. Meeks (1968) treated a 1.61 ha marsh with 0.22 kg of **DDT/ha** and reported that during the **15-month** study the highest concentrations detected among the reptiles, birds, and mammals sampled occurred in fat from a Northern water snake (*Natrix sipedon sioedon*, 23.66  $\mu\text{g/g}$ ), a Virginia rail (*Rallus limicola*, 15.96  $\mu\text{g/g}$ ), and a common snapping turtle (*C. sernentina*, 13.04  $\mu\text{g/g}$ ). Two Blanding's turtles (*Emys blandingii*) and seven painted turtles (*Chvsemys picta*) contained concentrations lower than that detected in the snapping turtle. Concentrations detected in fish were generally lower than that reported in birds and reptiles. Carnivorous species such as the Northern water snake, Virginia rail and snapping turtle reached their peak concentrations 13 to 15 months following initial exposure to the DDT.

This study effectively illustrates the **bioaccumulation and biomagnification** of DDT over time.

## 2.2 POLYCHLORINATED BIPHENYLS

Turtles appear to be excellent monitors of PCB contamination in aquatic environments. Polychlorinated biphenyls, like organochlorine pesticides, concentrate primarily in adipose tissue (Stone et al. 1980, Helwig and Hora 1983, Watson et al. 1985, Bryan et al. 1987a). Reproductive organs in turtles from **PCB-contaminated** sites, also contain relatively high concentrations of **PCBs** compared to other soft tissues (Bryan et al. 1987a). The highest reported concentration of **PCBs** in turtles is 4,530  $\mu\text{g/g}$  in the fat of a C. serpentina collected from a pond near a liquid waste disposal site (Watson et al. 1985). The concentration in the fat of this turtle was  $10^4$  times greater than that in whirligig beetles (Gyrinidae) and  $1.7 \times 10^4$  times that detected in three species of fish, the pumpkinseed sunfish (Lepomis gibbosus), golden shiner (Notemionius crysoleucas), and brown bullhead (Ictalurus nebulosus). The concentration in turtle muscle, however, was lower than detected in fat and is only 6.27 times greater than the average PCB concentration in whole fish. These data suggest that **PCBs** were biomagnified in the pond ecosystem. Several other studies have reported PCB concentrations in the fat of field-collected turtles that exceed 1000  $\mu\text{g/g}$  (Olafsson et al. 1983, Stone et al. 1980, Bryan et al. 1987a). In comparison with other vertebrates, fish are the only other **taxa** where concentrations exceeding 1  $\text{mg/g}$  have been detected in species from the wild (**Eisler** 1986b). These fish were collected from the Hudson River in an area of known PCB **contamination** (Brown et al. 1985). The ability of turtles to store high concentrations of **PCBs** in their fat without apparent adverse effects makes this **taxa** extremely useful for the biological monitoring of **PCBs** in freshwater ecosystems.

Polychlorinated biphenyls can be transferred **from** a gravid turtle to her eggs in utero (Stone et al. 1980). Concentrations in the eggs appear to be dependent on whether fat reserves are present in the gravid females. Stone et al. (1980) reported PCB concentrations in the eggs and tissues of five gravid C. serpentina collected from the

Hudson River. Liver and muscle were analyzed from each of these turtles, however, a fat sample was only analyzed from one turtle. Although not discussed in the paper, it is possible that fat reserves were not present in four of the females and, therefore, could not be analyzed. Female turtles will fast during the nesting season. Such an activity would result in the mobilization of the stored **PCBs** that could alter the ratio of PCB concentrations in different tissues. Muscle contained 60.8% of the concentration detected in the liver of the turtle that had fat and the concentration in the eggs slightly exceeded that detected in muscle. The eggs from this turtles contained 2.9% of the concentration detected in the adipose tissue of the female. In contrast, the remaining four turtles contained muscle concentrations of 10.5% of that detected in liver; and PCB concentrations in eggs exceeded those reported in muscle by 10 to 64 times. It may be that the utilization of fat reserves resulted in the mobilization and preferential deposition of **PCBs** into the lipid-rich egg yolks. Toxic forms of **PCBs** are transferred to the eggs, the majority (95%) of which is concentrated in yolk (5% is partitioned into albumin and shell) (Bryan et al. 1987b). Because high concentrations of **PCBs** can be transferred to turtle eggs in utero, studies are needed to determine the concentrations of **PCBs** that are lethal to the development of turtle eggs and that may subsequently result in population decline in highly contaminated areas.

There is some indication that sex differences occur in the ability of turtles to concentrate **PCBs**. Albers et al. (1986) reported that males contained significantly higher concentrations of **PCBs** than females. The mean concentration in the fat from C. sernentina was 40.1  $\mu\text{g/g}$  in males and 8.41  $\mu\text{g/g}$  in females. This difference may be due to the elimination of **PCBs** into eggs by mature females. If a sex difference does exist and is due to elimination of **PCBs** into eggs, the sampling and analysis of exclusively adult males may result in a more uniform set of data among the turtles sampled throughout the active months of the year. In any event, the reproductive status of all turtles should be noted for comparative purposes.

### 2.3 METALS

Heavy metals have been monitored extensively in both terrestrial and aquatic systems, however, only a few studies have used turtles as monitors of metal **contamination in** the environment. The metals that have been measured in either field-collected turtles or turtle eggs are lead, mercury, cadmium, chromium, copper, zinc, nickel, molybdenum, iron, cobalt, aluminum, strontium, and barium. The majority of the information available focuses on residue concentrations and tissue distributions of these metals with no reference made to contaminant concentrations in the environment.

Tissue distributions of metals in turtles are similar to those reported in mammals. Bone and shell contain the highest concentrations of lead, followed by liver and kidney tissues (Beresford et al. 1981, Krajicek and Overman 1988). Exposure to lead can be measured in turtles from samples of carapace and blood that can be obtained without harm to the animal and are therefore recommended as tissues to be used for routine monitoring of lead (Krajicek and Overman 1988). Among the soft tissues, highest concentrations of cadmium were detected in kidney tissue (Robinson and Wells 1975). Mercury, chromium, nickel and zinc have been measured in kidney and liver tissues of turtles (Albers et al. 1986, **Helwig** and Hora 1983, Flickinger and King 1972). These tissues usually contain the highest concentrations of metals (Hammond and Beliles 1979) and are generally used in the monitoring of metals in mammals and birds (Eisler **1985a, 1985b, 1986a**, 1987).

Concentrations of copper were found to vary with the sex of the turtle. Albers et al. (1986) noted significantly higher concentrations of the metal in the livers of male **C. serpentina** than in the females from the same site. No other metal **concentrations** were correlated with the sex of the turtle. The sex related difference reported by Albers et al. (1986) may be attributed to the elimination of copper by gravid females into their eggs or differences in exposure due to activity patterns or feeding habits that may vary during the breeding season.

Several metals have been measured in the eggs of **C. caretta** (Stoneburner et al. 1980, Hillestad et al. 1974). Significantly higher concentrations of cadmium, copper, and lead were found in egg yolk than

in albumin (Hillestad et al. 1974). In addition, of the metals analyzed in the eggs, the highest concentrations were of zinc (25.6 to 28.0  $\mu\text{g/g}$ ), iron (24.8 to 26.0  $\mu\text{g/g}$ ), and strontium (23.0 to 25.8  $\mu\text{g/g}$ ) (Stoneburner et al. 1980). The high concentrations of zinc and iron may be attributed to their requirement as an essential metal. Strontium is an analog of calcium, and although not an essential element for life, it may have been, to some extent, transferred to the egg in place of calcium. Because the eggs were collected in nests, concentrations detected in the eggs do not provide conclusive evidence that metals can be transferred to eggs in utero. Because C. caretta is protected under the United States Endangered Species Act, adults cannot be legally killed to determine metal concentrations of eggs in utero in order to evaluate the hypothesis of metal transference to eggs. Eggs from nonendangered freshwater turtles collected in utero should be analyzed for metals and compared with oviposited eggs to establish the contribution of the mother to contaminants in eggs.

#### 2.4 RADIONUCLIDES

Radionuclide contaminants, whether in the environment as fallout products from nuclear weapons testing, as nuclear waste, or used in isotope tracer studies, have been detected in both freshwater and terrestrial turtles. A comprehensive review of radionuclides in reptiles and amphibians has been prepared by Hinton and Scott (in press). The present review, however, emphasizes radionuclides as they pertain to the use of turtles as biological monitors of environmental contamination. The radionuclides that have been detected in turtles are strontium-90, cesium-137, cobalt-60, strontium-85, zinc-85, and iodine-131.

Strontium-90 concentrations have been measured in whole turtles and in shells (Scott et al. 1986, Towns 1987, Holcomb et al. 1971, Jackson et al. 1974). Dissection and analysis of various tissues revealed 99% of the whole body concentration of  $^{90}\text{Sr}$  was contained in the shell and bone (Towns 1987). This is expected because strontium is a chemical analog of calcium and, as such, should occur primarily in calcified tissues. Elimination rates for  $^{90}\text{Sr}$  in I. scripta were

seasonal and were highest during the spring breeding season (Scott et al. 1986). The average yearly biological half-life of  $^{90}\text{Sr}$  in T. scripta is approximately 365 days (Scott et al. 1986). This information indicates that once a turtle is contaminated-with  $^{90}\text{Sr}$ , several years are required before the majority of the radionuclide is eliminated or decays to another form.

Geographic differences were found in  $^{90}\text{Sr}$  concentrations of shells collected from numerous locations throughout the southeastern United States and an inverse correlation between size (age) and  $^{90}\text{Sr}$  concentration was detected in Terrapene carolina from Mississippi and Tennessee (Holcomb et al. 1971). The inverse correlation of age with  $^{90}\text{Sr}$  concentration, is in agreement with that reported in humans (Kulp 1965) and in deer (Farris et al. 1969). Rapid growth and deposition of calcium, and its chemical analog strontium, into bone and shell of juvenile turtles may result in higher concentrations of  $^{90}\text{Sr}$  in juveniles than adults. Correlations between age and  $^{90}\text{Sr}$  concentration were not found in T. Carolina collected from two other states or in other species collected from the region sampled (Holcomb et al. 1971); however, sample sizes were small (less than six) in the latter studies.

Differences in  $^{90}\text{Sr}$  concentrations between species have been reported and may be related to differences in **food habits**. A comparison of  $^{90}\text{Sr}$  in the herbivorous Gonherus polyphemus (gopher tortoise) to that in the omnivorous T. carolina revealed higher concentrations of the radionuclide in the former species (Holcomb et al. 1971). Because higher concentrations of  $^{90}\text{Sr}$  are usually detected in terrestrial plants than in non-calcified **animal tissues**, the herbivorous species would be expected to contain higher concentrations of  $^{90}\text{Sr}$  than the omnivorous, species. A **trophic** level difference was not observed among the aquatic species examined.

Strontium-90 concentrations detected in the **shells** of turtles sampled in several areas throughout the southeastern **United States were** found, in many cases, to be equivalent to or to exceed concentrations of  $^{90}\text{Sr}$  in the bones of black-tailed jackrabbits (Lepus californicus) collected 32 km north of the **Yucca Flat Nevada Test Site at Groom Valley** (Holcomb et al. 1971, Jackson et al. 1974). The only known source of

$^{90}\text{Sr}$  in the turtles' environments was from nuclear fallout. The ability of turtles to accumulate and apparently concentrate  $^{90}\text{Sr}$  from these areas suggests that the shells of turtles may be very useful in the detection of this radionuclide in the environment.

Cesium-137 has also been studied in turtles (Brungs 1967, Scott et al. 1986, Peters 1986, Towns 1987, Peters and Brisbin 1988). Analysis of various tissues from *T. scripta* collected from an area contaminated with  $^{137}\text{Cs}$  revealed that  $^{137}\text{Cs}$  was distributed throughout the body, however, the highest concentration of the radionuclide was in muscle (Towns 1987). The distribution of  $^{137}\text{Cs}$  in turtles is similar to that reported in mammals (Stribling et al. 1986). Seasonal variations have been reported in the elimination of  $^{137}\text{Cs}$  from *T. scripta* (Scott et al. 1986). Like  $^{90}\text{Sr}$ , elimination rates were highest during the spring through early summer months, which corresponded with the breeding season and the period of highest metabolic activity of the species (Scott et al. 1986). The elimination rate of  $^{137}\text{Cs}$  during the spring and summer months was approximately  $7.2 \times 10^{-3} \pm 4.4 \times 10^{-3}$  kBq/day (Peters and Brisbin 1988). Positive correlations exist between the weight of the turtle and the elimination rate, and between weight and the concentration of the radionuclide in the turtle (initial body burden) (Peters and Brisbin 1988). No relationship was found between elimination rate and the sex of the turtle (Peters and Brisbin 1988) which is consistent with that reported in birds (Fendley et al. 1977). Because  $^{137}\text{Cs}$  is a chemical analog of potassium and is not concentrated in hard tissues as is  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$  would be expected to have a much faster elimination rate than  $^{90}\text{Sr}$  and, consequently, a shorter biological half-life. The biological half-life of  $^{137}\text{Cs}$  in *T. scripta* is 64 days (Scott et al. 1986), several times less than the biological half-life of  $^{90}\text{Sr}$ . The time required for *T. scripta* to reach an equilibrium radiocesium concentration with its environment has been calculated to be 320 days (Peters and Brisbin 1988). A comparison of the biological half-life of  $^{137}\text{Cs}$  in turtles to that in several other taxa revealed that it was greater than that for birds (Fendley et al. 1977, Halford et al. 1983) and wild mammals (Jenkins et al. 1969). The longer retention of  $^{137}\text{Cs}$  in turtles is consistent with the slower metabolic rate in the

poikilothermic turtles. In general, the biological half-life of  $^{137}\text{Cs}$  is longer in poikilotherms than in homeotherms (Mailhot et al. 1989).

A limited amount of information exists on other **radionuclides in** turtles. An inverse relationship was reported to exist between the concentration of  $^{60}\text{Co}$  in an organism and its **trophic** level. Ophel and Fraser (1971) compared the concentration of  $^{60}\text{Co}$  in water (filtered with a **1.2- $\mu\text{m}$**  pore-sized filter, concentration in water based on **Bq/ml**) to that in plants, fish, reptiles, and amphibians from a contaminated pond. Concentration factors in-aquatic plants relative to water ranged from 270 to 2,790, whereas, concentrations in **whole** fish exceeded those in water by 9 to 130 times, and that in a whole ***C. seroentina*** was 90 times as great as the **concentration in water**. Although only one turtle was analyzed, it is apparent that primary producers concentrated  $^{60}\text{Co}$  to a greater extent than consumers. Turtles, there-fore, may not be the best **taxa** to use as an indicator of  $^{60}\text{Co}$  contamination.

Turtles have been shown to concentrate high concentrations of  $^{131}\text{I}$  into thyroid tissue (Shellabarger et al. 1956, Gibbs et al. 1964). In a laboratory study, adults were shown to retain a greater amount of the original dose than did juveniles (Gibbs et al. 1964). Turtles have not been used to monitor iodine in the environment. Among the vertebrates that inhabit wetlands, turtles (specifically adult turtles) may prove most useful in the assessment of radioiodine contamination (J. Eldridge, ORNL, personal communication). Turtles are long-lived and have larger thyroid glands than fish, factors that may facilitate the detection of the radionuclide. In addition, turtles have greater exposure to contaminants in water and sediment than do **birds or mammals**, which also supports the use of turtles as monitors of **radioiodine contamination**.

## 2.5 BIOCHEMICAL AND HISTOPATHOLOGICAL RESPONSES TO STRESS

Few turtle studies have been conducted that examine biochemical and tissue responses to chemical and physical perturbations. Laboratory studies have shown turtles to be more **tolerant to radiation than most** vertebrates (Atland et al. 1951, Cosgrove 1965, Cosgrove 1971). Atland et al. (1951) investigated the effects of whole-body irradiation on ***T. carolina*** with x-ray exposures ranging from 0.129 to 2.58 C/kg

(500 to 10,000 R). Turtles irradiated with as much as 0.258 C/kg (1000 R) showed 100% survival for four months, suggesting their ability to withstand higher acute doses than birds (Abraham 1972) and mammals (Dunaway et al. 1969). A partial explanation for the higher tolerance in turtles is probably the shielding effect of the shell which can reduce the average exposure by 21% (Cosgrove 1965). The low metabolic rate of turtles compared to mammals may also come into play. **LD<sub>50</sub>** values have been determined for turtles exposed to various intensities of x-rays. The **LD<sub>50(120)</sub>** values for three species of turtles were reported to range from 0.219 (Cosgrove 1965) to 0.267 C/kg (850 to 1035 R) for T. Carolina, to be slightly less than 0.258 C/kg (1000 R) for juvenile Chrysemys picta elegans (- T. scripta), and to be less than 0.206 C/kg (800 R) for juvenile C. seroentina (Cosgrove 1971). The principal effects observed were on blood forming tissues and reproductive organs (Atland et al. 1951, Cosgrove 1965). Species differences in responses to radiation indicate that juvenile C. seroentina may be more sensitive to radiation than the other two species thus, C. seroentina may be the turtle species of choice to detect genotoxic damage caused by radiation.

In a field study, Albers et al. (1986) revealed that blood and blood plasma characteristics may not be useful indicators of exposure to chemical contaminants in turtles. Differences in the levels of protein, albumin, and plasma glucose in C. seroentina may be attributed to differences in age and plasma glucose levels may be elevated by the stress of collection and handling. In addition, variations in the concentration of hemoglobin can be attributed to differences in the salinities of the waters from which the turtles were captured (Albers et al. 1986). This study places a cautionary note on the interpretation of results obtained from the use of non-specific biochemical indicators of stress.

Measurements of the DNA content in T. scripta collected from seepage basins containing radioactive and non-radioactive contaminants and from a reference site proved to be a very useful indicator of exposure to genotoxic agents in the environment (Bickham et al. 1988). Bickham et al. (1988) reported the only turtles to contain mosaic DNA

were four individuals from the contaminated site. A comparison of the coefficients of variation in DNA of turtles with normal DNA histograms (non-mosaics) revealed a significant difference between the DNA of turtles from the contaminated and reference sites. The higher coefficient of variation in turtles from the seepage basins may have been caused by mutations (deletions or duplications) in DNA induced by radiation and or chemical agents (Bickham et al. 1988). In addition, a positive correlation was detected **between** the coefficient of variation and plastron length in male turtles. The investigators proposed that larger, older turtles may be better indicators of environmentally induced mutagenic effects than younger turtles or shorter-lived species due to longer periods of exposure and the **accumulation** of mutations over time. The use of flow cytometry in the measurement of DNA content should be explored further and comparisons made with fish residing in contaminated waters to determine the sensitivity of turtle DNA to genotoxic agents.

## 2.6 **TURTLE GROWTH** RATES IN RELATION TO CONTAMINATION

Growth rates have been measured in turtles, however, in most instances, the data are inconclusive and differences in the growth of individual turtles cannot be attributed exclusively to contaminants in the environment. Growth rates may vary with age as noted by Gibbons (1968) and Kiviat (1980) who observed a lower growth rate in adult **C. serpentina** compared to juveniles collected from uncontaminated sites. Differences in growth rates may also be a response to the ingestion of nutrient deficient food items by turtles from one site (Albers et al. 1986). In addition, the diets of omnivorous species can **fluctuate with** the availability of foods resulting in higher growth rates in turtles,, with protein-rich diets (Knight and Gibbons 1968, Graham and Perkins 1976). In one study, the growth rates of four **T. Carolina** collected from a DDT-contaminated site were measured and compared to the growth rates of five **T. Carolina** from a reference site and found to **be very** similar (**Stickel** 1951). Although it appeared that the aerial application of DDT (from 1.24 to 2.25 **kg/ha**) over a four-year period did not impair the growth of individual turtles, the small sample size of

four turtles may not have presented a representative view of the entire population. Because of the inconclusive nature of growth rates as indicators of contaminant exposure, their use as biomarkers is discouraged.

## 2.7 DISCUSSION

A review of the literature on contaminants in turtles revealed several pertinent points. Both organochlorine pesticides and **PCBs** accumulate primarily in adipose tissue (e.g., Meeks 1968, Stone et al. 1980) and are biomagnified through food chains (e.g., Holcolmb and Parker 1979, Watson et al. 1985). Turtles contain large fat reserves in which chlorinated organics, especially **PCBs**, can accumulate at concentrations often exceeding those reported in mammals and birds and do so without apparent adverse effects on the turtles themselves (e.g., Watson et al. 1985, Olafsson et al. 1983). As such, turtles appear to be excellent monitors of **PCBs** and good monitors of organochlorine pesticides in the environment. There is a limited amount of evidence that PCB concentrations may be higher in male than in female turtles (Albers et al. 1986). This may be due to the transference of chlorinated organics from gravid females to the yolks of their eggs (e.g., Bryan et al. 1987b, Flickenger and King 1972).

Several metals have been detected in turtles, most of which were measured in kidney and liver tissues where many metals concentrate (e.g., Albers et al. 1986). Bone and shell, however, were found to contain the highest concentrations of lead (Krajicek and Overman 1988). Sex-related differences in metal concentrations were detected on occasion (e.g., copper), but were not observed in all cases for a given metal (Albers et al. 1986). Non-essential and essential metals have also been detected in sea turtle eggs (Stoneburner et al. 1980, Hillestad et al. 1974), indicating that metals may be transferred from the female to the developing ovarian follicles within her.

The radionuclides <sup>90</sup>Sr and <sup>137</sup>Cs have received the greatest amount of study with regard to the interaction of turtles and radionuclide contaminants in the environment. Strontium-90 was reported to accumulate almost exclusively in bone and shell (Towns 1987) and, thus,

its long biological half-life of approximately one year was not unexpected. Concentrations of  $^{90}\text{Sr}$  have been reported in turtles from several sites with no known history of contamination that exceed those reported in the bones of mammals collected from a contaminated site 32 km from a nuclear weapons test range (Holcolmb et al. 1971, Jackson et al. 1974). The high concentrations of  $^{90}\text{Sr}$  in turtles suggest this **taxon** as an excellent monitor of  $^{90}\text{Sr}$  in the environment. Cesium-137, when present, is distributed throughout the body of a turtle; however, the highest concentrations occur **in muscle**. Cesium-137 has a much shorter biological half-life in turtles (64 days) than  $^{90}\text{Sr}$  (Scott et al. 1986). The elimination rates for both radionuclides fluctuate with seasonal changes in turtle activity (Scott et al. 1986). Turtles are more resistant to external radiation than mammals and amphibians, primarily due to the shielding effects of the shell (Cosgrove 1965). This characteristic also favors the use of turtles as monitors of environments contaminated with radionuclides.

Few studies have been performed on turtles that examine the usefulness of biochemical and cellular/tissue parameters as indicators of exposure to chemical contaminants. Positive responses are often correlated with factors other than the degree of contamination such as age, diet, and nutritional status, which render interpretation of the results difficult. One study (Bickham et al. **1988**), however, did present positive **results on the usefulness** of a biochemical, indicator on field collected turtles. A significantly greater amount of **variation in DNA content** was detected in turtles from a **site** contaminated with radioactive and nonradioactive contaminants than in **the DNA of turtles** from the reference site. Evidence of aneuploid **mosaicism was also found** in the DNA of turtles from the contaminated site. These positive findings encourage the testing of other **"biomarkers"** on turtles in the wild.

Growth has also been measured in **turtles as an indicator of** exposure to chemical contaminants (e.g., Albers et al. 1986). Growth rates have not been shown to, be **good indicators of chemical stress**. They are variable and differences between populations may be detected that are caused by factors other than the contaminant, such as the

availability of protein-rich foods, age, and health of the organism. For these reasons, growth rates are not recommended as a biomonitoring index to be used on turtles.

Alternatives exist to using the soft tissues of turtles in order to monitor the accumulation of certain environmental contaminants in turtles. Lead and  $^{90}\text{Sr}$  concentrate in bone and shell. Samples of marginal **scutes** of the carapace can be removed and used to assay for these contaminants without harming the turtle. A few milliliters of blood can also be removed from a turtle and analyzed for the activities of certain enzymes that respond to contaminants (e.g.,  **$\delta$ -aminolevulinic acid dehydrase** and acetyl cholinesterase) or to obtain DNA for analysis of genotoxic damage. In addition, most contaminants detected in adults are also present in the eggs, albeit at lower concentrations. Although eggs of most species are not easily located in the field, this alternative may be useful as in the monitoring of contaminants in the eggs of endangered sea turtles.

Three species of turtles are frequently used to detect contaminants in the environment. *Terrapene Carolina* is most often used in terrestrial investigations. It is omnivorous and thus has exposure to contaminants that concentrate in plants (e.g.,  $^{90}\text{Sr}$ ) and animal matter (e.g., chlorinated **organics**). With reference to freshwater species, *Chelvdra sernentina* has been used primarily to detect **PCBs**, organochlorine pesticides, and heavy metals, whereas *Trachemys scripta* has been used primarily in radionuclide investigations. *Trachemys scriota* and *C. seroentina* have different food and habitat preferences that may influence their exposure to certain chemical contaminants.

A final comment on the literature focuses on the lack of comparative information on contaminant concentrations in the water, soil or sediment, other biota, and turtles. Such information would aid in assessing the usefulness of turtles as indicators of environmental contamination and would assist in the determination of the routes by which turtles are exposed to specific contaminants. Data on the concentration and availability of chemical contaminants in the environment can also aid in the interpretation of results obtained from the use of biochemical and physiological indicators of stress in biota

from the site in question. Surveillance data on contaminants in turtles have much greater significance when concentrations in the **abiotic** environment are recorded.

### 3. SITE DESCRIPTIONS

#### 3.1 WHITE OAK LAKE

White Oak Lake, a 6.88 ha (Cox et al. in press) eutrophic lake located on the Department of Energy's Oak Ridge Reservation near Oak Ridge Tennessee, was utilized as the contaminated study site. The lake, which has an average depth of 0.64 m (Cox et al. in press), has been used as a settling basin for low-level radioactive and non-radioactive waste generated by Oak Ridge National Laboratory (ORNL) since 1943. Cesium-137, cobalt-60, strontium-90, and tritium contribute to most of the radioactivity within the lake. Ruthenium-106 and **trans-uranic** nuclides are also present in the lake at above background levels. Selenium-75 was detected in lake water during 1986 (Blaylock, B. G. and M. L. Frank, unpublished) and was not present in the lake prior to or after 1986. Water and sediment concentrations of the predominant radionuclide contaminants are presented in Table 1. Tritium is found in highest concentration (10,000 **Bq/l**) in the lake water followed by radiostrontium (4.8 **Bq/l**) (Rogers et al. 1988). Cesium-137 is the radionuclide of highest concentration in the lake sediment (14,700 **Bq/kg** wet weight, samples taken at a depth of 2.5 cm). Mercury is present in White Oak Lake sediment at between 3 and 5.9 **µg/g** dry weight (Hoffman et al. 1984). Elevated levels of polychlorinated biphenyls (**PCBs**) in the lake were evidenced by above background concentrations of the organic contaminant in tissues of fish from White Oak Lake (Southworth 1987).

White Oak Lake supports a variety of wildlife species. The most abundant fish species within the lake include carp (*Cyprinus carpio*), blue gill sunfish (*Lepomis macrochirus*), yellow bullhead (*Ictalurus natalis*) and gizzard shad (*Dorosoma cepedianum*) (Ryon et al. 1988). Common waterfowl that utilize the lake during most of the year are primarily mallards (*Anas platyrhynchos*), American coots (*Fulica americana*), wood ducks (*Aix sponsa*) and Canada geese (*Branta canadensis*). Mammalian species commonly encountered near the shore and upland from the lake are white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), muskrats (*Ondatra zibethica*) and white-footed mice (*Peromyscus leucopus*). With regard to lake vegetation, the

Table 1. Concentrations of predominant radionuclides in White Oak Lake

Radionuclide	Water <sup>a</sup> Bq/l	Sediment Bq/kg
<sup>137</sup> Cs	<0.4	14,700 <sup>b</sup>
<sup>60</sup> Co	1.8	4,900 <sup>b</sup>
Total Sr	4.8	1,360 <sup>b</sup>
<sup>3</sup> H	10,000	630 <sup>c</sup>

<sup>a</sup>Rogers *et al.* 1988.

<sup>b</sup>Concentration based on **wet** weight for samples taken at a depth of 2.5 cm, Oakes *et al.* 1982.

<sup>c</sup>Concentration based on dry weight, Blaylock and Frank 1979.

dominant emergent plants located along the lake margins and in shallow areas include sedges (Scirous sp.), cattails (**Typha latifolia**), smartweed (**Polygonum** sp.) and arrowhead (Saeittaria sp.). Floating and submergent macrophytes are less diverse and abundant than the emergent plants and are comprised primarily of duckweeds (Lemna minor and **Spirodilla** sp.) and water milfoil (**Myriophyllum** sp.), respectively.

### 3.2 **BEARDEN** CREEKEMBAYMENT

**Bearden** Creek embayment, an embayment on Melton Hill Reservoir, is located 5.23 km from White Oak Lake, was used as the reference site for this study (Figure 1). There is no documented history of contamination within or in close proximity to the embayment. Water enters the embayment via **Bearden** Creek and the Clinch River. The water level of **Bearden** Creek embayment reaches a maximum depth of 1.5 to 2 m and is indirectly controlled by the Tennessee Valley Authority as it regulates the flow of the Clinch River. Wildlife common to the marshy embayment include muskrats, white-tailed deer and raccoons. Wood ducks and mallards are frequently seen in the area. The vegetation along the margins of the embayment are similar to those in White Oak Lake. Water milfoil is the most common submergent aquatic macrophyte.

