THE DETERMINATION OF UPTAKE AND DEPURATION RATE KINETICS AND BIOCONCENTRATION FACTOR OF NAPHTHALENE AND LINDANE IN BLUEGILL SUNFISH, LEPOMIS MACROCHIRUS

THESIS

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By

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Bluegill were exposed to 3 and 30 µg/L lindane and 20 and 200 µg/L naphthalene to determine uptake rate constants, K_1, depuration rate constants, K_2, and bioconcentration factors, BCF. Correlations were determined between lipid normalized and non-lipid normalized BCFs, and between observed K_1, K_2 and BCFs and predicted values.

The K_1 values for both chemicals and concentrations were similar. The K_2 values were different (1.04 day^{-1}, 0.46 day^{-1}). Naphthalene was more rapid. BCFs for lindane (315) and naphthalene (98) were different. Lipid normalized BCFs for naphthalene were more variable than non-lipid normalized BCFs. The reverse was observed for lindane BCFs. Predicted K_1, K_2, and BCFs were in agreement with observed values.

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INTRODUCTION

Background

The bioconcentration of chemicals by aquatic organisms is of concern to scientists because it is a process by which some organic chemicals accumulate to potentially harmful levels. Also, it is an important fate process for removal of chemicals from water.

Fate is the transport, deposition or degradation of a chemical which has been released into the environment. Knowledge of a chemical's behavior is of value in predicting potentially detrimental effects to an ecosystem (organisms and environment) by suggesting avenues of entrance to the various biotic components, possible degradation to toxic intermediate substances and types and rates of reactions.

Not all chemicals which are released into a water system remain in the water. Predictions as to where in the environment a chemical is likely to move can be made based on the physical-chemical properties of the chemical. Generally, chemicals with high vapor pressure and low water solubilities will be associated with sediments (adsorption) and chemicals with high solubilities will remain in the water. Others may evaporate into the atmosphere (3).

Chemical concentrations in water are decreased by microbial biotransformation and biodegradation. Aquatic organisms such as

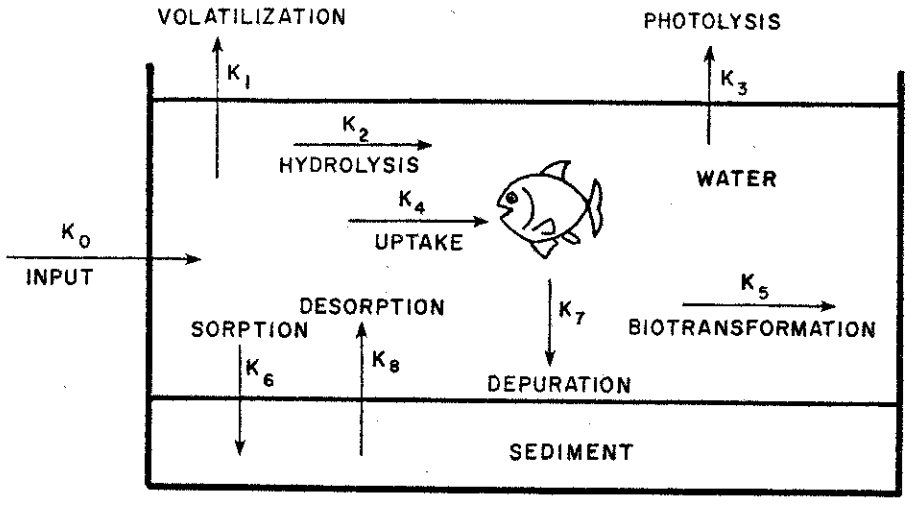
insects and fish bioconcentrate and metabolize chemicals thereby removing them from aquatic system. For some chemicals sunlight is the most significant degradation mechanism (eg., ethylenediaminetetra-acetic acid, EDTA) (3).

The research reported in this thesis was part of a larger research program to determine the rate constants for the chemical processes of volatilization, sorption, hydrolysis, photolysis, biotransformation/degradation, and bioconcentration. These derived rate constants can be used in environmental fate modeling.

One approach for predicting fate is the "environmental rates approach" in which laboratory data are expressed as rates which can be incorporated into material balance models that predict exposure concentrations from a given rate (Figure 1-1). Branson (3) suggests that the main advantages of the environmental rate constant approach are the predictability of concentrations at various points in time and an understanding of what happens to a chemical in the environment over a period of time rather than at a single point in time. These can be used to rank chemicals by relative acceptability in the environment by determining which chemicals are most and least likely to be persistent and accumulative (3).

Bioconcentration

Bioconcentration of organic chemicals by aquatic organisms is an important process investigated to determine the fate of chemicals in aquatic environments. Bioconcentration is usually the dominant process by which fish accumulate organic chemical residues (9). Fish probably



MATERIAL BALANCE EQUATION

$$V\frac{dCw}{dt} = K_0 - K_1 ACw - K_2 VCw - K_3 ACw - K_4 FCw - K_5 BCw - K_6 SCw + K_7 FC_f + K_8 SCw$$

INPUT; VOLATILIZATION; HYDROLYSIS; PHOTOLYSIS; FISH UPTAKE; BIOTRANSFORMATION; SORPTION; FISH DEPURATION; DESORPTION

where V= Volume of water , ℓ
A= Surface area , m^2
F= Fish mass , kg
S= Sediment mass , kg
B= Microbial biomass , kg
Cw= Concentration of chemical in water , mg/ℓ
K= Rate constant
C_f = Concentration of chemical in fish , mg/kg
C_s = Concentration of chemical in sediment , mg/kg

Figure 1-1. Schematic diagram of a reservoir showing the environmental fate processes and a mass balance equation (after Branson 1979).

do not significantly decrease the concentration of a chemical in water. The extent to which a chemical bioconcentrates in fish is an important consequence to the fish and consumers of the fish, including man and wildlife. The ability of a chemical to move to higher levels in the food chain (biomagnification) is of importance from an environmental standpoint because the acute toxicity of a substance may be low and the physiological effects unnoticed until the chronic effects are evident. By this time it may be too late to correct or reverse the effects. For this reason prior knowledge of the bioconcentration potential of new or existing chemicals is desirable. Examples of chemicals which biomagnify and have been known to cause ecological problems are DDT and the polychlorinated biphenyls, (PCBs) (7).

In this study bioconcentration is defined as an increase in the concentration of a chemical residue relative to water in or on a fish by transport directly from the water through gills or other membranes. This excludes bioaccumulation which is an increase of a chemical on or in the organism resulting from consumption and direct uptake through membranes (5).

Evaluation of the potential of a chemical to bioconcentrate requires the determination of an uptake phase, a depuration (elimination) phase and a steady state equilibrium. The uptake phase (UP) is that period of time the organism is exposed to a chemical and that chemical is bioconcentrating. Depuration is the clearance of the chemical from the organism (5). Steady state is that point when the

concentration of the test chemical in the fish no longer increases upon constant exposure.

The bioconcentration potential of a chemical in fish is usually evaluated by some modification of the plateau approach of testing (1). Under this approach fish are continuously exposed to a constant concentration of the test material until a steady state or plateau is reached as determined by periodical analysis (Figure 1-2). The time needed to reach steady state has been arbitrarily set at twenty-eight days but may range from weeks to months depending on the physical characteristics of the chemical (1). The advantage of the plateau approach is that the bioconcentration factor (ratio of chemical in fish to the chemical in the water) is derived from experimental observation and is based on measured steady state equilibrium concentrations.

Although the uptake and depuration rates are ideally derived from experimental data, such data may be lacking at a time when an evaluation of an uptake pattern that can be expected of an organism is needed. A major problem with this experimental approach is that some hydrophobic chemicals may reach steady state levels only after long periods of continuous exposure. Branson et al. (2) developed an "accelerated test" which assumes that the bioconcentration mechanism can be described kinetically by the model

$$C_w \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C_f \qquad (2)$$

where C_w is the concentration of the chemical in the water, C_f is the

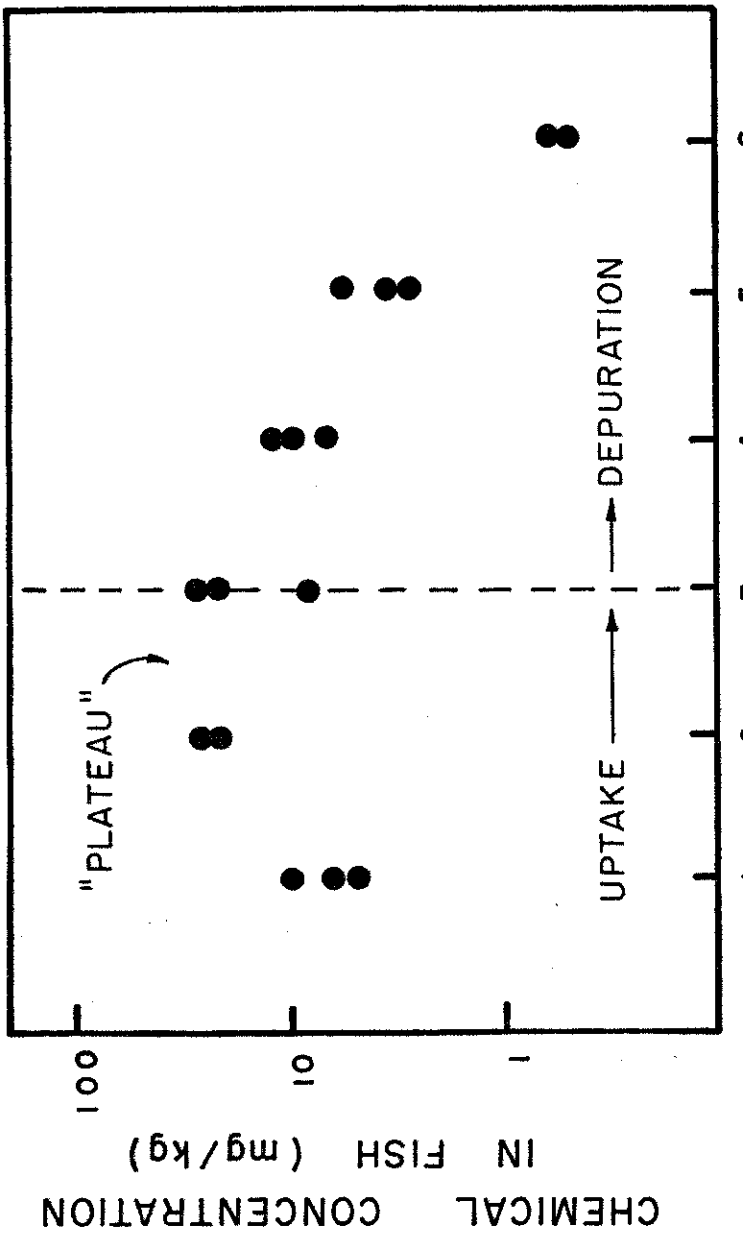


Figure 1-2. Plateau region where an equilibrium concentration is reached.

concentration of the chemical in the fish and K_1 and K_2 are the uptake and depuration rate constants, respectively. A non-linear regression (7) is used to estimate K_1 and K_2. From these kinetic rate constants the steady state or plateau concentrations can be calculated and bioconcentration factor (BCF) derived.

