USE OF THE TOTAL MAXIMUM DAILY LOAD
CONCEPT IN ASSESSING DELETERIOUS
ECOLOGICAL EFFECTS IN A
RESERVOIR

By

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CHAPTER I

INTRODUCTION

The effects of pesticides on wildlife populations, through incidental contact, have been devastating (Woodwell 1984, Andersson et al. 1988). Nowhere is this more evident than in piscivorous bird populations. In fact, there has been a documented decline in piscivorous birds over the last 30 years (Ludwig and Kurita 1988). A direct correlation between the decreases in population and high levels of organochlorine insecticides found in birds' tissue has been established [Oklahoma Department of Wildlife Conservation (ODWC) 1989]. The source of elevated insecticide levels has been attributed primarily to birds' diet, specifically, to the consumption of contaminated fish. Certain lipophilic chemicals such as dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) pose a greater toxic threat to consumers of fish than to fish themselves. Situations in which fish are exposed to apparently safe water concentrations result, over time, in residue levels that are hazardous to predators upon consumption (Veith et al. 1980). Fish accumulate insecticides primarily through their environment, yet the water concentrations that ultimately are hazardous to predators do not have such an immediate effect in fish. Most likely, this is due to small quantities of toxicants that are absorbed over time and stored in lipid tissues. Unfortunately, when predators eat whole fish, they are exposed to all accumulated insecticides at once.

Bioaccumulation describes the process by which biota absorb insecticides directly from the environment and indirectly through their food source (Biddinger and Gloss 1984). It has been defined by the Environmental Protection Agency (EPA) as "the net accumulation of a chemical from combined exposure to water, food, and sediment by an organism" (1992).

Pesticides enter water systems through nonpoint source (NPS) pollution routes by which stormwater runoff transports sediment, nutrients, and organic and toxic substances into lakes and streams. The source of runoff may be from certain land-use activities or from the atmosphere (Novotny and Chesters 1981). NPS pollution is prevalent and the consequences are detrimental. Thompson cited an EPA list of "toxic hotspots" comprised of 17,000 river segments, streams, lakes, and estuaries which failed to meet water quality standards or designated uses due to toxicant contamination. Of these, 16,435, or 96.5%, were determined to be caused by NPS pollution (1989). Due to the nature of NPS pollution, minute quantities are added to a water system over time which escape routine detection using standard sampling methods. Thus, it is nearly impossible to monitor the continuing influx of NPS toxicants.

This research project was part of a larger study conducted by the Oklahoma State University (OSU) Water Quality Research Laboratory on Tenkiller Ferry Lake (hereafter Lake Tenkiller). The study was funded through an EPA Clean Lakes Project to monitor several physical and chemical parameters of Lake Tenkiller over time. The goal of this particular project was to analyze the concentration of organochlorine insecticides in water, sediment and fish in order to gain knowledge of the current and historic toxicant contamination in Lake Tenkiller. The concept of a total maximum daily load (TMDL) was developed as a management tool for watershed managers to allocate potential loading of organochlorine insecticides into a lake ecosystem. Although organochlorine insecticides are not readily in use today, they were chosen in order to provide a worst-case scenario of toxicant contamination. The goal is to enable application of TMDLs to any similar situation.

The concepts of a TMDL, rather than actual methodology, were used in this project because of their applicability to the toxicant bioaccumulation problem. Traditional TMDL methodology was not applied since the calculations require information that is currently inaccessible. The TMDL process was established by the Clean Water Act, Section 303(d). The law instructs each state to "determine the greatest amount of a pollutant a water body can receive each day without violating the state's water quality standards." (Thompson 1989). One advantage of using TMDLs are their applicability as water quality based processes; thereby accounting for contributions from all pollution sources, yet requiring in-stream standards be achieved (EPA 1991). Another advantage of the TMDL concept is that toxicity effects are considered based upon an ecosystem perspective rather than effects shown by a single species. Kimball and Levin stress the importance of toxicity evaluation on an ecosystem level in addition to traditional bioassay testing (1985). An ecosystem viewpoint assesses effects that might not otherwise be seen. The most critical consequence, indirect effects, i.e., reproductive abilities, is considered, as well as interaction among and between species, abiotic factors, and effects over several hierarchical stages (Kimball and Levin 1985).

The results of two studies, National Academy of Science (NAS) 1973 and Newell et al. 1987, were set as standards upon which comparisons with data were based and TMDL values calculated. Both studies focused on developing permissible numeric values for the medium of concern that would not pose a toxic risk.

In Water Quality Criteria 1972, the NAS prescribed admissible levels of organochlorine insecticides in whole fish on a wet weight basis (1973). These recommendations allowed for the protection of fish predators. Even though a precise method was not presented, it is assumed that the recommended levels were derived after examining the available literature for toxic effects on piscivorous birds. A residue concentration of 1.0 mg/kg in fish was recommended for DDT, DDD, and DDE. The residue concentration for aldrin, dieldrin, endrin, heptachlor, and lindane was 0.1 mg/kg, either singly or in combination.

Newell et al. made use of published laboratory animal toxicology data in order to derive fish flesh criteria which would prevent toxic accumulation in predators (1987). Final results of experiments were extrapolated to become fish flesh criteria. The general formula used to convert the data to these criteria follows:

NOEL/LOEL/Cancer Risk Dose (mg/kg/day) x AF/UF (s) x Target Species Weight (kg) ÷ Target Species Daily Intake (kg/day) = Criterion (mg/kg)

where:

NOEL (no observed effect level),
LOEL (lowest observed effect level), or

Cancer Risk Dose is the result of a chronic or subacute toxicity test, or
The lower 95% confidence limit for the 1 in 1,000 or 1 in 100 risk calculated from dose-response data from a carcinogenicity assay with a lab species;
AF/UF is one or more application or uncertainty factors.

Fish flesh criteria were determined as follows:

Aldrin & Dieldrin	0.12 mg/kg
DDT, DDD, & DDE	0.2 mg/kg
Endrin	0.025 mg/kg
Heptachlor	0.2 mg/kg
Lindane	0.1 mg/kg.

In this project, the TMDL value represents a calculated water concentration of a specific insecticide that may enter the Tenkiller lake ecosystem without posing a threat. The daily concentration will be such that any insecticide accumulation in fish will not exceed a determined threshold criteria, thereby eliminating toxic risk to fish predators.