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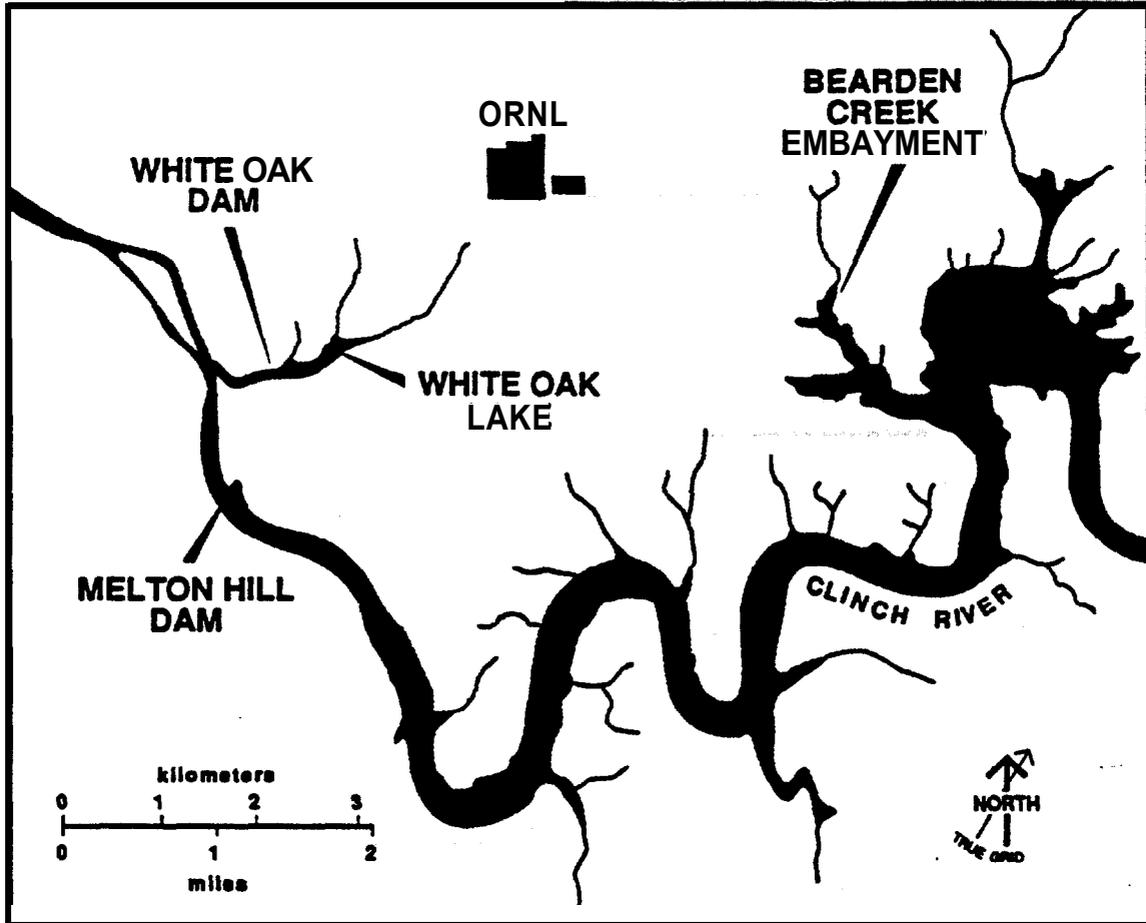


Fig. 1. White Oak Lake and **Bearden** Creek embayment in relation to Oak Ridge National Laboratory and the Clinch River.

#### 4. PRELIMINARY SURVEYS

##### 4.1 APPROACH

Initial reconnaissance of the study sites included the trapping of turtles to determine the species composition and abundance of each species in White Oak Lake and **Bearden** Creek embayment. Several individuals from White Oak Lake and a few from **Bearden** Creek embayment were marked and released at the site of capture. The mark and subsequent recapture of turtles within the site generated information on the movement of individuals and produced a rough estimate of the abundance and trapability of each species.

Data on the food habits of three species of White Oak Lake turtles were collected to determine differences in **trophic** levels among the species that inhabit White Oak Lake. These data were acquired by the examination of the gastrointestinal tract contents of sacrificed turtles. A criterion in the selection of the two species for study was that one be an herbivore and the other a carnivore. These survey studies were used to focus the study on two such species.

In order to determine the key tissues to be used for residue analyses, several turtles were trapped from White Oak Lake and analyzed for specific contaminants. Liver, muscle, bone, shell, fat and eggs were removed from these turtles to determine the distributions of radionuclide contaminants within the bodies of the animals. This was done to determine the key tissues to be used for residue analysis. Since **PCBs** have been reported in fish from White Oak Lake (Southworth **1987**), fat was removed from a few turtles to evaluate whether these organochlorines were contaminants of concern in the turtles.

This study required the selection of a reference site for comparison with White Oak Lake. **Bearden** Creek embayment appeared ecologically suitable, however, no data existed on contaminant concentrations within the area. Sediment samples were collected from **Bearden** Creek embayment to determine the concentrations of gamma emitting radionuclide contaminants, <sup>90</sup>Sr, and mercury in the embayment. Background concentrations of these contaminants would support the use of **Bearden** Creek embayment as a reference site.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Field Techniques

Turtles were trapped from White Oak Lake (June 4 to September 12, 1985, April 21 to August 14, **1986**, April 21 to October 7, 1987, and April 19 to May 16, 1988) and **Bearden** Creek embayment (August 1 to October 2, 1986, June 1 to October 7, 1987, April 14 to September 5, 1988) during seasons of peak turtle activity. No attempts were made to set traps during winter months, when turtles are inactive. Animals were captured using hoop nets, **0.625-cm** wire-mesh funnel traps, **2.5-cm wire-mesh** box traps (Tomahawk Company, Tomahawk, Wisconsin), and trotlines. Traps were usually baited with either rainbow trout or beef enclosed in wire-mesh bait packets. Pork liver, sardines, bluegill sunfish, shad and watermelon rinds were used on occasion. Commercial **fish attractants** were used during September 1987 and were not found to be useful in attracting turtles to the trotlines or to the traps. Baited traps were placed near the shore in water approximately 60 cm deep. Trotlines were only used in White Oak Lake during the 1986 and 1987 field seasons. Two to four lines which were strung across the lake were also checked daily.

Turtles were marked for recapture using a method developed by J. W. Gibbons of Savannah River Ecology Laboratory (Gibbons 1988). Each marginal **scute** on the carapace of the turtle was designated by a letter of the alphabet as shown in Fig. 2. A battery powered hand drill was used to put a small hole into one or more of the outer **scutes**. Each individual of a given species was marked with a unique code (i.e., A, AV, or AVK). In addition, the pigmented plastron patterns of ***T. scripta*** and ***Chrysemys picta*** were photocopied (by placing turtles on a clear sheet of plastic over the glass plate of a photocopying machine and covering the turtle with a towel to block out the light) to obtain supplemental diagnostic information (Gibbons 1986) to positively identify individuals.

Locations of the recaptured turtles were noted and used to map turtle movement within the lake. Turtles that were marked and released during the 1985 and 1986 field seasons and later recaptured were taken

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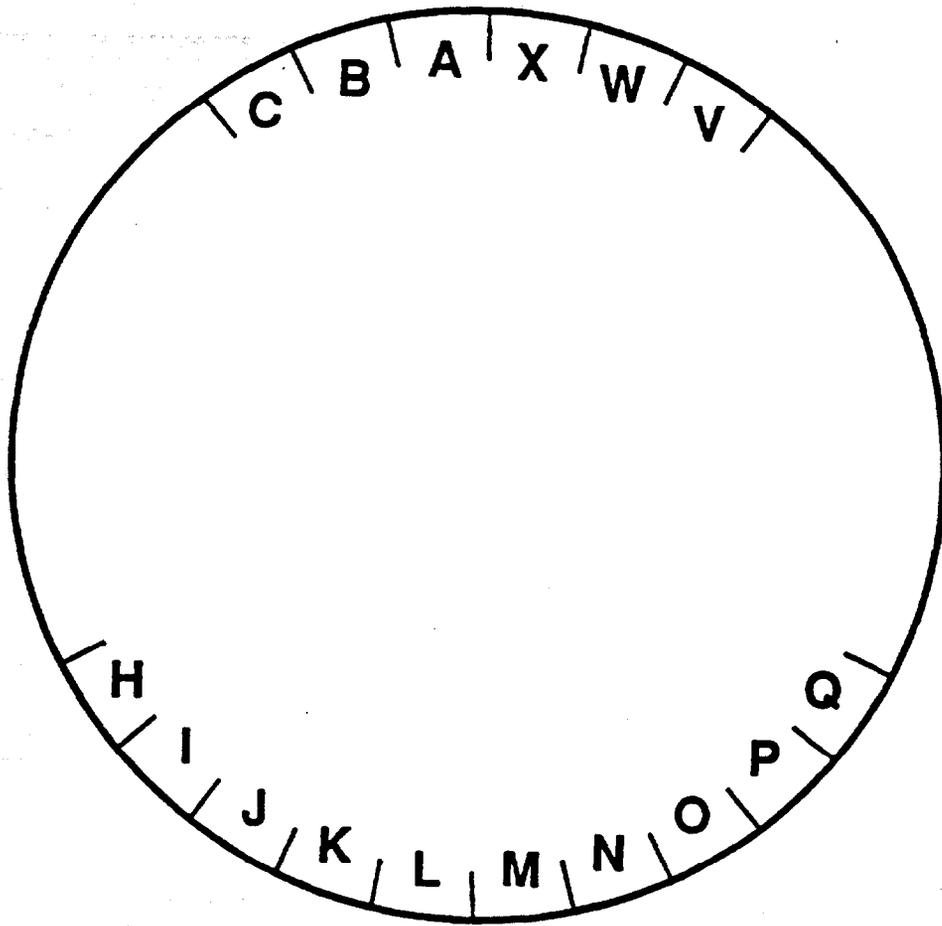


Fig. 2. Outer **scutes** of a *Trachemys scripta* carapace (ventral view) and the letters used to **designate** them.

to the laboratory, identified, weighed, and photocopied as appropriate. These turtles were released at the site of capture.

#### 4.2.2 Turtle Dissection and Sample Preparation

In order to evaluate the exposure of White Oak Lake turtles to contaminants, seven individuals that **were trapped** from the lake and not designated for mark-recapture study were taken to the laboratory for chemical and radiological analyses. Turtles were frozen and later dissected to obtain tissue samples. Samples of bone, muscle, plastron, carapace, liver, fat, and various internal organs were removed from the turtles. Carapace and plastron samples were scrubbed with a brush, scraped with a scalpel, and rinsed with tap water to remove any algae attached to the shell. The GI tracts of the animals were also removed, emptied of their contents, and the tissue washed with tap water to remove any ingested matter. All tissue samples were placed into glass jars and stored in the freezer for later analysis of radionuclides and **PCBs**.

The GI tract contents of several turtles were retained and examined to determine the diet composition. Stomach and intestinal contents were placed into labeled glass jars containing 70% ethanol. The contents of the jars were examined under a dissecting microscope and a record was made **of** the percent volume of each component in the diet for each sample.

#### 4.2.3 Sediment Collection and Preparation

Radiochemical and mercury analyses were made on sediment samples from **Bearden** Creek embayment to justify the use of the **embayment** as a reference site. Three surface grab samples taken on October 7, 1987, and six surface samples (approximately 5 cm deep) taken on January 31, 1987, were placed into plastic bags and brought back to the laboratory for processing. The sediment samples were air dried for approximately one week to remove excess water and **then oven-dried** for two days at 100 °C. Dried samples were weighed, sieved through a U.S. Standard sieve, series No. 20 (840  $\mu\text{m}$  openings), reweighed, and stored in either glass vials or plastic petri dishes for subsequent residue analyses.

#### 4.2.4 Radionuclide Analysis

Frozen tissue samples and oven-dried sediment samples were screened for gamma radioactivity using a gamma ray spectrometer (Nuclear Data Inc. 6620 microprocessor) coupled to an intrinsic germanium-lithium detector having a relative efficiency of 25% and a resolution of 1.8 keV for the  $^{60}\text{Co}$  1332 photon. Containers of predetermined counting geometry were filled with either specific tissues or sediment and placed into a lead encased detector. Samples were counted until error terms of less than or equal to 10% were obtained. Activities were corrected to the date of capture for each animal to adjust for the radioactive decay of the radionuclides.

Sediment samples were analyzed for  $^{90}\text{Sr}$  by the ORNL Analytical Chemistry Division. The procedure used required the leaching and digestion of the sample in nitric acid followed by the addition of a strontium carrier solution. Several steps follow that result in the removal of iron, radium, and barium from the sample. The strontium is isolated in a powder form that is placed into a planchet and counted for beta radioactivity.

#### 4.2.5 Polychlorinated Biphenyl Analysis

Fat samples from White Oak Lake turtles were analyzed for PCB concentrations using a two part procedure. The initial step is the extraction of **PCBs** from a 10-g sample of tissue via Soxhlet extraction. A packed column gas **chromatograph** with an electron capture detector is then used to separate and quantify the **PCBs**. The analysis of tissue samples for **PCBs** was performed by the Oak Ridge National Laboratory's Analytical Chemistry Division.

#### 4.2.6 **Mercury** Analysis

The oven-dried, sieved sediment samples from **Bearden** Creek embayment were analyzed for total mercury by the ORNL Analytical Chemistry Division. The protocol included digestion of the samples in nitric and perchloric acids followed by the addition of stannous

chloride to reduce the mercury. Total inorganic and organic mercury content was determined by cold vapor atomic absorption spectroscopy.

#### 4.3 RESULTS

##### 4.3.1 Diversity and Abundance of Turtles

White Oak Lake has a high diversity of turtles as evidenced by the capture of a total of 137 turtles of six species (Table 2). Trachemys scripta (Agassiz) (yellow-bellied slider) was the most abundant species captured. Twice as many T. scripta were captured than Chelydra sernentina (Linnaeus) (common snapping turtle), the second most abundant species. Trachemys scripta and C. sernentina comprised 53% and 26% of the turtles trapped, respectively. Other species in the lake included Chrysemys picta (Schnider) (painted turtle), Trionyx sniniferus (Le Sueur) (Eastern spiny softshell), Sternotherus odoratus (Latreille) (stinkpot), and Graptemys geographica (Le Sueur) (map turtle).

**Bearden** Creek embayment has a slightly higher diversity of turtles than White Oak Lake. A total of 247 turtles of 7 species were trapped from the embayment (Table 3). Trachemys scripta was once again the most abundant species and constituted 75% of the total number trapped. Chelydra sernentina constituted 9% of the total followed in abundance by T. s. sniniferus (7%). Species of lesser abundance included S. odoratus and C. picta. Two species found in **Bearden** Creek embayment but **not in** White Oak Lake were Sternotherus minor peltifer (Smith and Glass, stripe-necked musk turtle) and Chrysemys concinna concinna (Le Conte, river **cooter**). Musk turtles are notoriously difficult to trap. The S. m. peltifer caught may have been stimulated to roam more than usual due to the rapid and extreme decline in the water level during the period of their capture and were thus more likely to be trapped. The range of C. c. concinna is not reported to expand into eastern Tennessee. **In** the past, this species was sold in pet stores and it is possible that the individual trapped had been released into the embayment or adjacent Clinch River (A. S. Echternacht, University of Tennessee, personal communication). The fact that C. geographica was captured in White Oak Lake and not in **Bearden** Creek embayment may be because C. geographica is

Table 2. Capture frequency of White Oak Lake turtles collected from 1985 through 1988

Species	Total captures	Disposition			
		Marked	Recaptured	Not marked	
				Killed	Released
<b><i>Trachemys scripta</i></b>	73	15	8	24	26
<i>Chelydra</i> <b><i>serpentina</i></b>	35	0	0	16	19
<b><i>Chrysemys</i>, <i>picta</i></b>	16	8	2	1	5
<b><i>Trionyx spiniferus</i></b>	7	0	0	6	1
<i>Stemotheris</i> <i>odoratus</i>	<b>5</b>	0	0	3	2
<b><i>Graptemys</i> <i>geographica</i></b>	1	0	0	1	0

Table 3. Capture frequency of **Bearden** Creek embayment turtles collected from **1986 through 1988**

<b>Species</b>	Total captures	<b>Disposition</b>			
		<b>Marked</b>	Recaptured	<b>Not marked</b>	
				<b>Killed</b>	<b>Released</b>
<b><i>Trachemys scripta</i></b>	<b>185</b>	<b>4</b>	1	14	166
<i>Chelydra</i> <b><i>serpentina</i></b>	23	<b>0</b>	0	<b>10</b>	13
<i>Trionyx</i> <b><i>spiniferus</i></b>	18	0	<b>0</b>	2	16
<i>Stemotherus</i> <i>odoratus</i>	12	0	0	4	8
<b><i>Chrysemys pfecta</i></b>	6	<b>1</b>	0	2	3
<b><i>Sternotherus</i></b> <i>minor peltifer</i>	<b>2</b>	0	0	0	2
<b><i>Chrysemys concinna</i></b> <i>concinna</i>	1	0	0	1	0

reported to prefer large, permanent bodies of water, such that lakes are preferred over ponds (or embayments) (**Conant** 1975).

#### 4.3.2 Turtle Ranges From Mark-Recapture Data

Both **T. scrinta** and **C. picta** were found to be very mobile within White Oak Lake. The average recapture distance of **T. scrinta** was approximately 125 m within a year (Table 4). **Chrvsemvs picta** appeared to be more mobile than **T. scrinta**. The recapture distances for two **C. picta** were 244 m and 275 m over a 16-month and 6-month period, respectively (Table 4). Only one of the four **T. scrinta** that was marked in **Bearden** Creek embayment was recaptured. This individual was marked and released on August 1, 1986, and recaptured less than 3 m away on October 2 of the same year.

#### 4.3.3 Food Habits of Selected Turtles

The degree of **carnivory** was evaluated for three species of turtles that have been reported to consume large proportions of animal matter. Examination of the gastrointestinal contents of four **T. s. spiniferus** revealed the ingestion of primarily fish and crustaceans (88 to 90% by volume of the diet) by this species (Table 5). The only **C. geographica** examined appeared to have fed exclusively on snails (Table 5). The diet of **C. serpentina** was not easily discernible from the GI tract contents of the individual examined. This turtle had a large amount of detritus and sediment (95%) in its gut with very few identifiable food items (Table 5). This may indicate that the turtle had fed on carrion and incidentally ingested large amounts of sediment and detritus in the process. Parasitic roundworms were often abundant in the GI tracts of the turtles.

#### 4.3.4 Radionuclides in Turtles

Only three gamma emitting radionuclide contaminants were detected in the tissues of the turtles (one **C. serpentina** and two **T. s. spiniferus**) captured from White Oak Lake (Tables 6-8). Cesium-137 was detected in greatest concentration followed by <sup>60</sup>Co and <sup>75</sup>Se. Of the tissues examined in these preliminary studies, the highest concentration

Table 4. Recapture data for White Oak Lake turtles

Species	Sex	Capture date	Recapture distance (m)	Whole body weight (g)	Change in weight (g)
<i>Chrysemys picta</i>	F	5/6/86 9/10/87	244	139 NR*	
	M	8/14/86 2/26/87	275	236 251	+15
<i>Trachemys scripta</i>	F	6/18/86 7/21/86	84	197 274	+77
	F	9/12/85 7/21/8	<3	NR 140	
	M	9/12/85 7/21/86	213	NR 222	
	M	9/12/85 7/21/86	76	NR 260	
		9/3/87	267	364	+104
		9/11/87	<3	353	-11
		9/23/87	<3	NR	
M	6/25/86 9/10/87	73	404 456	+52	

\*Weight was not recorded.

Table 5. Gastrointestinal contents of individual turtles collected from White Oak Lake from June to August 1986

Species	<b>GI tract contents (% volume)</b>					Detritus and sediment
	Fish	<b>Snails</b>	<b>Crayfish</b>	Insects	Vegetation	
<b>Graptemys</b> <i>geographica</i>	0	100	0	0	0	0
<b>Chelydra</b> <i>serpentifera</i>	3	1.0	0	0.5	<b>0.5</b>	9s
<i>Trionyx spiniferus</i> <i>spiniferus</i>	0	0	88	10	2	0
<i>Trionyx spiniferus</i> <b>spiniferus</b>	0	0	9s	3	1	1
<b>Trionyx spiniferus</b> <i>spiniferus</i>	97	0	0	0	0	3
<i>Trionyx spiniferus</i> <b>spiniferus</b>	98	0	0	0	2	0

Table 6. Cesium-137 concentrations in tissues of three turtles  
from White Oak Lake

Species	<sup>137</sup> Cs (Bq/kg wet wt.)							
	Carapace	Plastron	Muscle	Bone	G.I. tract	Egg yolks	Liver	Fat
<i>Chelydra serpentine</i>	173	160	222	124	113	69.3	83.7	14.8
<i>Trionyx spiniferus</i> spiniferus A	70.4	— <sup>a</sup>	478	161	197	198	267	65.5
<i>Trionyx spiniferus</i> <i>spiniferus</i> B	48.5	127	252	160	156	96.7	134	33.7

<sup>a</sup>Not analyzed.

Table 7. Cobalt-60 concentrations in tissues of three turtles  
from White Oak Lake

species	<sup>60</sup> C (Bq/kg, wet wt.)							
	Carapace	Plastron	Muscle	Bone	G.I. tract	Egg yolks	Liver	Fat
<i>Chelydra serpentina</i>	26.7	21.5	n.d. <sup>b</sup>	5.6	11.1	69.3	83.7	14.8
<i>Trionyx spiniferus spiniferus A</i>	11.4	— <sup>a</sup>	5.2	5.6	n.d.	32.6	35.9	n.d.
<i>Trionyx spiniferus spiniferus B</i>	10.4	20.4	n.d.	11.9	24.4	20.7	38.5	6.7

<sup>a</sup>Not analyzed.

<sup>b</sup>n.d. = <3.7 Bq/kg wet weight.

Table 8. Selenium-75 concentrations in tissues of three turtles from White Oak Lake

Species	<sup>75</sup> Se (Bq/kg. wet wt.)							
	Carapace	Plastron	Muscle	Bone	G.I. tract	Egg yolks	Liver	Fat
<i>Chelydra serpentina</i>	n.d.	n.d.	n.d.	n.d.	n.d.	27.0	n.d.	n.d.
<i>Trionyx spiniferus spiniferus A</i>	n.d.	— <sup>b</sup>	n.d.	n.d.	n.d.	24.1	n.d.	n.d.
<i>Trionyx spiniferus spiniferus B</i>	n.d.	n.d.	n.d.	n.d.	n.d.	25.2	18.5	n.d.

<sup>a</sup>n.d. = <3.7 Bq/kg wet weight.

<sup>b</sup>Not analyzed.

of  $^{137}\text{Cs}$  was present in muscle tissue where concentrations ranged from 222 to 478 Bq/kg (wet weight) for the three turtles. The highest concentration of  $^{60}\text{Co}$  was detected in liver. Concentrations of the radionuclide in the livers of the three turtles ranged from 35.9 to 83.7 Bq/kg (wet weight). Egg yolks from premature eggs within the turtles contained the largest concentration of  $^{75}\text{Se}$  at concentrations of approximately 25 Bq/kg (wet weight) for each of the turtles examined.

A comparison of the tissue distribution of  $^{137}\text{Cs}$  in the turtles revealed both similarities and differences between the two species. Muscle tissue contained the highest concentration of the radionuclide, this was true for both T. s. sniniferus and C. sernentina. Concentrations of  $^{137}\text{Cs}$  in the carapace and plastron of C. sernentina were approximately 75% of that detected in muscle. Cesium-137 in the shell (relative to muscle) of T. s. sniniferus was lower than that detected in C. sernentina. In addition, concentrations of the radionuclide were higher in the plastron than in the carapace of T. s. sniniferus. This discrepancy is explained by differences in the composition of the two shell types. In T. s. sniniferus the carapace contains a greater amount of soft tissue and is much more pliable than the plastron. In C. sernentina, as in most other freshwater and terrestrial turtles, the carapace and plastron are hard, bony tissues of similar consistency and texture. Apparently,  $^{137}\text{Cs}$  has a higher affinity for calcified shell than for fleshy, noncalcified shell types. The relative concentration of  $^{137}\text{Cs}$  in bone compared to muscle was 0.56 in C. sernentina and averaged 0.49 in T. s. sniniferus. No trend was observed between the concentrations of the radionuclide in bone and plastron. Cesium-137 was detected in all soft tissues analyzed (Table 6). Concentrations of the radionuclide in liver relative to muscle were 0.38 for C. sernentina and 0.40 for T. s. sniniferus. The fraction of  $^{137}\text{Cs}$  detected in the emptied GI tracts was approximately 0.52 in both C. sernentina and T. s. sniniferus. Fat contained less than 15% of the  $^{137}\text{Cs}$  reported in muscle for both species analyzed. Egg yolks from C. sernentina contained 31% of the concentration detected in muscle. The percentage in the egg yolks of T. s. sniniferus averaged 40%.

Most of the  $^{60}\text{Co}$  detected in the tissues of the turtles was contained within **three body** compartments; the kidney, liver, and egg yolks (Table 7). Kidney tissue from one T. s. sasiniferus contained 53.3 Bq/kg (wet weight) of  $^{60}\text{Co}$ ; 14.8 Bq/kg (wet weight) greater than that detected in the liver of the same turtle. Unfortunately, kidney tissue was not removed from the **other turtles** and counted for **gamma** radioactivity. Liver was subsequently used as the reference tissue for  $^{60}\text{Co}$  because it contained the **second highest** concentration of the radionuclide and was analyzed in each of the **three** turtles.

Unlike  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$  was not uniformly distributed throughout all tissues. Very little, if any,  $^{60}\text{Co}$  was detected in muscle. Concentrations of the **radionuclide were much** less in bone than in shell. Shell from C. sernentina contained approximately 29% of the concentration detected in liver, and bone contained only 7%. As was true for  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$  concentrations differed in the plastron and carapace of T. s. sasiniferus. The fraction of the radionuclide **in plastron** and carapace relative to liver was 0.53 and 0.27. Bone from this species contained 24% of that detected in liver. Concentrations of  $^{60}\text{Co}$  in the gastrointestinal tracts varied from 13% of that in liver for C. sernentina to zero and 63% for the two T. s. sasiniferus. This variation may be explained by differences in the thoroughness of washing the GI tract. The **concentration of  $^{60}\text{Co}$**  in the ingested matter present in the gut prior to cleaning would also be reflected in **the** concentration of the radionuclide in the GI tract lining. Finally, egg yolks in the turtles contained a large fraction of  $^{60}\text{Co}$  relative to that **in liver**. The fraction present in **the egg yolks** of C. sernentina was 0.83 compared to 0.73 in the egg yolks of T. s. sasiniferus.

Selenium-75 was **concentrated in** the egg yolks of both C. sernentina and T. s. sasiniferus (Table 8). Concentrations **of the radionuclide in** the egg yolks of the turtles ranged from 24.1 to 27.0 Bq/kg (wet weight). The liver of one T. s. sasiniferus also contained  $^{75}\text{Se}$  at a concentration of 18.5 Bq/kg (wet weight). No other tissues were **found** to contain the radionuclide.

#### 4.3.5 Polychlorinated Biphenyls in Turtle Fat

Both PCB-1254 and PCB-1260 were detected in fat samples from *T. scripta* and *T. s. spiniferus* collected from White Oak Lake (Table 9). Concentrations of the PCBs appeared higher in *T. s. spiniferus* than in *T. scripta*. Fat tissue from a *T. scripta* collected from Bearden Creek embayment on September 1, 1987, was also analyzed for PCBs and found to contain 0.01  $\mu\text{g/g}$  PCB-1260 (wet weight) and 72  $\mu\text{g/g}$  PCB-1260 (wet weight). The low concentration of PCB-1254 detected in the *T. scripta* collected from White Oak Lake on July 27, 1987, and the high concentration of PCB-1260 in the Bearden Creek embayment turtle were unexpected. (Above background concentrations have been reported in fish from White Oak Lake (Southworth 1987), therefore elevated concentrations were expected in turtles from the lake.) Cross-contamination of the three 1986 *T. s. spiniferus* samples with other samples occurred during the tissue homogenization process (B. Grant, ORNL Analytical Chemistry Division, personal communication). The possibility therefore exists that the two *T. scripta* samples in question may have also been cross-contaminated. These data are not adequate to speculate on the relative concentrations of PCBs at the two sites or on differences in PCB concentrations in the resident turtle populations.

#### 4.3.6 Contaminants in Bearden Creek Embayment Sediment

Sediment samples taken from Bearden Creek embayment were found to contain background concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$  and mercury (Appendix B). The concentration of  $^{90}\text{Sr}$  in the sediment was less than or equal to 12.8 Bq/kg (dry weight). Cesium-137 concentrations in the sediment ranged from non-detectable (<1.85 Bq/kg dry weight) to 4.77 Bq/kg (dry weight). Cobalt-60 was not detected (Cl.85 Bq/kg dry weight) in any of the six samples analyzed, nor were any other gamma emitting radionuclide contaminants present. Total inorganic and organic mercury concentrations in sediment ranged from 0.03 to 1.13  $\mu\text{g/g}$  (dry weight) with a mean of  $0.19 \pm 0.36 \mu\text{g/g}$  (dry weight). Only one of nine samples contained an above background concentration of mercury (>1.0  $\mu\text{g/g}$ ).

Table 9. Polychlorinated biphenyl concentrations in fat tissue of turtles from White Oak Lake

Species	Date of capture	PCB ( $\mu\text{g/g}$ wet wt)	
		PCB-1254	PCB-1260
<i>Trachemys scripta</i>	7/27/87	<0.01	49
	8/11/87	45	49
<i>Trionyx spiniferus</i> <i>spiniferus</i>	9/10/87	82	86
	8/7/86	62	77
	8/8/86	45	52
	7/21/86	60	72

#### 4.4 DISCUSSION

The sampling of turtles from White Oak Lake and **Bearden** Creek embayment revealed the sites as having a high diversity of turtle species. Based on frequency of capture, the most abundant species in both areas was T. *scriota*. This species comprised 53% of the turtles trapped in White Oak Lake and 75% of the total number of turtles trapped in **Bearden** Creek embayment. The second most abundant turtle (based on trapping frequency) from both sites was C. *sernentina*. Although much less abundant than T. *scrinta*, this species constituted 26% of the turtles trapped in White Oak Lake and 9% of the turtles trapped in **Bearden** Creek embayment. Other species trapped from both sites included T. *s. sniniferus*, S. *odoratus* and C. *picta*. Based on abundance alone, T. *scrinta* and C. *seroentina* are excellent candidates for future ecotoxicological study.

Iwo highly aquatic turtle species, T. *scrinta* and C. *picta*, were found to be very mobile within White Oak Lake, Based on recapture data, individuals of both species traveled an average distance of over 100 m within a year. This provided evidence that these species are able to integrate contaminant exposure over time and space. Because of their mobility throughout the lake, it is reasonable to use existing data on average concentrations of contaminants in White Oak Lake water and sediment as indicators of turtle exposures rather than obtaining data from the precise point of capture for each animal. The mobility of T. *scrinta* throughout White Oak Lake supports the use of this species in a monitoring investigation.

Data obtained on the food habits of T. *s. sniniferus* and G. *geographica* revealed these species as almost exclusively carnivorous. Examination of the GI tract contents of an individual C. *sernentina* did not prove conclusive. The consumption of sediment and detritus by this turtle indicated that it may have fed on carrion. Based on the limited data obtained on the feeding habits of these species, T. *s. soiniferus* may be a useful species to detect contaminants that are biomagnified in aquatic ecosystems.

One purpose for the collection of preliminary data on turtles from White Oak Lake and **Bearden** Creek embayment was to obtain information

that would aid in focusing the study on two species of turtles that occupy different ecological niches within the aquatic sites. The species selected should **meet the following** criteria: (1) they are abundant in both study sites, (2) one species is primarily herbivorous and the other primarily carnivorous, and (3) differences **in** specific habitat types within a given aquatic site would also be useful.