The rate constant, K_2 is a mathematically derived value represented by the slope defined by least squares regression of log concentration of the chemical in the organism versus time (7). The uptake rate constant, K_1 is mathematically determined from K_2. It is a biphasic process in that the fish's elimination processes are removing a portion of the chemical from the fish during the uptake phase. The uptake-depuration mechanisms can be estimated by first order rate kinetic expressions (2). Uptake is pseudo-first order with respect to water concentration and K_2 and depuration is first order with respect to the concentration in the fish. Therefore, the rate of change of the chemical in the fish would be:

$$\frac{dC_f}{dt} = K_1 C_w = K_2 C_f \qquad (2).$$

When C_w is constant the increase in residue in the fish is given by the equation:

$$C_f = (K_1/K_2)C_w(1-e^{-K_2 t}) \qquad (2, 10).$$

Branson et al. (2) applied this model to the uptake and elimination of 2, 2'4,4'-tetrachlorobiphenyl by rainbow trout (Salmo gairdneri). The results of the accelerated five-day exposure testing compared favorably with those from a long-term, forty-two day exposure.

The advantage of this short term test is that it requires less test material and shorter exposure periods. A shorter time frame lessens the effect of complicating factors such as growth, lipid deposition or bioenergetics, yet adequate information is gained (8).

The concentration of the chemical retained by the fish is assumed to be a function of the chemical dissolved in the water (3). As exposure time increases a steady state level is attained, that is the residue level no longer changes with continued exposure. At this point the concentration of the chemical in the fish and in the surrounding water are at equilibrium. From this a constant of proportionality or bioconcentration factor (BCF) can be derived such that

$$C_f = C_w = BCF \qquad (2).$$

The BCF, then, is a measure of the potential of a chemical to accumulate in the tissues of aquatic organisms. The estimates of BCFs have become increasingly important in the assessment of environmental hazard. A BCF for each chemical is desired so chemicals can be ranked relative to one another for this characteristic.

Approaches for Estimating Bioconcentration

Bioconcentration testing can be costly ($3000 - 5000 per test or more depending on chemicals) (11) and may take considerable periods of time. Therefore, methods of screening chemicals are of great value. Chemicals that are not highly bioaccumulative can be quickly eliminated from the bioconcentration testing or used in more informative testing

and consideration be given to new chemicals or those posing greater hazards, such as toxicity or carcinogenicity (11).

Predictive, short term chemical tests such as that of Branson's accelerated test are more rapid and less costly than the plateau approach. Recently, n-octanol water partition coefficients and water solubilities have been used to estimate BCF (4, 7, 10). These characteristics can be used to calculate pre-test estimates of K_1 and K_2 thus optimizing sampling interval time to steady state. They can also be used to calculate BCFs by utilization of models.

Chemicals are continually being transported between the solid, liquid and gaseous phases in the environment. This relative tendency of chemicals to partition themselves between solid and liquid phases (e.g., biota, water) can be predicted using the n-octanol water partition coefficient (K_{ow}) and water solubility (S).

A partition coefficient is defined as the ratio of the equilibrium concentration of the chemical between an organic, non-polar solvent and a polar solvent, often water. The fat-solvent water partition coefficients have been determined using oil, hexane or n-octanol. n-Octanol is most often used because it is believed to most closely resemble the fatty tissues in plants and animals. Fatty tissues have been shown to be most responsible for storage of the organic chemicals in aquatic organisms (10). It is the ability of a chemical to bioconcentrate in the fatty tissues, or lipids, of fish that makes the correlation between K_{ow} and bioconcentration of organic chemicals so effective in BCF prediction. Several investigators (7, 10, 11), have shown the logarithms of

bioconcentration factor (log BCF) and the n-octanol water partition coefficient (log P) are linearly correlated.

The greatest factor affecting the correlation of K_{ow} with BCF is the relative water solubility of the chemical (4). Chemicals of low lipid solubility (log P $\leq$ 2.5) and relatively high water solubility (log 1/S $\geq$ 2.0) result in estimates of greater uncertainty (1). These chemicals, however, have low bioconcentration potential (BCF) and are therefore of less consequence in the environment (1).

Another factor affecting the correlation is that highly lipophilic chemicals (e.g., hexachlorobenzene, log P = 6.44) may have a low BCF but have a high log P. This non-linearity is the result of relatively slow uptake of the chemical (6). This point also illustrates the value of a flow-through test system in which the fish are exposed for long time periods to a constant level of the test material allowing a concentration equilibrium or steady state to be reached.

A correlation can also be made between log P and K_1 and K_2. Generally, the higher the log P the more rapid will be the uptake rate and slower the elimination of the chemical. Linear regression for the correlation has been derived by Neely *et al.* (7) and Konemann and Van Leeuwen (6). They suggest that for chemicals having log P in the range of approximately 2 to 6, BCF, K_1 and K_2 can be predicted using log P.

Water solubility (S) is also of value in prediction of the movement of a chemical between biota and water. Chemicals of high aqueous solubilities will be of low lipophilic nature resulting in lower

bioconcentration potential. Using bioconcentration data in rainbow trout from Neely et al. (7), Chiou et al. (4) correlated the water solubility of seven organic chemicals and their BCFs. The correlation coefficient equalled 0.93.

The n-octanol water partition coefficient expresses an equilibrium concentration ratio of an organic chemical partitioned between an organic liquid and water. This partitioning can be considered equivalent to partitioning of an organic chemical between itself and water (4), or the amount of the chemical that will be dissolved by the water. This suggests that a correlation exists between a partition coefficient and aqueous solubility. Chiou et al. (4) used the correlation to determine experimental K_{ow} from aqueous solubilities of thirty-four chemicals. Their work suggested that this correlation will help clarify questionable data reported for the K_{ow} of various chemicals (eg. DDT).

Hypotheses

The basis of this research is centered on bioconcentration of naphthalene and lindane in bluegill sunfish (Lepomis macrochirus) tissue and the rate kinetics and BCFs associated with their bioconcentration. These chemicals were selected because they exhibit a wide range of toxicity and persistence as well as wide diversity in physical-chemical characteristics such as water solubilities, molecular structure, partition coefficient and adsorption spectra. Each chemical represents a general group of chemicals similar in these and other properties. Thus, they can be considered benchmark chemicals whose rate constants can be of great utility in developing predictive models.

The following hypothesis were addressed and tested in this research.

H_o1 There is no significant difference between naphthalene and lindane uptake rate kinetics.

In testing this hypotheses one would expect to find a difference in the uptake kinetics of lindane and naphthalene. Each shows differences in physical and chemical properties which could affect rates. Neely et al. (7) measured uptake rate kinetics of various chemicals in trout muscle. Each chemical was shown to have a unique uptake rate.

H_o2 There is no significant difference in the depuration kinetics between lindane and naphthalene.

As in uptake rate kinetics one would expect to find a difference in the depuration kinetics of naphthalene and lindane. Neely (7) found depuration rates of various chemicals to differ when measuring rate kinetics in rainbow trout.

H_o3 The exposure concentration makes no significant difference in the uptake-depuration kinetics for lindane and naphthalene.

Various investigators have found results which are not in agreement as to the dependence or independence of the exposure concentration on the uptake-depuration rate kinetics for various chemicals (1). One would expect environmental or physiological factors, such as increased ventilation rates with increased temperatures, to be more instrumental in determining uptake than would the exposure concentration. Elimination is a more complex function of the exchange process at the gill, excretion via bile

or kidney and metabolism. These functions, too, are influenced by physiological and environmental factors. It would seem that these functions would operate at their own rate regardless of exposure concentration unless impaired by the exposure concentration.

H_o4 There is no significant difference in BCF between lindane and naphthalene.

Bioconcentration factors for many chemicals have been determined by several investigations. The results of these tests indicate that each chemical exhibits its own BCF (1). It appears that the structure and chemical characteristics (i.e., long chain alkanes and aqueous solubility) greatly influence the level to which a chemical will bioconcentrate within an organism. Since naphthalene and lindane vary considerably in their characteristics, one would expect them to have different BCFs.

H_o5 The exposure concentration makes no significant difference in BCF.

Several investigators have found BCF to be independent of exposure concentrations (1,11). The level to which the chemical concentration in the fish increases is influenced by the level of the chemical concentration and the availability of the chemical in the water. At maximum allowable toxicant concentration levels, physical and metabolic characteristics such as adiposity of elimination rates are also influential in determining to what level the chemical will accumulate before reaching an equilibrium state (8).

H_o6 There is no significant difference between lipid normalized values and weight normalized values for uptake and depuration rates or BCF for naphthalene or lindane.

When comparing bioconcentration rates of test materials, there is often considerable variation relative to weights of fish. It would seem that such differences would lessen the utility of the final analysis if a normalizing factor is not applied. Each of the tested chemicals displays a lipophilic character of its own and would be expected to be attracted to the fish lipid in relative portion to that content. However, lipids vary with species, life stage, growth, sex and other such variables so that uniformity in fish would be an important consideration in bioconcentration testing (11).

H_o7 There is no significant difference in predicted uptake rate kinetics, depuration rate kinetics and BCFs compared to experimentally derived values. The ability to predict reasonable BCF and rate kinetics is an important constituent in the hazard evaluation of chemicals in the environment. Two relationships used for these predictions, n-octanol-water partitioning and water solubilities are previously discussed in this thesis. Many investigations have shown these relationships to be useful in predicting bioconcentration but there are limitations and reservations (9, 11). These include use of chemicals with high log P (>6) or low log P (<2).

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CHAPTER II

MATERIALS AND METHODS

Chemicals

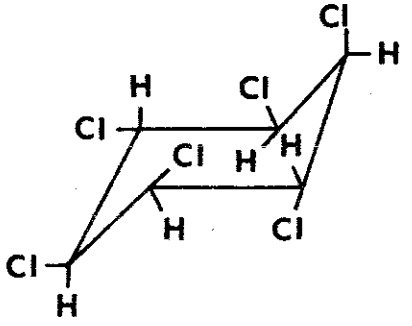
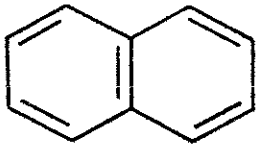
The uptake and depuration of two chemicals, naphthalene and lindane, were investigated in this research. These two chemicals exhibit a wide diversity of physical and chemical properties including water solubility, molecular structure, toxicity, partition coefficient, absorption spectra and persistence.

Naphthalene is a bicyclic aromatic hydrocarbon (Table 2-1). Naphthalene ($C_{10}H_8$) is the most abundant (ca. 5 percent) of all the constituents of coal tar and is produced from petroleum hydrocarbons (1). This compound is used as an intermediate in the production of dye compounds and the formulation of solvents, lubricants, motor fuels and plastics. It has also been used directly as a moth repellent, insecticide, vermicide and intestinal antiseptic. Naphthalene is non-polar, easily halogenated, stable and intermediate in biodegradability (1). The concentration in which 50 percent of the test organisms die during a 96 hr. test period (96 hr. LC_{50}) toxicity to mosquito fish (Gambusia affinis) is 21 mg/L (2). Solubility in distilled water is 20 - 28 mg/L (3).