Study Site Description

Tenkiller Ferry Lake is located in Cherokee and Sequoyah counties in northeastern Oklahoma (Figure 1). The Illinois River, with its headwaters in northwest Arkansas, meanders through Adair, Delaware, Cherokee, and Sequoyah counties in Oklahoma, eventually emptying into Lake Tenkiller. The drainage basin of the Illinois River includes 233,199 hectares in Oklahoma, alone. A Soil Conservation Service report estimated that 50% of the drainage basin is composed of forest, 42% grass, 3% urban, 1% crop land, 1% orchard and vineyard, and 3% includes other uses such as confined animal operations (USDA 1992). The Curtis Report reveals the "other" category also contains 0.12% plant

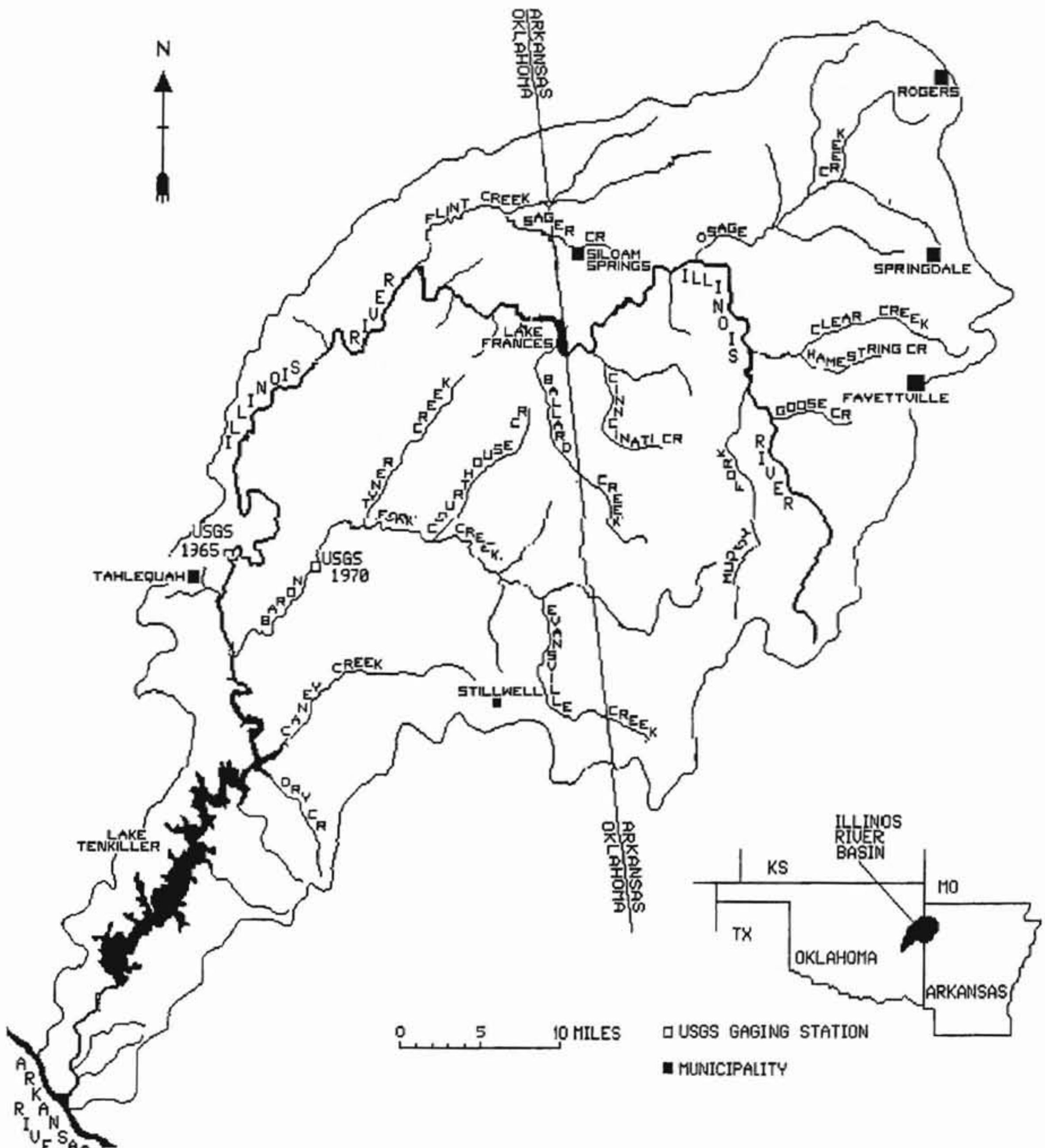

Figure 1. Watershed of the Illinois River

production by commercial nurseries (OSDA 1993). Thus, there are many diverse land uses in the watershed that could potentially generate pesticide runoff.

Piscivorous wildlife living in the Lake Tenkiller area consist of mink (Mustela vison), river otters (Lutra canadensis), raccoons (Procyon lotor), muskrats (Ondatra zibethicus), and shore birds (USDA 1992). In addition, endangered and threatened piscivorous species live in the region including bald eagles (Halieatus leucocephalus), of which 75 were sighted at Lake Tenkiller during a winter bird count (ODWC 1991), peregrine falcons (Falco peregrius), and interior least terns (Sterna albifrons) (USDA 1992).

Statistical Hypotheses

The null hypotheses tested were:

1) H_o: The organochlorine insecticide concentration was at an acceptable level in water.

2) H_o: There was no difference in the organochlorine insecticide concentration among the sediment sampling stations.

3) H_o: The organochlorine insecticide concentration was at an acceptable level in whole fish.

The first and third hypotheses tested the assumption that insecticide concentrations were at acceptable levels contingent upon the media examined. An acceptable level was defined as being equal to or lower than certain standards. For water, the standards from NAS 1973 and WHO 1993 were used; for fish, NAS 1973 and Newell et al. 1987 were utilized. Testing whether the data was equal to or below the standards implied that insecticides were not bioaccumulating at levels deleterious to piscivorous wildlife. The second hypothesis tested the assumption that insecticides entering from the river would partition primarily to the sediments located in the upper portion of the lake versus the lower area. Presuming this assumption was true, the sediment concentrations would be unequal among the stations sampled.

CHAPTER II

LITERATURE REVIEW

Dichlorodiphenyltrichloroethane (DDT) was the first pesticide of the organochlorine family to be formed in 1939 by Paul Müller (Rinella et al. 1993). At the time, DDT was hailed as the antidote for humanity's ailments. It was pronounced as the remedy for all insect control problems which in turn curbed the spread of insect-borne infectious diseases. DDT is readily absorbed through an insect's cuticle, but not through mammalian skin (Cremlyn 1978). Hence, a disparity exists between insect and mammalian toxicities, thereby increasing its usefulness. DDT possesses certain properties such as stability, persistence, low mammalian toxicity, and broad insecticidal toxicity which were thought to be beneficial as an insecticide (Cremlyn 1978). These same properties, however, promoted excessive and indiscriminate use of the insecticide which in turn stimulated the development of DDT resistance in many insect species. In response, other organochlorine pesticides with characteristics similar to DDT were developed in order to combat the resistant pests. Examples include lindane developed in 1942; heptachlor, aldrin, and dieldrin in 1948; endrin in the 1950's; and methoxychlor in 1969 (Smith et al. 1988). The primary use of these pesticides was to combat the spread of nuisance insects in households, crops, and livestock areas. However, it was soon discovered that these compounds were causing negative effects upon wildlife and non-target species rather than resistant pests. Also, many of the chemical compounds were suspected to be carcinogenic. EPA banned the use of DDT in the United States in 1972 (Woodwell 1984). Subsequently, the use of other organochlorines was either curtailed or banned. It is now known that many of the pesticides are, in fact, carcinogenic (EPA 1992). Unfortunately, residues of these pesticides still exist in the environment today due to their unique chemical properties.