Trachemys scripta was the most abundant species in both **White Oak Lake** and **Bearden** Creek embayment. According to the literature, adults of this species feed primarily on vegetation (Clark and Gibbons 1969).

Trachemys scripta is also a highly aquatic species (Carr 1952, Berry and Shine 1980). Chelydra serpentina, on the other hand, was the second most abundant species collected from the two sites. Although the data presented on the feeding habits of C. serpentina were inconclusive, **carnivory** in this species is well documented (Carr 1952, Hammer 1969). This species has been classified as a **"bottom-walking** species" because of its close association with the-sediment (Berry and Shine 1980). For these reasons, T. scripta and C. serpentina were selected as the species for further study of the influence of food habits and degree of sediment contact on exposure of turtles to **contaminants in White Oak Lake**.

Analysis of tissues from turtles collected from **White Oak Lake** showed specific tissue affinities for  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$  and  $^{75}\text{Se}$ . Of the tissues examined,  $^{137}\text{Cs}$  was detected in highest concentration in muscle. This **is** in agreement with the findings of Towns (1987) who reported  $^{137}\text{Cs}$  concentrations in T. scripta. Cobalt-60 was **detected** in highest concentration in kidney tissue; however, this is based on only one turtle. Other than kidney, liver also **contained relatively** high concentrations of  $^{60}\text{Co}$ . The distribution of  $^{60}\text{Co}$  in turtles **has not been** previously reported in the **literature**. In **avian** species, however, the **radionuclide** has been found to concentrate in the pancreas, liver and kidney (Koning et al. 1984). (Pancreas was not sampled in the turtles analyzed herein.) Selenium-75 had a very high affinity for the egg yolks within the gravid turtles. Published data on the **distribution of** this radionuclide **in other species** of turtles **is** lacking. It is suspected, however, that had the turtles not been gravid,  $^{75}\text{Se}$  would

have been concentrated in the liver and kidneys of the turtles as it is in birds (**Leonzio** et al. 1986) and mammals (Smith et al. 1937).

Although the analysis of turtle eggs can serve as an alternative method for monitoring radionuclide contaminants in the environment ( $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and  $^{75}\text{Se}$  were all detected in the eggs analyzed), the use of tissues from adult turtles is recommended. Turtle nests are difficult to locate and the egg-laying period only encompasses a small fraction of the year extending from late spring through the summer months. Adults are active from April through October in Tennessee. Data presented here on the distribution of gamma-emitting radionuclides in turtles were useful in the determination of key tissues for residue analyses. In the study that follows, muscle tissue was used for  $^{137}\text{Cs}$  analysis and liver for  $^{60}\text{Co}$  analysis (kidney tissue was reserved for mercury analysis). Unlike  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  with long physical half-lives of 30 and 5 years, respectively,  $^{75}\text{Se}$  has a half-life of only 120 days. Selenium-75 was detected in the White Oak Lake ecosystem during 1986. The release of the radionuclide ceased in the fall of that year. Due to the limited release of  $^{75}\text{Se}$  and its short physical half-life, this radionuclide was not designated as a major contaminant in the lake. It was not expected to be a radionuclide of concern during the 1987 and 1988 field seasons.

Polychlorinated biphenyls were detected in fat samples obtained from **T. scripta** and **T. s. sniniferus** collected from White Oak Lake:- Preliminary analysis showed high concentrations of **PCBs** in turtle fat (49 to 86  $\mu\text{g/g}$  PCB-1260); however, analytical errors make these values unreliable. If PCB concentrations in turtle fat are elevated ( $>2.0 \mu\text{g/g}$  wet weight), then steps should be taken to analyze muscle from White Oak Lake turtles to determine the degree of contamination and potential risk to humans who ingest the turtle meat.

A survey of contaminants in **Bearden** Creek embayment revealed the site as being relatively free of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and mercury. Sediment samples taken from the site contained only background **concentrations** of each of the above contaminants. Concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ , and mercury in sediment from **Bearden** Creek embayment were similar to those reported from noncontaminated sites in the vicinity of

the Oak Ridge reservation (Hoffman et al. 1984). Cobalt-60, unlike <sup>90</sup>Sr and <sup>137</sup>Cs, is not a product of nuclear fallout. Therefore the fact that <sup>60</sup>Co was not detected (< 1.85 Bq/kg) in the sediment samples is not unexpected. Results presented herein support the use of **Bearden Creek** embayment as a reference **site** to be compared with White Oak Lake.

## 5. TRACHEMYS SCRIPTA AND CHELYDRA SERPENTINA: A **COMPARATIVE** STUDY

### 5.1 NATURAL HISTORY

#### 5.1.1 Trachemys scripta (Agassiz)

The yellow-bellied slider turtle, Trachemys scripta, is a common turtle of the central and southeastern United States (**Conant** 1975). The turtle has a dark olive green to black carapace marked with vertical yellow bands. The plastron is yellow and marked with dark smudges; yellow stripes run along the legs and head of the turtle. Adults average from 12.5 to 20 cm in length (**Conant** 1975) with females generally larger than males. Females mature between six to ten years; males between three to five years (Gibbons et al. 1981). Females reach sexual maturity at a fixed age, whereas males reach maturity primarily at a fixed size (Gibbons et al. 1981). Adults follow a Type II survivorship curve with maximum longevity in the wild estimated at 30 years (Gibbons and Semlitsch 1982).

Trachemys scripta utilize a variety of habitats including small ponds, ditches, marshes, lakes, streams and rivers (Carr 1952, Cagle 1969, **Conant** 1975). They prefer shallow, still waters and are often the most abundant turtle species in such habitats (Cagle 1969, Congdon et al. 1986). This species has been classified into the category of "aquatic swimmer" because of its highly aquatic nature (Berry and Shine 1980). This species feeds in the water and moves within its habitat primarily by open-water swimming (Berry and Shine 1980). Trachemys scripta feeds on both plant and animal matter and is therefore considered omnivorous (Cagle 1969). There is evidence, however, that juveniles shift from primarily carnivorous to predominantly herbivorous in the adult stage (Clark and Gibbons 1969). The food habits of this species are largely explained by the age of the turtle and the type of food available (Clark and Gibbons 1969, Congdon et al. 1986).

The annual activity cycle of the turtle is regulated primarily by the water temperature. The species is active within a temperature range of **10°C** to **35°C** (Cagle 1969). At temperatures below 10°C turtles will hibernate in either mud or aquatic vegetation in shallow water (Neill 1948). Basking has been found to be an important thermoregulatory

behavior in *T. scripta* (Crawford et al. 1983). It also aids in the prevention of external **fungal** growths and in the **removal of** large ectoparasites (Cagle 1969).

Reproductive behavior and activity levels differ between sexes, such that males are more active than females in early spring and late autumn (Morreale et al. 1984). Females were found to have a more sedentary life style than males except during the nesting season (April through July) when females generally move greater **distances than males** (Morreale et al. 1984). Morreale et al. (1984) discovered that during early spring and late autumn males exhibited both terrestrial **and** aquatic movements of greater than one kilometer. No aggressive territoriality has been reported in male *T. scripta* (Gibbons et al. 1983).

The egg-laying period of *T. scripta* extends from April through mid July (Cagle 1969). During this time, females lay between five to eleven eggs (Congdon and Gibbons 1983) in each of one **to** three **clutches** (Cagle 1969). Gibbons et al. (1983) reported a reduction in reproductive output and an increase in emigration rates by females during drought conditions. Another factor that limits the number of young produced each year is mammalian predation. Such predators may destroy as many as 90% **of** the eggs in some nesting areas (Cagle 1969). The young turtles that do hatch either **leave the nest** in late fall or over winter in the ground and emerge the following summer (Cagle 1969).

#### 5.1.2 *Chelydra serpentina* (Linnaeus)

The common snapping turtle, *Chelydra serpentina*, is the most widely distributed turtle in North America, Their north-south range extends from southern Canada to the **Gulf** of Mexico (**Conant** 1975). They occur all along the eastern coast of the United States and extend as **far west** as the Rocky Mountains (**Conant** 1975). *Chelydra serpentina* is an aggressive turtle having a flattened, dark carapace and a comparatively small yellowish plastron. The turtle is equipped with a powerful jaw with a strong hook **at** its tip. The head is very dark in color as **are** the legs and tail. The tail is long and has **three rows of** tubercles (**Carr** 1952). The adult turtle is large, having a carapace length of between

20 to 30 cm (**Conant** 1975). The adults weigh between 4.5 to 16 kg and may reach a weight of 39 kg in captivity (**Conant** 1975). Males are generally larger than females (Mosimann and Bider 1960, Kiviat 1980). The sexes are not easily distinguishable in the field. There are no sexual dimorphisms in shell dimensions or in head width (Mosimann and Bider 1960). However, the distance from the plastron to the cloaca is longer in adult males than adult females (Mosimann and Bider 1980). The maximum longevity of this species in the wild has not been determined. Galbraith and Brooks (1989) reported 33.6 years as the mean age of 67 nesting females collected in a river system in Ontario, Canada. **This** species is suspected as living longer than **T. scripta**.

**Chelydra serpentina** utilize a variety of different habitats. **They** may be found in almost any permanent body of freshwater (**Conant** 1975) and may also be found in some brackish water habitats (Kiviat 1980). The species has a preference for muddy or soft substrates (Carr 1952). **Chelydra serpentina** has been categorized as a "bottom-walking species" because its primary means of locomotion is by walking and not swimming (Berry and Shine 1980). With reference to food habits, **C. serpentina** utilize small aquatic invertebrates, fish, reptiles, amphibians, birds, mammals, carrion and aquatic vegetation as food resources (Lagler 1943, Coulter 1957, **Conant** 1975). Although often classified as an omnivore, the majority of the turtle's diet usually consists of animal matter (Lagler 1943, Coulter 1957, and Congdon et al. 1986). Lagler (1943) examined the stomachs of 173 **C. serpentina** collected in Michigan and found an average of 63.8% of the diet (based on composition by volume) to be comprised of animal matter (35.3% fish, 19.6% carrion, 7.8% invertebrates and 1.1% other vertebrates).

Like **T. scripta**, the activity patterns of **C. serpentina** are largely influenced by temperature. Obbard and Brooks (1981) reported **C. serpentina** in Ontario, Canada to emerge from winter dormancy in early May when the water temperature reached 16 °C. This species rarely basks and spends much *of* its time buried in sediment in shallow water (**Conant** 1975). Differences in the thermoregulatory behavior patterns of **C. serpentina** and **T. scripta** may explain the differences in water temperature required to stimulate emergence from winter dormancy.

Chelvdra serpentina usually hibernate underwater either under a covering of mud or plant debris with several individuals congregated in one area (Carr 1952).

The size of the species' home range depends on the characteristics of the habitat, and the age and sex of the turtle (Kiviat 1980). Kiviat (1980) estimated immature C. serpentina to have a home range of 3.3 ha. Adult males and adult **non-nesting** females had home ranges of 8.9 ha and 7.2 ha, respectively. Nesting females are reported to wander a great deal out of their home-range even though suitable nesting sites may have been within close proximity (Kiviat 1980, Obbard and Brooks 1980). Nest site fidelity was observed by Obbard and Brooks (1980) who determined the mean round-trip distance traveled between the **home range** and nesting site as 10.6 km. Male-male aggression has been observed and there is some evidence that males may be territorial (Kiviat 1980).

Movement of C. serpentina within a lake or pond can contribute substantially to the disruption and resuspension of the sediment. Kiviat (1980) reported this species as disturbing at least one percent of the substrate in a bay to a depth of 15 cm by burrowing and from 20 to 25% of the substrate to a depth of 2 to 7 cm by treading, annually.

The breeding season for C. serpentina extends from April through November in the central and southern regions of North America (Carr 1952), and begins in June and ends in August or September in the northern United States (Kiviat 1980) and southern Canada (Obbard and Brooks 1980). The majority of female C. serpentina in the northern United States and southern **Canada nest** in June (Hammer 1969, Obbard and Brooks 1981, respectively) and lay an average of 25 eggs per clutch (Carr 1952). The incubation period for the eggs is between 91 and 125 days (Hammer 1969). Skunks (Spilogale putorius), raccoons (Procyon lotor), and mink (Mustela vison) are the major egg predators destroying as much as 59% of the nests within an area (Hammer 1969).

Chelvdra serpentina is of greater economic importance than perhaps any other species of turtle in **North America**. The species is actively hunted in some areas and the **meat from the turtles** prepared as steaks or used in soups or stews. Snapping turtle soup can be purchased by the

can in some grocery stores, and it is sometimes served at seafood restaurants.

## 5.2 MATERIALS **AND** METHODS

### 5.2.1 Field Techniques

Trachemys scripta was trapped in White Oak Lake from July 23 through September, 1987, and from **Bearden** Creek embayment from August 8 through September 16, 1987. Hoop nets and **0.6-cm** wire-mesh funnel traps were baited with either rainbow trout or pork liver contained in wire packets. The baited traps were placed in water to a depth of approximately 60 cm and checked each morning. A total of six male and six female unmarked **T. scripta** were collected from each site and dissected in the laboratory. Individuals were sacrificed by injecting 2.5 to 7.0 ml of a 10% solution of tricaine methane sulfonate, an anesthetic, into the neck before decapitation with a table-top cable cutter.

Chelydra serpentina was trapped from White Oak Lake from April 20 through May 15, 1988, and from **Bearden** Creek embayment from April 29 through July 12, 1988. Hoop nets baited with wire packets containing rainbow trout and beef were placed in water approximately 80 cm deep. Traps were set and checked daily. Attempts were made to capture six males and six females from each site; however the goal was not realized-twelve males were captured from White Oak Lake and six males and three females were trapped in **Bearden** Creek embayment. Trapping on **Bearden** Creek embayment was extended through September 2, 1988, but no **C. serpentina** were captured during this period. Captured **C. serpentina** were either decapitated or shot in the head using a 357 revolver with 0.38 caliber wadcutters.

### 5.2.2 Turtle Dissection and Sample Preparation

Individual **T. scripta** and **C. serpentina** were either dissected entirely in the laboratory (**T. scripta**) or partially in the field and partially in the laboratory (**C. serpentina**). Once dead, turtles were immediately dissected and a section of liver was removed and placed on ice for DNA analysis. Kidney and muscle samples were removed for

mercury analysis, and sections of **muscle and liver** were retained for determination of gamma radionuclides. **Samples** of bone (femur) and carapace designated for  $^{90}\text{Sr}$  analysis were removed from each of the turtles. Carapace samples were scrubbed with a brush, scraped with a scalpel, and rinsed with tap water to remove attached algae. Portions of fat were retained for future PCB analysis. All tissues, except the liver sample retained for DNA damage analysis, were placed in glass jars and stored in a freezer for later analyses. Analysis of liver samples designated for DNA damage assessment **was begun** immediately after removal.

The gastrointestinal contents of the turtles were retained and examined to establish the actual food habits of the two species at the study sites. **The** stomach and intestinal contents were **transferred to** glass jars to which a solution of **70% ethanol** was added as a preservative. The contents of the jars were examined under a dissecting microscope and the percent volume of fish, vegetation, insects, detritus, and rocks and mud was noted. The mean percent volume of components in the GI tracts of each species from both White Oak Lake and **Bearden** Creek embayment was converted to a weighted mean based on the total volume of GI tract contents examined for **each** turtle.

### 5.2.3 Radionuclide Analysis

Cesium-137 and  $^{60}\text{Co}$  were measured in frozen muscle and liver samples using a germanium-lithium (**GeLi**) crystal detector coupled to a gamma ray spectrometer (Nuclear Data Inc. 6620 microprocessor). Prior to counting, samples were thawed and placed in petri dishes of predetermined counting geometry. Samples within the petri dishes were refrozen and later placed into the **lead encased detector** to determine the activities of the gamma-emitting radionuclides present. Samples were counted for various lengths of time in order **to** obtain counting error terms of less **than or** equal to 10%. Samples that had nondetectable concentrations ( $< 3.7 \text{ Bq/kg}$ ) of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  were removed after two hours of counting time. All concentrations reported were **corrected to** the date **of** capture for each turtle. The limit of detection **was**  $3.7 \times 10^{-3} \text{ Bq/g}$ .

Bone and carapace samples were analyzed for  $^{90}\text{Sr}$  by Cerenkov counting following acid digestion of **ashed** samples. The actual radiation measured, the Cerenkov radiation effect, is produced by the high energy beta particles emitted by  $^{90}\text{Y}$ , a daughter product of  $^{90}\text{Sr}$  (Lauchli 1969, Larsen 1981). Bone and carapace samples were oven dried for two days at 100°C and then **ashed** in a muffle furnace at 550°C for one day. Weights of wet, dried and **ashed** samples were recorded. **Ashed** bone and carapace samples were ground to a fine powder using a mortar and pestle. Approximately 0.4 g of the **ashed** sample was digested in 4 M hydrochloric acid (enough to dissolve the sample) then heated to dryness. The sample was redissolved in 8 M nitric acid and once again heated to dryness. A few drops of 30% hydrogen peroxide were added to remove color. The sample was redissolved in approximately 10 drops of 4 M hydrochloric acid and transferred quantitatively to a **20-ml** plastic scintillation vial. Deionized water was added to make up the volume of all samples to 20 ml. Blanks contained deionized water and 10 drops of 4 M hydrochloric acid. The vials were counted for Cerenkov radiation in a Packard Tri-Carb scintillation spectrometer (Model 3002). The counting efficiency of the detector was determined by counting two  $^{90}\text{Sr}$  standard solutions each time a set of samples was counted. The concentration of  $^{90}\text{Sr}$  in each sample was corrected for the appropriate counting efficiency which ranged from 48 to 59%. The limit of detection was  $3.7 \times 10^{-2}$  Bq/g.

#### 5.2.4 Mercury Analysis

Frozen samples of kidney and muscle tissues from the turtles were analyzed for total mercury by the ORNL Analytical Chemistry Division. The procedure included the digestion of a 1-g (wet weight) sample in nitric and perchloric acids followed by the addition of stannous chloride to reduce the mercury. Total inorganic and organic mercury was determined by cold vapor atomic absorption spectroscopy.

#### 5.2.5 DNA Damage Analysis

DNA damage was used as a biochemical indicator of nonspecific exposure to genotoxic agents in the environment. An alkaline unwinding

technique (designed by Kanter and Schwartz **1982 and modified** by Shugart 1988) followed by a spectrofluorometric assay (outlined by Cesarone et al. 1979 and modified by Shugart 1988) was used to **measure single-** stranded breaks in DNA. Assessment of DNA damage by quantification of strand breaks has been tested on different tissue cells and, organisms as a means of determining damage to the DNA molecule **caused** by x-rays (**Ahnström** and Erixon 1973, Kanter and **Schwartz 1982**), and genotoxic compounds such as **benzo[a]pyrene** (Shugart **1988**), trichloroethylene (Nelson and Bull **1988**), the polychlorinated biphenyl Aroclor 1254 (Robbiano and **Pino 1981**), and-mercuric **chloride (Cantoni** and, Costa 1983). This assay was used to evaluate the integrity of DNA in turtles exposed to **contaminants in White Oak Lake** as compared to that in turtles from the reference *site*. This study illustrates one of **the earliest** attempts to test the DNA alkaline **unwinding** technique on populations exposed to genotoxic agents in the wild. The protocol outlined below is from Shugart 1988; modifications, however, were made **in the calculation** of strand breaks.

The initial step in the analysis of DNA **strand breaks is the** homogenization of the tissue. **All** reagents used for the assay were analytical grade or better. A **500-mg** sample of fresh turtle liver was placed into a ground glass homogenization tube (2 ml). The sample was kept on ice during the entire homogenization process. A cold (4 °C) 1-ml solution of 1 N ammoniumhydroxide in 0.2% **Triton X-100 was** added to the glass tube. Six to ten strokes were required to achieve complete homogenization. The homogenate was **transferred to a** plastic centrifuge tube with 2 ml of deionized water.

The sample was further processed (at 4 °C) by extracting the nucleic acids from the proteins. The extraction process included the addition of 6 ml of a chloroform/ isoamyl alcohol/phenol solution (**24/1/25 v/v**) to the centrifuge tube. The contents of the tube were mixed by inversion and allowed to stand for **15 minutes**. **The phases** were separated by placing the centrifuge tube into an **ultracentrifuge** at a setting of 16,000 rpm for 20 minutes at **4°C**. Following centrifugation, the aqueous phase (top layer) was removed with a **pipet** and transferred

to an Eppendorf vial. Vials were stored in a refrigerator at 4°C for up to a month.

The DNA was isolated by column chromatography. The sample contained within the Eppendorf tube was mixed by inversion, and 1.25 ml was placed onto a Sephadex G-50 column (1 cm internal diameter, 3.5 ml settled bed volume) equilibrated with G-50 buffer (150 mM sodium chloride; 10 mM Tris, pH 7.4; 1 mM magnesium chloride; and 0.5 mM EDTA). The sample was allowed to flow into the column, then 1.25 ml of G-50 buffer was added to the column and the eluate produced by this addition was collected in an Eppendorf vial. The eluate containing the DNA was stored in a refrigerator at 4 °C for no longer than 10 days. The concentration of DNA collected was estimated prior to the determination of strand breaks. A 25- $\mu$ l sample of the eluate (or 25  $\mu$ l of G-50 buffer for a blank) was placed into a test tube and 100  $\mu$ l of 0.1 N sodium hydroxide was added to the tube while vortexing. The tubes (sample and blank) were capped and placed into a test tube heating block set at 80 °C for one hour. A 0.2 M potassium phosphate buffer and Hoechst dye solution (Hoechst dye 33258, from Polyscience, Inc., 1 mg/ml of deionized water) was prepared by adding 1  $\mu$ l of Hoechst dye solution to 3 ml of buffer for every test tube. (The Hoechst dye complexes with both single-stranded DNA and double-stranded DNA, with greater enhancement of fluorescence occurring when the dye is bound to **double-stranded DNA**.) The dye-buffer solution was left in the dark at room temperature until use. After the samples had incubated for one hour, the test tubes were centrifuged at medium speed in a clinical centrifuge for one minute to concentrate all liquid into the bottom of the tubes. Once this was done, 3 ml of the dye-buffer solution was added to each test tube while vortexing. The test tubes were left in the dark at room temperature for 10 to 15 minutes. Fluorescence measurements of the samples were made using a Perkin-Elmer IS-5 fluorescence spectrophotometer set at excitation 360 nm and emission 450 nm. Fluorescence values recorded are measurements of the total DNA content of the sample (double-stranded DNA converted to single-stranded DNA). These values were compared to a standard DNA solution prepared from calf

**thymus** DNA (from Sigma Chemical Co., St. Louis, MO.) and adjusted to be within the range of the instrument.

The alkaline unwinding technique consists of three **subassays**, each performed in triplicate under specific **pH** and temperature conditions. In the double-stranded (ds) DNA sub-assay, the DNA is not **unwound or** denatured. The following reagents were added to each test tube; **50  $\mu$ l** of 0.05 N sodium hydroxide, **50  $\mu$ l** of 0.05 N hydrochloric acid, **5  $\mu$ l** of 0.2% sodium dodecyl sulfate (SDS) in **2 mM** EDTA (disodium ethylenediaminetetraacetate) and 3 ml of a potassium phosphate buffer-Hoechst dye solution (see previous paragraph) while vortexing. A **100- $\mu$ l** aliquot of the sample (or **100  $\mu$ l** of G-50 buffer for the blank) was added to the test tube while vortexing. The test tubes were immediately placed in the dark at room temperature for 15 minutes. **The fluorescence** was read as described in the previous paragraph.

The alkaline unwound (auw) DNA **subassay** causes partial unwinding of the DNA to expose single-stranded breaks. Fragments of **single-** stranded DNA are released from between **the breaks**, and the DNA is sheared to produce a combination of single-stranded and **double-stranded** DNA. Briefly, a **100- $\mu$ l** sample of the DNA (or **100  $\mu$ l** of G-50 buffer for the blank) was transferred to a test tube. While vortexing, **50  $\mu$ l** of 0.05 N sodium hydroxide was added. The sample was **vortexed** for 3 seconds. The test tubes were capped and incubated in a test tube heating block at **38 °C** for 30 minutes. This step results in the partial unwinding of the DNA thereby exposing single-stranded fragments of the DNA. Following incubation, the test tubes were centrifuged and **50  $\mu$ l** of 0.05 N hydrochloric acid added to the test, **tubes while** vortexing (for 3 seconds) to neutralize the solution and stop the unwinding process. To prevent the DNA from **reannealing** and clumping, **5  $\mu$ l** of 0.2% SDS in **2 mM** EDTA was added to **each test tube** and the DNA sheared by forcefully passing it through a 20-gauge syringe five times. Completion of this step was followed by the addition of 3 ml of the 'phosphate buffer-Hoechst dye solution to each test tube while vortexing. The test tubes were left in the dark at room temperature for 15 **minutes and the** fluorescence read as described earlier.

The single-stranded (ss) DNA **subassay** results in completely denaturing the DNA and recording the fluorescence. A **100- $\mu$ l** fraction of the sample (or 100  $\mu$ l of the G-50 buffer for the blank) was transferred to a test tube followed by the addition of 50  $\mu$ l of 0.05 N sodium hydroxide (while vortexing). The test tubes were then capped and incubated in a test tube heating block at 85 °C for 75 minutes. The DNA is completely unwound and denatured in this step. After the incubation period, the test tubes were centrifuged and 5  $\mu$ l of 2% SDS in 2 mM EDTA were added to the test tubes. Each of the samples were then forced through a **20-gauge** syringe five times to shear the DNA. Once sheared, 50  $\mu$ l of 0.05 N hydrochloric acid was added to each test tube, while vortexing the sample, to neutralize the base. The fluorescence of the samples was read following addition of 3 ml of potassium phosphate buffer-Hoechst dye solution to each test tube and incubation of the samples as described earlier.

To quantify the fraction of ds DNA present in each sample, the mean fluorescence values of the three replicates of each **subassay** (minus the blanks) were inserted into the following equation (Kanter and Schwartz 1982):

$$F = (\text{auwDNA value} - \text{ssDNA value}) / (\text{dsDNA value} - \text{ssDNA value}). \quad (1)$$

The fraction of ds DNA is related to the number of strand breaks (n) in the sample of DNA (expressed per alkaline unwinding unit, Kanter and Schwartz 1982). The relative number of breaks in the DNA of White Oak Lake **T. scripta** and **C. serpentina** was determined by using the fraction of **dsDNA** in **Bearden** Creek embayment (BCE) turtles of a given species as the control and the fraction of **dsDNA** in the White Oak Lake (WOL) turtles of the same species as the test population, such that

$$n = (\ln F_{\text{WOL}} / \ln F_{\text{BCE}}) - 1. \quad (2)$$

The single-stranded to double-stranded DNA ratio determined by this assay is both organ and species specific (based on the uniqueness of DNA for a given species and the binding specificity of the dye to DNA).

This ratio differed significantly (Student's t-test on **nontransformed** data,  $p < 0.001$ ) between sites for both ***T. scripta*** and ***C. serpentina***. Because this ratio differed, the possibility exists that a **contaminant** may have bound to the DNA of the turtles from White Oak Lake, thus altering the experimentally determined ratio. This problem was corrected for mathematically using a method **described by** Kanter and Schwartz (1982) whereby a new **dsDNA fluorescence value** is calculated based on the **ssDNA/dsDNA** ratio of the control population and the **ssDNA** fluorescence value of the experimental population. The corrected fluorescence values were used in the calculation of the fraction of double-stranded DNA **in White Oak** lake turtles.

#### 5.2.6 Statistical Tests

A variety of statistical **tests** were **used to evaluate** differences between and within sample sets. **Non-paired Student's t-tests** were performed on non-transformed and log-transformed data for most **site and** species comparisons. A Mann-Whitney U-test was used to compare data sets that were not in the form of a normal distribution. **A one-way** analysis of variance was used to **evaluate interactions** between contaminant concentration and turtle weight. A two-way analysis of variance (**ANOVA**) was used to test for interactions between the contaminant concentration, the animal's sex, and the site of capture.

Cesium-137 and <sup>60</sup>Co concentrations in turtles from **Bearden** Creek embayment were often below the limit of detection (which was set at  $3.7 \times 10^{-3}$  Bq/g for convenience). In order to **make** comparisons of the statistical significance of differences in the data, calculated minimum detectable activity values were used for samples below the limit of detection. The minimum detectable **activity** is the **amount of activity** that would have to be present in the sample in order for the detector to measure it.

### 5.3 RESULTS

#### 5.3.1 Food **Habits**

Examination of the GI tract **contents of *T. scripta*** from White Oak Lake and **Bearden** Creek embayment showed that the **turtles from the two**

sites feed primarily on plant matter (Table 10). Vegetation comprised  $93.1 \pm 7.0\%$  and  $86.0 \pm 59.4\%$  (weighted mean percent volume  $\pm$  weighted standard error of the mean) of the GI tract contents in turtles from White Oak Lake and **Bearden** Creek embayment, respectively. Trachemys scripta appeared to be an opportunistic feeder, consuming primarily vegetation in the water column and at the surface. Evidence of opportunism included the presence of fruits, seed heads, and algae in the GI tracts of the turtles. Cicadas (Homoptera: Cicadidae) were the dominant insect found in the GI tracts of T. scripta collected from **Bearden** Creek embayment. The **17-year** cicadas (Magicicada sp.) emerged in mass during the summer of 1987, and a large number of insects were probably floating on the surface of the water and consumed by both fish and turtles. Very little detritus and mud were found in the GI tracts of T. scripta from either site. Contact with contaminated sediment via ingestion is expected to be less in this species than in turtles that are primarily scavengers. Food habits data for individual T. scripta are listed in APPENDIX C.

Examination of the GI tract contents of C. serpentina confirmed that the species is omnivorous at both White Oak Lake and **Bearden** Creek embayment, however, the relative contribution of plants and animals to the diet differed between sites (Table 10). The percentage (weighted mean percent volume) of fish and of vegetation present in the GI tracts of the turtles differed between the two sites. A greater **percentage of** vegetation was ingested by C. serpentina from White Oak Lake ( $51.3 \pm 74.7\%$ , weighted mean percent volume  $\pm$  weighted standard error of the mean) than by the same species from the reference site ( $13.6 \pm 80.9\%$ ). The vegetation present in the GI tracts of C. serpentina from White Oak Lake was almost exclusively algae, whereas that in the turtles from **Bearden** Creek embayment were largely water milfoil (Myriophyllum sp.), a submergent aquatic macrophyte. Field observations indicated that algae was considerably more abundant in White Oak Lake than in **Bearden** Creek embayment. Although water milfoil was present at both sites, it was more abundant at the reference site. Chelydra serpentina may have fed preferentially on algae when **it** was available. Food habits data for individual C. serpentina are listed in APPENDIX C.