Lindane is the gamma isomer of benzene hexachloride (Table 2-1). The uses of lindane include household sprays and livestock dusts for controlling some pests on fruits and vegetables (4). Lindane is

TABLE 2-1

LINDANE AND NAPTHALENE - PHYSICAL CHEMICAL PROPERTIES

	Lindane	Naphthalene
· Structure		
· Molecular Weight	291	128.18
· Melting Point	112.9°C	85.55
· Vapor Pressure	9.4×10^{-6} to 1.6×10^{-4} torr.	0.0492 torr.
· Solubility in Water at 25° ± 1 C	6.8 to 7.8 mg/l	31.7 to 34.4 mg/l
· Log octanol/water partition coefficient	3.72	3.37
· pK_a	--	--

From: EPA (1979)

relatively persistent in natural waters and sediments; although, it is less than DDT, a chemical similar in characteristics, in toxicity and persistence (5). The maximum acceptable toxicant concentration (MATC) for bluegill is 9.1 - 12.5 µg/L (4). Ninety-six hour LC_{50} estimates of the acute toxicity of lindane to bluegill range from 26 - 75 µg/L (6). Solubility of lindane in distilled water is 7.3 - 10 mg/L (3). Lindane is present in the Mississippi River and some streams in the Western United States at concentrations ranging from 2.8 - 38.0 µg/L (6).

Pretest Calculations

Pretest calculations to estimate K_2 were based on the solubility (S) data and n-octanol water partition coefficient, k_{ow} (Table 2-2).

The equation used based on solubility is

$$\text{Log } K_2 = 0.43 \log (S) - 2.11 \quad (7).$$

The equation used based on n-octanol partition coefficient is

$$\text{Log } K_2 = 0.414 \log (k_{ow}) + 0.122 \quad (7).$$

The duration of the uptake phase (UP) is near the mid-point of an uptake curve plotted on semi-log paper, or $UP = 1.6/K_2$, but not more than $3.0/K_2$ which is equivalent to 95 percent of steady state (9). The duration of the uptake phase was estimated as

$$1.6/K_2 \geq UP \leq 3.0/K_2 \quad (8).$$

Pretest estimates of the BCFs were determined from the equation for whole fish (body burden)

$$\text{Log BCF} = 0.76 \log k_{ow} - 23 \quad (7).$$

TABLE 2-2

CALCULATED PRE-TEST ESTIMATES OF PARAMETERS FOR NAPHTHALENE AND LINDANE

Test	Log P	$K_{(ow)}$[a]	Solubility	K_2	$UP_{max.}$	$DP_{max.}$	BCF
Naphthalene	3.37[a]	2344[b]	20 - 27 mg/l[c]	(s) .034[g] (K_{ow}) .054[h]	4 d.	7 d.	212
Lindane	3.85[d]	7079[d]	7.3 - 10 mg/l[c]	(s) 0.021[g] (K_{ow}) 0.034[h]	6 d.	12 d.	496

Subscript indicates reference as found in literature citations

[a] Veith et al. 1979a.

[b] Kariskoff et al. 1979.

[c] Mackay and Leinonen.

[d] Veith et al. 1979b.

[g] $\log K_2 = 0.43 \log S) - 2.11$, where
S = solubility in water

[h] $\log K_2 = 0.414 \log (K_{ow}) + 0.122$, where
K_{ow} = octanol-water partition coefficient

Test Organism

Uptake-depuration rates and bioconcentration factors for lindane and naphthalene were determined using the bluegill sunfish, Lepomis macrochirus. Bluegill was the fish of choice because of the available literature and its convenient size. For the experiments, bluegill sunfish were obtained from the Lewisville hatchery, Denton County, Texas, which is operated by the Texas Parks and Wildlife Service.

Before testing, fish were held in a two sectioned flow-through tank for acclimation to water conditions and to assure the fish were in good health and disease-free. Tanks were aerated with oil-free air. Non-chlorinated well water was used for all tests. An analysis of the well water from the aquatic toxicology laboratory where the experiments were performed is in Appendix Table 1.

Exposure System

A continuous flow-through system was used for the bioconcentration testing (Figure 2-1). Dilution water from the well was piped into a Blue M water bath unit that served as a headbox. Dilution water was vigorously aerated in the headbox and water in the test chamber was aerated only during the depuration phase of testing. Water levels in the headbox were controlled by an overhead drain. One side of the headbox had twelve ports with glass tubing attached which connected the water supply to the test chambers. Each valve had the same rate of flow for delivery of water into the test chamber. The rate was adjusted to provide 15 volume additions per 24 hours.

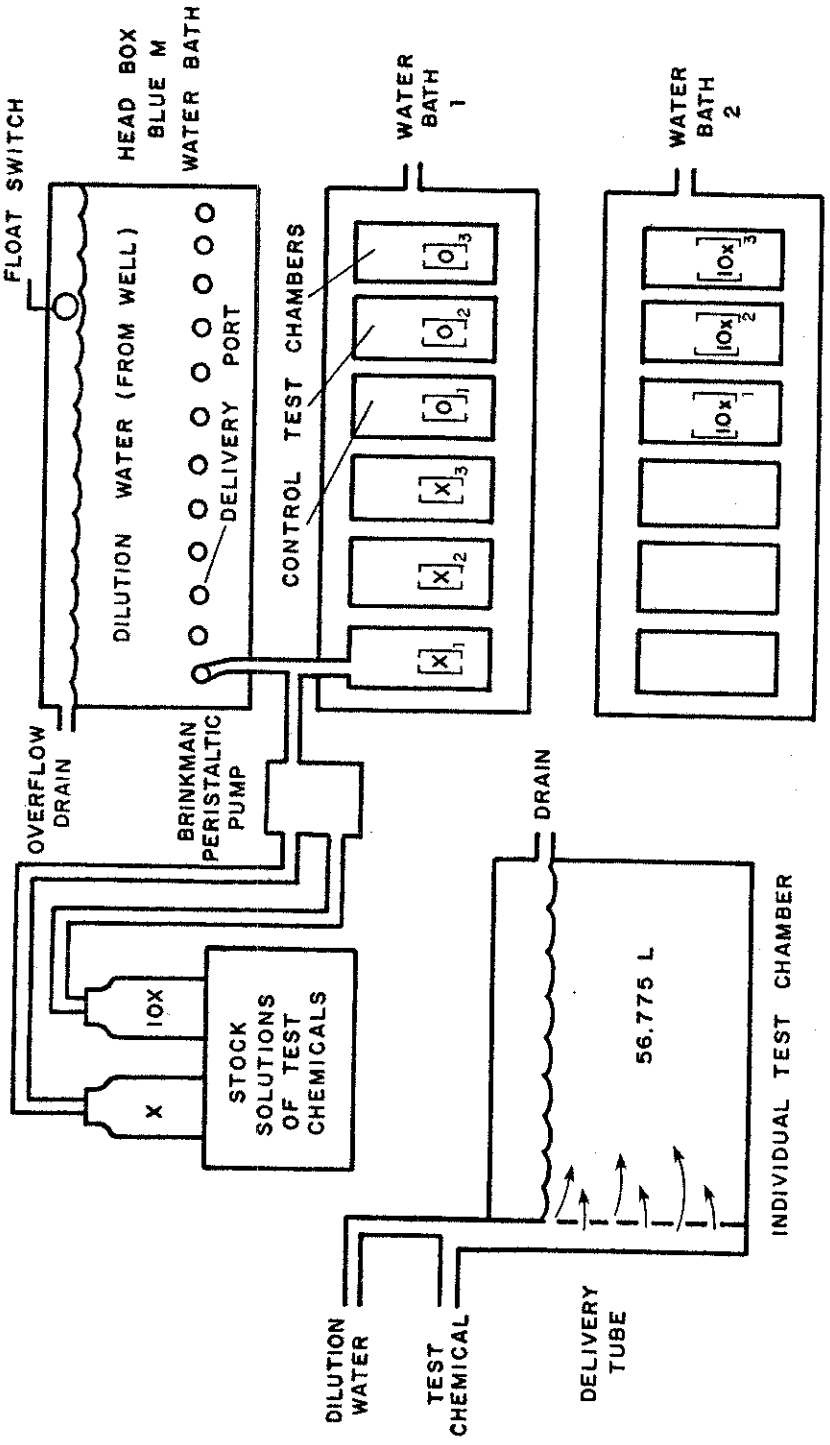


Figure 2-1. Flow-through system for bioconcentration tests.

Three 75.1 L aquaria, filled to 56.8 L of water, were used as test chambers. Thirty-six fish weighing approximately 8 grams each were placed in each chamber. Chemical solution and dilution water entered the side of the test chambers through a delivery tube which had a series of openings through which the test material and water were released into the test chambers.

Two holding tanks housed the test chambers. In each, water stabilized the temperatures within the test chambers. An opening near the top of the chamber allowed flow from the chamber into the holding tank. A lower positioned drain in the holding tank allowed water to drain from the tanks into a floor drain.

During exposure, chemical concentration, temperature and dissolved oxygen were monitored twice daily. Photoperiod equalled that existing at the time of experimentation. Alkalinity and hardness were determined using a Hach Kit on the first and last day of experimentation. Table 2 in the Appendix presents the general conditions of testing.

Test Chemical Concentrations

In the bioconcentration tests fish were continuously exposed to dilution water containing added test material at concentrations of 0, X, and 10X µg/L. In the testing with naphthalene the nominal X concentration was 20 µg/L and 10X, 200 µg/L. For lindane testing the nominal concentration was 3 µg/L and 30 µg/L. These concentrations were chosen after consideration of analytical and toxicological boundaries as well as MATC criteria.

Stock Solutions

Stock solutions used to dose the test chambers were prepared from commercial grade lindane (99 percent - Sigma Chemical Company No. H-4500) and reagent grade naphthalene (Baker 1-2718) for experiments. No solvents were used since all stock solutions were prepared at or below aqueous saturation concentrations.

Stock solutions of test chemical concentrations of 0, X and 10X mg/L were delivered by a Brinkman 12-channel peristaltic pump to the test chambers at a controlled rate that resulted in a concentration specific for each chamber. In the case of naphthalene X was 20 µg/L; for lindane X was 3 µg/L. Stock solution concentrations were determined using the equation

$$\text{Stock solution concentration (mg/L)} = \frac{\text{Dilution water (mL/min)} \times \text{Desired chemical concentration (mg/L)}}{\text{Dosing rate (mL/min.)}}$$

The stock solutions were made up in 19 L glass carboys to which 18 L of deionized water and the chemical were added. This was agitated by a motorized stir rod to facilitate complete mixing.

Sampling

At four times during uptake and five times during depuration four fish from each test chamber were sampled and analyzed to determine the concentration of the test chemical in the fish. Water samples were taken daily during uptake and again after fish were placed in untreated water. The sampling schedule is in Appendix Table 3 and Table 4.

Extraction

Fish were weighed, measured and samples frozen until time of extraction. Whole fish were macerated and dried with sodium sulfate. Lipids were removed by soxhlet extraction using 23 percent anhydrous ether and 77 percent pentane. Florisil absorbent column chromatography with anhydrous ether elution was used to clean the extract of lipids, oils and other potentially interfering organics. The volume of the ether used was 30 percent of the total extract volume. A Kuderna-Danish concentrator with graduated collection tip was placed under the column to receive the eluate. Each sample was condensed by hot water bath to 10 mLs. A 3-ball Synder column was used during the condensing. Pentane was the solvent used in all the steps of the procedure because of its expediancy in condensing the samples as well as its efficiency (9).