For convenience, several coefficients of organochlorine pesticides are provided in Table 1. Succinctly, these pesticides are infamous for extensive contamination and persistence in the environment. This is attributed, in part, to their hydrophobic nature and high affinity for soil, sediment, and biotic lipid tissue (Smith et al. 1988). Consequently, an organochlorine that enters a water system will partition into the sediment or biota and not remain in the water column. Persistent pesticides that partition into soil and sediment may slowly be released back to the ecosystem over many years (NAS 1973), and thus, act as a continuing source of contamination. As an example, agriculture soil was determined to be the source of elevated concentrations of total DDT in streams of the Yakima River Basin (Rinella et al. 1993).

Biodegradation of chlorinated insecticides does not readily occur as is true with most highly chlorinated compounds (Smith et al. 1988). For example, the degradation products of DDT are DDE and dichlorodiphenyldichloroethane (DDD), but DDE and DDD are persistent and prevalent compounds in the environment. In aquatic systems, DDE's half-life was found to be more than 120 years at pH 5 and 27 °C (EPA 1992).

Table 1. Coefficients of Organochlorine Pesticides [a]

Insecticide	Empirical Formula	Log K_{ow}	Log K_{oc}	Log BCF [d]	Henry's (atm $-m^3$/mole)
Aldrin	$C_{12}H_9Cl_6$	6.5	5.36	4.71	0.496×10^{-3}
DDT	$C_{14}H_9Cl_5$	6.36 [b]	5.38 [c]	4.60	5.13×10^{-4}
DDD	$C_{14}H_{10}Cl_4$	5.99 [b]	5.89 [c]	4.32	No Data
Dieldrin	$C_{12}H_8Cl_6O$	4.32	4.08	3.05	5.8×10^{-5}
Endrin	$C_{12}H_8Cl_6O$	4.56	4.53	3.24	7.52×10^{-6}
Heptachlor	$C_{10}H_5Cl_7$	5.27	4.48	3.78	1.48×10^{-3}
Lindane	$C_6H_6Cl_6$	3.80	3.81	2.66	1.06×10^{-5}
Methoxychlor	$C_{16}H_{15}Cl_3O_2$	5.08	2.79	3.63	1.58×10^{-5}

[a] All information was obtained from Howard 1991 unless otherwise noted.
[b] Smith et al. 1988
[c] U.S. Department of Health & Human Services 1992
[d] Veith, G. D., K. J. Macek, S. R. Petrocelli, and John Carroll 1980.

Lindane is the only chlorinated insecticide found to biodegrade significantly which may be accounted for by its relatively high water solubility (Moore and Ramamoorthy 1984). Oxidation is also not an effective process in decomposing these compounds with the exception of aldrin's aerobic conversion to dieldrin (Smith et al. 1988). However, dieldrin is known to be resistant to further degradation (EPA 1992). Thus, the properties of these pesticides ensures their persistence in the environment.

Difficulties arise when pesticides persist in aquatic communities, are lipophilic, and are not readily susceptible to an organism's depuration (Macek 1970). When the toxicant concentrates faster than it can be metabolized, if even susceptible to metabolism, it bioaccumulates within the organism's tissues (NAS 1973). A high octanol-water partition coefficient coupled with organochlorines' previous characteristics assures bioaccumulation in biotic fatty tissue (Cremlyn 1978). Insignificant aqueous concentrations of these insecticides often result in unacceptable toxic levels in aquatic organisms (Smith et al. 1988). Higher trophic predators such as eagles, minks, or even humans risk the potential of consuming contaminated biota and accumulating the toxicants in their own tissues.

The same properties which ensure organochlorines' persistence and potential for accumulation also make these compounds toxic to biota. The accumulated toxicants may reach such levels as to be lethal or to affect reproductive abilities (EPA 1992). Biota of aquatic ecosystems appear to be exceedingly vulnerable to toxicant accumulation. After a thorough literature review, Dillon concluded that aquatic organisms are more sensitive to the toxicity of chlorinated hydrocarbons than to heavy metals or petroleum hydrocarbons (1984). A toxicant's presence in an ecosystem often is unknown until toxic effects are observed within top predators. At this point, however, irreversible damage may have occurred to the prey and predator populations as well as the ecosystem.

Piscivorous birds experience this phenomenon frequently (Woodwell 1984). An early and classic example of this occurred at Clear Lake in California. In 1949, DDD was applied to the lake area in summer to control swarms of gnats. The lake was sprayed again in 1954 and 1957. Each time after spraying, the water's concentration of 0.020 mg/l dissipated within 2 weeks and no immediate harmful effects were observed. However, in December of 1954, large numbers of dead western grebes (Aechmophorus occidentalis) were found. Upon analysis, some of the western grebes contained 1600 mg/l of DDD in lipid tissue. This was a magnification of 80,000 times the original concentration in water. In addition, no young were hatched from 1950-1961. It was not until twenty years after the first DDD application that the western grebes' reproduction became successful at this site (Cremlyn 1978).

The repercussions of organochlorines' accumulation in birds has been especially well documented. In fact, DDT has been held responsible for the decline of brown pelicans, bald eagles, ospreys, and peregrine falcons across the United States (ODWC 1989). The mechanism of these pesticides' action appears to directly or indirectly affect birds' reproductive capabilities, through hormonal influences (Ludwig and Kurita 1988). As a consequence, new generations of chicks are not hatched successfully. Common obstructions to reproductive success are egg infertility, thin egg shells, embryotoxicity, structural embryonic deformities, edema, and lack of yolk sac absorption before hatching (Ludwig and Kurita 1988). EPA reported that concentrations greater than only 0.2 mg/L will cause eggshell thinning in brown pelicans (1992). Liver porferia, liver enlargement, small body size, wasting syndrome, thyroid and Vitamin A abnormalities, edema, and altered immune systems are characteristics of young and adult birds who endure chronic toxicant exposure (Ludwig and Kurita 1988). In addition, it has been estimated that toxicants will "concentrate in birds 25 million fold above what is present in water, and 21-39 times above what is present in fish these birds eat" (Ludwig and Kurita 1988). Finally, EPA (1992) reported the following insecticides to be lethal in birds at the concentration indicated:

DDE	250 mg/l

Dieldrin	5 mg/l
Endrin	0.8 mg/l
Heptachlor	8 mg/l.

CHAPTER III

METHODS

Sampling Locations

The sampling locations for this project were chosen from the previously selected stations for the OSU EPA Clean Lakes Project. The OSU project has eight stations situated in the lake ecosystem. Station 1 is located in the Illinois River just above confluence of the river with Lake Tenkiller and downstream from the confluence with Baron Fork. Stations 2, 3, 4, 5, 6, and 7 are representative of the various transitional zones throughout the lake, while station 8 is located in the Illinois River south of the dam. A map of the sampling stations is provided in Figure 2.