Table 10. Gastrointestinal tract contents of Trachemys scripta and Chelydra serpentina from the Oak Ridge reservation

	N	GI tract contents (% volume) <sup>a</sup>					
		Fish	Vegetation	Insects	Clams	Detritus	Mud and rocks
WhiteOak Lake							
<i>Trachemys scripta</i>	11	0.1	93.1	0.5	0	6.3	0.0
<i>Chelydra serpentina</i>	12	48.1	51.3	0	0	0	0.6
Btarden Creek embayment							
<i>Trachemys scripta</i>	12	3.2	86.0	8.8	0	2.0	0
<i>Chelydra serpentina</i>	9	83.7	13.6	0	0.2	0	2.5

<sup>a</sup>Weighted mean percent volume.

A comparison of the food habits of T. scripta and C. serpentina showed the former species to be more herbivorous (Table 10). Trachemys scripta from White Oak Lake consumed approximately 93% vegetation and less than 1% fish, whereas C. serpentina from the same site ingested 51% vegetation and 48% fish. The difference is even more striking when the two species from **Bearden** Creek embayment are compared (Table 10). At this site, T. scripta ingested approximately 86% vegetation and 3% fish. In contrast, C. serpentina from the same site consumed 14% vegetation and 84% fish. Because the diet of C. serpentina consists of a higher proportion of animal than plant matter, this species is likely *to* be a better monitor than T. scripta for contaminants that are biomagnified through food chains.

### 5.3.2 Cesium-137 and Cobalt-60

Cesium-137 and  $^{60}\text{Co}$  concentrations were greater in T. scripta from White Oak Lake than in those from **Bearden** Creek embayment (Tables 11 and 12). No relationship was detected between whole body weight and radionuclide concentration. In addition, a two-way ANOVA of these data did not reveal significant differences between males and females at each site, thus the data for both sexes were pooled at each site. Statistically significant differences between the two sites were found in the concentrations of  $^{137}\text{Cs}$  in both muscle and liver (Student's t-test on log-transformed data using minimum detectable activities for **Bearden** Creek embayment samples,  $p < 0.001$ ) and in the concentration of  $^{60}\text{Co}$  in liver (Student's t-test on nontransformed data using minimum detectable activity values for **Bearden** Creek embayment samples,  $p < 0.01$ ).

The mean concentration of  $^{137}\text{Cs}$  in muscle tissue of T. scripta from White Oak Lake was  $44.9 \pm 144$  Bq/g, wet weight. Concentrations of  $^{137}\text{Cs}$  in muscle tissues of individual T. scripta are listed in APPENDIXD. Two turtles from the lake had  $^{137}\text{Cs}$  concentrations that greatly exceeded those of the remaining 10 turtles. A male turtle collected on August 11, 1987, had a concentration of  $^{137}\text{Cs}$  in muscle of 502 Bq/g wet weight. Cobalt-60 was not detected ( $< 3.7 \times 10^{-3}$  Bq/g) in

Table 11. Cesium-137 in muscle tissue of Trachemys scripta and Chelydra serpentina from White Oak Lake

Species	N	<sup>137</sup> Cs (Bq/g. wet wt)	
		Mean $\pm$ 1 SD	Range
<i>Trachemys scripta</i>	12	44.9 $\pm$ 144 <sup>a</sup>	12.4 x 10 <sup>-2</sup> to 502
<i>Chelydra serpentina</i>	12	39.6 x 10 <sup>-2</sup> $\pm$ 31.3 x 10 <sup>-2a</sup>	20.3 x 10 <sup>-2</sup> to 1.37

<sup>a</sup>Means not significantly different (Student's t-test, p > 0.05).

Table 12. Cesium-137 and cobalt-60 in livers of Trachemys scripta and Chelydra serpentina from White Oak Lake

<u>Species</u>	N	<u><math>^{137}\text{Cs}</math> (Bq/g wet wt.)<sup>a</sup></u>	<u><math>^{60}\text{Co}</math> (Bq/g wet wt.)<sup>a</sup></u>
<i>Trachemys scripta</i>	12	5.84 $\pm$ 19.0 <sup>b</sup>	6.03x10 <sup>-2</sup> $\pm$ 6.03x10 <sup>-2</sup> <sup>c</sup>
<i>Chelydra serpentina</i>	12	17.44x10 <sup>-2</sup> $\pm$ 1.10x10 <sup>-2</sup> <sup>b</sup>	4.76x10 <sup>-2</sup> $\pm$ 2.72x10 <sup>-2</sup> <sup>c</sup>

<sup>a</sup>Mean  $\pm$  1 standard deviation from the mean.

<sup>b,c</sup>Means with the same superscript are not significantly different at p > 0.05 (Student's t-test).

the muscle of this turtle nor in any of the **other muscle samples** from **T. scripta** collected from **White Oak Lake**. **Bone from this individual also** contained  $^{85}\text{Sr}$  (4.44 Bq/g wet weight). Because  $^{85}\text{Sr}$  is not known to be present in White Oak Lake, it is likely that the turtle migrated from the Old Hydrofracture Pond near Melton Branch; the pond was spiked with  $^{85}\text{Sr}$  in March 1987 (O. M. Sealand, ORNL, personal communication). The Old Hydrofracture Pond is located approximately 2.5 km from the point of capture in White Oak Lake and is **the only** known environmental source of  $^{85}\text{Sr}$  within the area. A **second male turtle collected from White Oak Lake** on July 27, 1987, contained 33 Bq/g wet weight of  $^{137}\text{Cs}$  in muscle. Cobalt-60 was not detected ( $< 3.7 \times 10^{-3}$  Bq/g) in the muscle **nor was**  $^{85}\text{Sr}$  detected in the bone. A Dixon **statistical test for determining** outliers indicated that the  $^{137}\text{Cs}$  concentration in the muscle of this turtle was significantly different ( $p < 0.01$ ) from the concentrations of the radionuclide detected in the muscles of the remaining ten turtles. Although it cannot be proven, this individual probably emigrated from a settling pond on the reservation containing higher concentrations of  $^{137}\text{Cs}$  than White Oak Lake (e.g., Pond 3513 or Pond.3524). Nonetheless, these two turtles were not deleted from the data set, **since** they were, in fact, captured in White Oak Lake and constituted a portion of the sample of turtles within the lake.

Both  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  were detected in the liver samples of **T. scripta** from White Oak Lake. **Mean concentrations of the two radionuclides were**  $5.84 \pm 19.0$  Bq/g and  $6.03 \times 10^{-2} \pm 6.03 \times 10^{-2}$  Bq/g (wet weight), respectively. Concentrations of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  in liver tissues were highest in the two turtles of suspect origin. Concentrations of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  in the livers of individual **T. scripta** from White Oak Lake are listed in APPENDIX D.

**Trachemys scripta** collected from Bearden Creek embayment contained expected background concentrations ( $< 3.7 \times 10^{-2}$  Bq/g) of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$ . Only one of the twelve **T. scripta** from the site had a concentration of  $^{137}\text{Cs}$  that exceeded the detection limit of  $3.7 \times 10^{-3}$  Bq/g. This single turtle contained  $1.44 \times 10^{-2}$  Bq/g (wet weight)  $^{137}\text{Cs}$  in muscle. Cobalt-60 was not detected ( $< 3.7 \times 10^{-3}$  Bq/g)

in any of the muscle samples. Neither radionuclide was detected ( $< 3.7 \times 10^{-3}$  Bq/g) in livers of T. scripta from **Bearden** Creek embayment.

Muscle samples from C. sernentina collected from White Oak Lake contained above background concentrations of  $^{137}\text{Cs}$  and a significant difference in the mean  $^{137}\text{Cs}$  concentration was detected between the two populations (Student's t-test on non-transformed data,  $p < 0.01$ ). No interaction was detected between  $^{137}\text{Cs}$  concentration in liver or muscle and whole body weight. The radionuclide was present in all White Oak Lake muscle samples at a mean of  $39.6 \times 10^{-2} \pm 31.3 \times 10^{-2}$  Bq/g, wet weight. Cesium-137 was not detected ( $< 3.7 \times 10^{-3}$  Bq/g) in muscle samples from C. sernentina collected in **Bearden** Creek embayment, nor was  $^{60}\text{Co}$  detected ( $3.7 \times 10^{-3}$  Bq/g) in any of the muscle samples. Liver samples from C. sernentina captured in White Oak Lake contained elevated concentrations ( $> 3.7 \times 10^{-3}$  Bq/g) of both  $^{137}\text{Cs}$  and  $^{60}\text{Co}$ . Concentrations of  $^{137}\text{Cs}$  in liver ranged from  $7.63 \times 10^{-2}$  to  $50.3 \times 10^{-2}$  Bq/g (wet weight) with a mean and one standard deviation from the mean of  $17.4 \times 10^{-2} \pm 11.0 \times 10^{-2}$  Bq/g, wet weight. The mean concentration of  $^{60}\text{Co}$  in the livers of these turtles was  $4.76 \times 10^{-2} \pm 2.72 \times 10^{-2}$  Bq/g, wet weight. Cesium-137 and  $^{60}\text{Co}$  concentrations detected in individual C. sernentina are listed in APPENDIX D. Neither  $^{137}\text{Cs}$  nor  $^{60}\text{Co}$  was detected ( $< 3.7 \times 10^{-3}$  Bq/g) in the livers of **Bearden** Creek embayment C. sernentina.

Comparisons of T. scripta and C. sernentina did not reveal significant differences (Student's t-test and Mann-Whitney U-test on nontransformed and log-transformed data for both species,  $p < 0.05$ ) in the concentrations of the radionuclides between the two species of turtles (Tables 11 and 12). If, however, the two T. scripta that are believed to have immigrated to White Oak Lake from other contaminated sites are removed from each of the comparisons, a difference is seen between the two species. The difference (Student's t-test on nontransformed and log-transformed data,  $p < 0.05$  and  $p < 0.01$ , respectively) occurs in the concentration of  $^{137}\text{Cs}$  in liver tissue. No species difference (Student's t-test on non-transformed and log-transformed data,  $p > 0.05$ ) was detected for  $^{137}\text{Cs}$  in muscle or  $^{60}\text{Co}$  in liver upon removal of the two outliers.

### 5.3.3 Strontium-90

Concentrations of  $^{90}\text{Sr}$  in the calcified tissues of T. scripta were higher in turtles from White Oak Lake than in turtles from **Bearden** Creek embayment (Mann-Whitney U-test,  $p \leq 0.05$ ). Strontium-90 concentrations in the bone and carapace of T. scripta from White Oak Lake ranged from 2.31 to  $4.83 \times 10^3$  Bq/g (wet weight) with a mean of  $4.26 \times 10^2 \pm 1.39 \times 10^3$  Bq/g and from 1.65 to  $4.07 \times 10^3$  Bq/g (wet weight) with a mean of  $3.66 \times 10^2 \pm 1.17 \times 10^3$  Bq/g, respectively (Table 13). As was observed for the  $^{137}\text{Cs}$  data, a two-way ANOVA did not indicate a significant difference in the mean concentration for males and females within a given site ( $p > 0.05$ ). In addition, concentrations were not influenced by whole body weight in T. scripta or C. serpentina from either site. The radionuclide was detected in several turtles from **Bearden** Creek embayment. Concentrations of  $^{90}\text{Sr}$  in the bones of T. scripta from this site ranged from nondetectable ( $<3.7 \times 10^{-2}$  Bq/g) to 1.04 Bq/g, wet weight. Concentrations in the carapace were similar, ranging from nondetectable to 1.18 Bq/g, wet weight. Concentrations of  $^{90}\text{Sr}$  from individual T. scripta from both sites are listed in APPENDIX E.

Strontium-90 concentrations in bone and carapace were greater in C. serpentina from White Oak Lake than in those from the reference site (Mann-Whitney U-test,  $p < 0.05$ ). The mean concentration of  $^{90}\text{Sr}$  in the bones of C. serpentina from White Oak Lake was  $16.5 \pm 13.6$  Bq/g (wet weight), several orders of magnitude greater than that detected in turtles from **Bearden** Creek embayment. Only three of the nine C. serpentina from **Bearden** Creek embayment contained detectable concentrations of  $^{90}\text{Sr}$  ( $> 3.7 \times 10^{-2}$  Bq/g). These three turtles averaged  $12.4 \times 10^{-2} \pm 6.74 \times 10^{-3}$  Bq/g (wet weight) of  $^{90}\text{Sr}$  in bone. Strontium-90 in the carapace of C. serpentina from White Oak Lake averaged  $16.6 \pm 11.7$  Bq/g (wet weight). Concentrations of this radionuclide in C. serpentina from **Bearden** Creek embayment ranged from non-detectable ( $<3.7 \times 10^{-2}$  Bq/g) to  $17.7 \times 10^{-2}$  Bq/g, wet weight. A listing of  $^{90}\text{Sr}$  concentrations in individual C. serpentina from both White Oak Lake and **Bearden** Creek embayment is provided in APPENDIX E.

Table 13. Strontium-90 in skeletal tissues of Trachemys scripta and Chelydra serpentina from White Oak Lake

<u>Species</u>	N	<u><sup>90</sup>Sr (Bq/g wet wt)<sup>a</sup></u>	
		Bone	Carapace
<u>Trachemys scripta</u>	12	$4.26 \times 10^2 \pm 1.39 \times 10^3$ <sup>b</sup>	$3.66 \times 10^2 \pm 1.17 \times 10^3$ <sup>c</sup>
<u>Chelydra serpentina</u>	12	$16.5 \pm 13.6$ <sup>b</sup>	$16.6 \pm 11.7$ <sup>c</sup>

<sup>a</sup>Mean  $\pm$  1 standard deviation from the mean.

<sup>b,c</sup>Means with the same superscript are not significantly different at  $p > 0.05$  (Student's t-test).

No species differences were found between the  $^{90}\text{Sr}$  concentrations of T. scripta and C. sernentina from White Oak Lake (Student's t-test on non-transformed and log-transformed data,  $p < 0.05$ ). This finding was true for the **concentrations in both bone and carapace** (Table 15). The two highest concentrations of  $^{90}\text{Sr}$  detected in these turtles were in the two T. scripta that were thought to have immigrated to White Oak Lake from sites of **higher** radioactivity than the lake. These turtles contained approximately 140 and 4,400 **Bq/g** (wet weight) of  $^{90}\text{Sr}$  in their calcified tissues. The elimination of these two turtles from the comparison, however, did not alter the outcome of the species comparison. Differences were not detected in  $^{90}\text{Sr}$  concentration between C. sernentina and T. scripta from White Oak Lake (Student's t-test on non-transformed **and log-transformed** data,  $p > 0.05$ ).

#### 5.3.4 Mercury

Total inorganic and organic mercury in kidney and muscle tissues of T. scripta from White Oak Lake and **Bearden** Creek embayment are summarized in Table 14. Mercury residues in the kidneys of the White Oak Lake and **Bearden** Creek embayment T. scripta averaged  $0.64 \pm 1.11 \mu\text{g/g}$  and  $0.12 \pm 0.13 \mu\text{g/g}$  wet weight, respectively. At both sites, mean mercury concentrations in muscle were less than  $0.1 \mu\text{g/g}$  wet weight. Statistical analysis of the data revealed a significant difference (Student's t-test on log-transformed data,  $p < 0.01$ ) in mercury concentrations between the two populations. *This was true* for both kidney and muscle tissues. A two way **ANOVA** did not show the sex of the turtle as having any influence on the concentration of mercury within the turtle. In addition, the concentration of mercury in a turtle was not dependent upon its weight. Mercury concentrations within individual T. scripta are listed in APPENDIX F.

As in T. scripta, inorganic and organic mercury concentrations in C. serpentina were significantly higher in the turtles collected from White Oak Lake than in those collected from **Bearden** Creek embayment (Student's t-test on **non-transformed and** log-transformed data,  $p < 0.025$  and  $p < 0.01$ , respectively). This was true for both kidney and muscle tissues (Table 14). No significant difference was detected between the

Table 14. Mercury concentrations in tissues of Trachemys scripta and Chelydra serpentina from the Oak Ridge reservation

Species	N	Hg ( $\mu\text{g/g}$ wet wt) <sup>a</sup>	
		Kidney	Muscle
White Oak Lake			
<i>Pseudemys scripta</i>	12	0.64 $\pm$ 1.11 <sup>b,f</sup>	0.10 $\pm$ 0.13 <sup>c,s</sup>
<i>Chelydra serpentina</i>	12	1.30 $\pm$ 1.16 <sup>d,f</sup>	0.34 $\pm$ 0.28 <sup>e,s</sup>
Bearden Creek embayment			
<i>Pseudemys scripta</i>	12	0.12 $\pm$ 0.13 <sup>b</sup>	0.03 $\pm$ 0.03 <sup>c</sup>
<i>Chelydra serpentina</i>	9	0.17 $\pm$ 0.07 <sup>d</sup>	0.10 $\pm$ 0.13.

<sup>a</sup>Mean  $\pm$  1 standard deviation from the mean.

<sup>b,c,d,e,f</sup>Means with the same superscript are significantly different at  $p < 0.01$  (Student's t-test).

<sup>s</sup>Weans significantly different at  $p < 0.05$  (Student's t-test).

Table 15. Fraction of double-stranded DNA in liver samples of Trachemys scripta and Chelydra serpentina from the Oak Ridge reservation

Species and Site	DNA (fraction ds)			Relative no. breaks per alkaline unwinding unit'
	Mean	SE	N	
<i>Trachemys scripta</i>				
White Oak Lake	0.49 <sup>b</sup>	0.43	12	4.42 <sup>d</sup>
Bearden Creek embayment	0.88 <sup>b</sup>	0.31	12	1.0
<i>Chelydra serpentina</i>				
White Oak Lake	0.61 <sup>c</sup>	0.11	12	3.66 <sup>d</sup>
Bear & Creek embayment	0.89 <sup>c</sup>	0.06	9	1.0

<sup>a</sup>ln F<sub>ds</sub> (WOL)

ln F<sub>ds</sub> (reference site)

<sup>b,c</sup>Means with the same superscript are significantly different p < 0.001 (Student's t-t&t).

<sup>d</sup>Mean number of breaks not significantly different p > 0.05 (Student's t-test).

concentrations of mercury in male versus female turtles from **Bearden** Creek embayment. Mean concentrations of mercury in the kidneys of **C. serpentina** from White Oak Lake and **Bearden** Creek embayment were  $1.30 \pm 1.16 \mu\text{g/g}$  and  $0.34 \pm 0.28 \mu\text{g/g}$  wet weight, respectively. Mercury concentrations in muscle were below  $1 \mu\text{g/g}$  (wet weight) in turtles collected from both sites. As indicated by analysis of variance tests, concentrations were not dependent on the weight or sex of the turtles. Mercury concentrations in individual **C. serpentina** are reported in APPENDIX F.

Higher mercury concentrations were found in kidney and muscle tissues of **C. serpentina** than in **T. scripta** from White Oak Lake (Table 14). The difference in kidney tissue was found to be statistically significant (Student's t-test on log-transformed data,  $p < 0.01$ ) as it was for muscle tissue (Student's t-test on non-transformed and log-transformed data,  $p < 0.05$  and  $p < 0.01$ , respectively). All mean concentrations of mercury were below  $1 \mu\text{g/g}$  (wet weight) except for that in the kidneys of **C. serpentina**.

#### 5.3.5 DNA Damage

Examination of the DNA from livers of **T. scripta** for single-stranded breaks revealed differences in the integrity of the DNA from the two **populations** of turtles (Student's t-test on nontransformed data,  $p < 0.001$ ). The mean fraction of double-stranded DNA obtained using the alkaline unwinding assay was 0.877 for **Bearden** Creek embayment turtles and 0.492 for White **Oak** Lake turtles (Table 15). The DNA of **T. scripta** from White Oak Lake contained approximately 4.4 times the number of breaks detected in the DNA of turtles from **Bearden** Creek embayment. Data from the assay for the individual turtles are reported in APPENDIX G.

A difference in the fraction of double-stranded DNA was also detected between the contaminated and reference site populations of **C. serpentina** (Student's t-test on non-transformed data,  $p < 0.001$ ). The mean fraction of double-stranded DNA detected under the conditions of the assay was 0.61 and 0.89 for the White Oak Lake and **Bearden** Creek embayment turtles, respectively (Table 15). The DNA from White Oak Lake

turtles contained approximately 3.7 times the **number of breaks** detected in C. seroentina from the reference site. Data taken during the assay on individual C. seroentina from both **sites** are reported in APPENDIX G.

A comparison of the relative number of **breaks per** alkaline unwinding unit for T. scripta and C. seroentina from White Oak Lake indicated a similar level of DNA damage in the two species (Table 15). No statistically significant difference ( Student's t-test on nontransformed data,  $p > 0.05$ ) was detected between the two species. Both species apparently received higher exposures to genotoxic agents in White Oak Lake than in **Bearden Creek embayment**.

#### 5.4 DISCUSSION

Two species of freshwater turtles **that occupy** different ecological niches were compared for their usefulness as indicators of chemical contamination in aquatic ecosystems. Radionuclide and mercury concentrations were monitored in Trachemys scripta and Chelydra seroentina from a contaminated and a non-contaminated reference site. A DNA alkaline unwinding assay that measures **breaks in** single-stranded DNA was used as an indication of exposure to genotoxic agents in the environment. Species differences within sites were consistent with differences in diet and mobility. The influence, of, the degree of sediment contact on exposure of the turtles to **contaminants could not be** evaluated by this study.

The abilities of T. scripta and C. seroentina to accumulate chemical contaminants from an aquatic environment were **evidenced by** elevated tissue **concentrations of** radionuclides and mercury. Higher concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and mercury were detected in the turtles from White Oak Lake than in those from **Bearden Creek embayment**, the reference site. Cobalt-60 was not detected ( $3.7 \times 10^{-3}$  Bq/g) in the turtles from the embayment. Mercury was detected, however, at concentrations below the background **concentration of 1  $\mu\text{g/g}$**  (Eisler 1987). Cesium-137 and  $^{90}\text{Sr}$ , fallout products **from nuclear weapons testing (Langham 1965)**, were also present in turtles from **Bearden Creek embayment**. Only one turtle, a T. scripta, contained a detectable **amount** of  $^{137}\text{Cs}$  and the **concentration of the radionuclide** was well below

$3.7 \times 10^{-2}$  Bq/g. Concentrations of  $^{90}\text{Sr}$  greater than  $3.7 \times 10^{-2}$  Bq/g were detected in four T. scriota and three C. seroentina from the reference site. Sediment from the site, however, did not contain elevated concentrations of  $^{90}\text{Sr}$ . In T. scriota,  $^{90}\text{Sr}$  has an average yearly biological half-life of 365 days (Scott et al. 1986) and approximately 99% of the total  $^{90}\text{Sr}$  within an individual animal is contained in the bone and shell (Towns 1987). Thus, once  $^{90}\text{Sr}$  is assimilated, it is incorporated into bone and shell and eliminated at a very slow rate. Therefore, the source of the  $^{90}\text{Sr}$  in the bones and shells of these turtles may be attributed to the accumulation and retention of the fallout product or exposure to  $^{90}\text{Sr}$  contaminated areas elsewhere on the ORNL reservation.

A comparison of contaminant concentrations between T. scriota and C. seroentina from White Oak lake indicated both species as effective monitors of contaminants in the lake. Differences in  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and  $^{90}\text{Sr}$  concentrations were not statistically significant between the two species from White Oak Lake. However, less variation was evident in the C. seroentina data than in the T. scriota data. In addition, radionuclide spectral analysis provided evidence for the movement of one T. scriota from the Old Hydrofracture Pond about 2.5 km from White Oak Lake, indicating that T. scriota may be a highly mobile species. Less variability in the C. seroentina data and evidence of the movement of one (possibly two) T. scriota from contaminated sites outside of White Oak Lake, suggest that C. seroentina had a more uniform exposure to  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and  $^{90}\text{Sr}$  in the lake. Consequently, C. seroentina may serve as a more useful biological monitor of these radionuclides than T. scriota.

An interesting difference emerges between the two species when the two T. scriota that appear to have immigrated to White Oak Lake from other contaminated sites on the Oak Ridge reservation are removed from each of the comparisons. These turtles contained radionuclides and mercury at concentrations statistically higher than those detected in the other ten T. scriota. A species difference found upon removal of the two outliers is in the concentrations of  $^{137}\text{Cs}$  in liver tissue, concentrations being significantly higher in C. seroentina than in

T. scripta. No difference was detected, however, for  $^{137}\text{Cs}$  in muscle upon removal of the two **outliers**. According to Vanderploeg et al. (1975), the primary route of exposure and accumulation of  $^{137}\text{Cs}$  in aquatic organisms is by ingestion. There is also a limited amount of data indicating biomagnification **of the radionuclide through** aquatic food chains (Kolehmainen et al. 1966, Spigarelli 1971, Gustafon 1969). If the biomagnification process is **assumed valid in aquatic** systems, then fish would be expected to **contain higher concentrations**  $^{137}\text{Cs}$  than vegetation collected from **the same site**. It is possible that because C. serpentina ingested a greater proportion of **fish than did** the herbivorous T. scripta, a greater **concentration** of  $^{137}\text{Cs}$  reached the liver of the former species.

Cesium-137 concentrations in muscle, which are higher than those in liver, did not differ significantly between species even upon removal of the outliers. **Cesium-137 is** an analog of potassium and as such, reacts metabolically like potassium (Hobbs and McClellan 1980). Under steady-state conditions, the concentration of  $^{137}\text{Cs}$  in muscle may show little variability among closely related species. The average yearly biological half-life of  $^{137}\text{Cs}$  in T. scripta is 64 days (Scott et al. 1986), indicating the radionuclide as **being** in a rapidly exchanged metabolic pool. Peters and Brisbin (1988) estimated the time required for T. scripta to reach an equilibrium **concentration** of  $^{137}\text{Cs}$  during a chronic period of exposure to be between 213 to 643 days. Because all turtles used in the present study were greater than two years old (**at** least six years old for T. scripta and at least 12 years old for C. serpentina), the concentration of  $^{137}\text{Cs}$  in turtles collected from, - White Oak Lake should have been at equilibrium with **their environment** providing the concentration of  $^{137}\text{Cs}$  in the lake remained constant during this period. **The steady-state concentration of**  $^{137}\text{Cs}$  in muscle of the two species may explain why concentrations of  $^{137}\text{Cs}$  in muscle tissue did not differ **between** species. Because the **radionuclide will** reach the liver before.. **it is distributed to other tissues**, the species difference in  $^{137}\text{Cs}$  concentrations in liver tissue is probably attributed to the ingestion of higher concentrations of  $^{137}\text{Cs}$  by C. serpentina. Explanations only hold true, however, if

'biomagnification does occur for  $^{137}\text{Cs}$  in White Oak Lake and if the  $^{137}\text{Cs}$  in T. scripta and C. sernentina collected from the lake are at steady-state equilibrium with the concentration of  $^{137}\text{Cs}$  in the lake.

The lack of difference between the  $^{90}\text{Sr}$  concentrations in T. scripta and C. sernentina may be due to differences in the routes of uptake utilized by turtles. Strontium-90 may enter a turtle by two means--by ingestion and by direct uptake from water. Hinton and Whicker (1985) administered  $^{85}\text{Sr}$  to T. scripta via gavage and determined that 40 to 60% of the radioisotope was absorbed by the gastrointestinal tract, a higher percentage than reported in mammalian species. Jackson *et al.* (1974) examined  $^{90}\text{Sr}$  concentrations in the shells of turtles from the southeastern United States and attributed the difference between two terrestrial species to differences in food habits. Higher concentrations were in the herbivorous Gonherus polyphemus (gopher tortoise) than in the omnivorous Terrapene Carolina (Eastern box turtle). These studies provide evidence for the ability of turtles to assimilate strontium by ingestion. Aquatic species may have an additional mode of uptake. Fish acquire  $^{90}\text{Sr}$  primarily from the water by direct uptake of the radionuclide across the gill membrane (Ophel and Judd 1962, Nelson 1966). Dunson (1969) and Dunson and Weymouth (1965) reported the influx of sodium ions from water across the cloacal and pharyngeal membranes of freshwater turtles. The possibility exists for the uptake of strontium ions across these membranes. A species of turtle that lives in close contact with radiostrontium contaminated sediment, such as C. sernentina, may be exposed to higher concentrations of the radionuclide than a more free-swimming species, such as T. scripta, due to the resuspension of  $^{90}\text{Sr}$  at the sediment-water interface. If absorption across the cloacal and pharyngeal membranes are the major routes of uptake for  $^{90}\text{Sr}$  in freshwater turtles, then a more sedentary species (*e.g.*, C. sernentina) should contain higher concentrations of  $^{90}\text{Sr}$  in its tissues. The importance of food habits is less clear because  $^{90}\text{Sr}$  is not biomagnified in aquatic systems and the assimilation of the radionuclide is highly dependent on the availability of calcium (Vanderploeg *et al.* 1975). If most of the  $^{90}\text{Sr}$  acquired by a turtle is by ingestion of plant material, as it is in terrestrial

species, then an herbivorous species (e.g., T. scripta) should contain the higher concentration of **the radionuclide**. Because no significant difference in <sup>90</sup>Sr concentration was detected between T. scripta and C. serpentina, the process of <sup>90</sup>Sr uptake in aquatic turtles is believed to be a complicated one involving both uptake from food and uptake from water across the cloacal and perhaps pharyngeal membranes.

As was found for <sup>90</sup>Sr, no difference was detected in the concentration of <sup>60</sup>Co in T. scripta and C. serpentina from White Oak Lake. Cobalt is contained within the cobalamin (vitamin B<sub>12</sub>) molecule which is required by animals for **normal biological** function. The metal is not biomagnified in aquatic systems as evidenced by the low absorption efficiency of cobalt from food (approximately 5%) in fish (Vanderploeg *et al.* 1975). Algae and submergent macrophytes have among the highest bioaccumulation factors **for cobalt in freshwater ecosystems** (Vanderploeg *et al.* 1975, Ophel and Fraser 1971). Ophel and Fraser (1971) examined fish in a pond contaminated with <sup>60</sup>Co and found the highest concentrations of the radionuclide to **be in the herbivorous** species. Because T. scripta fed almost **exclusively** on vegetation, this species would be expected to have had a greater potential for exposure to <sup>60</sup>Co than C. serpentina. The fact that C. serpentina also consumed a large amount of vegetation (approximately 48% by volume in the White Oak Lake population) results in an overlap between **trophic** levels making it difficult to evaluate the influence of food habits on the concentrations of <sup>60</sup>Co in the two species of turtles. In addition, if the radionuclide is as poorly absorbed by the GI tracts of turtles **as it is in fish**, then it may be even more unlikely to detect a species difference in <sup>60</sup>Co concentration. Although the absorption efficiency of <sup>60</sup>Co by direct uptake from water has not been **determined for turtles**, it is not suspected as being a major route of uptake.