Efficiency Determination

Because of the numerous steps in the total analysis, (Figure 2-2), great care was taken to assure maximum recovery of the chemical residue in the fish tissue. For this reason the efficiency of the procedure was determined. Two methods were used in the determination of efficiency. The most common technique used is to determine "recovery efficiency." In this method macerated fish tissue is 'spiked' with a known concentration of the test chemical. Then all procedures of the system (soxhlet extraction, florisil column, condensing, G.C. analysis) are implemented as in actual testing. From these data the percent recovery of the chemical can be determined, thereby indicating the efficiency of the system. In the actual determination of recovery efficiency, two

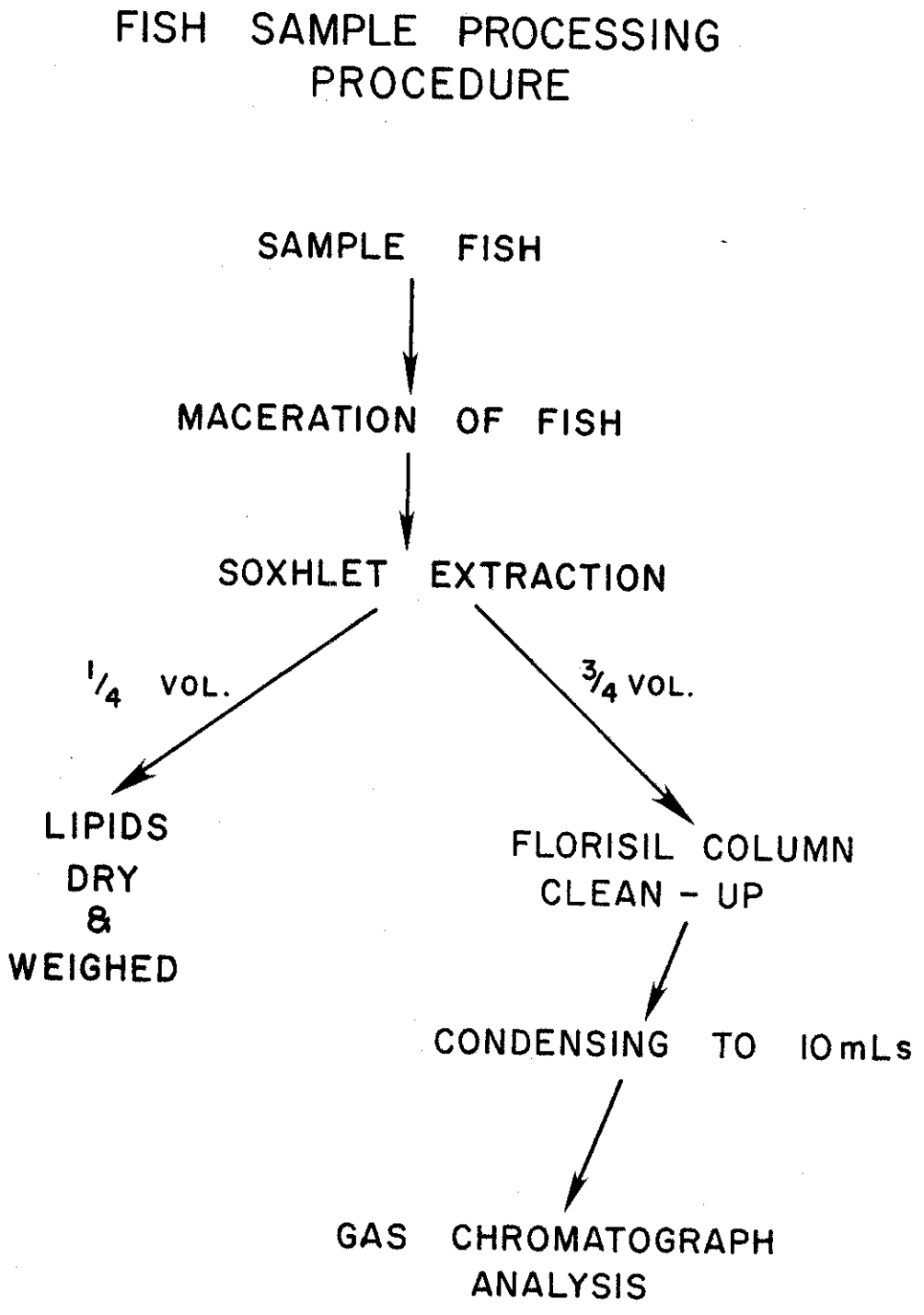


Figure 2-2. Flow-chart for fish extraction and processing.

concentrations of the test chemicals were used as the spikes. These corresponded to higher and lower levels of concentrations expected after exposure of fish to the test chemicals.

The generally accepted criterion in determining how long the extraction should be conducted for optimum efficiency is 80 - 120 flushes during the extraction (10). When the condensing solvent drips into the thimble containing the fish tissue, the solvent rises to an overflow level at which time it flushes through the tissue back into the flask from which it originated. As it does so it is removing the lipids from the fish. The flushing is a function of the temperature at which the extraction occurs. If the flushing is too rapid reducing the heat input will slow the cycle and vice versa. This guideline, however, appeared to be ineffective with our system in that the flushing cycle was variable due to differences in fish size, heating mantles and other mechanical variations. More important is the problem that as the condensation dripped down into the thimble, the solvent was dripping through the thimble pores, the overflow level was never reached and flushing did not occur. This problem could not be ameliorated; therefore, it was necessary to choose a time frame in which complete extraction was assured, instead of using the suggested number of flushes. To ascertain this efficiency, a method referred to as "system efficiency" was employed. This method involved sequential time measurements of the extraction of the chemical from the tissue of a single fish.

The fish used had been exposed to the chemical in actual testing and was one of the largest of the test fish (16.7 grams). In addition,

it had been exposed to the higher of the two test concentrations (200 g/L naphthalene) and was exposed for the complete uptake phase of the testing (4 days). Preparation of the tissue and early soxhlet extraction was as with all test fish. However, after 5 hours the extract was removed, new solvent added and the extraction continued. This procedure occurred after 9 hours, 12 hours and 15 hours. The extracts were processed as in actual testing. Through gas chromatograph analysis the efficiency of the extraction for that time period could be determined and the optimum time needed to extract within 0.1 mg/Kg body burden of naphthalene residues could be derived. This value is near the limits of the analytical instrument. Efficiency results are in the Appendix Table 5.

Lipids

Lipid content of the fish was determined by taking one-fourth of the extract after soxhlet extraction, water bath drying each sample, drying in a dissicator and weighing routinely until constancy at milligrams for 24 hours (10). Lipid content of samples is in Appendix Table 6.

Water Samples

Water samples for lindane and naphthalene analysis taken from each chamber were 500 MIS. Samples were filtered through fired Schleicher and Schuell 55 mm glass fiber filters. This step removed bacterial or other particulate matter from the sample that might contain the test chemical and therefore would not be available for uptake by fish.

After filtering, water samples containing naphthalene were placed in a volumetric flask which was modified with a screw cap. These were layered with 5 mLs of reagent grade pentane and a 6 cm stir bar added. The flask was sealed with a cap containing a teflon coated septa and samples were then stirred on a Corning PC-353 magnetic stirred for ten minutes at a speed high enough to produce a vortex. The naphthalene was removed from the pentane layer at the time of analysis.

For lindane extraction, 5 mLs of the filtered water sample was pipetted into a small vial and 5 mLs of hexane was added to this. The lindane was extracted by vigorous agitation for approximately one minute on a Sybron Thermolyne maxi-mix.

Analytical Determinations

A Hewlett-Packard 5710A Gas Chromatograph with flame ionization detecror was used to determine naphthalene concentrations in water and fish samples. Oven temperature program was set at 90^{o}C for 2 minutes and increased at the rate of 8^{o}C per minute to 160^{o}C. Attenuation setting ranged from 1 - 8. Carrier gas (He) flow rate was set at 40 cc/min. The 2.44 m glass column had an inside diameter (id) of 0.5 mm and was packed with SP 2100. The lower detection limit for naphthalene in fish was 0.03 mg/Kg and in water was 5 µg/L.

A Tracor 560 Gas Chromatograph with electron capture detector was utilized in determining lindane concentrations in water and fish samples. The oven temperature was 210^{o}C, detector temperature 350^{o}C

and the injection port temperature 200°C. Attenuation ranged from 20 - 5000. Carrier gas (Argon/methane) flow rate was set at 10 cc/min. The column used was glass 180 cm x 2 mm id packed with 10 percent SP 2100 on Supelco port 100/120. The lower detection limit for lindane in fish was 0.002 mg/kg and in water was 0.1 µg/L.

In all analyses a 10 µL Hamilton syringe was used to inject 5 µL into the gas chromatograph. The concentration of test chemicals in all the samples was determined by comparing peak heights to those obtained from prepared standards. Naphthalene standards were prepared by a series of dilutions from stock standards. The stock standard was prepared from naphthalene crystals dissolved in 10 mLs of methanol, then raised to volume in distilled water using a volumetric flask. From this aliquots were added to reagent pentane solvent to produce working standards ranging from 40 mg/L to 0.1 µg/L.

A stock standard having a concentration of 200 mg/L lindane in isooctane was used to prepare lindane working standards. Lindane working standards were aliquots diluted in reagent pentane and ranged from 4 µg/L to 5 mg/L. All mixtures were made up in volumetric flasks and were teflon tape sealed for storage. Chemical concentrations in the fish are in Appendix Table 6.

Data Analysis

All calculations and data analysis were accomplished using an AS 5000 computer and software package, The Statistical Analysis System (SAS) (11).

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CHAPTER III

RESULTS AND DISCUSSION

Data were collected on the uptake and depuration rates of lindane and naphthalene in bluegill sunfish. From these data, the uptake rate constants, K_1, depuration rate constants, K_2, and bioconcentration factors, BCF, were derived. Fish lipid content was determined and this information was used to correlate lipid normalized BCF and non-lipid normalized BCF with the ability of lindane and naphthalene to bio-concentrate in fish. Correlation was also determined between observed K_1, K_2 and BCF values and predicted K_1, K_2 and BCF values based on the log of n-octanol-water partition coefficient (log P). In the following section, results are discussed as they relate to the proposed hypotheses tested.