River water and river sediment samples were collected from station 1. This station was included in order to determine the quality and quantity of pesticides actually entering the lake from the river.

Lake water and lake sediment samples were collected from four previously selected stations, 2, 4, 5, and 6. Stations 2 and 4 were chosen since they represent a region of dynamic change; the area in which river water collides with lake water. In addition, stations 2 and 4 are upstream and downstream of Greenleaf Nursery. Thus, any

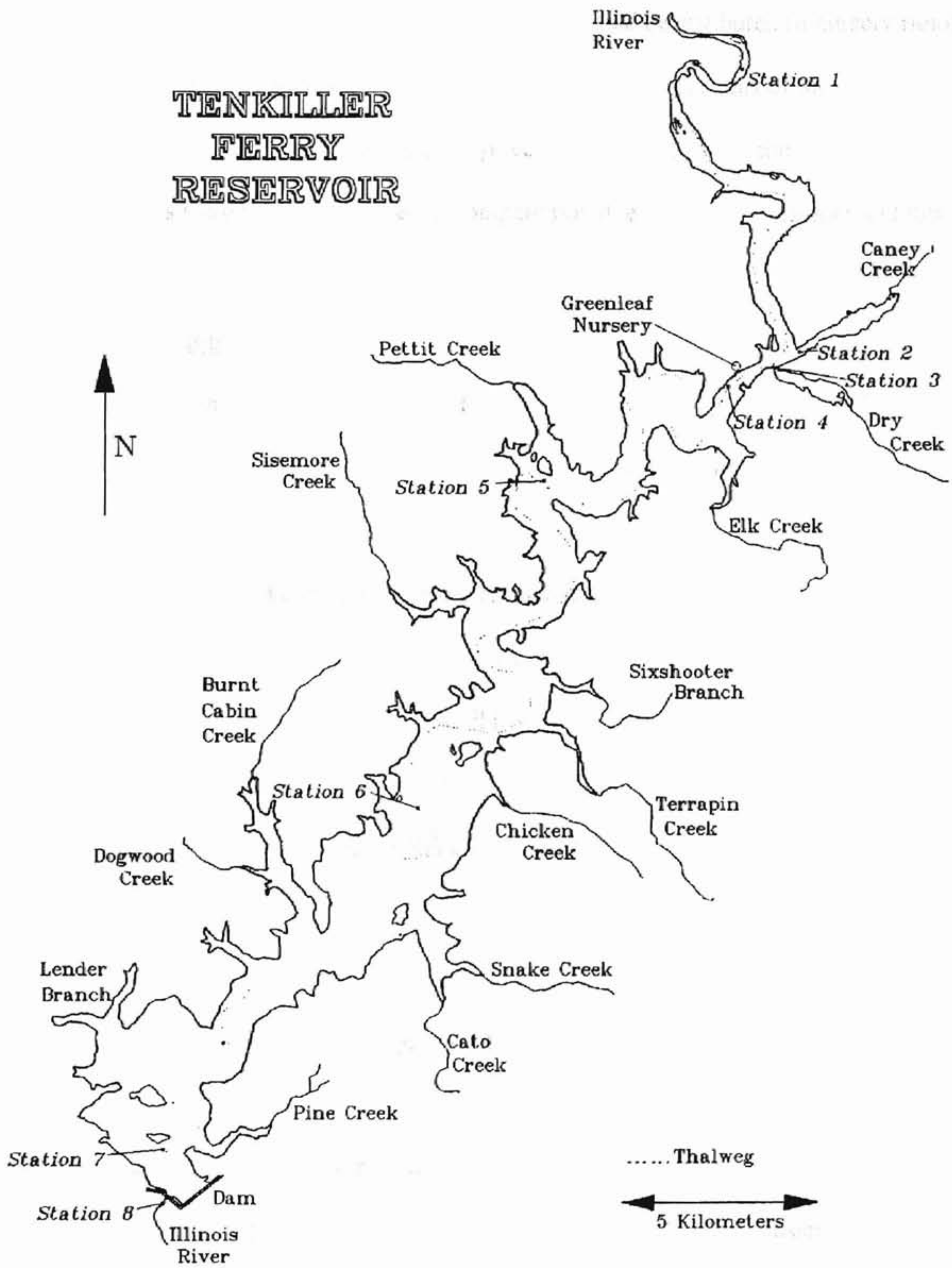

Figure 2. Sampling locations of Tenkiller Ferry Reservoir

discrepancies in the quantity or quality of pesticides could be attributed to nursery runoff. Stations 5 and 6 were chosen as representatives of the lower portions of the lake.

Channel catfish (Ictalurus punctatus) were collected from stations 2, 5, and 6. These stations were selected in order to compare possible trends for the upper and lower portions of the lake.

Sample Handling

All glassware used in collection of water and sediment samples was acid-rinsed and dried in an oven. The glassware was then rinsed with the appropriate solvents prior to use. Aluminum foil was placed over the glass rim to prevent contamination from the lid of the collection vessel. Water samples were extracted as soon as possible and analyzed within 40 days. Sediment samples were stored at 4° C and then extracted and analyzed within 40 days. Fish tissue was frozen until it was extracted and analyzed.

Quality Control

A field blank and procedure blank were used to insure quality control in the analyses for water samples. Procedure blanks were also used in sediment and fish analyses. In addition, each medium contained a representative spiked sample.

Water and Sediment Collection and Analyses

All water samples were collected from 0.5 m depth below the surface in 3.8 liter amber glass bottles. Three samples were taken at each of 4 stations from the sides and front of the boat to obtain a measure of spatial homogeneity. Three subsamples were also taken from one bottle to provide a measure of analytical variability. A total of 17 water samples were analyzed. The samples were prepared for analysis following EPA's standard method for extraction of organochlorine pesticides, Method 608. The extraction disks used were Empore® C18 Disks.

Sediment samples were taken with an Ekman dredge and placed in acid-rinsed and dried Mason glass jars. Three samples were taken at each location, and three subsamples were taken from one jar. Since the environment at a lake bottom is not expected to change drastically over a short period of time, a single sampling period was judged adequate for this project. Therefore, a total of 17 sediment samples were analyzed. The samples were extracted following EPA's Sonication procedure, Method 3550 (EPA 1986).

Fish Collection and Analysis

Channel catfish were chosen for this study for several reasons. In a sense, they represent a worst-case scenario for toxicant accumulation among fish species. Channel catfish risk exposure to accumulated pesticides through sediment because they are bottom dwellers. They are also carnivores which promotes the possibility of pesticide accumulation through the food chain. In addition, channel catfish possess value as a recreational fishing species. A report by the Oklahoma State Department of Health (OSDH) (1987) revealed more pesticide accumulation in this species of fish than others obtained from Lake Tenkiller. Channel catfish contained detectable amounts of chlordane, DDT, and PCBs in 1980, 1983, 1987, and 1992. In the 1980 sampling period, channel catfish contained chlordane values in excess of the OSDH Concern Level. All of the examined fish tissue residues were obtained from fillets only. No samples of excretory organs or adipose tissue where toxicants are expected to concentrate were examined in the OSDH study.