Mercury concentrations **were** significantly higher in C. serpentina than in T. scripta from White Oak Lake, a **difference that is consistent** with the differences in **the food habits of the two species**. Chelydra Chelydrana was found to be more, **omnivorous** than T. scripta. serpentina ingested (by volume) an average of 52% fish and 48%

vegetation compared to an average of 0.1% fish and 93% vegetation in T. scrinta. Inorganic and elemental mercury are converted to methylmercury by methylating bacteria in sediment, and it is this methylated form that is biomagnified in aquatic food chains (Boudou and Ribeyre 1983). According to Eisler (1987), almost all of the mercury accumulated in the tissues of organisms at the top of an aquatic food chain is in the form of methylmercury. Because methylmercury can be biomagnified through the food chain, a carnivore or omnivore such as C. serpentina would be expected to have higher concentrations of the metal than an herbivore such as T. scrinta. Although mercury concentrations in White Oak Lake C. sementina and T. scripta exceeded those detected in the same species from the reference site, the average mercury concentrations in muscle of the White Oak Lake turtles was less than the 1  $\mu\text{g/g}$  FDA action limit (FDA 1984). Concentrations of mercury in White Oak Lake sediment ranged from 3 to 5.9  $\mu\text{g/g}$  (Hoffman et al. 1984). This metal is present in White Oak Lake at above-background concentrations ( $> 1.0 \mu\text{g/g}$ ) but appears to be in a form that is not readily taken up by biota. Albers et al. (1986) reported similar results where concentrations of mercury in sediment ranged from 66 to 107  $\mu\text{g/g}$ , but the concentration in C. sernentina averaged less than 1.30  $\mu\text{g/g}$  in liver and less than 0.60  $\mu\text{g/g}$  (wet weight) in kidney tissue. As in White Oak Lake, the mercury in the sediment from the Albers et al. (1986) study site did not appear to be in a biologically available form.

Results of the alkaline unwinding assay indicate that both T. scrinta and C. sernentina from White Oak Lake have a higher frequency of DNA breaks than does the same species from **Bearden** Creek embayment. No statistically significant difference was found between the amount of DNA damage in the two species from White Oak Lake. Although exposure to genotoxic agents is the probable cause of the observed DNA damage in White Oak Lake turtles, other environmental stressors can produce similar effects. Poor nutritional status (e.g., **protein** deficient diet) of White Oak Lake turtles could result in a decline in the synthesis of enzymes required in DNA repair, and thus a greater amount of DNA damage would be seen in the turtles from White Oak Lake than in those from the reference site. Other environmental stressors such as extreme

temperatures and disease can also weaken the animal **and may** result in an elevated amount of DNA damage. Because turtles from White Oak Lake did not appear malnourished, diseased, or in any way unhealthy, contaminants in the lake are likely the primary causative agents of the DNA damage. Although physical (radiation, **Ahnström** and Erixon 1973, Kanter and Schwartz 1982) and chemical agents (certain forms of mercury, **Cantoni** and Costa 1983, and **PCBs**, Robbiano and **Pino** 1981) can produce breaks in DNA, the specific genotoxic agent cannot be established from **this** assay. The finding of elevated DNA damage in White Oak Lake turtles does, however, suggest that biota in the **lake are exposed** to above-background concentrations of genotoxicants. Also, the lack of difference in DNA damage between **T. scripta** and **C. sernentina** from White Oak Lake reveals, that exposure to genotoxic agents in the lake is nearly equivalent between the two species. Bickham *et al.* (1988) also detected DNA damage in turtles occupying seepage basins containing radioactive and nonradioactive contaminants. Turtles from **the** seepage basins contained significantly greater variation in DNA content in red blood cells than turtles from the reference site. Differences in DNA content were attributed to mutations produced by low-level radiation and or an unknown chemical contaminant. **Both** studies promote the use of long-lived turtles in the detection of genotoxic agents in contaminated aquatic environments.

Of the two species of freshwater **turtles examined in this study**, **C. sernentina** appears to be a better monitor **of the contaminants** measured. Data obtained on **this** species can be compared with biological monitoring data on fish and vegetation from **White Oak Lake**. The **concentration of  $^{137}\text{Cs}$  in the muscle of the piscivorous C. sernentina averaged  $39.6 \times 10^{-2}$  Bq/g (wet weight) and was within the range reported in White Oak Lake fish. Concentrations of  $^{137}\text{Cs}$  in fillets of eight species of fish from White Oak Lake ranged from  $29.2 \times 10^{-2}$  (warmouth, **Lepomis gulosus**) to  $61.8 \times 10^{-2}$  Bq/g wet weight (largemouth bass, **Micropterus salmoides**) (**Blaylock** and Mohrbacher, unpublished). Concentration in muscle were 790 times greater in **C. sernentina** and 588 to 1233 times greater in White Oak Lake fish than that in White Oak Lake**

water (Filtered water concentration of 0.50 **Bq/l** measured in the fall of 1987, Mohrbacher, D. A. and B. G. Blaylock, unpublished.).

Concentration factors for macrophytes in White Oak Lake occur over a wide range and are dependent upon collection site, species, the part of the plant analyzed, and the age of the plant. Common cattails (*Typha latifolia*) collected from the upper end of the lake in 1988 (Mohrbacher, D. A. and B. G. Blaylock, unpublished) contained 1.24 **Bq/g** (wet weight) <sup>137</sup>**Cs**, 2480 times greater than that in filtered lake water. Water milfoil (*Myriophyllum* sp.) collected from the middle and lower end of the lake in 1987 (Mohrbacher, D. A. and B. G. Blaylock, unpublished), however, contained  $49.7 \times 10^{-2}$  **Bq/g** (wet weight) <sup>137</sup>**Cs**, 994 times greater than that detected in filtered lake water. Whether or not biomagnification of <sup>137</sup>**Cs** occurs in the lake cannot be ascertained from these data. It would be interesting to determine whether differences exist in the concentrations of <sup>137</sup>**Cs** in liver tissues of fish species as appeared *to* be the case for *T. scrinta* and *C. sernentina*.

Strontium-90 concentrations in *C. sernentina* were greater than those measured in fish from White Oak Lake. The mean concentration of 16.5 **Bq/g** (wet weight) in *C. sernentina* bone was greater than the 4.8 **Bq/g** (air-dried weight) detected in channel catfish (*Ictalurus punctatus*) bone (Southworth 1988) and the 1.35 **Bq/g** (wet weight) measured in bone from carp (*Cyprinus carpio*) (Blaylock, B. G., and D. A. Mohrbacher, unpublished). Concentrations in bone were 1500 and 123 times greater than that in lake water (Unfiltered concentration of 11 **Bq/l**, Blaylock et al. 1987.) for *C. sernentina* and carp, respectively. Differences in mode of uptake and bone composition may account for these differences. Common cattails, *Typha latifolia*, collected from the upper end of White Oak Lake in 1987 contained 1.12 **Bq/g** (wet weight) <sup>90</sup>**Sr** (Mohrbacher, D. A. and B. G. Blaylock, unpublished), a concentration slightly less than that in the bone of carp. In aquatic systems the calcified tissues of animals may serve as better indicators of <sup>90</sup>**Sr** contamination than plants.

The concentrations of mercury in *C. sernentina* and fish from White Oak Lake were similar. *Chelydra serpentina* contained 0.17 **µg/g** (wet weight) of mercury in muscle, whereas redbreast (*Lepomis auritus*) and

bluegill sunfish (Lepomis macrochirus) contained 0.16  $\mu\text{g/g}$  (wet weight) of mercury in muscle (Southworth 1987). Mercury partitions largely into sediment (The concentrations in White Oak Lake water ranges from less than  $5.0 \times 10^{-5}$  to  $10^{-4}$  mg/l (Blasing et al. 1989), whereas concentrations in sediment range from 3 to 5.9  $\mu\text{g/g}$  (Hoffman et al. 1984.) and, therefore, the low concentration of mercury detected in both fish and turtles from White Oak Lake support the hypothesis that mercury in the lake sediment may be, to a large extent, biologically unavailable.

Cobalt-60 was only detected in the livers of the turtles from White Oak Lake and comparable data are not available on fish from the same site. The concentration of  $^{60}\text{Co}$  in C. serpentina liver was 216 times greater than that in filtered lake water (0.220 Bq/l, fall 1987, Mohrbacher, D. A. and B. G. Blaylock, unpublished) indicating that this radionuclide is concentrated by turtles in White Oak Lake. Macrophytes from the lake also accumulate  $^{60}\text{Co}$ . Typhaolia collected in 1987 contained  $34.1 \times 10^{-2}$  Bq/g (wet weight)  $^{60}\text{Co}$ , whereas Myriophyllum sp. collected in 1988 contained  $7.4 \times 10^{-2}$  Bq/g (wet weight)  $^{60}\text{Co}$  (Mohrbacher, D. A. and B. G. Blaylock, unpublished). Concentration factors for these plants were 1550 and 336, respectively, and were greater than that for C. serpentina. Other plant species and livers from fish need to be analyzed for  $^{60}\text{Co}$  before any relationship between trophic level and concentration can be drawn.

The data obtained on turtles from White Oak Lake served as a useful supplement to that reported in fish from the same site. The only contaminant that was detected in higher concentration, & turtles than in fish was  $^{90}\text{Sr}$ , indicating that turtles may be more useful than fish in the monitoring of this radionuclide. Both species of turtles from White Oak Lake showed evidence of DNA damage, indicating the presence of genotoxic agents in the lake. Evidence of DNA damage was also found in sunfish collected from the lake (Adams et al. 1989). Although fish are usually more abundant and often easier to sample than turtles, turtles, specifically C. serpentina, may be better indicators of contaminants with high biomagnification potentials (e.g., PCBs and mercury) due to

their trophic position and large fat reserves where high concentrations of chlorinated organic compounds can be stored.

The data presented suggest C. sernentina as a better indicator of certain contaminants than T. scrinta, however, advantages and disadvantages exist in using snapping turtles. Chelydra sernentina is larger in size than T. scrinta, so ample quantities of specific tissues for residue analyses (e.g., fat for PCB analysis) are readily obtained. Chelydra sernentina is a more carnivorous species and, therefore, is expected to contain higher concentrations of contaminants that are biomagnified through food chains. Analysis for mercury and <sup>137</sup>Cs support this supposition. Overland movement between ponds was greater in T. scrinta than in C. sernentina, making the latter species a better monitor for contaminants within a given pond or lake.

Disadvantages to the use of C. sernentina focus on the sampling and handling of these animals. Chelydra sernentina have large home ranges and are often not as abundant as the smaller pond turtles (e.g., Trachemys and Chrysemys). Thus, it may be difficult to capture enough C. sernentina to constitute a sufficient sample size. Also, C. sernentina are very aggressive and dangerous to handle. If the pond or lake is large and sufficient manpower is available for field work, C. sernentina is recommended because of the large tissue samples and its trophic position in the ecosystem. Alternatively, T. scrinta is preferable in small ponds where a sample size of greater than three turtles is required. In any case, the characteristics of the site, the number of animals needed, and the type of contaminant to be monitored should all be considered in selecting an appropriate monitoring species.

An additional reason for including turtles in biological monitoring activities is that turtle meat is often utilized as a supplementary food source and, as such, the presence of contaminants in muscle tissue is of importance in the evaluation of a human health risk. Of the contaminants examined in White Oak Lake turtles, only <sup>137</sup>Cs was detected in muscle at above-background concentrations. Mercury concentrations in muscle were less than 20% of the FDA action limit. Cobalt-60 was not detected in muscle, and although <sup>90</sup>Sr concentrations were not measured in muscle, concentrations are expected to be low

because the radionuclide has a stronger affinity for calcified tissues, such as bone and shell. Therefore, the only contaminants in turtle muscle that may be of a human health **concern are  $^{137}\text{Cs}$** , and possibly **PCBs**.

## 6. SUMMARY

Several criteria, such as species abundance and food habits, must be considered in the selection of an appropriate indicator species for the monitoring of contaminants in the environment. The work presented here compared two species of turtles that occupy different ecological niches for their usefulness as monitors in contaminated freshwater ecosystems. Trapping of turtles from the contaminated and reference site revealed Trachemys scripta (Agassiz) and C. sernentina (Linnaeus) as the most abundant species at both locations. In addition, a comprehensive review of the literature on contaminants in turtles indicated these species as the turtles most frequently used in the surveillance of contaminants in freshwater environments. Also, natural history background on T. scripta and C. sernentina revealed that the species differ in their food habits and in their degree of contact with sediments. For these reasons, T. scripta and C. sernentina were selected as the species for comparison as monitors of contaminants in freshwater ecosystems.

The sites selected for study were White Oak Lake, a settling basin for low-level radioactive and nonradioactive contaminants, and **Bearden** Creek embayment. Data obtained from chemical and radiological analyses of sediment samples from **Bearden** Creek embayment supported the use of the embayment as a reference site.

Trachemys scripta and C. sernentina were trapped from the contaminated and reference site and analyzed for concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and mercury in specific target tissues (Target tissues were selected based on preliminary studies and information from the literature.). In addition, liver samples from the turtles were analyzed for single-stranded DNA breaks, a non-specific indicator of possible exposure to genotoxic agents in the environment. Significantly higher concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ,  $^{60}\text{Co}$ , and mercury were detected in turtles from White Oak Lake than in turtles from **Bearden** Creek embayment. Furthermore, turtles from White Oak Lake contained a significantly greater amount of DNA damage than those from the reference site. The specific genotoxic agent or agents responsible for the enhanced number

of breaks in the DNA of turtles from the contaminated site was not determined.

A comparison of T. scripta and C. serrentina from White Oak Lake indicated no difference in the amount of DNA damage between the two species. Also, no differences **were detected** in the concentrations of  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ , and  $^{60}\text{Co}$  between T. scripta and C. serrentina. Concentrations of  $^{137}\text{Cs}$  in liver tissues, however, were significantly higher in C. serrentina than in T. scripta upon the removal of two T. scripta outliers from the comparison. Radiochemical analyses of these two turtles indicated that they may have immigrated from more highly contaminated ponds. Removal of the outliers from the data, however, did not alter the outcome of the other comparisons. Mercury concentrations were significantly higher in C. serrentina than in T. scripta. Examination of the gastrointestinal **contents of the** turtles indicated C. serrentina as ingesting a greater amount of animal matter than T. scripta. Differences in the concentrations of mercury, and to a lesser extent  $^{137}\text{Cs}$ , may be attributed to differences in the food habits of the species and the high biomagnification potential of methyl mercury. Because of the higher **trophic** position of C. serrentina and less variability detected in the C. serrentina data than in the T. scripta, snapping turtles are recommended as the **turtle species** of choice in the monitoring of contaminants that are biomagnified through food chains. Prior to any biological monitoring investigation, however, the geographic distribution of **the** turtles, relative abundance of the species, **nature of** the contaminant of concern, and available manpower should all be **considered**.

## LITERATURE CITED

- Abraham, R. L. 1972. Mortality of mallards exposed to gamma radiation. Radiat. Res. **49:322-327.**
- Adams, S. M., K. L. Shepard, B. D. Jimenez, and L. R. Shugart. 1989. Biological indicators of contaminant related stress. Section 5, pp. 5.1-5.49. IN: J. M. Loar (ed.), Third Annual Report on the ORNL Biological Monitoring and Abatement Program. ORNL/TM-DRAFT. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Ahnström**, G. and K. Erixon. 1973. Radiation induced strand breakage in DNA from mammalian cells. Strand separation in alkaline solution. Int. J. Radiat. Biol. **23:285-289.**
- Albers, P. H., L. **Sileo**, and B. M. Mulhern. 1986. Effects of environmental contaminants on snapping turtles of a tidal wetland. Arch. Environ. Contam. Toxicol. **15:39-49.**
- Atland**, P. D., B. **Highman**, and B. Wood. 1951. Some effects of x-irradiation on turtles. J. Exp. Zool. **118:1-19.**
- Belisle**, A. A., W. L. Reichel, L. N. Locke, T. G. Lamont, B. M. Mulhern, R. M. Prouty, R. B. **DeWolf**, and E. Cromartie. 1972. Residues of organochlorine pesticides, polychlorinated biphenyls, and mercury and autopsy data for bald eagles, 1969 and 1970. Pestic. i t J. **6:133-138.**
- Beresford, 'W., M. P. Donovan, J. M. Henninger, and M. **P. Waalkes**. 1981. Lead in the bone and soft tissues of box turtles caught near smelters. Bull. Environ. Contam. Toxicol. **27:349-352.**
- Berry, J. F. and R. Shine. 1980. Sexual size dimorphism and sexual selection in turtles (order Testudines). Oecologia **44:185-191.**
- Bickham**, J. W., B. G. Hanks, M. J. Smolen, T. Lamb, and J. W. Gibbons. 1988. Flow cytometry analysis of the effects of low-level radiation exposure on natural populations of slider turtles (Trachemys scripta). Arch. Environ. Contam. Toxicol. **17:837-841.**

- Blasing**, T. J., K. J. Daniels, P. Y. Goldberg, B. M. Hordwedell, I. L. **McCollough**, F. R. O'Donnell, A. E. Osborne-Lee, M. F. Tardiff, S. W. Teeters, C. K. Valentine, and D. A. Wolf. 1989. Environmental surveillance **data report** for the third quarter of 1988. **ORNL/M-534**. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Blaylock, B. G. and M. L. Frank. 1979. Distribution of **tritium** in a chronically contaminated lake. pp. 247-256. IN: Behavior of Tritium in the Environment. IAEA-SM-232/74. International Atomic Energy Agency, Vienna, Austria.
- Boudou A. and F. Ribeyre. 1983. Contamination of aquatic biocenoses by mercury compounds: an experimental ecotoxicological approach. pp. 73-116. IN: J. O. **Nriagu** (ed.). Aquatic Toxicology. John Wiley and Sons, New York. 525 pp.
- Brown, **M. P.**, M. B. Werner, R. J. Sloan, and K. W. Simpson. 1985. Polychlorinated biphenyls in the Hudson River. Environ. Sci. Technol. **19:656-661**.
- Brungs, W. A. 1967. Distribution of cobalt 60, zinc 65, strontium 85, and cesium 137 in a freshwater pond. U. S. Department of Health, Education, and Welfare. **Public Health Service**, National Center for Radiological Health. **Rockville**, Maryland.
- Bryan, **A. M.**, P. G. Olafsson, and W. B. Stone. 1987a. Disposition of low and high environmental concentrations of **PCBs in snapping turtle tissues**. Bull. Environ. Contam. Toxicol. **38:1000-1005**.
- Bryan, A. M., W. B. Stone, and P. G. Olafsson. 1987b. Disposition of toxic PCB **congeners** in snapping turtle eggs: expressed as toxic equivalents of TCDD. Bull. Environ. Contam. Toxicol. **39:791-796**.
- Cagle, F. R. 1969. The life history of the slider turtle, Trachemys scripta troostii (Holbrook). Ecol. Monogr. **2:33-54**.
- Cantoni**, O. and M. Costa. 1983. Correlations of **DNA strand breaks** and their repair with cell survival following acute exposure to **mercury(II)** and x-rays. Molec. Pharmacol. **24:84-89**.
- Carr, A. 1952. Handbook of Turtles. Cornell University Press, Ithaca, NY. 542 pp.

- Cesarone, C. F., C. Bolognesi, and L. Santi. 1979. Improved microfluorometric DNA determination in biological material using 33258 Hoechst. Anal. Biochem. **100:188-197.**
- Clark, D. B. and J. W. Gibbons. 1969. Dietary shift in the turtle Trachemys scripta (Schoepff) from youth to maturity. Copeia **4:704-706.**
- Clark, D. R., Jr., and A. J. Krynitsky. 1980. Organochlorine residues in eggs of loggerhead and green sea turtles nesting at Merritt Island, Florida- July and August 1976. Pestic. Monit. J. **14:7-10.**
- Clark, D. R., Jr., and A. J. Krynitsky. 1985. DDE residues and artificial incubation of loggerhead sea turtle eggs. Bull Environ. Contam. Toxicol. **34:121-125.**
- Conant, R. 1975. A Field Guide to Reptiles and Amphibians of Eastern/Central North America, 2nd ed. Houghton Mifflin Company, Boston. 427 pp.
- Congdon, D. and J. W. Gibbons. 1983. Relationships of reproductive characteristics to body size in Trachemys scripta. Herpetologica **39:147-151.**
- Congdon, J. D., J. L. Greene, and J. W. Gibbons. 1986. Biomass of freshwater turtles: a geographic comparison. Am. Midl. Nat. **115:165-173.**
- Cosgrove, G. E. 1965. The radiosensitivity of snakes and box turtles. Radiat. Res. **25:706-712.**
- Cosgrove, G. E. 1971. Reptilian radiobiology. J. Am. Vet. Med. Assoc. **159:1678-1684.**
- Coulter, M. W. 1957. Predation by snapping turtles upon aquatic birds in Maine marshes. J. Wildl. Manage. **21:17-21.**
- Crawford, K. M., J. R. Spotila, and E. A. Standora. 1983. Operative environmental temperature and basking behavior of the turtle, Trachemys scripta. Ecology **64:989-999.**

- Dunaway**, P. B., L. L. Lewis, J. D. Story, J. A. Payne, and J. M. Inglis. 1969. Radiation effects in the Soricidae, Cricetidae and Muridae. pp. 173-184. IN: D. J. Nelson and F. C. **Evans** (eds.), Proceedings of the Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967. USAEC Report CONF-670503.
- Dunson**, W. A. 1969. Concentration of **sodium** by freshwater turtles. pp. 191-197. IN: D. J. Nelson and F. C. Evans (eds.), Proceedings of the Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967. USAEC Report CONF-670503.
- Dunson**, W. A. and R. D. Weymouth. 1965. Active uptake of sodium by softshell turtles (Trionyx sooinifer). **149:67-69**.
- Eisler, R. 1985a. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report **85(1.2)**. U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland.
- Eisler, R. 1985b. Selenium hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report **85(1.5)**. U.S. Fish and Wildlife **Service, Patuxent** Wildlife Research Center, Laurel, Maryland.
- Eisler, R. 1986a. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report **85(1.6)**. **U.S. Fish** and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland.
- Eisler, R. 1986b. Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: a synoptic review. **85(1.7)**. U. S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland.
- Eisler, R. 1987. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report **85(1.10)**. U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland.
- Ewert, M. A. 1979. The embryo and its egg: development and natural history. pp. 333-413; IN: M. Harless and H. Morlock (eds.), Turtles: Perspectives and Research. John Wiley and Sons, New York. 695 pp.

- Farris, G. C., F. W. **Whicker**, and . H. Dahl. 1969. Strontium-90 levels in mule deer and forage plants. pp. 602-608. IN: D. J. Nelson and F. C. Evans (eds.), Second National Symposium on Radioecology, Oak Ridge, Tennessee, May 15-17, 1967. USAEC Report CONF-670503.
- Fendley, T. T., M. N. **Manlove**, and I. L. Brisbin, Jr. 1977. The accumulation and elimination of radiocesium by naturally contaminated wood ducks. Health Phys. **32:415-422.**
- Ferguson, D. E. 1963. Notes concerning the effect of heptachlor on certain poikilotherms. Copeia **163:441-443.**
- Flickinger, E. L. and K. A. King. 1972. Some effects of **aldrin**-treated rice on Gulf Coast wildlife. J. Wild. Manage. **36:706-727.**
- Flickinger, E. L. and Mulhern. 1980. Aldrin persists in yellow mud turtle. Herpetol. Rev. **11:29-30.**
- Food and Drug Administration (FDA). 1984. Action level for methylmercury in fish. Fed. Regist. **49:45663.**
- Galbraith, D. A. and R. J. Brooks. 1989. Age estimates for snapping turtles. J. Wildl. Manage. **53:502-508.**
- Gibbons, J. W. 1968. Growth rates of the common snapping turtle, Chelydra serpentina, in a polluted river. Herpetologica **24:266-267.**
- Gibbons, J. W. 1986. Movement patterns among turtle populations: applicability to management of the desert tortoise. Herpetologica **42:104-113.**
- Gibbons, J. W. 1987. Why do turtles live so long? Bioscience **37:262-269.**
- Gibbons, J. W. 1988. Turtle population studies. Carolina Tips **51:45-47.**
- Gibbons, J. W. and R. D. **Semlitsch**. 1982. **Survivorship** and longevity of a long-lived vertebrate species: how long do turtles live? J. **51:523-527.**
- Gibbons, J. W., J. L. Greene, and J. D. Congdon. 1983. **Drought**-related responses of aquatic turtle populations. J. Herpetol. **17:242-246.**

- Gibbons, J. W., R. D. Semlitsch, J. L. Greene, J. P. Schubauer. 1981. Variation in age and size at maturity of the **slider turtle** (*Trachemys scripta*). *Am. Nat.* **117:841-845**.
- Gibbs, W., E. Wilson, H. Hodges, and C. C. Lushbaugh. 1964. Comparative study of thyroid and whole body retention of iodine in box turtles. pp. 20-22. Second Annual **Oak** Ridge Radioisotope Conference, Oak Ridge National Laboratory, Oak Ridge, Tennessee. USAEC Report TID-7689.
- Graham, T. E. and R. W. Perkins. 1976. Growth of **the common snapping** turtle, *Chelvdra s. serpentina*, in a polluted marsh. *Bull. Md. Herpetol. Soc.* **12:123-125**.
- Gustafon, P. F. 1969. Cesium-137 in freshwater fish during 1954-1965. pp. 249-257. IN: D. J. Nelson and F. **C. Evans** (eds.) Proceedings of the Second National Symposium on Radioecology, held at Ann Arbor, Michigan, May 15-17, 1976. USAEC Report CONF-670503.
- Halford**, D. K., O. D. Markham, and G. C. White. 1983. Biological elimination rates of **radioisotopes** by mallards **contaminated at a** liquid radioactive **waste disposal** area. *Health Phys.* **45:745-756**.
- Hall, R. J. 1980. Effects of environmental contaminants **on reptiles: a** review. U. S. Fish and Wildlife Service Special Scientific Report - Wildlife No. 228. United States Department **of the** Interior, Washington D.C.
- Hammer, D. A. 1969. Parameters **of** a marsh snapping turtle population Lacreek Refuge, South Dakota. *J. Wild. Manage.* **33:995-1005**.
- Hammond, P. B. and R. P. Beliles. 1980. Metals. pp. 409-467. IN: J. Doull, C. D. Klaassen, and M. O. Amdur (eds.), *Casarett and Doull's Toxicology: The Basic Science of Poisons*, Macmillan Publishing Co., Inc., New York. 778 pp.
- Helwig, **D. D.** and M. E. Hora. 1983. Polychlorinated biphenyl, mercury, and cadmium concentrations **in Minnesota snapping** turtles. *Bull. Environ. Contam. Toxicol.* **30:186-190**.
- Hillestad, H. O., R. J. Teimold, R. R. Stickney, H. L. **Windom**, and **J. H. Jenkins**. 1974. Pesticides, heavy metals **and radionuclide** uptake in loggerhead sea turtles from Georgia and South Carolina. *Herpetol. Rev.* **5:7**.

- Hinton, T. G.** and D. E. Scott. in press. Radioecological techniques used in herpetology with an emphasis on freshwater turtles. IN: J. W. Gibbons (ed.). The Life History and Ecology of the Slider Turtle. Smithsonian Institution Press.
- Hinton, T. G.** and F. W. **Whicker**. 1986. The kinetics of radium and strontium in Trachemys scripta kept at 18 and 28 °C. *NERP* Report. Savannah River Ecology Laboratory, Aiken, South Carolina.
- Hobbs, C. H. and R. O. McClellan. 1980. Radiation and radioactive materials. pp. **497-530**. IN: J. D. Doull, C. D. Klassen, and M. O. Amdur (eds.), Casarett and Doull's Toxicology The Basic Science of Poisons, second edition. Macmillan Publishing Co., Inc., New York. 778 pp.
- Hoffman, F. O., B. G. Blaylock, C. C. Travis, **K. L. Daniels**, E. L. Etnier, K. E. **Cowser**, and C. W. Weber. 1984. Preliminary screening of contaminants in sediments. ORNL/TM-9370. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Holcomb, C. M. and W. S. Parker. 1979. Mirex residues in eggs and livers of two long-lived reptiles (Chrysemys scripta and Terrapene carolina) in Mississippi, 1970-1977. Bull. Environ. Contam. ~~23:369-371~~.
- Holcomb, C. M., C. G. Jackson, Jr., **M. M. Jackson**, and S. Skaidrite. 1971; Occurrence of radionuclides in the exoskeleton of turtles. pp. 385-393. IN: D. J. Nelson (ed.), Proceedings of the Third National Symposium on Radioecology, Oak Ridge, Tennessee, May 10-12, 1971. **CONF-710501-P1**.
- Huckabee, J. W., J. W. Elwood, and S. G. Hildebrand. 1979. Accumulation of mercury in freshwater **biota**. pp. 277-302. IN: J. O. Nriagu (ed.), The Biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Biomedical Press, New York.
- Jackson, C. G., Jr., C. M. Holcomb, S. Kleinberg-Krisans, and M. M. Jackson. 1974. Variation in strontium-90 exoskeleton burdens of turtles (Reptilia: Testudines) in southeastern United States. Herpetologica **30:406-409**.

- Jenkins, J. H., J. R. Monroe, and F. B. Golley. 1969. Comparison of fallout  $^{137}\text{Cs}$  accumulation and excretion in certain southeastern mammals. pp. 623-626. IN: D. J. Nelson and F. C. Evans (eds.), Second National Symposium on Radioecology, Oak Ridge, Tennessee, May 15-17, 1967. USAEC Report CONF-670503.
- Kanter, P. M., and H. S. Schwartz. 1982. A fluorescence enhancement assay for cellular DNA damage. Molec. Pharmacol. **22:145-151.**
- Kiviat, E. 1980. A Hudson River tidemars, snapping turtle population. Trans. Northeast Fish Wildl. Conf., Ellenville, New York, April 27-30, 1980. pp. 158-168.
- Knight, A. W., and J. W. Gibbons. 1968. Food of the painted turtle, Chrysemys picta, in a polluted river. Am. Midl. Nat. **80:558-562.**
- Kolehmainen, S., E. Häsänen, and J. K. Meittinen. 1966.  $^{137}\text{Cs}$  levels in fish of different limnological types of lakes in Finland during 1963. Health Phys. **12:917-922.**
- Koning, J., R. Kirchmann, G. B. Gerber, R. Van Bruwaene, and J. Colard. 1984. Distribution of  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  in mallard ducks. Health **46:684-687.**
- Krajicek, J. J. and S. R. Overmann. 1988. The common snapping turtle (Chelydra seroentina): a bioindicator species for lead (Pb) contamination in aquatic environments. Abstract, Society of Environmental Toxicology and Chemistry Ninth Annual Meeting. Arlington, Virginia, November 13-17, 1988.
- Kulp, J. L. 1965. Radionuclides in man from nuclear tests. pp. 247-284. IN: E. B. Fowler (ed.), Radioactive Fallout. Soils. Plants. Food. Man. Elsevier Publishing Co., Amsterdam. 317 pp.
- Lagler, K. F. 1943. Food habits and economic relations of the turtles of Michigan with special reference to game management. Am. Midl. Nat. **29:257-312.**
- Langham, W. H. 1965. Considerations of biospheric contamination by radioactive fallout. pp.3-8. IN: E. B. Fowler (ed.), Radioactive Fallout. Soils. Plants. Foods. Man. Elsevier Publishing Company, New York, New York. 317 pp.