Determination of Uptake Kinetics

Semi-log plots of the concentrations of the chemicals in fish are shown in Figures 3-1, 3-2, 3-3 and 3-4. With both naphthalene and lindane, a ten-fold increase in exposure concentrations resulted in a similar increase in concentration of test chemicals in fish tissues. The uptake of lindane and naphthalene at all exposure concentrations was rapid. In determining the plateau or steady state, Duncan's Multiple Range Test showed no significant difference ($p=0.05$) in the concentration of the chemicals in the fish after the first day of

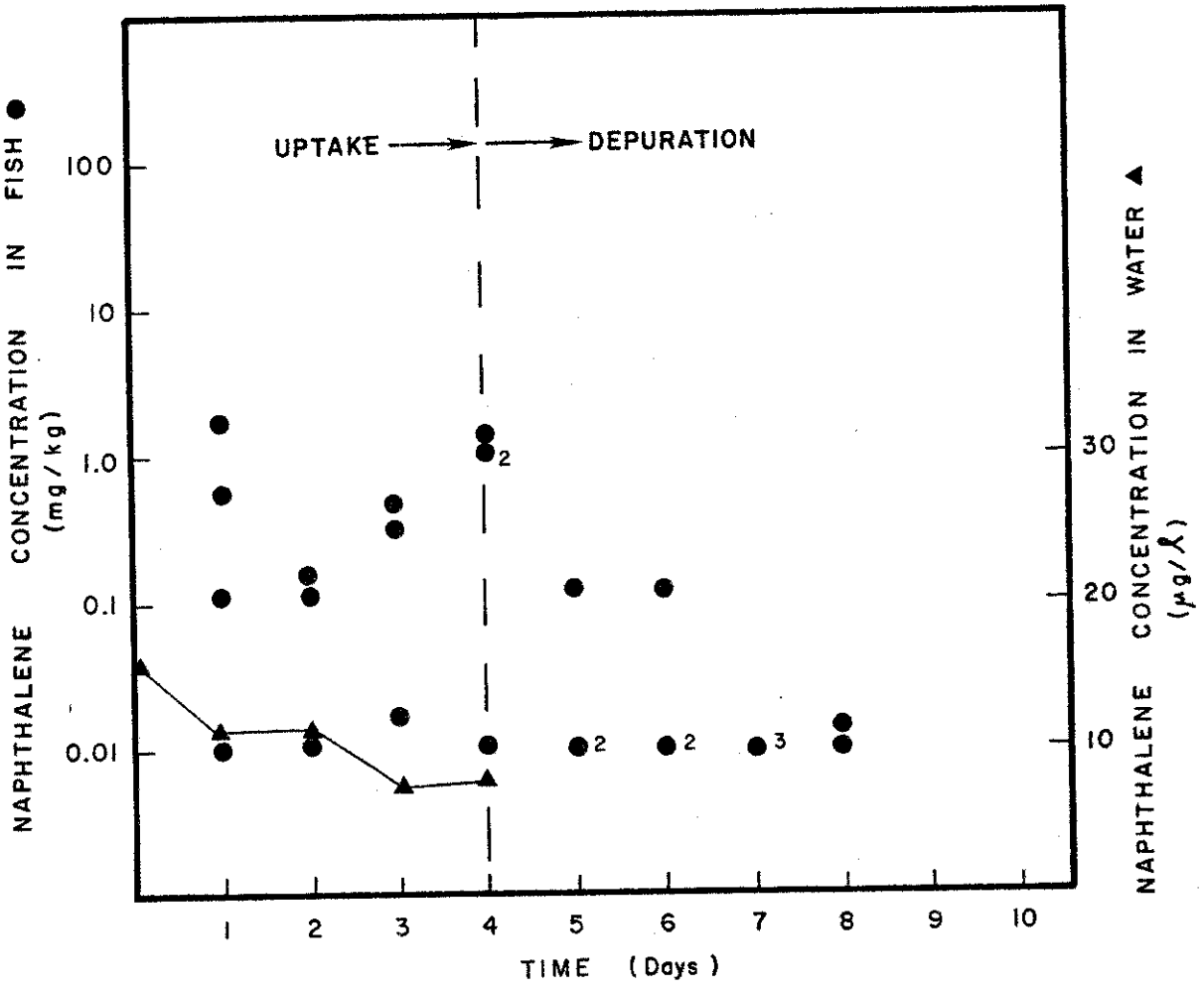

Figure 3-1. Semilog plot of uptake and depuration of naphthalene in bluegill sunfish at a mean exposure concentration of 9.5 μg/l. The numerals at data points refer to the number of values located at that point.

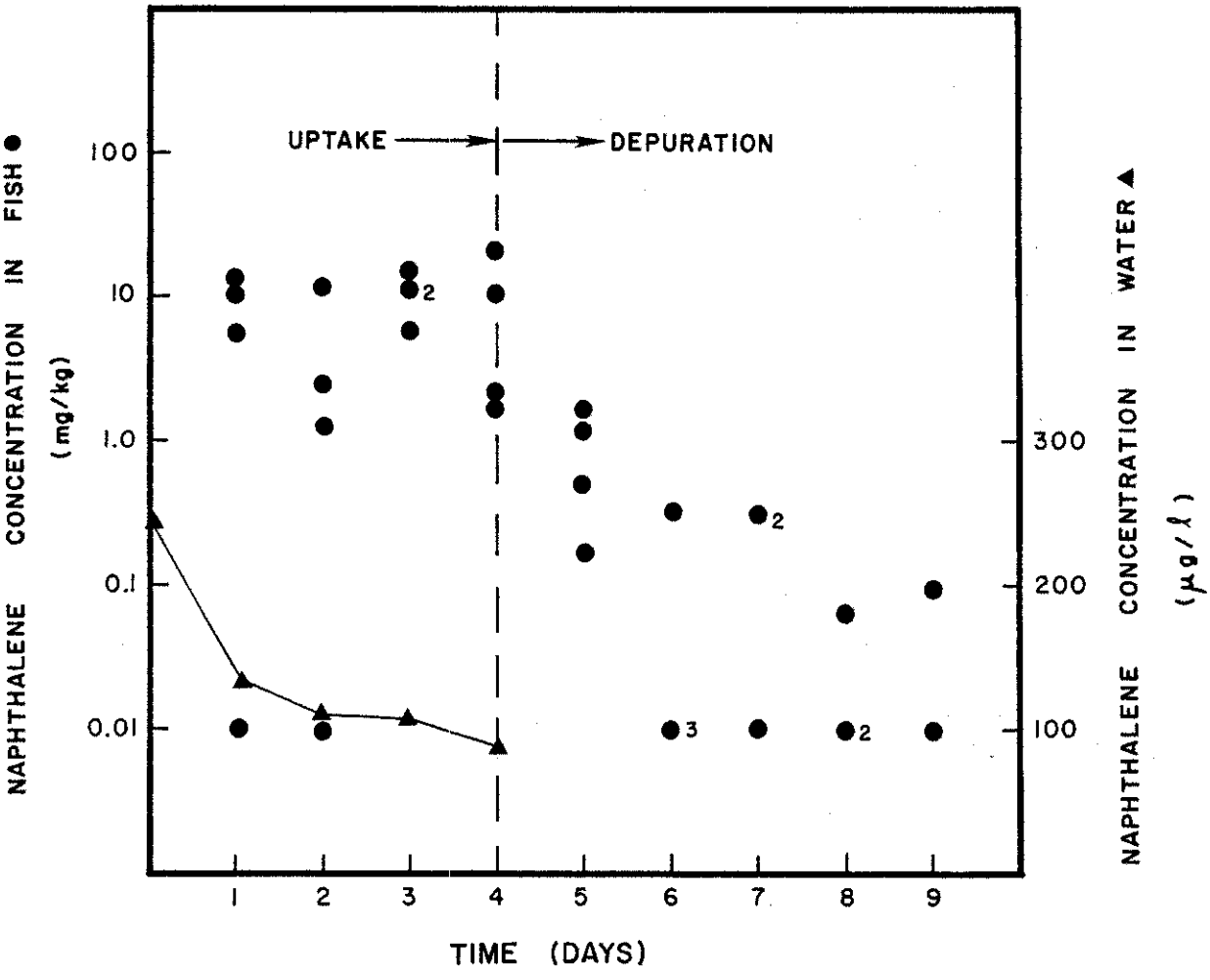

Figure 3-2. Semilog plot of uptake and depuration of naphthalene in bluegill sunfish at a mean exposure concentration of 111.6 µg/l. The numerals at data points refer to the number of values located at that point.

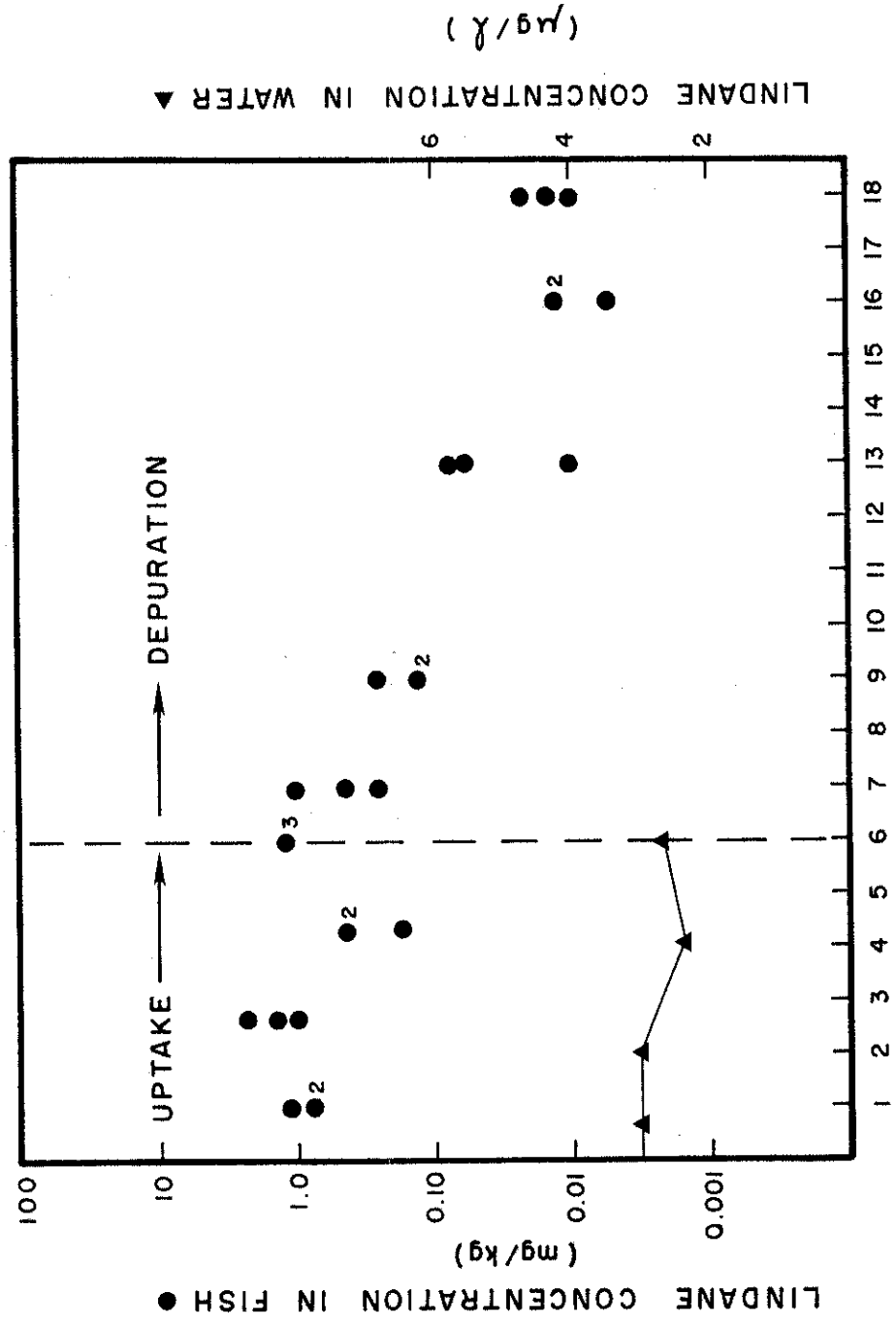

Figure 3-3. Semilog plot of the uptake and depuration of lindane in bluegill sunfish at a mean exposure concentration of 2.77 µg/l. The numerals at data poynts refer to the number of values located at that point.