Whole fish samples, rather than fillets or specific organs, were analyzed in this project. A primary objective was to evaluate effects of pesticides at the ecosystem level. Thus, whole fish samples served as an appropriate indicator of toxicant residue bioaccumulation in the ecosystem.

Six fish were collected with vertical gill nets from the sampling sites. The fish were wrapped in aluminum foil, placed on ice, and stored in a freezer until analyses could be completed. The length, sex, and location of capture was recorded for each fish. Whole fish samples were ground using a stainless steel meat grinder. The lipid content of each was determined following the Food and Drug Administration's (FDA) section 211.13f (FDA 1982). Three aliquots of 25-50 g from each fish was extracted and analyzed using petroleum ether-acetonitrile partitioning following section 211.14a (FDA 1982). Next, the alternative Florisil clean-up method was used following section 252 (FDA 1982). Therefore, a total of 18 fish aliquots were analyzed. Finally, the mean and standard deviation of insecticide content for each fish was reported.

Gas Chromatography

Water, sediment, and fish samples were analyzed for lindane, heptachlor, aldrin, dieldrin, endrin, DDD, DDT, and methoxychlor of the organochlorine pesticide family (Table 1). All samples were analyzed using a Perkin-Elmer Gas Chromatograph and a Tracor 560 Gas Chromatograph. The Perkin-Elmer chromatography column was an Alltech® silica packed column 4.000 mm ID by 1.8 m long filled with 1.5% OV-17 + 1.95% QF-1 liquid phases coated on Chromosorb WHP 80/100 mesh as the stationary support. The Tracor 560 possessed a J & W Scientific® fused silica capillary column

0.053 mm ID by 15.0 m long containing Durabond-608 with 0.83 μm film thickness. Both chromatographs were equipped with electron-capture detectors. An Argon/Methane mixture was used as the carrier gas with the flow rate set at 55 psi. The injection port, column, and detector temperatures for the Perkin-Elmer were 275, 237, and 300 °C, respectively. Likewise, the temperatures for the Tracor were 275, 210, and 300 °C. Methods for analysis followed standard EPA practice, Method 8080 (EPA 1986). The detection limits of the gas chromatographs were determined to be in the ng range, and are presented together with the results for each examined media.

Statistical Analyses

No statistical analyses could be calculated for water due to the low number of samples with detectable quantities of insecticides.

Statistical Analysis System© (SAS) was used to complete the analyses for sediment and fish (1990). All statistical analyses had a confidence level of 95%, or α was equal to 0.5. The SAS univariate procedure for one population *t*-tests was used to determine significance for the fish samples as compared with NAS 1973 and Newell et al. 1987. For the sediment samples, the data was first tested for equal variance by using Levene's test. Once variances were determined to be equal, analysis of variance (ANOVA) was used to test for significance among the various stations. If significance was found, then Tukey's test was used to ascertain where the significance lay among the stations.

Mean Daily Flow Calculation

The mean daily flow of the Illinois River into Lake Tenkiller was calculated in order to determine an approximate TMDL value. By combining the water flow measured at two United States Geological Survey (USGS) gauging stations, USGS 1965 and USGS 1970, the majority of flow into Lake Tenkiller was estimated. USGS 1965 is located on the Illinois River east of Tahlequah, and USGS 1970 is located on Baron Fork 13 km prior to confluence with the Illinois River (Figure 1). The mean river water flow at these two stations consisted of monthly averaged samples from October 1, 1979- September 30, 1992. WQSTAT II© software was used to calculate the monthly flow values for each station (Phillips et al. 1988). The flow data was then classified into three flow groups: low, mean, and high. The low flow was equivalent to the 0.25 quartile of the combined flow values as calculated by Quatro-Pro©. Likewise, the mean value was comparable to the 0.50 quartile, and the high value equal to the 0.75 quartile of the flow values.

Calculation of Bioconcentration Factors

Many variables affect the bioconcentration of insecticides in aquatic organisms. In fish species alone, the age, surface-to-volume ratio, species, season, sex, lipid content, and vertical distribution of individuals influence the rate at which toxicants are absorbed (Biddinger and Gloss 1984). Due to the inherent variability of measured bioconcentration factors (BCF) and the inability to locate a study which determined BCFs for all of the organochlorine insecticides discussed here, a calculated BCF was used. BCF is defined as the partition coefficient established between a chemical and an organism exposed to the chemical through water only (EPA 1992). Essentially, the BCF provides an estimate of the bioaccumulation potential of toxicants in aquatic organisms. The calculated BCFs were estimated from the partition coefficient (log K_{ow}) using the regression equation

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

proposed by Veith et al. 1980. The regression coefficient "r" was determined to be 0.907 for the 84 tests performed (Veith et al. 1980). Table 1 contains the calculated BCFs.

<u>TMDL Calculation</u>

Ecological risk assessment was accomplished by using the TMDL concept. The daily allowable concentration of chlorinated insecticides that may flow into a reservoir via river water was determined. This level is safe since it will not directly or indirectly result in injury to the biota, due to the consequences of bioaccumulation.

The concentrations derived by NAS 1973 and Newell et al. 1987 were substantiated as the ecologically relevant levels and used to compare the insecticide residues of Lake Tenkiller fish. Thus, the fish were defined as either fit or unfit for piscivorous wildlife consumption based upon the ecologically relevant levels. These levels are threshold criteria not to be exceeded in fish in order to preserve the biological integrity of predators. Since 14 years elapsed between the levels' inception, the values are dissimilar. For that reason, the more conservative of the two standards was used to calculate the safe water concentration. Since 0.1 mg/kg was the most conservative value and was consistent with the other values, it was used as the ecologically relevant level for methoxychlor. Using the log BCF of a specific insecticide (Table 1), we were able to back-calculate the ecologically relevant level to a corresponding safe water concentration. These concentrations were determined by using the simple algebraic equation, $x = y * z$, where:

x is the water concentration;
y is the ecologically relevant level, either NAS 1973 or Newell et al. 1987;
z is the calculated BCF.

The daily flow of the river into the lake was then multiplied with the safe water concentration resulting in an ecologically-based TMDL value. The data for daily low, mean, and high flows were utilized to formulate TMDL values based upon fluctuating river water conditions. Thus, there are a total of 24 TMDL values. Each of the eight insecticides has three values for the three different rates of water flow.

CHAPTER IV

RESULTS & DISCUSSION

Water Samples

Water samples were collected after it had rained for two days in the watershed. The rainfall presented an ideal time to collect samples since runoff would have flushed all insecticides into the receiving water.

Field and procedure blanks did not reveal any contamination due to sampling error or extraction procedure error. Overall, the majority of the analyzed water samples did not contain any toxicants. In fact, only 2 of the 17 samples contained detectable quantities of insecticides. Those two samples were taken from stations 1 and 2 (Table 2). Thus, as expected, insecticides were detected at the first 2 stations only and not at stations farther down the lake. This would be due to the natural biodegradation of the insecticides and their absorption to biotic or abiotic material soon after entering the water.