- Larsen, I. L. 1981. Strontium-90 determinations by Cerenkov radiation counting for well monitoring at Oak Ridge National Laboratory. ORNL/TM-7760. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Lauchli, A. 1969. Radioassay for emitters in biological materials using Cerenkov radiation. Int. J. Appl. Radiat. Isot. **20:265-270.**
- Leonzio, c., C. Fossi, and S. Focardi. 1986. Heavy metals and selenium variation in a migratory bird wintering in a mercury polluted lagoon. Bull. Environ. Contam. Toxicol. **37:219-225.**
- McKim, J. M., Jr. and K. L. Johnson. 1983. Polychlorinated biphenyls and p,p'-DDE in loggerhead and green postyearling Atlantic sea turtles. Bull. Environ. Contam. Toxicol. **31:53-60.**
- Mailhot, H., R. H. Peters, and R. J. Comett. 1989. The biological half-time of radioactive Cs in poikilothermic and homeothermic animals. Health Phys. **56:473-484.**
- Matsumura, F. 1975. Toxicology of Insecticides. Plenum Press, New York. 503 pp.
- Meeks, R. L. 1968. The accumulation of <sup>36</sup>Cl ring-labeled DDT in freshwater marsh. J. Wildl. Manage. **32:376-398.**
- Morreale, S. E., J. W. Gibbons, and J. D. Congdon. 1984. Significance of activity and movement in the yellow-bellied slider turtle (Trachemys scripta). Can. J. **2001. 62:1038-1042.**
- Mosimann, J.E. and J. R. Bider. 1960. Variation, sexual dimorphism, and maturity in a Quebec population of the common snapping turtle, Chelydra serpentina. Can. J. Zool. **38:19-38.**
- Mulhem, B. M., W. L. Reichel, L. N. Locke, T. G. Lamont, A. Belisle, E. Cromartie, G. E. Bagley, and R. M. Prouty. 1970. Organochlorine residues and autopsy data from bald eagles 1966-1968. Pestic. Monit. J. **4:141-144.**
- Murphy, S. D. 1980. Pesticides. pp. 357-408. IN: J. D. Doull, C. D. Klassen, and M. O. Amdur (eds.), Casarett and Doull's Toxicology The Basic Science of Poisons, second edition. Macmillan Publishing Co., Inc., New York. 778 pp.
- National Research Council. 1986. Ecological Knowledge and Problem Solving. National Academy Press, Washington, D.C. 388 pp.

- Neill, W. T. 1948. Hibernation of amphibians and reptiles in Richmond. County, Georgia. Herpetologica 4:107-114.
- Nelson, D. J. 1966. The prediction of strontium-90 uptake in fish using data on specific activities **and biological** half-lives. pp. 843-858. IN: B. **Aberg** and F. P. **Hungate** (eds.). Proceedings of the International Symposium on Radioecological Concentration Processes, Stockholm, Sweden, April 25-29, 1966.
- Nelson, M. A. and R. J. Bull. 1988. Induction of strand breaks in DNA by trichloroethylene and metabolites in-rat and **mouse** liver **in vivo**. Toxicol. Appl. Pharmacol. 94:45-54.
- Oakes, T. W., B. A. Kelly, W. F. Ohnesorge, J. S. Eldridge, J. C. Bird, K. E. Shank, and F. S. Tsakeres. 1982. Technical background information for **the environmental** safety report Vol. 4: White Oak Lake and Dam. ORNL-5681. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Obbard, M. E. and R. J. Brooks. 1980. Nesting migrations of the snapping turtle (Chelydra serpentina). Herpetologica 36:158-162.
- Obbard, M. E. and R. J. Brooks. 1981. A radio-telemetry and mark-recapture study of activity in the common snapping turtle, Chelydra serpentina. i \_\_\_\_ a \_\_\_\_ 1981:630-637.
- Olafsson, P. G., A. M. Bryan, B. Bush, and W. Stone. 1983. Snapping turtles - a biological screen for **PCBs**. Chemosphere 12:1525-1532.
- Ohlendorph, H. M., D. J. Hoffman, M. K. Saiki, and T. W. Aldrich. 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impact by selenium from irrigation drainwater. Sci. Total 52:49-63.
- Ophel. I. L. and C. D. Fraser.. 1971. The fate of **cobalt-60 in a** natural freshwater ecosystem. pp.323-327. IN: D. J. Nelson (ed.). Third National Symposium on Radioecology, Oak Ridge, Tennessee May 10-12, 1971. USAEC Report **CONF-710501-P1**.
- Ophel, I. L. and J. M. Judd. 1962. Absorption of **radiostrontium** by the gills of freshwater fish. Nature 194:1187-1188.
- Owen, P. J. and M. R. Wells. 1976. Insecticide **residues in two** turtle species following treatments **with DDT**. Bull. Environ. Contam. 115:406-411.

- Pearson, J. E., K. Tinsley, T. Hernandez. 1973. Distribution of dieldrin in turtles. Bull. Environ. Contam. Toxicol. **10:360-364.**
- Peters, E. L. 1986. Radiocesium kinetics in the yellow-bellied turtle. M.S. Thesis. University of Georgia, Athens. 51 pp.
- Peters, E. L. and I. L. Brisbin, Jr. 1988. Radiocesium elimination in the yellow-bellied turtle (Trachemys scripta). J. Appl. Ecol. **25:461-471.**
- Phillips, J. B., and M. R. Wells. 1974. Adenosine triphosphatase activity in liver, intestinal mucosa, cloacal bladder, and kidney tissue of five turtle species following in vitro treatment with **1,1,1-trichloro-2,2-bis** (p-chlorophenyl)ethane (DDT). J. Agric. Food Chem **22:404-407.**
- Punzo**, F., J. Laveglia, D. Lohr, and P. A. Dahm. 1979. Organochlorine insecticide residues in amphibians and reptiles from Iowa and lizards from Southeastern United States. Bull. Environ. Contam. **~~21:842~~-848.**
- Reeves, R. G., D. W. **Woodham**, M. C. Ganyard, and C. A. Bond. 1977. Preliminary monitoring of agricultural pesticides in a cooperative Tobacco Pest Management Project in North Carolina, **1971-first** year study. Pestic. Monit. J. **11:99-106.**
- Robbiano, L. and A. **Pino**. 1981. Induction in rats of liver DNA **single-**strand breaks by the polychlorinated biphenyl Aroclor 1254. Boll. Soc. Ital. Biol. Sper. **57:407-412.**
- Robinson, K. **M.** and M. R. Wells. 1975. Retention of a single oral dose of cadmium in tissues of the softshell turtle, Trionyx sninifer. Bull. Environ. Contam. Toxicol. **14:750-752.**
- Rogers, J. G., K. L. Daniels, S. T. Goodpasture, and C. W. Kimborough. 1988. Environmental surveillance of the U.S. Department of Energy Oak Ridge reservation and surrounding environs during 1987. **ES/ESH-4/V2.** Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Rosene, W., Jr., P. Stewart, and V. Adomaitis. 1961. 'Residues of heptachlor epoxide in wild animals. Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm. **15:107-113.**
- Ryon, M. G. 1988. Fishes. Section 6.2, pp. 211-239. IN: J. M. Loar (**ed.**), Second Annual Report on the ORNL Biological Monitoring and

- Abatement Program. ORNL/TM-DRAFT. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Schmitt, C. J., J. L. Zajicek, and M. A. **Ribick**. 1985. **National** Pesticide Monitoring Program: residues of organochlorine chemicals in freshwater fish, 1980-1981. Arch. Environ. **Contam. Toxicol.** **14:225-260.**
- Scott, D. E., . W. **Whicker** and J. W. Gibbons. 1986. Effect of season and the retention of <sup>137</sup>Cs and 90Sr by the yellow-bellied slider turtle (Trachemys scripta). Can. J. Zool. **64:2850-2853.**
- Shellabarger, C. J., A. Gorbman, F. C. Schatz, and D. McGill. 1956. Some quantitative and qualitative aspects of I<sup>131</sup> metabolism in turtles. Endocrinology **59:331-339.**
- Shugart, L. R. 1988. Quantification of chemically induced damage to DNA of aquatic organisms by alkaline unwinding assay. Aquat. Toxicol. **13:43-52.**
- Smith, M. I., B. B. Westfall, and E. F. Stohlam, Jr. 1937. The elimination of selenium and its **distribution** in the tissues. U.S. Health Rep. **52:1171-1177.**
- Southworth, G. R. 1987. Bioaccumulation studies. Section 4, pp. 86-114. IN: J. M. Loar (ed.), First Annual Report on the ORNL Biological Monitoring and Abatement Program. **ORNL/TM-DRAFT.** Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Southworth, G. R. 1988. Bioaccumulation studies. **Section 4,** . pp. 99-142. IN: J. M. Loar (ed.), Second Annual Report on the **ORNL** Biological Monitoring and Abatement Program. ORNL/TM-DRAFT. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

- Spigarelli, S. A. 1971. Ecological factors affecting the accumulation of cesium-137 fallout by a natural population of largemouth bass (Micronterus salmoides). pp. 328-333. IN: D. J. Nelson (ed.). Proceedings of the Third National Symposium on Radioecology, Oak Ridge, Tennessee, May 10-12, 1971. USAEC Report **CONF-710501-P1**.
- Stickel**, L. F. 1951. Woodmouse and box turtle populations in an area treated annually with DDT for five years. J. Wildl. Managee. **15:161-164**.
- Stone, W. B., E. Kiviat, and S. A. Butkas. 1980. Toxicants in snapping turtles. N. Y. Fish Game J. **27:39-50**.
- Stoneburner, D. L., M. N. Nicora, and E. R. Blood. 1980. Heavy metals in loggerhead sea turtle eggs (Caretta caretta): evidence to support the **hypothesis that** demes exist in the western Atlantic Population. J. Herpetol. **14:171-175**.
- Stribling, H. L., I. L. Brisbin, Jr., and J. R. Sweeney. 1986. Radiocesium concentrations in two populations of feral hogs. Health Phys. **50:852-854**.
- Suter, G. W. 1989. Ecological endpoints. pp. 21-28. IN: W. Warren Hicks, B. R. Parkhurst, and S. S. Baker, Jr. (eds.), Ecological Assessment of Hazardous Waste Sites: A Field and Laboratory Reference. **EPA/600/3-89/013**. United States Environmental Protection Agency.
- Thompson, N. P., P. W. **Rankin**, and D. W. Johnston. 1974. Polychlorinated biphenyls and **p,p'**-DDE in green turtle eggs from Ascension Island, South Atlantic Ocean. Bull. Environ. Contam. Tox. **399-406**.
- Towns, A. L. 1987. <sup>137</sup>Cs and <sup>90</sup>Sr in turtles: a whole-body measurement technique and tissue distribution. M.S. Thesis, Colorado State University, Fort Collins, Colorado.
- Vanderploeg, H. A., D. C. Parzyck, W. H. Wilcox, J. R. **Kercher**, and **S. V. Kaye**. 1975. Bioaccumulation factors for radionuclides in freshwater biota. ORNL-5002. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

- Watson, M. R., W. B. Stone, J. C. Okoniewski, L. M. Smith. 1985. Wildlife as monitors of the **movement of** polychlorinated biphenyls and other organic organochlorine compounds from a hazardous waste site. Trans. Northeast. Fish Wildl. Conf., Hartford, Connecticut, May 5-8, 1985. pp. 91-104.
- Wells, M. R., J. B. Phillips, and G. G. Murphy. 1974. **ATPase** activity in tissues of the map turtle, *Graptemys geographica*, following in vitro treatment with aldrin and dieldrin. Bull. Environ. Contam. Toxicol. **11:572-576.**
- Woodwell, G. M., C. F. Wurster, Jr., and P. A. Isaacson. 1967. DDT residues in an east coast estuary: a case of biological concentration of a persistent insecticide. Science **156:821-824.**

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APPENDIX A

SUMMARY OF CONTAMINANT CONCENTRATIONS IN TURTLES



Table A.1. Pesticide concentrations in field-collected turtles

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference		
Aldrin	<i>Kinosternon flavescens</i>	Flooded rice field, TX	F	4.00 whole body*	1	Elevated concentration detected.	Flickinger and Mulhern 1980		
	<i>Kinosternon flavescens</i>	Klco field, TX	unkn.	n.d. <sup>b</sup> whole body	3	Contaminant not detected.	Flickinger and King 1972		
	<i>Trachemys scripta</i>	Rice field, TX	unkn.	4.9 whole body 0.2 unlaidd eggs*	2 4	Concentrations higher than in <i>K. flavescens</i> . lower concentrations detected in eggs than in adults.	Flickinger and King 1972		
Chlordane	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	1.30 fat	8	Trans, cis, and oxy forms measured oxy-chlordane was the predominant form, concentrations reported here. Males generally had higher concentrations than the females.	Aibers et al. 1986		
		Contaminated wetland #2, NJ	F	0.52 fat	8				
		Reference wetland, MD	M	1.21 rot 1.09 fat	1 6				
	<i>Caretta caretta</i>	National Wildlife Refuge, FL	..	n.d. - <del>0.001</del> 0.001	9			Trace amounts present in 2 eggs.	Clark and Krynsky 1980
DDT and metabolites	<i>Caretta caretta</i>	East Coast, FL	unkn.	n.d.-0.040 muscle n.d.-0.051 liver	9 8			Trace amounts detected.	McKim and Johnson 1983
	<i>Caretta caretta</i>	National Wildlife Refuge, FL	..	0.047 eggs	9			DDE in 11 eggs with the mean reported here. DDT was detected in 2 eggs (n.d.-0.048).	Clark and Krynsky 1990
	<i>Caretta caretta</i>	National Wildlife Refuge, FL	..	0.099 eggs	56	DDE measured. DDE concentrations did not decline during incubation.	Clark and Krynsky 1985		

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
DDT and metabolites (cont. )	<i>Caretta caretta</i>	Seashores, CA, SC	--	0.058-0.305 <sup>a</sup> egg yolks	unkn.	Total of DDT, DDE, and TDE reported.	Hillestad <i>et al.</i> 1974
	<i>Chelonia mydas</i>	cut Coast, FL	unkn.	n.d. muscle n.d. liver	4 4	Not detected at < 0.005 $\mu\text{g/g}$ .	McKim and Johnson 1983
	<i>Chelonia mydas</i>	National Wildlife Refuge, FL	--	n.d.-0.042 eggs	2	DDT detected in 1 egg (reported here). DDE not detected in 1 egg at 0.005. DDE higher in <i>C. caretta</i> than <i>C. mydas</i> .	Clark and Krynitzky 1980
	<i>Chelonia mydas</i>	Ascension Island -	-	n.d.-0.009 egg yolks	10	DDE detected in 7 eggs	Thompson <i>et al.</i> 1974
	<i>Chelydra</i> ♀♂♂♂♂♂	Contaminated wetland #1, NJ	M	0.02 fat	8	DDT, DDE, and TDE measured. DDE was the only form detected (values reported here in all turtles). TDE at 0.1 ppm was detected in turtles from NJ site 02.	Albers <i>et al.</i> 1986
		Contaminated wetland #2, NJ	M	0.06 fat	3		
		Reference wetland, MD	M	1.49 fat	8		
			M	0.26 fat	7		
		F	0.01 fat	6			
<i>Chelydra</i> ♂	rponclw Hudson River, NY	M	n.d.-57.5 fat n.d.-17.4 liver n.d.-0.26 muscle	6 11 12	DDE measured with highest concentrations reported in fat.	Stone <i>et al.</i> 1980	
		F	0.47 liver	5			
			0.17 muscle	5			
			<0.18 <sup>a</sup> um	5			
<i>Chelydra</i> ♂	rpmtlna Various waters in NY other than Hudson River	M	n.d.-81.3 fat n.d.-3.58 liver 0.033 muscle	4 4 2	Highest DDE concentration was in a male collected from Irondequoit gay.	Stone <i>et al.</i> 1980	
		F	4.43 fat	5			
			0.29 liver 0.016 muscle	3 3			
<i>Chelydra serpentina</i>	Agricultural area, IO	F	n.d. fat	1	DDE not detected in <i>C. serpentina</i>	Punzo <i>et al.</i> 1919	
		--	n.d.unlaid <sup>a</sup> gg	1			

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
DDT and metabolites (cont)	<i>Chelydra serpentina</i>	Tobacco fields (site #1), NC	unkn.	0.31 whole body	2	DDT, DDE, and DDE were measured in all samples. DDE was the only metabolite at a concentration greater than 0.04 ppm. Only DDE concentrations are reported here. There was no apparent difference.	Reeves et al. 1977
		tobacco fields (site #2), NC	unkn.	0.16 whole body	6		
	<i>Chelydra serpentina</i>	DDT radiolabeled marsh, OH	M	0.1 brain	1	DDT residue concentrations were highest in fat. C. # serpentina contained the greatest amount of DDT compared to <i>E. blandingii</i> and <i>C. picta</i> .	Heeks 1968
1.1 liver				1			
0.3 kidney				1			
n.d. heart				1			
13.0 fat				1			
0.2 lung				1			
2.2 testes				1			
0.1 muscle				1			
0.2 blood				1			
0.2 spleen				1			
0.2 pancreas				1			
0.2 shell	1						
	<i>Chrysemys picta</i>	Tobacco fields (site #1), NC	unkn.	0.43 whole body	3	See <i>Chelydra serpentina</i> , Reeves et al. 1977.	Reeves et al. 1977
		Tobacco fields (site #2), NC	unkn.	0.61 whole body	3		



Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
DDT and metabolites (cont.)	<i>Emys blandingii</i>	DDT radiolabeled marsh, OH	M	n.d. brain	1	See <i>Chelydra serpentina</i> , Meeks 1968.	Meeks 1968
				0.9 liver	1		
				0.4 kidney	1		
				0.1 hoort	1		
				3.8 tot	1		
				0.1 lungs	1		
				1.5 <sup>o</sup> testes	1		
				n.d. muscle	1		
				0.2 blood	1		
				n.d. spleen	1		
			P	0.1 pancreas	1		
				0.1 shell	1		
				0.3 brain	1		
				0.9 liver	1		
				0.2 kidney	1		
				0.1 heart	1		
				4.b fat	1		
				02. lung	1		
				0.1 muscle	1		
				0.5 ovary	1		
0.1 muscle	1						
0.6 shell	1						
	<i>Kinosternon flavescens</i>	Rice field, TX	unkn.	1.2 whole body	3	Higher concentrations detected in <i>K. flavescens</i> than <i>T. scripta</i> .	Flickinger and King 1972
	<i>Sternotherus odoratus</i>	Tobacco fields (site #1), NC Tobacco fields (site 02), NC	unkn.	0.36 whole body 0.19 whole body	4 b	See <i>Chelydra serpentina</i> , Reeves et al. 1977.	Reeves et al. 1977
	<i>Terrapene carolina carolina</i>	Forest area treated both with DDT, MD	both	not measured	86	No difference in population size was detected. Growth of 4 juvenile turtles from the DDT area appear normal.	Stickel 1951
		Reference forest, NO both	both	not measured	82		

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference
DDT and metabolites (cont.)	<i>Trachemys scripta</i>	Rice field, TX	unkn.	co.1 whole body 0.2unlaid ● aa'	2 4	Higher concentrations detected in the eggs.	Flickinger and King 1972
	<i>Trachemys scripta</i>	Tobacco fields (site ● ), NC	unkn.	0.12 whole body	7	See <i>Chelydra serpentina</i> , Reeves ● al. 1977.	Reeves ● et al.1977
		Tobacco fields (site #2), NC	unkn.	0.71 whole body	4		
Dieldrin	<i>Caretta caretta</i>	National Wildlife Refuge, FL	--	n.d.-0.0280 <sup>1</sup> y <sub>2</sub> ⊕	9	Trace amounts detected in 4 eggs.	Clark and Krynitsky 1980
	<i>Caretta caretta</i>	Seashords, GA, S C	--	Trace - 0.0564 <sup>1</sup> egg yolks	unkn.	trace amounts detected.	Hillestad et al. 1974
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	n.d. fat	8	Trace amounts detected in males from the freshwater NJ site.	Albers ● et al.1986
		Contaminated wetland #2, NJ	F	n.d. fat 0.05 fat	8		
	<i>Chelydra serpentina</i>	Reference wetland, ND	M F	0.02 fat n.d. fat	7 b	Trace amounts detected in ● ales only.	Albers ● et al.1986
	<i>Chelydra ● oqrtn4</i>	Agricultural area, 10	F --	n.d. fat n.d. ● U	1 1	Of the reptiles ● d amphibians ● xulwd, highest concentrations were reported in snakes.	Funzo et al. 1979
	<i>Chelydra ● orpontina</i>	Tobacco fields (site #1), NC	unkn.	<0.01 whole body	2	Not detected.	Reeves ● et al.1977
	Tobacco fields (site #2), NC	unkn.	<0.01 whole body	6	Not detected.		

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	P	Observation	Reference
Dieldrin (cont.)	<i>Chelydra serpentina</i>	Hudson River, NY	M	0.41 fat	6	Highest concentrations detected in fat samples in both sexes.	Stone et al. 1980
				n.d.-0.086 llvor	5		
			F	n.d.-0.034 muscle	6		
				17.0 fat	1		
			.	n.d.-0.026 llvor	3		
				n.d. muscle	2		
	<i>Chelydra serpentina</i>	Various waters in NY other than the Hudson River	M	n.d.-34.1 fat	4	Highest concentrations detected in fat from male collected from Irondequoit Bay (Lake Ontario).	Stone et al. 1980
				n.d.-0.99 llvor	4		
			F	n.d.-0.16 muscle	2		
				n.d.-2.40 fat	5		
			.	0.06 llvor	3		
				n.d.-0.01 muscle	3		
<i>Chrysemys picta bellii</i>	Agricultural roo, IO	M	0.074 fat	1	See <i>Chelydra serpentina</i> , Punzo et al. 1979.	Punzo et al. J. 1979	
<i>Chrysemys picta</i>	Tobacco fields (site #1), NC	unkn.	do.01 whole body	3	Not detected.	Reeves et al. J. 1977	
			<0.01 whole body	8	Not detected.		
<i>Kinosternon flavescens</i>	Flooded rice field, TX	F	47 whole body	1	Epoxidation of dieldrin to dieldrin may be a slow process in turtles.	Flickinger and Mulhern 1980	
<i>Kinosternon flavescens</i>	Rice field, TX	unkn.	0.6 whole body	3	Higher concentrations detected in <i>T. scripta</i> .	Flickinger and King 1972	
<i>Sternotherus odoratus</i>	Tobacco fields (site #1), NC	unkn.	<0.01 whole body	4	Not detected.	Reeves et al. 1. 1977	
			Tobacco fields (site #2), NC	unkn.	4.01 whole body		6

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Dieldrin (cont.)	<i>Trachemys scripta</i>	Tobacco foidr (site #1), NC	unkn.	<0.01 whole Body	6	Not dotected.	Rawer et al. 1977
		Tobacco foidr (alto #2), WC	unkn.	<0.01 uholo Body	4	lot detected.	
Endrin	<i>Trachemys scripta</i>	Rice field, TX	unkn.	1.2 whole body	2	Higher concentrations in eggs than in adults.	Flickinger and King 1972
			--	2.6 unlaid eggs	4		
	<i>Chelydra</i> sp. orpmtina	Contaminated wetland, #1, NJ	M	n.d. fat	8	Contaminant was net detected in turtles from this site.	Albers, et al. 1986
			F	n.d. fat	3		
	M	n.d. fat	8				
	<i>Chelydra serpentina</i>	Reference wetland, no	M	n.d. fat	7	Not detected	Albers et al. 1986
			F	n.d. fat	6		
	<i>Chelydra serpentina</i>	Tobacco fields (site #1), NC	unkn.	<0.01 whole body	2	Not dotected.	Reeves et al. 1977
			unkn.	a.01 whole body	1	Not detected.	
	<i>Chrysemys picta</i>	tobacco fields (site #1), NC	unkn.	<0.01 whole body	3	Not detected.	Reeves et al. 1977
unkn.			a.01 whole body	8	Not dotected.		
<i>Kinosternon flavescons</i>	Flooded rice field, TX	F	1.1 whole body	1	Above background concentration detected.	Flickinger et al. Mulhern 1960	
<i>Sternotherus odoratus</i>	Tobacco fields (site #1), NC	unkn.	<0.01 whole body	4	Not detected.	Reeves et al. 1977	
		unkn.	<0.01 uholo body	6	Not detected.		
<i>Trachemys scripta</i>	Tobacco foidr (site #1), NC	unkn.	<0.01 whole body	8	lot detected.	Reeves et al. 1977	
		unkn.	<0.01 whole body	4	Not detected.		
		Tobacco fields (site #2), NC					

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference
Heptachlor (Heptachlor epoxide)	<i>Caretta caretta</i>	National Wildlife Refuge, FL	--	n.d.-0.006 eggs	9	Detected in only 2 ♀♀	Clark and Krynitsky 1980
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	n.d. fat	8	Contaminant detected in males from the freshwater alto.	Albers et al. 1986
		Contaminated wetland #2, NJ	F	n.d. fat	3		
		Contaminated wetland #3, NJ	M	0.2. fat	8		
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.11 for	7	Trace amounts of the contaminant were detected in both sexes.	Albers et al. 1986
			F	0.03 fat	6		
	<i>Chelydra serpentina</i>	Agricultural area, IO	F	n.d. fat	1	Heptachlor epoxide was not detected in the samples.	Funzo et al. 1979
			--	n.d. eggs	1		
<i>Chelydra serpentina</i>	Land treated with heptachlor, CO	unkn.	not measured	1	Dead after 2 treatments of 0.33 kg/ha.	Ferguson 1963	
<i>Chrysemys picta bellii</i>	Agricultural area, IO	M	n.d. fat	1	Concentrations were higher in snakes than turtles from the site.	Funzo et al. 1979	
<i>Terrapene carolina</i>	Land treated with heptachlor, CO	unkn.	not measured	1	Dead after 2.25 kg/ha land treatment	Ferguson 1963	
<i>Trachemys scripta</i>	Land treated with heptachlor, GA & AL	unkn.	172.0 whole body	1	Dead after 279 days after a 2.29 kg/ha application to cotton.	Rosene et al. 1961	
Dioxin	<i>Caretta caretta</i>	National Wildlife Refuge, FL	--	n.d.	9	Only detected in 1 ♀	Clark and Krynitsky 1980.

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Dieldrin (cont.)	<i>Terrapene carolina</i>	Land treated with ● IroX over 7 years, MI	unkn.	1.1 llvor	6	Concentrations higher in <i>T. carolina</i> livers than in thoro of <i>T.</i> <i>scripta</i> . Also true for rho ● Rga.	Holcomb and Parker 1919
			unkn.	1.7 llvor	3		
			unkn.	1.3 llvor	1		
			unkn.	0.29 liver	5		
			unkn.	1.4 <sup>1</sup> unlaid ● ggr	unkn.		
			unkn.	1.6 <sup>4</sup> unlaid ● RRr	unkn.		
			unkn.	2.5 <sup>1</sup> unlaid egge	unkn.		
			unkn.	1.4 <sup>1</sup> unlaid egge	unkn.		
	<i>Trachemys scripta</i>	Land treated with ● IroX over 7 years, MI	unkn.	0.41 llvor	2	Peak residue levels in livers of both ● pocloa occurred 2.5 years after last treatment. Application 4 years after initial treatment had little effect.	Holcomb and Parker 1979
			unkn.	0.19 llvor	3		
			unkn.	0.16 llvor	4		
			unkn.	0.05 liver	9		
			unkn.	co.01 liver	6		
			..	1.9 <sup>1</sup> unlaid ● RRr	unkn.		
			..	2.2 <sup>1</sup> unlaid egge	unkn.		
..	0.15 <sup>4</sup> unlaid egge	unkn.					
		..	0.16 <sup>4</sup> unlaid ● UO	unkn.			
		..	0.04 <sup>4</sup> unlaid ● UI	unkn.			
Monachlor	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	1.05 fat	6	Trans and cis forms detected. Trans isomer slightly higher concentration. Total of the two isomers reported horo. Males had higher concentrations than females.	Albers et al. 196b
			F	0.39 fat	3		
			M	0.73 fat	8		
			M	0.92 fat	7		
		Contaminated wetland #2, NJ	M	0.46 fat	6		
		Reference wetland, MD	F				
	<i>Caretta caretta</i>	National Wildlife Refuge, FL	..	n.d.  ● ††	9	Detected in only one egg.	Clark and Krynitsky 1980
Toxaphene	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	n.d. fat	6	Contaminant not detected in ● nlur from contaminated and reference sites.	Albers et al. 1986
			F	n.d. fat	3		
			M	n.d. fat	8		
			A	n.d. fat	7		
			F	n.d. fat	6		
		Contaminated wetland ● 2, NJ					
		Reference wetland, MD					

Table A.1 (continued)

Pesticide	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Toxaphene (cont.)	<i>Kinosternon flavescens</i>	Flooded rice field, TX	F	0.3 whole body	1	Contaminant present	Flickinger and Mulhern 1980
	<i>Kinosternon flavescens</i>	Rice field, TX	unkn.	n.d. whole body	3	Contaminant not detected in any of the turtles.	Flickinger and King 1972
	<i>Trachemys scripta</i>	Rice field, TX	unkn.	n.d. whole body n.d. unslaid eggs	2 -4	Contaminant not detected.	Flickinger and King 1972

\*Shell was removed   • removed to have been removed prior to analysis in all cases.

\*Not detected.

\*Weight basis (dry or wet) not reported by author.

\*Concentration based on dry weight.