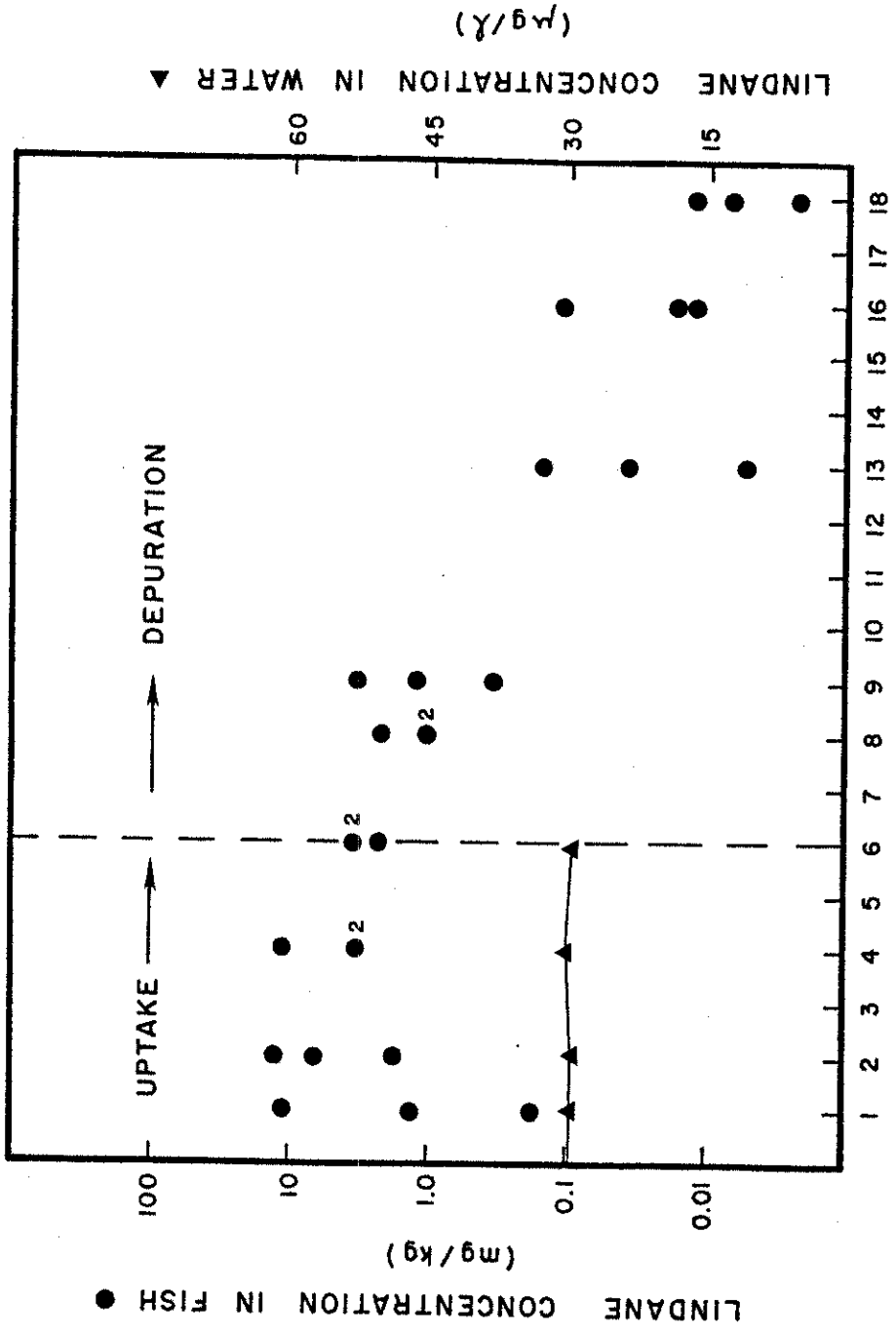

Figure 3-4. Semilog plot of the uptake and depuration of lindane in bluegill sunfish at a mean exposure concentration of 31.57 μg/l. The numerals at data points refer to the number of values located at that point.

exposure. This would suggest that all of the chemical was taken up before the end of the first day.

Because the uptake rate content, K_1 is calculated from the depuration rate constant K_2, regression analysis was not appropriate for the determination of the uptake rate constant, K_1. The uptake rate constants were calculated from the equation

$$K_1 = \frac{C_f}{C_w} \frac{K_2}{(1 - e^{-K_2 t})} \quad (15),$$

The term $(1 - e^{-K_2 t})$ is a factor to account for the amount of elimination occurring during the uptake phase. Calculated values of K_1 for naphthalene were 180 $\pm$ 174, (mean $\pm$ two standard deviations) and 298 $\pm$ 316 with mean exposure of 9.7 and 111.6 µg/L, respectively. Lindane K_1 values were 505 $\pm$ 253 and 272 $\pm$ 201 for mean exposure concentrations of 2.77 and 31.6 µg/L, respectively.

The standard error about K_1 was estimated from the standard error of the estimate of the observed bioconcentration factor BCF. It is not possible to obtain a standard error about K_1 because it is calculated from K_2. Because BCF and K_1 are both from portions of the uptake phase and both are two compartmental it would be expected that their standard error would be similar. The standard error about K_1 was calculated from the equation

$$K_1 \text{ standard error} = \frac{\text{standard error of BCF}}{\text{BCF}} \times K_1.$$

$H_0 1$. There is no difference in uptake rate kinetics between lindane and naphthalene.

Overlapping, 95 percent, confidence intervals were used to determine differences in uptake rate constants between lindane and naphthalene (Table 3-1). The 95 percent confidence intervals were estimated to be $\pm$ 2 times the standard error of K_1 around the mean of K_1. All intervals overlapped so there was no significant difference in uptake rate constant for the two chemicals.

Utilizing a flow-through system, Gakstatter et al. (5) demonstrated that decreasing insecticide concentration in the water was the result of uptake by the fish. During a five hour exposure of bluegill, he found a decrease of dieldrin in the water from 25.5 to 10.3 ppb, whereas DDT concentration decreased from 25 to 8 ppb. In the same test, lindane was removed from the water at a slower rate than DDT or dieldrin and after a few hours lindane concentration in the water appeared to be in equilibrium with the fish. This rate is similar to those observed in this study.

DiMichelle and Taylor (4) reported naphthalene uptake rates for the mummichog, Fundulus heteroclitus, of doubling the concentration every four hours. This rate is also consistent with uptake values found in this study.

H_O2. Exposure concentration makes no difference in uptake rate kinetics for lindane and naphthalene.

To test the hypothesis of no difference in uptake rate constants between exposure concentrations within the two levels of each chemical, overlapping 95 percent confidence intervals were also used (Table 3-1).

TABLE 3-1

RESULTS OF DERIVED CONFIDENCE INTERVALS FOR K_1

Chemical	Concentration µg/L	Uptake Rate Constant K_1	Lower Confidence Limit L_1	Upper Confidence Limit L_2
Naphthalene	20	180 ± 348[a]	-168	528
	200	298 ± 632	-334	930
Lindane	3	505 ± 506	-1	1011
	30	272 ± 402	-130	674

[a]95 percent confidence limits.

No difference was found between the rate constants at the two concentrations of naphthalene or between the rate constants at the two concentrations of lindane.

Studies have shown K_1 to be constant over a wide range of exposure concentrations (15). It seems that K_1 is also the same for a wide range of chemicals. When the log of the uptake rate constant (log K_1) is plotted against the log of the n-octanol water partition coefficient (log P) the slope of the regression line is close to zero (Figure 3-5). This would suggest that no matter what log P is for a chemical, log K_1 will be nearly the same as it is for other chemicals having varying log Ps.

Determination of Depuration Kinetics

Depuration rate constants were determined through regression analysis for each concentration of lindane and naphthalene.

Duncan's Multiple Range Test showed naphthalene depuration to be complete within two days. In determining the depuration rate constant the slope of the line fitted to concentration was determined for that time frame (day 4-6). The depuration rate constants for naphthalene were 1.4 ± 0.828 and 2.87 ± 0.505 day^{-1} at mean exposure concentrations of 9.7 and 111.6 µg/L, respectively.

The depuration rate constants for naphthalene determined in this study were more rapid than those reported in literature. Melancon and Lech (10) exposed fingerling rainbow trout, Salmo gairdneri, to ^{14}C naphthalene and ^{14}C methylnaphthalene in a flow-through system for four weeks. They found a depuration rate constant of about 0.1

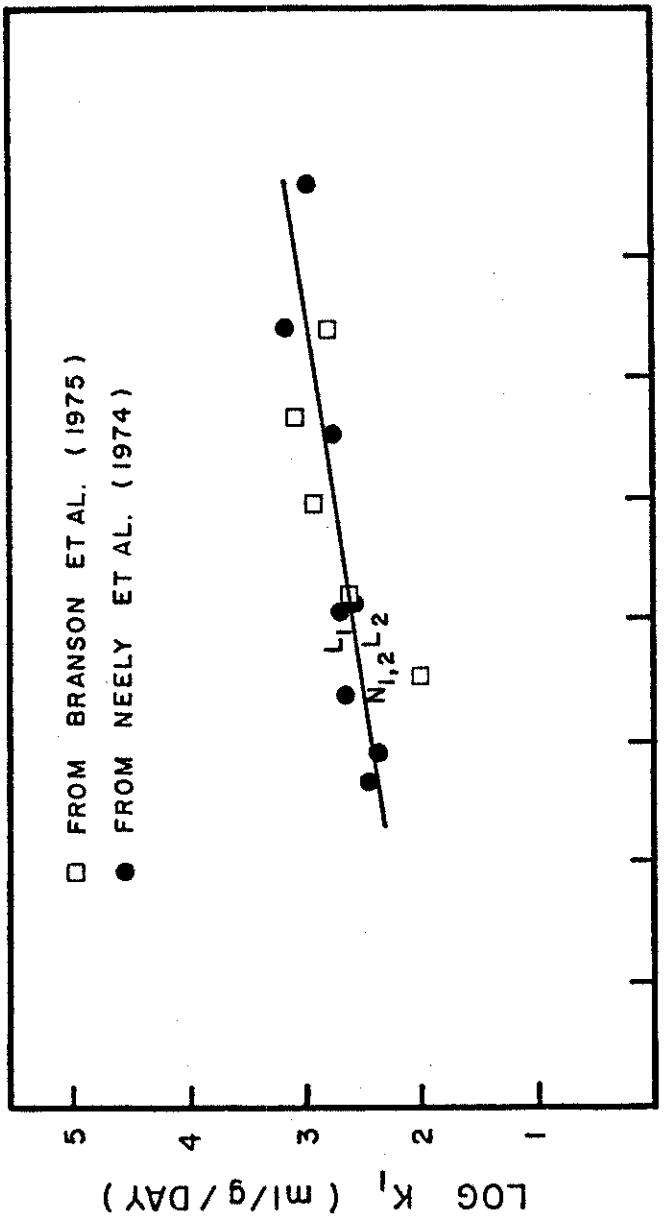

Figure 3-5. Uptake rate constants exhibiting relatively little scatter around the regression line that has only slight slope.

day^{-1} for the trout. This is nearly an order of magnitude slower than the rates for bluegill in this study. Roubal et al. (14) reported rate constants of greater than 0.2 day^{-1} for coho salmon, Oncorhynchus kisutch, and less than 0.02 day^{-1} for starry founder, Platichthys stellatus. These, too, are considerably slower than naphthalene depuration rates observed in this study.