The detection limits were as follows. Lindane, heptachlor, and aldrin were detectable to 250 ng/l; while endrin, dieldrin, DDD, DDT, and methoxychlor were detectable to 500 ng/l. Aldrin was used as a spike in one sample and yielded an average recovery of 43.13% between the two machines.

Table 2. Stations' Insecticide Data and Guideline Concentrations

Insecticide	1 TK 2 (μg/l)	2 TK 1 (μg/l)	NAS (μg/l)	WHO (μg/l)
Lindane	1.5071	2.4708	0.02	2.0
Heptachlor	2.9842	<0.250	0.01	0.03
Aldrin	<0.250	0.7445	0.01	0.03
DDD	<0.500	0.4822	0.002	2.0

Even though statistical analyses could not be calculated for water, we can still answer the #1 null hypothesis. Since insecticide levels were undetectable, we may assume the water levels of insecticides are at acceptable levels. Therefore, we fail to reject the null hypothesis. However, the insecticides detected did exceed recommended guidelines (Table 2).

NAS water values were set as the maximum concentration that would still allow for the protection of aquatic life (1973). The World Health Organization (WHO) determined specific drinking water guidelines for organochlorine insecticides (1993). The difference in standard concentrations for aquatic biota exposure as compared with human health exposure is quite evident (Table 2). It was surprising to find that lindane, heptachlor, aldrin, and DDD were at or above the recommended values. Potentially, this could have caused concern as to whether the insecticides were bioaccumulating at levels deleterious to biota.

<u>Sediment Samples</u>

There were several physical differences among the various sediment stations. To begin with, sediment composition differed from the river station as compared with the lake stations. Undoubtedly, this was due to the action of the river's flowing water which causes the sediment to be more like sand than clay as was found in the lake. Another discrepancy existed between the samples from station 2 as compared with the rest of the stations. Samples from station 2 emitted a manure-like odor and lacked the ability to bind together. In a sense, the sample was "runny" as compared with the other sediment samples.

The procedure blank did not reveal any insecticide contamination, yet, they were detected in every sample. Aldrin and dieldrin were the most prevalent toxicants with heptachlor, lindane, and DDT following. A few samples contained DDD, and only one sample contained endrin. Methoxychlor was not detected in any of the samples. Sample pH ranged from 5.3 to 7.1 with the median equal to 6.42. Table 3 contains the pH and insecticide concentrations of the sediment samples. Table 3 also reveals that there were some rather high insecticide concentrations in sediments. The potential release of those insecticides back into the overlaying water and their subsequent bioaccumulation would be a realistic concern as attempts are made to preserve the biological integrity of the lake.

The detection limits as determined for the sediment samples were: lindane, heptachlor, and aldrin detectable to 250 ng/kg; while endrin, dieldrin, DDD, DDT, and methoxychlor detectable to 500 ng/kg. Following are the insecticides used as spikes and their averaged percentage of recovery: aldrin 102.9%, DDT 91.2%, dieldrin 38.5%, endrin 94.3%, heptachlor 104.8%, and lindane 124.6%.

Statistical analyses were calculated for lindane, heptachlor, aldrin, dieldrin, and DDT only, since the sample size for the other insecticides was inadequate. Levene's test

Table 3. Insecticide Concentrations (mg/kg) of Sediment Samples

Sample	pH	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	DDD	DDT	Methoxychlor
1 TK 1	5.3	0.0009	<0.0003	0.0008	0.0042	<0.0005	<0.0005	0.0154	<0.0005
1 TK 2	7.1	0.0010	<0.0003	0.0015	0.0079	<0.0005	<0.0005	<0.0005	<0.0005
1 TK 3	6.7	<0.0003	0.0010	0.0014	0.0161	<0.0005	<0.0005	<0.0005	<0.0005
2 TK 1	6.6	<0.0003	0.0038	0.0065	<0.0005	<0.0005	<0.0005	0.2430	<0.0005
2 TK 2	6.2	0.0022	0.0009	0.0022	0.0038	<0.0005	0.0024	0.0016	<0.0005
2 TK 3	6.4	<0.0003	0.0021	0.0038	<0.0005	0.0075	0.0031	0.0018	<0.0005
4 TK 1	6.6	0.0005	0.0004	0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
4 TK 2	6.2	0.0010	0.0005	0.0013	0.0016	<0.0005	<0.0005	<0.0005	<0.0005
4 TK 3a	6.4	0.0011	0.0006	0.0008	0.0028	<0.0005	<0.0005	<0.0005	<0.0005
4 TK 3b	6.3	0.0030	0.0016	0.0027	0.0026	<0.0005	0.0018	<0.0005	<0.0005
4 TK 3c	6.1	0.0005	0.0004	0.0005	0.0031	<0.0005	<0.0005	<0.0005	<0.0005
5 TK 1	6.5	0.0012	<0.0003	0.0026	0.0234	<0.0005	<0.0005	<0.0005	<0.0005

Table 3. Insecticide Concentrations (mg/kg) of Sediment Samples--Continued

Sample	pH	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	DDD	DDT	Methoxychlor
5 TK 2	6.0	<0.00025	0.0017	0.0023	0.0041	<0.0005	<0.0005	<0.0005	<0.0005
5 TK 3	7.0	0.0015	<0.0003	0.0022	0.0056	<0.0005	0.0385	<0.0005	<0.0005
6 TK 1	7.1	<0.00025	<0.0003	0.0044	0.0264	<0.0005	<0.0005	0.0634	<0.0005
6 TK 2	6.3	0.0044	0.0027	<0.0003	0.0048	<0.0005	0.0054	0.0168	<0.0005
6 TK 3	6.4	0.0026	<0.0003	0.0024	0.0054	<0.0005	<0.0005	0.0020	<0.0005

determined that the variances were equal for each of the five insecticides examined. Since the ANOVA for lindane yielded borderline significance, Tukey's test was also employed. However, no significance was found among any of the tested stations for lindane. Heptachlor's ANOVA revealed no significance; thus there was no difference in heptachlor's concentration among the five stations. The same was true for both dieldrin and DDT. The ANOVA for aldrin was significant, and Tukey's test showed a significant difference between stations 1 and 2 and also stations 2 and 4.

The results demonstrated there was not a significant difference in insecticide accumulation among the sampling stations for lindane, heptachlor, dieldrin, and DDT. There was a significant difference in aldrin's sediment concentration, however. Stations 1 and 2 differed significantly as well as stations 2 and 4, but there was not a significant difference between stations 1 and 4, as might be expected. Therefore, it could be perceived that station 2 contained especially high levels of aldrin as compared with the rest of the stations. Station 2 is located at the confluence of Lake Tenkiller with Caney Creek (Figure 2). Aldrin has a high K_{ow}, 6.5, (Table 1) which readily explains its affinity to bind.

Excluding aldrin, all stations exhibited equal concentrations of insecticides. Therefore, we fail to reject the #2 null hypothesis for those insecticides. In the case of aldrin, we reject the null hypothesis and fail to reject the alternative hypothesis. The alternative hypothesis simply states that there was a difference in the insecticide concentration among the sediment stations sampled.