Table A.2. Polychlorinated biphenyl concentrations in field-collected turtles

PCBs	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference
Aroclors 1248 & 1254	<i>Chelonia mydas</i>	Ascension Island	-		10	Aroclor 1242 & 1254 major PCB contaminants. Mean concentration of both reported.	Thompson et al. 1974
Aroclors 1260 & 1254	<i>Chelydra serpentina</i>	5 rivers and 1 lake site, MN	M	Co.025 muscle	10	Higher concentrations in fat than muscle.	Helwig and Hora 1983
			F	<0.025-0.086 muscle	9		
				3.2 fat	5		
			unkn.	Co.025 muscle	2		
				<0.2 fat	1		
Aroclor 1260	<i>Caretta caretta</i>	National Wildlife Refuge, FL	--		9	37% of the eggs sampled contained detectable levels of PCBs the mean of which is reported.	Clark and Krynitisky 1990
Total	<i>Caretta caretta</i>	East Coast, FL	unkn.	0.001 muscle	9	Higher concentrations in liver than muscle.	McKim and Johnson 19.3
				n.d.-0.133 liver	8		
Total	<i>Caretta caretta</i>	East Coast, FL	unkn.	0.0019 muscle	4	Slightly higher PCB concentrations in <i>C. caretta</i> .	McKim and Johnson 1983
				n.d.-0.070 liver	4		
Total	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	40.41 fat	8	Male turtles from the brackish-water NJ site (1) had the highest concentrations of PCBs and were 100% higher than that in males from the freshwater MN site (2) and the MD site.	Albers et al. 1986
			F	a.41 fat	3		
		Contaminated wetland #2, NJ	M	17.2. fat	8		
		Reference wetland, MD	M	26.08 fat	7		
			F	25.68 fat	6		
Total	<i>Chelydra</i>	Upper Hudson River, NY		3,608 fat	1	Elevated concentrations in turtles from both sites.	Olafsson et al. 19.3
		Lake Ontario	M	633 fat	1		

TABLE A. 0 / . . .

PCBs	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference
Total	<i>Chelydra serpentina</i>	Ponds near industrial chemical disposal area, NY.	unkn.	4,530 fat 185 liver 17 muscle 81 fat	2 1 1 1	PCB concentrations reported for numerous biota with the highest concentration reported in the fat of the turtle from pond number 1.	Watson et al. 1985
Total	<i>Chelydra serpentina</i>	Upper Hudson River, NY	..	1.98 egg yolks 0.30 egg whites & shell	2 2	Values are the total of 5 isomers. 95% of the total toxicity (based on toxic equivalent of TCDD) was in the yolk.	Bryan et al. 1987b
Total	<i>Chelydra serpentina</i>	Contaminated dump site, NY	M	1600 fat 100 testes 82 brain 72 liver 49 heart 48 kidney 48 pancreas 13 lungs	1 1 1 1 1 1 1 1	Highest concentration in fat followed by testes, brain and liver.	Bryan et al. 1987a
Total	<i>Chelydra serpentina</i>	Reference pond, NY	M	4.2 fat 1.6 testes 1.0 brain 1.0 liver 0.64 heart 1.2 kidney 1.2 pancreas 0.41 lungs	1 1 1 1 1 1 1	Contaminant detected at very low concentrations.	Bryan et al. 1987a
Total	<i>Chelydra serpentina</i>	Hudson River, NY	M	3560 fat 82.2 liver 3.3 muscle 1.123 fat 38.5 liver 5.4 muscle 28.9 unfiled eggs	6 14 15 1 7 6 6	Highest concentrations reported in fat.	Stone et al. 1980

Table A.2 (continued)

PCBs	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference
Total	<i>Chelydra</i> ♀ □ □ ♦ ◆ ● ■ ☉	Various waters in NY other than the Hudson River	F	745 fat 1.4 liver 0.42 muscle 240 fat 0.1 liver 0.46 muscle	4 4 2 5 4 4	Highest concentrations reported in fat concentrations in muscle were within the FDA limit <del>×</del> □ □ ● diblo fish.	Stone et al. 1910

\*Shell was removed or assumed to have been removed prior to analysis in all cases.

Table A.3. Metal concentrations in field-collected turtles

Metal	Species	Location	Sex	Concentration (µg/g wet weight) N	Observation	Reference	
Aluminum	<i>Caretta caretta</i>	National Seashores, FL, GA, NC	--	1.23-2.19 egg yolks 9 6	Range of mean concentrations from 4 sites. Highest mean concentration in eggs from Canaveral National Seashore, FL.	Stoneburner et al. 1980	
Barium	<i>Caretta caretta</i>	National Seashores, FL, GA, NC	--	0.73-2.39 egg yolks 9 6	Range of mean concentrations from 4 sites. Highest mean concentration in eggs from Cape Lookout National Seashore, NC.	Stoneburner et al. 1980	
Cadmium	<i>Caretta caretta</i>	National Seashores, FL, GA, NC	--	0.01-0.07 egg yolks 9 6	Range of mean concentrations from 4 sites. Highest mean concentration above 0.1 µg/g reported in eggs from Cumberland Island National Seashore, GA and Canaveral National Seashore, FL.	Stoneburner et al. 1980	
	<i>Caretta caretta</i>	Seashores, GA, AL	--	0.17 µg U yolks 0.56 µg lbuln	unkn. Concentration in lbuln higher than in yolk.	Hillestad et al. 1974	
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	0.25 kidney 0.10 liver	8	Cd detected in low concentrations in all tissues. Highest concentrations were in kidney tissues of turtles from the NJ brackish water sites (#2).	Albers et al. 1986
			F	0.10 kidney 0.08 liver	3		
		Contaminated wetland #2, NJ	M	0.09 kidney 0.00 liver	8		
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.01 kidney 0.01 liver	7	The metal was detected in turtles from all sites.	Albers et al. 1986
			F	0.07 kidney	6		

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Cadmium (conc.)	<i>Chelydra serpentina</i>	5 river & 1 lake sites, MN	M F	0.010 muscle 0.012 unclo	8 4	total range of values from 0.002 to 0.025.	Helwig and Norm 1983
	<i>Trionyx spiniferus</i>	River that received effluent from plating industries, TN	F	9.07 kidney 0.19 small intestine	12 12	Of the tissues analyzed, concentrations were highest in kidney and lowest in small intestine.	Robinson and Wells 1915
Chromium	<i>Caretta caretta</i>	National Seashores, - - FL, CA, NC		0.36-0.60 egg yolkm	96	Range of mean concentrations from 4 to 11mm. Highest mean concentration reported in egg from Cumberland Island National Seashore, CA.	Stoneburner et al. 1960
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	2.97 kidney 0.10 liver	8 8	Highest concentrations in kidney tissues of turtles from two NJ brackish water sites.	Albers et al. 1966
			F	2.70 kidney	3		
		Contaminated wetland #2, NJ	M	0.60 liver 1.13 kidney 0.36 liver	3 8 8		
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.93 kidney 1.00 liver	7 7	See also Albers et al. 1916.	Albers et al. 1966
F			1.26 kidney 1.97 liver	6 6			
Cobalt	<i>Caretta caretta</i>	National seashores, - - FL, CA, NC		0-0.024 $\mu\text{g}$ U yolkm	96	Range of mean concentrations from 4 sites. Low concentrations detected.	Stoneburner et al. 1960
Copper	<i>Caretta caretta</i>	National seashores, - - FL, CA, NC		1.73-2.30 $\mu\text{g}$ S yolkm	96	Eggs from 11 sites contained elevated copper concentrations.	Stoneburner et al. 1960

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference	
Copper (cont.)	<i>Caretta caretta</i>	Soomhorom, CA, SC --		2.00* egg yolks 6.0* albumin		Concentration in unkn. • Ibum higher than in yolk.	Hillestad C01. 1974	
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	1.81 kidney 9.72 liver	8 8	Higher concentration reported in liver than in kidney tissue. Jax-rotod difference detected in NJ block water turtle, with the concentration detected in the liver of males • significantly higher than that in the turtle.	Abers et al. 1986	
			F	1.21 kidney 5.17 liver	3 3			
		Contaminated wetland # 2, NJ	M	1.73 kidney 2.00 liver	8 8			
			F	1.07 kidney 1.57 liver	6 6			
	<i>Chelydra serpentina</i>	Reference wetland, Ho	M	0.02 kidney 1.20 liver	7 7			
			F	1.07 kidney 1.57 liver	6 6			
	Iron	<i>Caretta caretta</i>	National Seashores, - FL, BA, WC		24.8-26.0 • g(yolks 96		Range of mean concentration from 4 • from. Second highest concentration of the metals analyzed (Zn, Fe, Sr).	Stonoburner et al. 1960.
	Lead	<i>Caretta caretta</i>	National Seashores, - FL, CA, NC		0.39-0.76 • SS yolkm 96		Range of mean concentration from 4 sites. Highest mean concentration reported in • sp from Canaveral National Seashore, FL.	Stonoburner et al. 1980
		<i>Caretta caretta</i>	Seashores, CA, SC --		2.87* egg yolks 12.0* • hln		Concentration in unkn. albumin higher than in yolk.	Hillestad et al. 1974
<i>Chelydra serpentina</i>		Pb contaminated river, HO Less contaminated upstream • Ho, HO Non-contaminated upstream • Ho, HO	both	13.7 carapace 5.6 blood		unkn. <i>C. serpentina</i> • good indicator unkn. • species for Pb. Shell unkn. and blood served • unkn. useful non-toxic tissue-type samples.	Krajicek and Overman 1988	
	both		0.4 carapace 1.1 blood					
	both		0.5 carapace 0.1 blood					

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration (µg/g wet weight)	N	Observation	Reference		
Lead (cont.)	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	0.19 kidney	8	Background concentrations of Hg detected in turtles from the contaminated and reference sites. No consistent relationship between Pb concentrations in liver and kidney tissues.	Albers et al. 1986		
			F	n.d. liver	8				
		Contaminated wetland #2, NJ	F	n.d. kidney	3				
			M	n.d. liver	3				
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.10 kidney	8			See <i>Chelydra serpentina</i> , Albers et al. 1966.	Albers et al. 1966
			F	0.12 liver	8				
			M	0.01 kidney	7				
			F	0.01 liver	7				
	<i>Terrapene carolina</i>	Woodland area near Pb smelter, NO	M	0.16 kidney	6	Highest concentrations in bone followed by kidney and liver.	Beresford et al. 1981		
				n.d. liver	6				
				51.6 humerus	4				
				64.5 femur	4				
				21.6 liver	4				
				24.3 kidney	4				
	<i>Terrapene carolina</i>	Reference woodland, W	M	6.00 blood	4	Pb concentrations in liver, kidney skin, blood and bone were significantly higher in turtles near the smelter. Pb levels were similar in liver and kidney tissues.	Beresford et al. 1961		
				0.35 skin	4				
0.20 lung				4					
4.51 humerus				1					
5.55 femur				1					
2.21 liver				1					
4.03 kidney				1					
0.22 blood				1					
n.d. skin				1					
n.d. lung				1					
<i>Terrapene carolina</i>	Reference woodland, W	F	2.41 humerus	3					
			3.21 femur	3					
			0.00 liver	3					
			0.11 kidney	3					
			n.d.-0.15 blood	3					
			a.d.-0.16 kidney	3					
			n.d. lung	3					

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Mercury	<i>Caretta caretta</i>	National Seashores, FL, GA, NC	--	0.14-0.48 • U yolks	96	Range of mean concentrations from 4 • • • • • Highest concentrations detected in • '6r from Canaveral National Seashore, FL.	Stoneburner et al. 1980
	<i>Chelydra serpentina</i>	5 river & 1 lake sites, MN	M	$\leq 0.02$ fat	9	Highest concentrations of Hg detected in the muscle of the females.	Helwig and Hora 1963
				0.12 muscle	10		
			F	0.03 fat	5		
				0.21 muscle	5		
			unkn.	0.03 fat	1		
				0.1 muscle	2		
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ Contaminated wetland • 2, NJ	M	0.55 kidney	6	Highest concentrations were reported in liver. Although Hg was detected in • adlrnta (0.4-2.80 ppm), Hg concentrations were relatively low in the turtles.	Albers • cal. 1916
			1.28 liver	8			
F			0.41 kidney	3			
			1.27 liver	3			
		M	0.39 kidney	8			
			0.60 liver	6			
<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.44 kidney	7	Hg detected in • nlula from the reference site.	Albers • c • J.1986	
		F	0.90 liver	7			
			0.56 kidney	6			
			0.41 liver	6			
<i>Kinosternon flavescens</i>	Rice Hold, 7X	unkn.	0.12 whole body	3	Low concentrations of Hg detected in both species.	Flickinger and King 1972	
<i>Trachemys scripta</i>	Rice field, TX	unkn.	0.08 whole body'	2	Higher concentrations detected in the adults than eggs.	Flickinger and King 1972	
			n.d. unlaid • UI	4			

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference	
Molybdenum	<i>Caretta caretta</i>	National Seashores, -- FL, GA, WC		1.09-6.23	6	Sryolks 96	Range of mean concentrations from 4 sites. Highest mean concentration reported in SS* from Canaveral National Seashore, FL.	Stoneburner et al 1960
Nickel	<i>Caretta caretta</i>	National Seashores, -- FL, GA, NC (4 sites)		0-0.79	96	SSyolks	Range of mean concentrations from 4 sites. Only SSr from Cape Lookout National Seashore, NC contained concentrations greater than 0.09 $\mu\text{g/g}$ .	Stoneburner et al 1980
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	1.24 kidney	8		Highest mean concentration reported in kidney tissues from turtles collected from the NJ brackish water sites (#2).	Albers et al. 1966
		Contaminated wetland #2, NJ	F	0.24 liver	8			
			M	1.01 kidney	3			
		F	0.27 liver	3				
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	0.45 kidney	8			
			F	0.13 liver	8			
				0.35 kidney	7		See above, Albers et al. 1966.	Albers et al. 1966
				0.44 liver	7			
				0.43 kidney	6			
				0.99 liver	6			
Strontium	<i>Caretta caretta</i>	National Seashores, -- FL, GA, NC		23.9-25.8	96	&g yolks	Range of mean concentrations from 4 sites. Third highest concentration of the metals analyzed. (Zn, Fe, Sr).	Stoneburner et al. 1980
Zinc	<i>Caretta caretta</i>	National Seashores, -- FL, GA, NC		25.6-28.0	96	egg yolk	Range of mean concentrations from 4 sites. Of the heavy metals analyzed, highest concentrations were reported for zinc.	Stoneburner et al. 1980

Table A.3 (continued)

Metal	Species	Location	Sex	Concentration ( $\mu\text{g/g}$ wet weight)	N	Observation	Reference
Zinc (cont.)	<i>Caretta caretta</i>	Seashores, GA, S C	- -	2.06 <sup>a</sup> egg yolks 6.0 <sup>a</sup> albumin	unkn.	Concentration in albumin higher than in yolk.	Hillestad et al. 1914
	<i>Chelydra serpentina</i>	Contaminated wetland #1, NJ	M	9.93 kidney 50.4 liver	6 6	Highest concentrations reported in liver tissue. Highest concentrations in the livers of wild turtles from the NJ brackish- water site (#2).	Albers et al. 1966
		Contaminated wetland #2, NJ	F	9.79 kidney 38.0 liver	3 3		
			M	10.5 kidney 30.7 liver	6 6		
	<i>Chelydra serpentina</i>	Reference wetland, MD	M	8.80 kidney 21.1 liver	7 7		
			f	9.60 kidney 28.3 liver	6 6	5.. above, Albers et al. 1966.	Albers et al. 1966

<sup>a</sup>Weight basis (dry or wet) not reported by authors.

<sup>b</sup>Not detected.

<sup>c</sup>All whole body ● .lyar did not include the shell.

Table A.4. Radionuclide concentrations in field-collected turtles

Radionuclide	Species	Location	sax	Concentration (Bq/g wet weight)	N	Observation	Reference
<sup>137</sup> Cs	<i>Chelydra serpentina</i>	Spiked pond, OH	unkn.	2.61 <sup>a</sup> whole body.	2	1.48 x 10 <sup>3</sup> Bq <sup>137</sup> Cs added to pond. Turtles were immigrants.	Brungs 1967
	<i>Trachemys scripta</i>	Savannah River Plant seepage basins, SC	both	2.11 whole body	36	Initial total body burden reported here. Seasonal variation in elimination rates of <sup>137</sup> Cs. Average yearly biological half-life 64 days.	Scott et al. 1986
	<i>Trachemys scripta</i>	Reference ponds, SC	both	0.0014 whole body	26	Turtles contained low levels of <sup>137</sup> Cs.	Scott et al. 1986
	<i>Trachemys scripta</i>	Savannah River Plant seepage basins, SC	both	9.0 whole body	15	Initial total body burden reported here. (213-64) day. vs. determined at the time to reach an equilibrium <sup>137</sup> Cs concentration. Elimination rate was 7.2 x 10 <sup>-3</sup> ± 4.4 x 10 <sup>-3</sup> kBq/day.	Peters and Brisbin 1988 Peters 1986
	<i>Trachemys scripta</i>	Savannah River Plant seepage basin, SC	unkn.	5.18 whole body	7	Concentrations in organs and tissues were normalized to that in muscle. Cesium in muscle was twice that in other soft tissues. Actual concentrations not reported.	Towns 1981
	<i>Trachemys scripta</i>	Contaminated reservoir, SC	unkn.	0.71 whole body	10	See above, Towns 1981.	Towns 1987

Table A.4 (continued)

Radionuclide	Species	Location	Sex	Concentration (Bq/g wet weight)	N	Observation	Reference
<sup>60</sup> Co	<i>Chelydra serpentina</i>	Spiked pond, OH	unkn.	0.94 <sup>a</sup> whole body	2	1.4 <sup>a</sup> x 10 <sup>3</sup> Bq <sup>60</sup> Co added to the pond. Turtles were 1ml grant*.	Brungs 1967
	<i>Chelydra serpentina</i>	Lake contaminated with <sup>60</sup> Co from a nearby disposal pit, Ontario, Canada	unkn.	34.1 uhol. body 140.4 carapace 4.1 whole body ● inuicarapace	1 1 1	0.37 Bq/l in lake water. Inverse relationship between concentration in organism and trophic level.	Ophel and Fraser 1911
	<i>Chelydra serpentina</i>	Uncontaminated ● t.l.s. MI	unkn.	0.79 <sup>a</sup> ah.11	2	<sup>90</sup> Sr detected.	Holcomb et al. 1971
<sup>90</sup> Sr	<i>Chelydra serpentina</i>	Uncontaminated sites, FL	unkn.	0.3 <sup>a</sup> ah.11	2	Higher concentration detected in <i>C. serpentina</i> from CA sites.	Jackson et al. 1974
	<i>Chelydra serpentina</i>	Uncontaminated sites, CA	unkn.	1.1. ah.11	2		
	<i>Chrysemys concinna peninsularis</i>	Uncontaminated sites, FL	unkn.	0.96 <sup>a</sup> ah.11	8	Minus one individual collected from another site ● o.n-0.36 Bq/g.	Jackson et al. 1974
	<i>Chrysemys concinna swanniensis</i>	Uncontaminated site, FL	unkn.	0.04 <sup>a</sup> ah.11	3	Low concentration detected.	Jackson et al. 1974
	<i>Chrysemys nelsoni</i>	Uncontaminated site, FL	unkn.	1.7 <sup>a</sup> shell	2	Higher concentration than other <i>Chrysemys</i> .	Jackson et al. 1974
	<i>Deirochelys reticularia</i>	Uncontaminated site, FL	unkn.	0.64 <sup>a</sup> Shell	3	<sup>90</sup> Sr detected.	Jackson et al. 1974
	<i>Gopherus polyphemus</i>	Uncontaminated site, AL	unkn.	4.77 <sup>a</sup> ah.11	1	This turtle had the second highest concentration of <sup>90</sup> Sr among all turtles ● uplod.	Holcomb et al. 1971

Table A.4 (continued)

Radionuclide	Species	Location	S.X	Concentration (Bq/g wet weight)	N	Observation	Reference
<sup>90</sup> Sr (cont.)	<i>Gopherus polyphemus</i>	Uncontaminated sites, FL	unkn.	4.50 <sup>a</sup> ah.11	2	Species with th. highest <sup>90</sup> Sr concentration. Explanation based on herbivorous diet of th. species.	Jackson <i>et al.</i> 1974
		Uncontaminated ● it., CA	unkn.	6.66 <sup>b</sup> shell	6		
		Uncontaminated alto., AL	unkn.	3.57 <sup>a</sup> ah.11	3		
	<i>Kinosternon bauri palmarum</i>	Uncontaminated sites, FL	unkn.	0.67 <sup>c</sup> ah.11	3	<sup>90</sup> Sr detected.	Jackson <i>et al.</i> 1914
	<i>Kinosternon subrubrum</i>	Uncontaminated sites, MI	unkn.	1.31 <sup>d</sup> ● lwil	8	No correlation between size and <sup>90</sup> Sr concentration.	Holcomb <i>et al.</i> 1911
	<i>Kinosternon subrubrum</i>	Uncontaminated ● lto., FL	unkn.	1.44 <sup>e</sup> shell	7	Higher concentrations detected in <i>K. subrubrum</i> than <i>K. bauri</i>	Jackson <i>et al.</i> 1914
		Uncontaminated sites, CA	unkn.	1.63 <sup>a</sup> ah.11	1		
	<i>Malaclemys terrapin macrospilota</i>	Uncontaminated site, FL	unkn.	0.02 <sup>f</sup> Shell	1	Low concentration detected.	Jackson <i>et al.</i> 1974
<i>Pseudemys floridana hoyi</i>	Uncontaminated site, MI	unkn.	1.76 <sup>a</sup> ah.11	1	Concentration higher than in subspecies from CL.	Holcomb <i>et al.</i> 1911	
<i>Pseudemys f. floridana peninsularis</i>	Uncontaminated sites, FL	unkn.	0.03 <sup>a</sup> ah.11		No correlation between size and <sup>90</sup> Sr concentration.	Holcomb <i>et al.</i> 1971	

Table A.4 (continued)

Radionuclide	Species	Location	Sex	Concentration (Bq/g wet weight)	N	Observation	Reference
<sup>90</sup> Sr (cont.)	<i>Sternotherus minor</i>	Uncontaminated sites, FL	unkn.	0.02 <sup>a</sup> shell	2	No correlation between size and <sup>90</sup> Sr concentration.	Holcomb et al. 1971
	<i>Sternotherus minor</i>	Uncontaminated sites, FL	unkn.	0.04 <sup>a</sup> shell	5	Low levels detected.	Jackson et al. 1974
	<i>Sternotherus odoratus</i>	Uncontaminated sites, FL	unkn.	0.05 <sup>a</sup> shell	2	Higher concentration in Mississippi turtle.	Holcomb et al. 1971
		Uncontaminated sites, MI	unkn.	1.0 <sup>a</sup> shell	1		
	<i>Sternotherus odoratus</i>	Uncontaminated sites, FL	unkn.	0.06 <sup>a</sup> shell	8	Higher concentrations reported in the CA population.	Jackson et al. 1974
		Uncontaminated sites, CA	unkn.	1.21 <sup>a</sup> shell	14		
	<i>Terrapene carolina</i>	Uncontaminated sites, MI	unkn.	1.0 <sup>a</sup> shell	31	Inverse correlation between size and <sup>90</sup> Sr concentration in MI and TN populations.	Holcomb et al. 1971
		Uncontaminated sites, TN	unkn.	1.14 <sup>a</sup> shell	11		
		Uncontaminated sites, CA	unkn.	1.48 <sup>a</sup> shell	7		
		Uncontaminated sites, AR	unkn.	0.73 <sup>a</sup> shell	3		
<i>Terrapene carolina</i>	Uncontaminated sites, FL	unkn.	0.78 <sup>a</sup> shell	2	<sup>90</sup> Sr detected.	Jackson et al. 1974	
<i>Trachemys scripta</i>	Savannah River Plant seepage basins, SC	both	94.2 whole body	36	Initial total body burden reported here. Seasonal variation in <sup>90</sup> Sr. Average yearly biological half-life was 165 days.	Scott et al. 1986	
<i>Trachemys scripta</i>	Reference ponds, SC	both	0.39 whole body	26	Turtles contained low levels of <sup>90</sup> Sr.	Scott et al. 1986	

Table A.4 (continued)

Radionuclide	Species	Location	Sex	Concentration (Bq/g wet weight)	N	Observation	Reference
<sup>90</sup> Sr (cont.)	<i>Trachemys scripta</i>	Savannah River Plant ● seepage basins, SC	unkn.	130.7 whole body	7	99% of the whole body concentration was ● attributed to shell and bone.	Towns 1987
	<i>Trachemys scripta</i>	Contaminated reservoir, S C	unkn.	1.37 whole body	10	See above, Towns 1987	Towns 1987
	<i>Trachemys scripta</i>	Uncontaminated sites, MI	unkn.	0.19 <sup>a</sup> rho11 10.4 <sup>b</sup> shell	29 1	No correlation between size and <sup>90</sup> Sr concentration. Male contained the highest concentration detected among the turtles sampled.	Holcomb et al. 1971
<sup>90</sup> Sr	<i>Chelydra</i> ●	orpointu Spikod pond, OH	unkn.	9.44 <sup>a</sup> whole body	2	1.48 x 10 <sup>3</sup> Bq <sup>90</sup> Sr added to the pond. Turtles were immigrants.	Brungs 1967
<sup>65</sup> Zn	<i>Chelydra serpentina</i>	Spikod pond, OH	unkn.	2.87 <sup>a</sup> whole body	2	1.40 x 10 <sup>3</sup> Bq <sup>65</sup> Zn added to the pond. Turtles were immigrants.	Brungs 1967

<sup>a</sup>Radionuclide concentration based on dry weight.

<sup>b</sup>Whole body includes the shell.

● Radionuclide concentration based on ● whole rho11 weight.

APPENDIX B  
CONCENTRATIONS OF CONTAMINANTS  
IN **BEARDEN** CREEK **EMBAYMENT** SEDIMENT



Table B.1. Concentrations of cesium-137 and strontium-90 in **Bearden** Creek embayment sediment

$^{137}\text{Cs}$ (Bq/kg. dry wt)	$^{90}\text{Sr}$ (Bq/kg. dry wt)
3.59	0.5 $\pm$ 2.2
3.11	< 4
4.1s	4.0 $\pm$ 8.8
<b>n.d.<sup>a</sup></b>	<b>&lt; 6</b>
n.d.	<b>5.5 <math>\pm</math> 4.4</b>
4.7	0.2 $\pm$ 2.4

Wondetectable at < 1.85 Bq/kg.

Table B.2. Mercury (inorganic and organic) concentrations in  
**Bearden** Creek embayment sediment

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<b>Sample Date</b>	<b>Sample Number</b>	<b>Hg(<math>\mu</math>g/g. dry wt.)</b>
<b>10/7/87</b>	1	0.04
	2	0.038
	3	0.037
<b>1/31/89</b>	1	0.08
	2	0.04
	3	1.13
	4	0.26
	S	0.03
	6	0.07

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APPENDIX C

GASTROINTESTINAL TRACT CONTENTS OF TRACHEMYS SCRIPTA AND  
CHELYDRA SERPENTINA FROM THE OAK RIDGE RESERVATION



Table C.1. Gastrointestinal tract contents of Trachemys scripta  
from White Oak Lake

Capture date	Sex	<u>GI tract contents (% volume)</u>					Mud and rocks
		$V_T^a$	Vegetation	Fish	Insects	Detritus	
7/27/87	M	73	90.0	0.0	2.5	7.5	0.0
7/27/87	F	100	92.0	<0.5	0.0	7.5	0.0
7/27/87	F	7.5	95.0	0.0	0.0	5.0	0.0
8/11/87	M	<b>38</b>	95.0	0.5	0.0	4.5	0.0
8/12/87	F	120	94.5	0.0	0.5	5.0	0.0
8/17/87	F	90	<b>94.5</b>	0.0	0.0	5.0	0.5
8/27/87	M	40	84.5	0.0	0.5	15.0	0.0
9/3/87	M	<b>15</b>	97.0	0.0	0.5	2.5	0.0
9/3/87	M	30	93.0	0.0	1.0	5.0	0.0
9/3/87 <sup>b</sup>	M	---	0.0	0.0	0.0	0.0	0.0
9/10/87	F	<b>55</b>	95.0	0.0	0.0	5.0	0.0
9/16/87	F	10	100.0	0.0	0.0	0.0	0.0

<sup>a</sup>Total volume of GI tract contents in milliliters.

<sup>b</sup>Empty GI tract not included in calculation.

Table C.2. Gastrointestinal tract contents of male ~~Trachemys scripta~~  
from **Bearden** Creek embayment

Capture date	Sex	V <sub>T</sub> <sup>a</sup>	GI tract contents (% volume)					
			Vegetation	Fish	Insects	Detritus	Mud and rocks	Crayfish
<b>8/31/87</b>	<b>M</b>	30	97.5	0.0	0.5	2.0	0.0	0.0
<b>9/1/87</b>	<b>M</b>	92	24.0	0.0	75.0	1.0	0.0	0.0
<b>9/1/87</b>	F	190	100.0	0.0	0.0	0.0	0.0	0.0
<b>9/1/87</b>	F	103	97.0	0.0	<b>0.5</b>	2.0	0.0	<b>&lt;0.5</b>
<b>9/14/87</b>	<b>M</b>	<b>68</b>	98.0	0.0	1.0	1.0	0.0	0.0
<b>9/14/87</b>	<b>M</b>	60	98.0	0.0	0.0	2.0	0.0	<b>0.0</b>
<b>9/14/87</b>	<b>M</b>	204	79.s	0.0	20.0	<b>&lt;0.5</b>	0.0	0.0
<b>9/15/87</b>	<b>M</b>	<b>85</b>	99.0	0.0	0.0	<b>&lt;0.5</b>	<b>&lt;0.5</b>	0.0
<b>9/15/87</b>	F	160	87.5	2.9	0.0	10.0	0.0	0.0
<b>9/16/87</b>	F	120	as.5	14.0	<b>0.0</b>	0.5	0.0	0.0
<b>9/17/87</b>	F	LOS	94.s	0.0	<b>5.0</b>	0.5	0.0	0.0
<b>9/18/87</b>	F	110	77.0	20.0	<b>0.5</b>	<b>2.5</b>	<b>0.0</b>	<b>0.0</b>

<sup>a</sup>Total volume of GI tract contents in milliliters.

Table d.3. Gastrointestinal tract contents of male Chelydra serpentina from White Oak Lake

Capture date	$V_T^a$	<u>GI tract contents (% volume)</u>		
		Fish	Vegetation	Mud and rocks
<b>4/20/88</b>	100	<b>80</b>	20	0
<b>4/29/88</b>	120	60	40	0
<b>4/29/88</b>	63	so	so	0
<b>5/1/88</b>	120	1s	as	0
<b>5/4/88</b>	<b>105</b>	so	so	0
S/6/88	68	100	0	0
<b>5/7/88</b>	<b>65</b>	60	40	0
<b>5/7/88</b>	4s	S	9s	0
<b>5/8/88</b>	113	10	as	S
<b>5/9/88</b>	30	100	0	0
<b>5/14/88</b>	88	<b>40</b>	60	0
<b>5/14/88</b>	90	so	so	0

<sup>a</sup>**Total** volume of **GI** tract contents in milliliters.