Lindane depuration rate constants were 0.38 $\pm$ 0.0333 and 0.53 $\pm$ 0.059 day^{-1} at mean exposures of 2.77 and 31.6 µg/L, respectively.

Gakstatter (5) found depuration rate constants (K_2) for lindane of 0.78 day^{-1} for goldfish, Carassius auratus, and 1.35 day^{-1} for bluegill. These depuration rates are considerably faster than those found in this study.

$H_0$3. There is no difference in depuration rate kinetics between naphthalene and lindane.

Depuration rate constants of the two chemicals were markedly different (Table 3-2). In the case of naphthalene, depuration was complete in less than half of a day. Lindane depuration proceeded at a slower rate and lindane half-life would be about 2 days. The mean of the naphthalene depuration rate constants was significantly different (P=0.05) from the mean of the depuration lindane rate constants. Several other investigators (1, 12) have found the depuration rate constants of chemicals to be different. Neely et al. (12) tested seven chemicals having a wide range of partition coefficients. Depuration ranged from 0.082 hr^{-1} for 1,1,2,2-tetrachlorethylene which has a n-octanol water partition coefficient of 2.88, to

TABLE 3-2

SUMMARY OF RESULTS OF BIOCONCENTRATION TESTS WITH NAPHTHALENE AND LINDANE
MEAN EXPOSURE AND FISH CONCENTRATIONS ARE THE AVERAGE TEST CHEMICAL
CONCENTRATIONS DURING PLATEAU OF THE TESTS

Chemical	Mean Exposure Concentration (µg/l)	K_1 (ml $g^{-1}da^{-1}$)	K_2 (day^{-1})	BCF_{K_1/K_2}	$BCF_{\overline{Cf}/\overline{Cw}}$	Mean Fish Concentration (mg/kg)
Naphthalene	9.7	180 ± 174[a]	1.40 ± 0.828[b]	160	97 ± 94	0.930
	111.6	298 ± 316	2.87 ± 0.505	140	98 ± 104	10.639
Lindane	2.77	505 ± 253	0.38 ± 0.033	1329	420 ± 210	1.188
	31.57	272 ± 201	0.53 ± 0.059	513	211 ± 156	6.659

[a] Calculated variance

[b] Mean ± 1 standard deviation.

0.00099 hr^{-1} for 2,2',4,4'-tetrachlorophenyloxide which has a n-octanol water partition coefficient of 7.62. The other chemicals evaluated had depuration rates between these two values. Gakstatter and Weiss (5) found elimination rates of lindane, dieldren and DDT to be quite different. Nearly all of the initial lindane (approximately 7.5 ppm) was eliminated in less than two days after fish were placed in untreated water. This rate is similar to that observed in this research. More than ninety percent of the initial dieldren (approximately 3.8 ppm) was eliminated within two weeks and less than fifty percent of the DDT was eliminated in two weeks.

$H_0$4. Exposure concentration makes no difference in depuration rate kinetics for lindane and naphthalene.

The Students t-Test showed there was a statistically significant (P=0.05) difference between the depuration rates constants for the two levels of naphthalene exposure concentration and between the two levels of lindane tested.

Both lindane and naphthalene depuration rates at lower exposure concentrations were slower than those observed at the higher exposure concentration. This suggests that depuration of these chemicals are dependent on exposure concentration. This would particularly be true in the case of naphthalene where the K_2 at the high exposure (200 µg/L) was nearly double that of the lower exposure (20 µg/L).

Various investigators have reported conflicting results on the dependence or independence of exposure concentration to the uptake and depuration rate kinetics for various chemicals. Bishop and Maki

(1) used four chemicals to compare short-term kinetic experiments with twenty-weight day plateau tests. Uptake-depuration rate constants calculated for ^{14}C-EDTA (Ethylenediaminetetraacetic acid) were independent of exposure concentrations of 0.76 and 0.08 mg/L. In the plateau tests, however, uptake rates were dependent on exposure and an apparent difference between the two exposure levels in the rate of elimination of ^{14}C-EDTA was noted. Fish exposed to 0.76 mg/L eliminated 81 percent of the accumulated residues within 336 hours. For fish exposed to 0.08 mg/L only 60 percent of the accumulated residues were eliminated in the same time period. The uptake of LAS (sodium dodecylbenzenosulfonate) demonstrated a marked concentration dependence at exposure levels of 0.64 and 0.063 mg/L. The uptake rate constant for an exposure level of 0.063 mg/L was approximately twice as large as the rate constant for 0.64 mg/L. However, for another chemical AE (tetradecylheptoethoxylate) Bishop and Maki (1) found in both the kinetic and the plateau tests, the uptake rate constant and depuration rate constant were independent of exposure concentrations of 0.154 and 0.014 mg/L.

Branson et al. (2) found similar values of depuration rates following exposure of rainbow trout (Salmo gairdneri) to 1.6 and 9.0 µg/L of 2,2'-4,4'-tetrachlorobiphenyl. Mayer (9) found elimination half-lives of di-2-ethylhexyl phthalate (DEPH) from fathead minnows (Pimephales promelas) to be reasonably constant over a ten-fold range of exposure concentrations.

Determination of BCFs

The bioconcentration factor of the chemicals in test fish were determined by dividing the mean concentrations of chemical in the fish by the mean exposure concentration during the plateau (Table 3-2). Results of Kilomagorov-Smirnov D-statistics showed that the BCF data was normally distributed (P=0.01).

$H_0$5. There is no difference in BCFs for lindane and naphthalene.

Naphthalene BCFs were 97 and 98 at mean exposure concentrations of 9.7 µg/L and 113.1 µg/L, respectively. Lindane BCFs were 420 and 211 at mean exposure concentrations of 2.77 and 31.57 µg/L day^{-1}, respectively. In bioconcentration testing it is generally accepted that orders of magnitude is the range used to show relative differences. For bioconcentration test results one can say that there is no great difference between BCFs at different exposure concentrations for lindane or for naphthalene. Statistically, however, Duncan's multiple range test found a significant difference in BCFs between lindane and naphthalene (P=0.05).

$H_0$6. Exposure concentration makes no difference in BCFs for lindane and naphthalene.

The Student's t-test showed the BCFs between exposure concentrations of naphthalene were not statistically different, (P=0.05). The BCFs between exposure concentrations of lindane, however, were statistically different, (P=0.05). Although the BCFs are statistically different between exposure concentrations of lindane, they are within an order of magnitude of each other.

Bishop and Maki (1) found bioconcentration factors ranging from essentially zero for EDTA, very low for the surfactants LAS and AE and very high for DDT (1.0, ≈ 100-200, ≈ 700-800, > 25,000, respectively).

Veith et al. (16) tested thirty chemicals using fathead minnows. Each chemical was shown to have a unique BCF ranging from very low, 2.7 for tris-(2,3, dibromo propyl) phosphate, to very high, 194,000 for PCBs.

The BCFs obtained experimentally in this study are similar to literature values of BCFs for lindane in bluegill. Reported values ranged from 30 (8) to 768 ± 441 (6). Similar BCFs of lindane are reported for other species; 560 for the mosquito fish, (Gambusia affinis) (11) 75 for brook trout (Salvelinus fontinalis) and 4000 for fathead minnows (8). Literature values for naphthalene BCFs are 430 for fathead minnow (1) and 40-300 for rainbow trout (3).

Correlation of Lipid Normalized and Non-Lipid Normalized BCFs

Many investigators (3, 13) have shown correlations between the ability of a chemical to accumulate in aquatic organisms and its lipophilicity. Lipophilic chemicals are associated with lipoidal tissues and their metabolism and clearance should be directly related to the mobilization and turnover of tissue lipids (13). Roberts et al. (13) showed tissue retention of chlordane fed to northern redhorse suckers (Moxostoma macrolepidotum) to be directly related to the lipid

pool. Lean fish did not bioaccumulate as much chlordane as fish with higher lipids.

Lieb et al. (7) measured the lipid content of rainbow trout starting with 14-week old fish and continuing over a period of 32 weeks. Lipid content increased in this time from 4.4 percent to 8.4 percent. Relative amounts (ppm) polychorinated biphenyls (PCB) fed the trout increased in direct proportion as the percent lipid increased. The greatest percent, 92.8, was located in the viscera adipose.

DeFoe et al. (3) found wet-weight residue of Aroclor 1248 (a PCB) in fathead minnor tissue to be directly proportional to the concentration of Aroclor in the Lake Superior test water. Females accumulated approximately twice as much PCBs. This was attributed to greater lipid content in the female fish. However, the concentration of Aroclor 1248 was essentially the same in males as in females when expressed on a lipid normalized basis and was in direct proportion to Aroclor in the water.

Besides sex and growth, other factors influence lipid content. Lipid content differ among species and lipid content appears to increase in cooler temperatures or seasons. These factors, too, could be assumed to affect the uptake or depuration rates of the chemical.

In this study the coefficients of variation, C.V., for non-normalized BCF and lipid normalized BCF were compared because, by definition, the greater the coefficient of variation the greater the variability relative to the mean of the sample. The C.V. for lipid normalized BCFs for the naphthalene samples were considerably higher

than non-lipid normalized BCFs. For lindane samples, however, the C.V.s for lipid normalized BCF are somewhat less than BCF coefficient of variation values (Table 3-3).

It would seem that the chemical itself may influence the effectiveness of lipid normalizing BCF values. Of the two chemicals used in this study, lindane is more lipophilic with higher n-octanol water partition coefficient (log P = 3.85) and BCF. Generally, chemical having higher log P are more lipophilic.

The fish used in this study had a considerable variability in weights and age class. Fish used in naphthalene testing ranged from 1.11 gms to 39.29 gm with a mean percent lipid content of 3.37 $\pm$ 4.51. In the lindane test fish weight range was 1.02 gm to 11.20 gm with a mean lipid content of 2.98 $\pm$ 2.24. The age classes of this fish would be from young of the year to juveniles. These ranges could contribute to the variation in fish concentration because the lipid content would vary proportionally to the weight of the fish. In the lindane tests the lipid normalized BCFs had a lower C.V. than the non-lipid normalized BCFs. This may be the result of less variability in weights of the fish tested. Percent lipid values determined in this research are similar to those reported in literature (3, 13). The fish were not sexed so no comparison of chemical concentrations in the fish can be made on that basis.

In the study of Aroclor 1248 by DeFoe *et al.* (3) concentrations were expressed on lipid basis to normalize values between male and female fish. Aroclor 1248 had a log P of 6.11 and a BCF of 70,500.