Equal insecticide concentrations in sediment may be due to non-point source input from creeks located along the entire length of the lake. Generally, organochlorine insecticides are quick to absorb to biotic or abiotic material. For that reason, it would be highly unlikely for insecticides transported only by the river to be equally deposited throughout the lake. We can only assume there are sources of insecticides other than the river contaminating the lake.

Fish Samples

Table 4 contains relevant information about the fish used in this study. Fish were caught in the fall which is ideal since % lipid content is typically higher at that time of the year than any other season. Depending on the age and length of fish, the lipid percentage ranged from 1.61 to 6.12%.

Table 4. Station, Length, Sex, and % Lipid of the Fish Samples

Fish	Station	Length (mm)	Sex	% Lipid
1	5	153	M	1.61
2	2	305	M	4.17
3	5	385	M	3.6
4	6	396	M	4.57
5	6	285	M	5.3
6	2	462	F	6.12

Fortunately, the procedure blank again yielded clean results with no toxicant contamination. Lindane, heptachlor, and aldrin were the most common insecticides. DDT and methoxychlor were present in several samples, some at very high quantities. Fewer samples contained dieldrin and DDD, and endrin was not present in any of the samples. Table 5 presents the fish insecticide concentrations.

Fish samples were detectable to the following levels: lindane, heptachlor, and aldrin detectable to 250 ng/kg; while endrin, dieldrin, DDD, DDT, and methoxychlor detectable to 500 ng/kg. Following are the insecticides used as spikes and their averaged percentage of recovery: aldrin 100.7%, lindane 113.1%, heptachlor 102.7%, DDT 95.3%, dieldrin 76.9%, and endrin 91.9%.

One population testing using Student's *t*-test was used to compare the fish insecticide data with NAS 1973 and Newell et al. 1987. Lindane, heptachlor, aldrin, dieldrin, DDD, and DDT were all significantly lower than the standards. Thus, we fail to reject the #3 null hypothesis; those insecticides are not bioaccumulating to deleterious levels in fish. There were not any standard levels for methoxychlor so 0.1, 0.2, and 1.0 mg/kg were used due to their comparability with other standards. At 0.1 mg/kg, there was not a significant difference between the methoxychlor data and that level. However, since the data did not exceed the value, it is still acceptable. Thus, we again fail to reject the #3 hypothesis. The methoxychlor data was significantly lower than 0.2 and 1.0 mg/kg, clearly not presenting a hazard, as well. Overall, Lake Tenkiller fish would be fit for piscivorous consumption.

Table 5. Insecticide concentrations (mg/kg) of fish samples. The mean (x) and standard deviation (s) is provided for each insecticide from each fish.

Sample	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	DDD	DDT	Methoxychlor
1 Fish 1	0.0043	0.0045	<0.0003	<0.0005	<0.0005	0.0287	0.1079	0.1073
1 Fish 2	<0.0003	<0.0003	0.0017	<0.0005	<0.0005	<0.0005	0.0676	0.0622
1 Fish 3	0.0030	0.0035	0.0042	<0.0005	<0.0005	0.0186	0.0298	0.3915
x	0.0036	0.0040	0.0029	0	0	0.0236	0.0684	0.1870
s	(0.002)	(0.002)	(0.002)	0	0	(0.039)	(0.001)	(0.001)
2 Fish 1	0.0279	0.0102	0.0100	0.0303	<0.0005	<0.0005	0.0162	<0.0005
2 Fish 2	0.0146	0.0110	0.0099	0.0387	<0.0005	<0.0005	0.0096	<0.0005
2 Fish 3	0.0151	0.0099	<0.0003	0.0295	<0.0005	<0.0005	<0.0005	0.1004
x	0.0192	0.0104	0.0099	0.0328	0	0	0.0129	0
s	(0.007)	(0.000)	(0.006)	(0.005)	0	0	(0.008)	0
3 Fish 1	0.0305	0.0089	0.0055	0.0749	<0.0005	<0.0005	<0.0005	0.4085
3 Fish 2	0.0106	0.0116	0.0071	0.0478	<0.0005	<0.0005	0.0127	0.0613
3 Fish 3	0.0215	0.0097	0.0064	0.0584	<0.0005	<0.0005	0.0955	0.1286
x	0.0208	0.0100	0.0063	0.0604	0	0	0.0541	0.1994
s	(0.009)	(0.000)	(0.000)	(0.014)	0	0	(0.052)	(0.184)

Table 5. Insecticide concentrations (mg/kg) of fish samples. The mean(x) and standard deviation (s) is provided for each insecticide from each fish.--Continued

Sample	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	DDD	DDT	Methoxychlor
4 Fish 1	0.0264	0.0131	0.0115	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
4 Fish 2	0.0355	0.0136	0.0195	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
4 Fish 3	0.0414	0.0136	0.0321	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
x	0.0334	0.0134	0.0211	0	0	0	0	0
s	(0.007)	(0.000)	(0.010)	0	0	0	0	0
5 Fish 1	0.0206	0.0068	0.0113	<0.0005	<0.0005	<0.0005	0.0110	<0.0005
5 Fish 2	0.0061	0.0035	0.0133	<0.0005	<0.0005	<0.0005	0.0055	<0.0005
5 Fish 3	0.0695	0.0096	0.0134	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
x	0.0321	0.0066	0.0126	0	0	0	0.0057	0
s	(0.033)	(0.003)	(0.001)	0	0	0	(0.006)	0
6 Fish 1	0.0174	0.0218	<0.0003	0.0348	<0.0005	<0.0005	0.2576	0.0543
6 Fish 2	0.0081	0.0349	0.0214	0.0493	<0.0005	0.0235	0.2128	0.0751
6 Fish 3	0.0102	0.0255	0.0379	0.0246	<0.0005	0.0013	0.1576	0.0348
x	0.0119	0.0274	0.0296	0.0246	0	0.0083	0.2093	0.0547
s	(0.005)	(0.007)	(0.019)	(0.024)	0	(0.013)	(0.003)	(0.037)

Calculated Mean Daily Flow

WQSTAT II© software calculated the monthly mean discharge for both USGS stations, 1965 and 1970, by using 142 months of data (over the period October 1, 1979 - September 30, 1992). The monthly mean discharge for USGS 1965 was 940.245 cubic feet per second (cfs) with a standard deviation of 967.657 cfs. USGS 1970 had a monthly mean discharge of 346.620 cfs with a standard deviation of 404.729 cfs. The total discharge into Lake Tenkiller would then be approximately 1,286.865 cfs every month. The daily total discharge into the lake, as calculated by Quatro-Pro©, would be: low flow 280 cfs, mean flow 629 cfs, and high flow 1482 cfs.

A benefit of using this approach is that the number of monthly averages is high, n = 142, which corresponds with many years of data. The negative aspect of this procedure is that the mean accounts for fluctuations over 13 years which results in a high standard deviation. Thus, calculating low, mean, and high flow values is an attempt to compensate for the constant river fluctuations.