Table C.4. Gastrointestinal tract contents of *Chelydra serpentina* from Bearden Creek embayment

Capture date	Sex	$V_T^a$	GI tract contents (% volume)				
			Vegetation	Fish	Insects	Mud and rocks	Clams
<b>4/29/88</b>	F	<b>65</b>	0	100	0	0	0
<b>5/1/88</b>	<b>F</b>	80	10	89.9	0.1	0	0
<b>5/9/88</b>	<b>F</b>	70	7	93	0	0	0
<b>5/10/88</b>	<b>M</b>	3s	0	<b>98</b>	0	2	0
S/27/88	<b>M</b>	<b>70</b>	0	96	0	2	2
<b>5/30/88</b>	<b>M</b>	as	s	<b>80</b>	0	1s	0
<b>6/7/88</b>	<b>M</b>	60	0	100	0	0	0
<b>7/7/88</b>	<b>M</b>	70	90	10	0	0	0
<b>7/12/88</b>	<b>M</b>	60	1	99	0	0	0

<sup>a</sup>Total volume of GI tract contents in milliliters.

APPENDIXD

CESIUM-137 AND COBALT-60 IN TRACHEMYS SCRIPTA AND  
CHELYDRA SERPENTINA FROM WHITE OAR LAKE



Table D.1. Cesium-137 in muscle tissue of Trachemys scripta from White Oak Lake

Capture date	Whole body wet wt (g)	Sex	<sup>137</sup> Cs (Bq/g, wet wt)
7/27/87	989	M	33.0
7/27/87	1,865	F	16.6 x 10 <sup>-2</sup>
7/27/87	619	F	38.4 x 10 <sup>-2</sup>
8/11/87	932	M	5.02 x 10 <sup>2</sup>
8/12/87	1,820	F	12.4 x 10 <sup>-2</sup>
8/17/87	1,687	F	30.0 x 10 <sup>-2</sup>
8/27/87	419	M	41.5 x 10 <sup>-2</sup>
9/3/87	369	M	36.7 x 10 <sup>-2</sup>
9/3/87	351	M	55.2 x 10 <sup>-2</sup>
9/3/87	a34	M	32.4 x 10 <sup>-2</sup>
9/10/87	2,750	F	38.5 x 10 <sup>-2</sup>
9/16/87	1,487	F	27.7 x 10 <sup>-2</sup>

Table D.2. Cesium-137 and cobalt-60 in liver tissue of Trachemys scripta from White Oak Lake

Capture date	Whole body wet wt (g)	Sex	<sup>137</sup> Cs (Bq/g) <sup>a</sup>	<sup>60</sup> Co (Bq/g) <sup>a</sup>
7/27/87	989	<b>M</b>	3.10	19.1 x 10 <sup>-2</sup>
7/27/87	1,865	<b>F</b>	a.41 x 10 <sup>-2</sup>	<b>8.75 x 10<sup>-2</sup></b>
7/27/87	619	<b>F</b>	11.9 x 10 <sup>-2</sup>	4.9 x 10 <sup>-2</sup>
8/11/87	932	<b>M</b>	66.1	17.2 x 10 <sup>-2</sup>
8/12/87	1,820	F	5.96 x 10 <sup>-2</sup>	1.21 x 10 <sup>-2</sup>
8/17/87	1,687	F	4.63 x 10 <sup>-2</sup>	2.82 x 10 <sup>-2</sup>
8/27/87	419	<b>M</b>	12.9 x 10 <sup>-2</sup>	1.65 x 10 <sup>-2</sup>
9/3/87	369	<b>M</b>	9.41 x 10 <sup>-2</sup>	1.39 x 10 <sup>-2</sup>
9/3/87	<b>351</b>	<b>M</b>	13.1 x 10 <sup>-2</sup>	2.9 x 10 <sup>-2</sup>
9/3/87	834	<b>M</b>	<b>5.56 x 10<sup>-2</sup></b>	3.64. x 10 <sup>-2</sup>
9/10/87	2,750	<b>F</b>	7.93 x 10 <sup>-2</sup>	3.86 x 10 <sup>-2</sup>
9/16/87	1,487	<b>F</b>	6.11 x 10 <sup>-2</sup>	4.98 x 10 <sup>-2</sup>

\*Based on wet weight.

Table D.3. Cesium-137 in muscle tissue of male Chelydra serpentina from White Oak Lake

Capture date	Whole body wet wt (kg)	<sup>137</sup> Cs (Bq/g, wet wt)
<b>4/20/88</b>	6.80	23.6 x 10 <sup>-2</sup>
<b>4/29/88</b>	13.6	24.8 x 10 <sup>-2</sup>
<b>4/29/88</b>	11.1	29.3 x 10 <sup>-2</sup>
<b>5/1/88</b>	9.07	1.37
S/4/88	<b>15.0</b>	28.3 x 10 <sup>-2</sup>
S/6/88	11.3	38.9 x 10 <sup>-2</sup>
S/7/88	9.07	32.1 x 10 <sup>-2</sup>
<b>5/7/88</b>	3.86	20.3 x 10 <sup>-2</sup>
<b>5/8/88</b>	11.8	41.7 x 10 <sup>-2</sup>
<b>5/9/88</b>	<b>8.85</b>	37.0 x 10 <sup>-2</sup>
<b>5/14/88</b>	8.16	33.7 x 10 <sup>-2</sup>
S/14/88	7.26	28.2 x 10 <sup>-2</sup>

Table D.4. Cesium-137 and cobalt-60 in liver tissue of male Chelydra serpentina from White Oak Lake

Capture date	Whole body wet wt (kg)	<sup>137</sup> Cs (Bq/g) <sup>a</sup>	<sup>60</sup> Co (Bq/g) <sup>a</sup>
4/20/88	6.80	19.3 x 10 <sup>-2</sup>	1.74 x 10 <sup>-2</sup>
4/29/88	13.6	7.93 x 10 <sup>-2</sup>	7.41 x 10 <sup>-2</sup>
4/29/88	11.1	17.6 x 10 <sup>-2</sup>	3.89 x 10 <sup>-2</sup>
5/1/88	9.07	50.3 x 10 <sup>-2</sup>	11.2 x 10 <sup>-2</sup>
5/4/88	15.0	15.6 x 10 <sup>-2</sup>	5.67 x 10 <sup>-2</sup>
5/6/88	11.3	15.2 x 10 <sup>-2</sup>	4.26 x 10 <sup>-2</sup>
5/7/88	9.07	17.3 x 10 <sup>-2</sup>	5.67 x 10 <sup>-2</sup>
5/7/88	3.86	7.63 x 10 <sup>-2</sup>	7.59 x 10 <sup>-2</sup>
5/8/88	11.8	19.1 x 10 <sup>-2</sup>	5.33 x 10 <sup>-2</sup>
5/9/88	8.85	14.5 x 10 <sup>-2</sup>	2.1 x 10 <sup>-2</sup>
5/14/88	8.16	11.4 x 10 <sup>-2</sup>	5.42 x 10 <sup>-2</sup>
5/14/88	7.26	13 x 10 <sup>-2</sup>	1.81 x 10 <sup>-2</sup>

<sup>a</sup>Based on wet weight.

APPENDIXE

STRONTIUM-90 IN TRACHEMYS SCRIPTA AND CHELYDRA SERPENTINA  
FROM **THE** OAR RIDGE RESERVATION



Table E.1. Strontium-90 in bone and carapace of Trachemys scripta  
from White Oak Lake

Capture date	Sex	Whole body wet wt (g)	<sup>90</sup> Sr(B 'g. wet wt)	
			Bone	Carapace
<b>7/27/87</b>	<b>M</b>	989	144	142
<b>7/27/87</b>	F	1,865	29.6	42.4
<b>7/27/87</b>	F	619	10.5	12.5
<b>8/11/87</b>	<b>M</b>	932	<b>4,830</b>	4,070
<b>8/12/87</b>	F	1,820	2.31	<b>1.65</b>
<b>8/17/87</b>	F	1,687	14.8	21.4
<b>8/27/87</b>	<b>M</b>	419	<b>14.5</b>	20.0
<b>9/3/87</b>	<b>M</b>	369	12.6	14.7
<b>9/3/87</b>	<b>M</b>	<b>351</b>	11.4	14.3
<b>9/3/87</b>	<b>M</b>	834	12.5	17.5
<b>9/10/87</b>	F	2,750	16.5	<b>18.5</b>
<b>9/16/87</b>	F	1,487	16.1	17.4

Table E.2. Strontium-90 in bone and carapace of Trachemys scripta from **Bearden** Creek embayment

Capture date	Sex	Whole body wet wt (g)	<sup>90</sup> Sr (Bq/g. wet wt)	
			Bone	Carapace
8/31/87	M	43s	12.0 x 10 <sup>-2</sup>	11.4 x 10 <sup>-2</sup>
9/1/87	M	412	ND <sup>a</sup>	ND
9/1/87	F	2,865	28.0 x 10 <sup>-2</sup>	ND
9/1/87	F	a34	ND	ND
9/14/87	M	1,079	1.04	1.18
9/14/87	M	477	4.7 x 10 <sup>-2</sup>	16.0 x 10 <sup>-2</sup>
9/14/87	M	1,162	ND	ND
9/15/87	M	746	ND	ND
9/15/87	F	1,312	7.0 x 10 <sup>-2</sup>	ND
9/16/87	F	1,350	ND	ND
9/17/87	F	1,101	5.8 x 10 <sup>-2</sup>	ND
9/18/87	F	642	ND	4.7 x 10 <sup>-2</sup>

<sup>a</sup>Not detected at < 3.7 x 10<sup>-2</sup> Bq/g.

Table E.3. Strontium-90 in bone and carapace of male Chelydra  
serpentina from White Oak Lake

Capture date	Whole body wet wt (kg)	<u><sup>90</sup>Sr (Bq/g. wet wt)</u>	
		Bone	Carapace
<b>4/20/87</b>	6.80	1.70	1.32
<b>4/29/87</b>	13.6	41.4	30.5
<b>4/29/87</b>	11.1	17.7	22.5
<b>5/1/87</b>	9.07	47.2	46.0
S/4/87	15.0	10.6	9.82
S/6/87	<b>11.3</b>	8.97	12.5
<b>5/7/87</b>	9.07	10.4	9.73
S/7/87	3.86	13.6	15.2
<b>5/8/87</b>	11.8	12.2	13.4
S/9/87	8.85	12.2	14.5
<b>5/14/87</b>	8.16	11.8	12.8
<b>5/14/87</b>	7.26	9.9s	10.8

Table E.4. Strontium-90 in bone and carapace of Chelydra serpentina from **Bearden** Creek embayment

Capture date	Sex	Whole body wet wt (kg)	<sup>90</sup> Sr (Bq/g. wet wt.)	
			Bone	Carapace
<b>4/29/88</b>	F	4.99	ND'	ND
<b>5/1/88</b>	<b>F</b>	4.54	ND	ND
<b>5/9/88</b>	F	4.31	$17.5 \times 10^{-2}$	$8.25 \times 10^{-2}$
<b>5/10/88</b>	<b>M</b>	11.6	ND	ND
<b>5/27/88</b>	<b>M</b>	9.07	ND	ND
<b>5/30/88</b>	<b>M</b>	10.4	ND	ND
<b>6/7/88</b>	<b>M</b>	4.99	ND	ND
<b>7/7/88</b>	<b>M</b>	7.94	$15.0 \times 10^{-2}$	$15.0 \times 10^{-2}$
<b>7/12/88</b>	<b>M</b>	6.80	$4.77 \times 10^{-2}$	$17.7 \times 10^{-2}$

'Not detected at c  $3.7 \times 10^{-2}$  Bq/g.

**APPENDIX F**

MERCURY IN TRACHEMYS SCRIPTA AND CHELYDRA SERPENTINA  
FROM THE OAR RIDGE RESERVATION

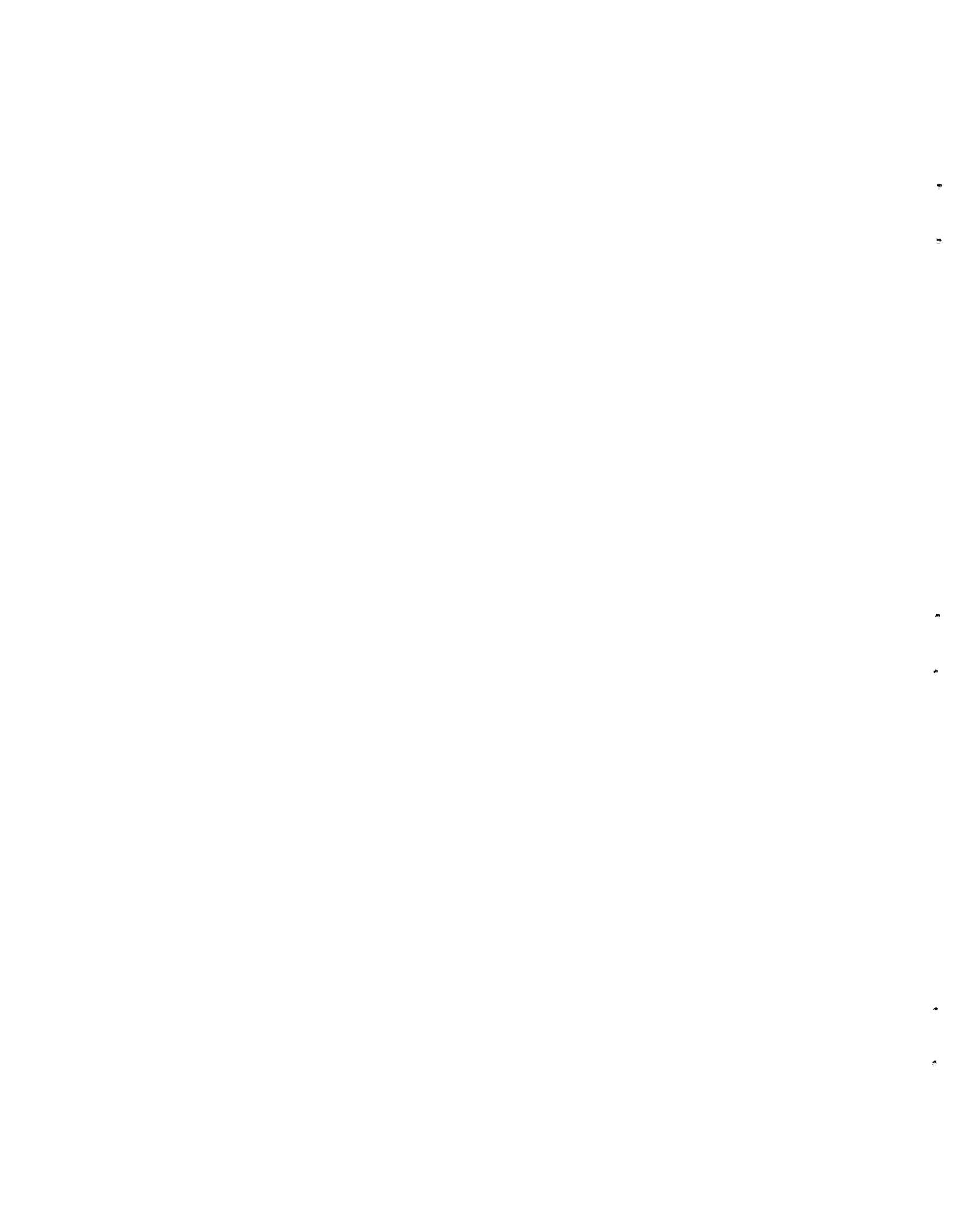


Table F.1. Mercury (inorganic and organic) concentrations in tissues of Trachemys scripta from White Oak Lake

Capture date	Whole body wet wt (g)	Tissue	Hg ( $\mu\text{g/g}$ , wet wt)
7/27/87	989	Kidney	4.00
		Muscle	0.48
7/27/87	1865	Kidney	0.15
		Muscle	0.14
7/27/87	619	Kidney	0.14
		Muscle	0.04
8/11/87	932	Kidney	1.81
		Muscle	0.25
8/12/87	1820	Kidney	0.18
		Muscle	0.05
8/17/87	1687	Kidney	0.17
		Muscle	0.03
8/27/87	419	Kidney	0.15
		Muscle	0.04
9/3/87	369	Kidney	0.24
		Muscle	0.07
9/3/87	351	Kidney	0.27
		Muscle	0.05
9/3/87	<b>834</b>	Kidney	0.19
		Muscle	0.02
9/10/87	2750	Kidney	0.18
		<b>Muscle</b>	0.04
9/16/87	1487	Kidney	0.23
		Muscle	0.04

Table F.2. Mercury (inorganic and organic) concentrations in tissues of Trachemys scripta from **Bearden** Creek embayment

Capture date	Whole body wet wt (g)	Tissue	Hg ( $\mu\text{g/g}$ , wet wt)
8/31/87	435	Kidney	0.16
		Muscle	0.02
9/1/87	412	Kidney	0.11
		Muscle	0.04
9/1/87	2865	Kidney	0.36
		<b>Muscle</b>	0.10
9/1/87	834	Kidney	0.04
		<b>Muscle</b>	0.01
9/14/87	1079	Kidney	0.40
		Muscle	0.06
9/14/87	477	Kidney	0.05
		<b>Muscle</b>	0.01
9/14/87	1162	Kidney	0.15
		Muscle	0.02
9/15/87	746	Kidney	0.05
		<b>Muscle</b>	0.02
9/15/87	1312	Kidney	0.08
		Muscle	0.02
9/16/87	1350	Kidney	0.04
		Muscle	0.01
9/17/87	1101	Kidney	0.03
		<b>Muscle</b>	0.01
9/18/88	642	Kidney	0.01
		<b>Muscle</b>	0.002

Table F.3. Mercury (inorganic and organic) concentrations in tissues of male Chelydra serpentina from White Oak Lake

Capture date	Whole body wet wt (kg)	Tissue	Hg ( $\mu\text{g/g}$ , wet wt)
4/20/88	6.80	Kidney	0.32
		Muscle	0.11
4/29/88	13.6	Kidney	1.28
		Muscle	0.15
4/29/88	11.1	Kidney	4.38
		Muscle	0.10
5/1/88	9.07	Kidney	1.11
		Muscle	0.10
5/4/88	15.0	Kidney	0.57
		Muscle	0.18
5/6/88	11.3	Kidney	1.35
		<b>Muscle</b>	0 . 3 2
5/7/88	9.07	Kidney	2.06
		Muscle	0.24
5/7/88	3.86	Kidney	0.56
		<b>Muscle</b>	0.11
5/8/88	11.8	Kidney	2.24
		tiuscle	0.27
5/9/88	8.85	Kidney	0.25
		<b>Muscle</b>	0 . 1 5
5/14/88	8.16	Kidney	0.97
		<b>Muscle</b>	0.12
5/14/88 .	7.26	Kidney	0.51
		<b>Muscle</b>	0.17

Table F.4. Mercury (inorganic and organic) concentrations in tissues of Chelydra serpentina from **Bearden** Creek embayment

Capture date	Sex	Whole body wet wt (kg)	Tissue	Hg ( $\mu\text{g/g}$ , wet wt)
<b>4/29/88</b>	F	4.99	Kidney	0.16
			Muscle	0.07
<b>5/1/88</b>	F	4.54	Kidney	0.19
			Muscle	0.06
<b>5/9/88</b>	F	4.31	Kidney	0.28
			Muscle	0.06
<b>5/10/88</b>	M	11.6	Kidney	0.24
			Muscle	0.10
<b>5/27/88</b>	<b>M</b>	9.07	Kidney	0.24
			Muscle	0.07
<b>5/30/88</b>	M	10.4	Kidney	1.07
			Muscle	0.20
<b>6/7/88</b>	<b>M</b>	4.99	Kidney	0.24
			Muscle	0.12
<b>7/7/88</b>	<b>M</b>	7.94	Kidney	0.33
			Muscle	0.12
<b>7/12/88</b>	<b>M</b>	6.80	Kidney	0.33
			Muscle	0.08

APPENDIX G  
FRACTION OF DOUBLE-STRANDED DNA IN LIVERS OF  
TRACHEMYS SCRIPTA AND CHELYDRA SERPENTINA  
FROM THE OAR RIDGE RESERVATION

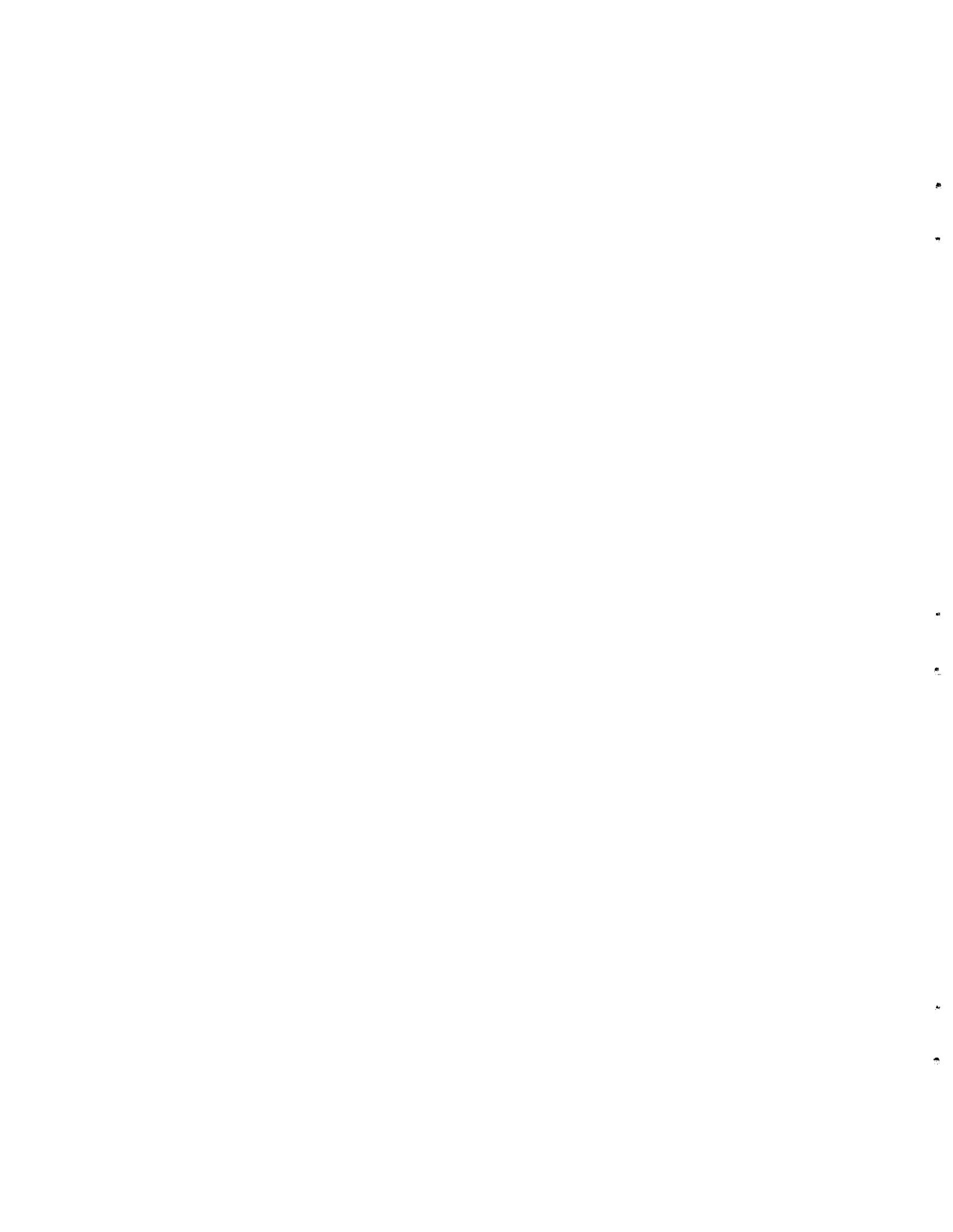


Table G.1. Fraction of double-stranded DNA in liver samples from Trachemys scripta collected from **Bearden** Creek embayment

Capture date	DNA assay <sup>a</sup>			ss/ds DNA <sup>b</sup>	F ds DNA <sup>c</sup>
	auw	ss	ds		
8/31/87	668	153	558	0.274	1.0
9/1/87	621	167	583	0.286	1.0
9/1/87	349	71.2	360	0.198	0.96
9/1/87	616	161	623	0.259	0.99
9/14/87	582	165	611	0.271'	0.94
9/14/87	544	176	580	0.304	0.91
9/14/87	620	176	676	0.261	0.89
9/15/87	551	243	637	0.381	0.78
9/15/87	257	93.5	294	0.319	0.82
9/16/87	319 <sup>d</sup>	171 <sup>d</sup>	514 <sup>d</sup>	0.354 <sup>e</sup>	0.68'
	362	143	381		
9/17/87	476 <sup>d</sup>	245 <sup>d</sup>	601 <sup>d</sup>	0.391.	0.80 <sup>e</sup>
	359	139	372		
9/18/87	228	100	265	0.379	0.77

<sup>a</sup>Parameters determined from the alkaline unwinding assay.  
 auw = alkaline unwound DNA subassay. **ss** = single-stranded DNA subassay, ds = double-stranded DNA subassay. Values reported are means.

<sup>b</sup>Single-stranded DNA/double-stranded DNA.

<sup>c</sup>auw DNA • ss DNA/ds DNA • ss DNA = fraction double-stranded DNA.

<sup>d</sup>Entire assay repeated twice. (Different **DNA concentrations** result in different fluorescence readings.)

<sup>e</sup>Mean values from repeated assays

Table 6.2. Fraction of double-stranded DNA in liver samples from Trachemys scripta from White Oak lake

Capture date	DNA assay <sup>a</sup>			ss/ds DNA <sup>b</sup>	F ds DNA <sup>c</sup>	Corrected F ds DNA <sup>d</sup>
	auw	ss	ds			
7/27/87	296	130	496	0.263	0.46	0.56
7/27/87	615	211	687	0.307	0.85	0.85
7/27/87	346	212	621	0.342	0.33	0.28
8/11/87	228	100	303	0.330	0.63	0.56
8/12/87	682	343	785	0.436	0.77	0.45
8/17/87	384	181	410	0.470	1.0	0.56
8/27/87	607 <sup>e</sup>	324 <sup>e</sup>	601 <sup>e</sup>	0.435 <sup>e</sup>	1.0 <sup>e</sup>	0.56
	298	123	338			
	466	183	458			
9/3/87	394 <sup>e</sup>	195 <sup>e</sup>	417 <sup>e</sup>	0.505 <sup>f</sup>	0.91 <sup>f</sup>	0.40
	357	199	368			
9/3/87	341 <sup>e</sup>	159 <sup>e</sup>	377 <sup>e</sup>	0.417	0.76 <sup>f</sup>	0.47
	404	206	514			
9/3/87	402 <sup>e</sup>	197 <sup>e</sup>	454 <sup>e</sup>	0.434 <sup>f</sup>	0.85 <sup>f</sup>	0.48
	231	106	245			
9/10/87	365	191	392	0.486	0.86	0.40
9/16/87	275	158	357	0.443	1.59	0.33

<sup>a</sup>Parameters determined from the alkaline unwinding assay. auw = alkaline unwound DNA subassay, ss = single-stranded DNA subassay, ds = double-stranded DNA subassay. Values reported are means.

<sup>b</sup>Single-stranded DNA/double-stranded DNA.

<sup>c</sup>auw DNA - ss DNA/ds DNA - ss DNA = fraction double-stranded DNA.

<sup>d</sup>Corrected fraction ds DNA based on the mean ss/ds DNA value from *Trachemys scripta* collected from Bearden Creek embayment.

<sup>e</sup>Assay repeated two or three times. (Different DNA concentrations resulted in different fluorescence readings.)

<sup>f</sup>Mean values from repeated assays.

Table G.3. **Fraction** of double-stranded DNA in liver **samples** from **Chelydra serpentina** from Bear & Creek embayment

Capture date	DNA assay <sup>a</sup>			ss/ds DNA <sup>b</sup>	F ds DNA <sup>c</sup>
	auw	ss	ds		
<b>4/29/88</b>	669	302	721	0.419	0.89
<b>5/1/88</b>	358	109	421	0.259	0.80
<b>5/9/88</b>	395	82.3	443	0.186	0.87
<b>5/10/88</b>	431	112	464	0.240	0.91
<b>5/27/88</b>	369	104	412	0.253	0.86
<b>5/30/88</b>	634	231	716	0.323	0.83
<b>6/7/88</b>	551	220	568	0.387	0.95
<b>7/7/88</b>	537	108	530	0.204	1.0
<b>7/12/88</b>	414	114	440	0.259	0.92

<sup>a</sup>Parameters determined from the alkaline unwinding assay.  
 auw = alkaline unbound DNA subassay, **ss = single-stranded**  
 DNA subassay, ds = double-stranded DNA subassay. Values  
 reported are means.

<sup>b</sup>Single-stranded DNA/double-stranded DNA.

<sup>c</sup>auw DNA - ss DNA/ds DNA - ss DNA = fraction double-stranded DNA.

Table G.4. Fraction of double-stranded DNA in liver samples from *Chelydra serpentina* from White Oak Lake

Capture date	DNA assay <sup>a</sup>			ss/ds DNA <sup>b</sup>	F ds DNA <sup>c</sup>	Corrected F ds DNA <sup>d</sup>
	auw	ss	ds			
4/20/88	383	143	426	0.336	0.85	0.65
4/29/88	423	201	455	0.441	0.87	0.43
4/29/88	413	148	456	0.324	0.86	0.70
5/1/88	414	184	414	0.388	0.80	0.49
5/4/88	352	117	387	0.303	0.87	0.78
5/6/88	394	160	427	0.376	0.88	0.57
5/7/88	440	158	463	0.341	0.93	0.70
5/7/88	379	133	419	0.317	0.86	0.73
5/8/88	528	248	560	0.443	0.90	0.44
5/9/88	592	228	650	0.351	0.86	0.62
5/14/88	429	170	460	0.370	0.89	0.60
5/14/88	395	156	430	0.363	0.87	0.60

<sup>a</sup>Parameters determined from the alkaline unwinding assay. auw = alkaline unwound DNA subassay, ss = single-stranded DNA subassay, ds = double-stranded DNA subassay. Values reported are means.

<sup>b</sup>Single-stranded DNA/double-stranded DNA.

<sup>c</sup> $\frac{\text{auw DNA} \cdot \text{ss DNA}}{\text{ds DNA} \cdot \text{ss DNA}}$  = fraction double-stranded DNA.

<sup>d</sup>Corrected fraction ds DNA based on the mean ss/ds DNA value from *Chelydra serpentina* collected from Bearden Creek embayment.

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