Calculated TMDLs

Table 6 contains the calculated safe insecticide water concentrations as well as TMDLs for low, mean, and high flow conditions. TMDL values were calculated in kg/day for ease of estimating runoff potential and monitoring. Values were intended to be ecologically-based estimates useful year round depending upon the rate of river flow. Since the calculations are based upon the allowable maximum level in water, the daily influx of insecticides must be equal to or less than the TMDL value. For instance, on a low flow day, there should be a maximum of only 0.032114 kg of lindane that are loaded to the lake on that particular day.

Table 6. Insecticides' Safe Water Concentrations and TMDL Values

Insecticide	Concentration (mg/l)	Low Flow (kg/day)	Mean Flow (kg/day)	High Flow (kg/day)
Aldrin	0.0255	0.0217	0.0489	0.1152
DDT	0.0435	0.0371	0.0834	0.1966
DDD	0.0463	0.03957	0.0889	0.2093
Dieldrin	0.0393	0.0336	0.0755	0.1779
Endrin	0.0077	0.0066	0.0148	0.0349
Heptachlor	0.0265	0.0226	0.0507	0.1196
Lindane	0.0376	0.0321	0.0721	0.1699
Methoxychlor	0.0275	0.0235	0.0528	0.1245

CHAPTER V

CONCLUSIONS

Assumptions and Errors

Several factors necessitated making assumptions in order to calculate TMDL values. In no particular order, the assumptions were:

1) Standards were accurate and correct.

2) Water flow into the lake was equal to water flow out of the lake.

3) River water was the only source discharging to the lake.

4) Fish accumulated insecticides through water exposure only, not through the food chain.

5) Fish were exposed equally to the insecticides.

6) Equal mixing occurred in the lake.

7) Fish were exposed to insecticides one at a time only, not to combinations of insecticides.

Even though the above assumptions had to be made, they do not necessarily illustrate a true portrayal of a real-world situation. Assumption #3 presumed that the river was the only source loading to the lake. The sediment data, however, indicated otherwise. Aldrin's significant difference in concentration at station 2 suggests that there is another source contributing to the lake. In all probability, the creeks load insecticides to the lake as well as the river.

Assumption #4 was crucial to formulating TMDL levels. The calculated TMDL value accounts for insecticide exposure through water only and not through sediment or food chain exposure. The latter two exposure scenarios could substantially increase insecticide residue, but would be equally difficult to predict resulting toxicant concentration levels.

Assumption #7 states that the TMDL value, as calculated, protects predators by assuming fish do not undergo multiple exposure scenarios to combinations of insecticides. Since the effect of such an exposure would be unknown, it is unaccountable. Also, the possible consequence of the toxicants working in synergy would be equally difficult, not to mention virtually impossible, to estimate.

A potential source of laboratory procedure error may be attributed to the blender used in mixing fish tissue with solvent. The first blender had a tendency to leak solvent so a second blender was employed. The difference in blenders and the small amount of leaked solvent would have minimal impact upon the results, but nonetheless must be counted. In addition, since data is continually susceptible to the fallibility of analysis equipment, the equipments' imperfections may contribute as a potential source of error. Thus, it should be remembered that the data is only as good as the equipment.

Ecological Consequences

The greatest difficulty in estimating ecological consequences is that once insecticides enter a lake ecosystem, accurate predictions cannot be made as to which abiotic area would pose the greatest toxic risk to biotic organisms. In all probability, the risk varies according to properties of the specific insecticide. As shown by the results of this project, the water and sediment data indicated there could be high insecticide levels present in fish. Yet, the fish captured for this study possessed very low concentrations that would most likely not be hazardous, as evidenced by comparisons with NAS 1973 and Newell et al. 1987 as standards.

This discrepancy between the abiotic and biotic insecticide levels could be due to several factors. Differences in water mixing, length of exposure, uptake rate, and depuration in fish could account for the disparity. Nonetheless, it is difficult to estimate toxicant concentrations in aquatic biota, and sampling water one time is not definitive enough to draw conclusions. The high water concentrations that were detected may have been an anomaly. Additionally, even though the sediment contained high insecticide levels, biota are not necessarily exposed to insecticides unless the insecticides are released back into the water, or the biota consume contaminated benthic organisms.

However, since the fish concentration levels were well below the standard levels and thus the corresponding TMDL levels, we could assume the overall fish population at Lake Tenkiller is not burdened with a toxicity problem. Furthermore, since fish often serve as indicator species of ecosystem contamination, we could cautiously presume there is not a deleterious bioaccumulation problem for fish predators at Lake Tenkiller as well.

The concepts and methodology presented in this project could prove useful to future watershed managers and scientists. Currently, in Washington State, 90% of eagle pairs nesting along Hood Canal are failing to produce offspring (News Tribune 1995).

Scientists suspect PCBs and other organochlorine chemicals as culprits. They have detected elevated levels of dioxins, furans, and PCBs in the eagles' eggs. One egg even contained levels of PCBs as high as 25 ppm. A representatives from the Fish and Wildlife Service stated that eagles are a "prime indicator of environmental contamination". In short, they serve as a warning for human health as well as ecosystem health.

Recommendations

Analyzing the piscivorous bird population at Tenkiller would be the most obvious route to confirm whether or not birds' tissue contains deleterious insecticide levels. In-depth studies containing observations and population counts over several seasons would be the most reasonable method to acquire this information. Tissue analysis would also allow the researcher to determine which toxicants, if any, are impairing the population. Both actions, however, are beyond the scope of this project.

Further study could be conducted by extensive insecticide sampling in the creeks, river, and lake to determine if the creeks are actually a contributing source of toxicants to the lake. This work could be coordinated with the acquisition of water flow levels to determine whether the toxicant concentrations are consistently below TMDL values for low, mean, or high flow periods. Baseline information such as this could prove invaluable to future watershed managers.

Traditionally, TMDL methodology is used to estimate the concentrations of phosphorus and nitrogen which may enter a water system without further stimulating the eutrophication process. Even in its original use, the TMDL method has several inherent difficulties which cast doubt as to its reliability. The principles of the method are practical and beneficial, but calculating the load allocation and wasteload allocation, which are necessary to derive the TMDL, are laborious. Due to the complexity of the calculations, many researchers doubt the accuracy and usefulness of this technique.

Likewise, TMDL methodology certainly does not account for several problems encountered in this research project. To begin with, it is extremely difficult to estimate residual pesticide concentrations in the abiotic component of the ecosystem, and it is difficult to predict possible exposure scenarios for the biotic component, as well. The uptake rate and depuration rate of pesticides in aquatic organisms are equally complicated to predict with accuracy. There are many variables, which have been previously addressed, that determine the actual residual concentration in the biotic component of an ecosystem. It is recommended that the estimated numbers in this project be verified with actual laboratory tests combined with field measurements and field tests before they are relied upon as valid. The concepts and methodology used in this project are ideal in formulating a procedure to protect the fauna of an ecosystem, but until this method has been improved upon, it should certainly not be relied upon for regulatory purposes, as most TMDL estimates are used.

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