TABLE 3-3

COMPARISON OF COEFFICIENTS OF VARIATION FOR LIPID NORMALIZED AND NON-LIPID NORMALIZED BCF VALUES

Chemical	Concentration µg/l	BCF	Coefficient of Variation for BCF	Coefficient of Variation for Lipid Normalized BCF
Naphthalene	20	97	96.64	124.00
	200	98	105.81	163.03
Lindane	3	420	49.96	45.15
	30	211	73.86	48.74

Table 3-4 shows the correlation between BCF, log P and bioconcentration potential as established by Veith et al. (16). The potential to concentrate is positively correlated with BCF and log P. It was noted that chemicals having high log P have a high bioconcentration potential. An exception is chlorinated paraffin having a log P of 7.05 but a BCF of only 49 and a bioconcentration potential of less than 0.1. Chlorinated paraffin is a long chain alkane and apparently steric hindrance reduces the uptake of chemicals of this structure (18).

Correlation of Observed and Predicted Values

Correlations between observed versus predicted values of uptake rate constants, depuration rate constants and BCFs obtained in this study for lindane and naphthalene are shown in Figures 3-6, 3-7 and 3-8.

Predictions based on log P for K_1, K_2 and BCFs for lindane and naphthalene are in good agreement with experimental values obtained in this study. When the data obtained are plotted with currently available data (Figure 3-9, 3-10), no obvious deviations from the predictive model are found. This correlation has been established by other investigators. Veith et al. (16) tested the correlation between the BCF and the n-octanol water partition coefficient (log P) for approximately 60 chemicals and found agreement for chemicals covering six orders of magnitude in the partition coefficient. In another study Veith et al. (17) tested 28 organic chemicals ranging from low to moderate lipid solubility, with BCF ranging from 2-3400.

TABLE 3-4

LOG BCF AND BIOCONCENTRATION POTENTIAL (DDE - 100*) FOR 30 ORGANIC CHEMICALS AS DETERMINED WITH THE FATHEAD MINNOW IN 32-DAY EXPOSURES

	Log BCF	BCF	Bioconcentration Potential	Log P
tris-(2,3-dibromopropyl) phosphate	0.44	2.7	<0.1	4.98
5-bromoindole	1.15	14	<0.1	2.97
hexachlorocyclopentadiene	1.47	29	<0.1	5.51
diphenylamine	1.48	30	<0.1	3.42
chlorinated paraffin	1.69	49	<0.1	7.05
toluene diamine	1.96	91	0.2	3.16
tricresyl phosphate	2.22	165	0.3	3.42
lindane	2.26	180	0.4	3.85
pentachlorophenol	2.89	770	1.5	5.01
2,4,6-tribromoanisole	2.94	865	1.7	4.48
1,2,4-trichlorobenzene	3.32	2800	5.5	4.23
methoxychlor	3.92	8300	16	4.30
heptachlor	3.98	9500	19	5.44
heptachloronorbornene	4.05	11100	22	5.28
heptachlorepoxide	4.16	14400	28	5.40
mirex	4.26	18100	35	6.89
hexachlorobenzene	4.27	18500	36	5.23
p,p'DDT	4.47	29400	58	5.75
octachlorostyrene	4.52	33000	65	6.29
chlordane	4.58	37800	74	6.00
p,p'DDE	4.71	51000	100*	5.69
Aroclor 1248	4.85	70500	138	6.11
Aroclor 1254	5.00	100000	196	6.47
Aroclor 1260	5.28	194000	300	6.91

From Veith et al. (1979).
*Bioconcentration potential calculated relative to p,p'DDE=100.

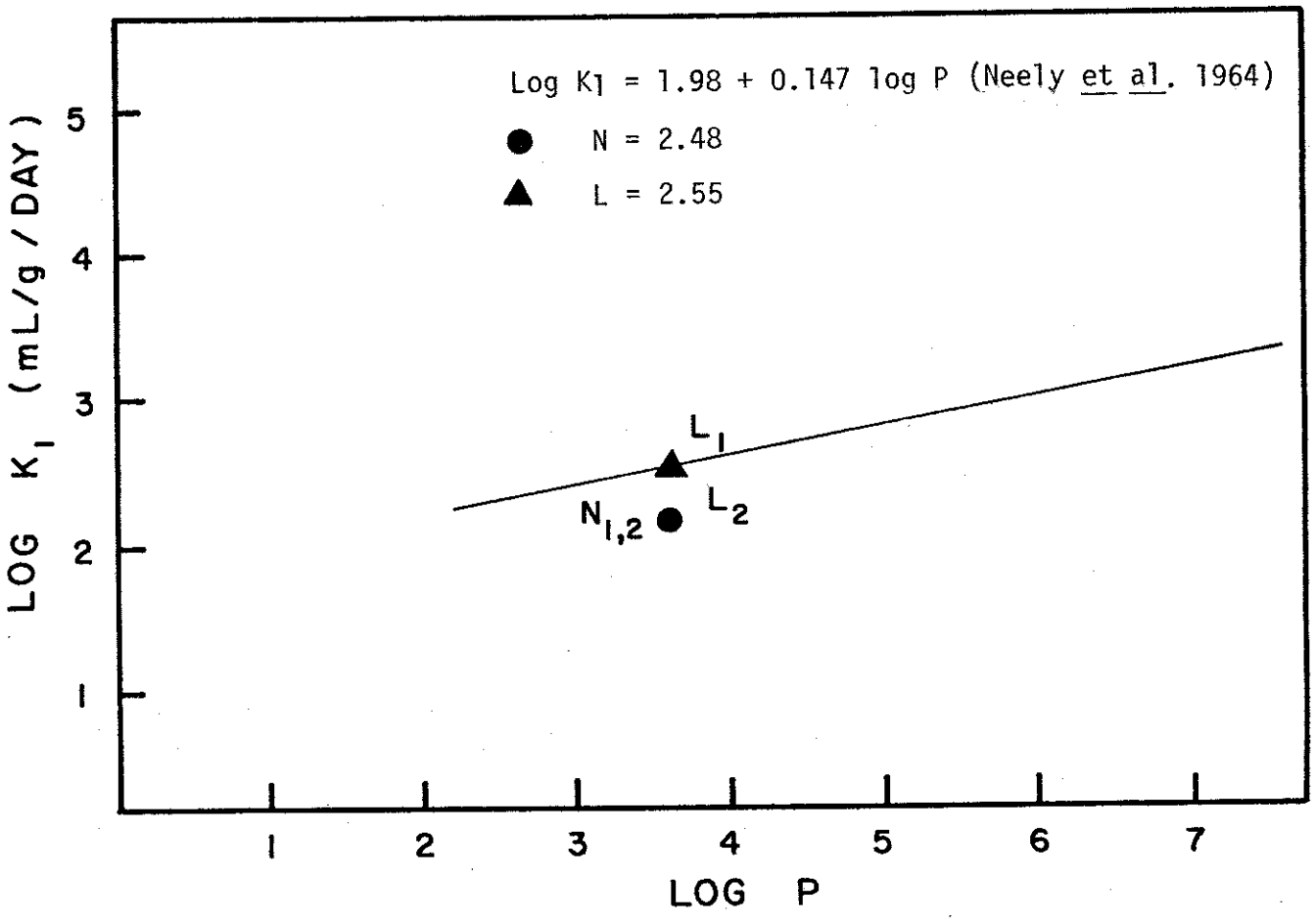

Figure 3-6. Correlation between observed vs. predicted values for K_1 and log P. N_1 and N_2, L_1 and L_2 are the experimentally derived K_1 values for lindane and naphthalene. Also shown are the predicted values from the equation for log K_1 based on log P.

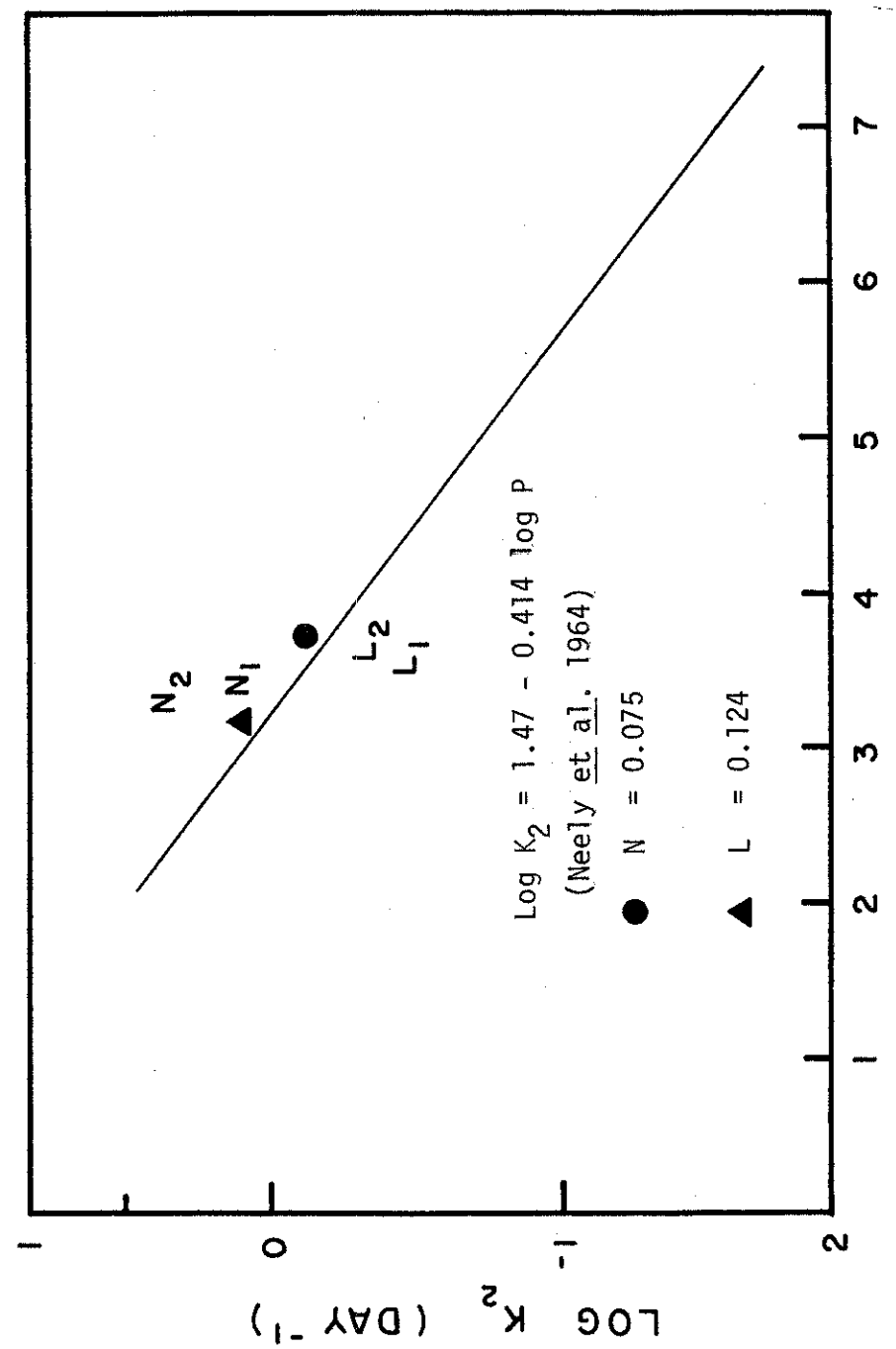

Figure 3-7. Correlation between observed vs. predicted values for K_2 and log P. N_1, N_2, L, and L_2 are the experimentally derived K_2 values for lindane and naphthalene. Also shown are the predicted values from the equation for log K_2 based on log P.

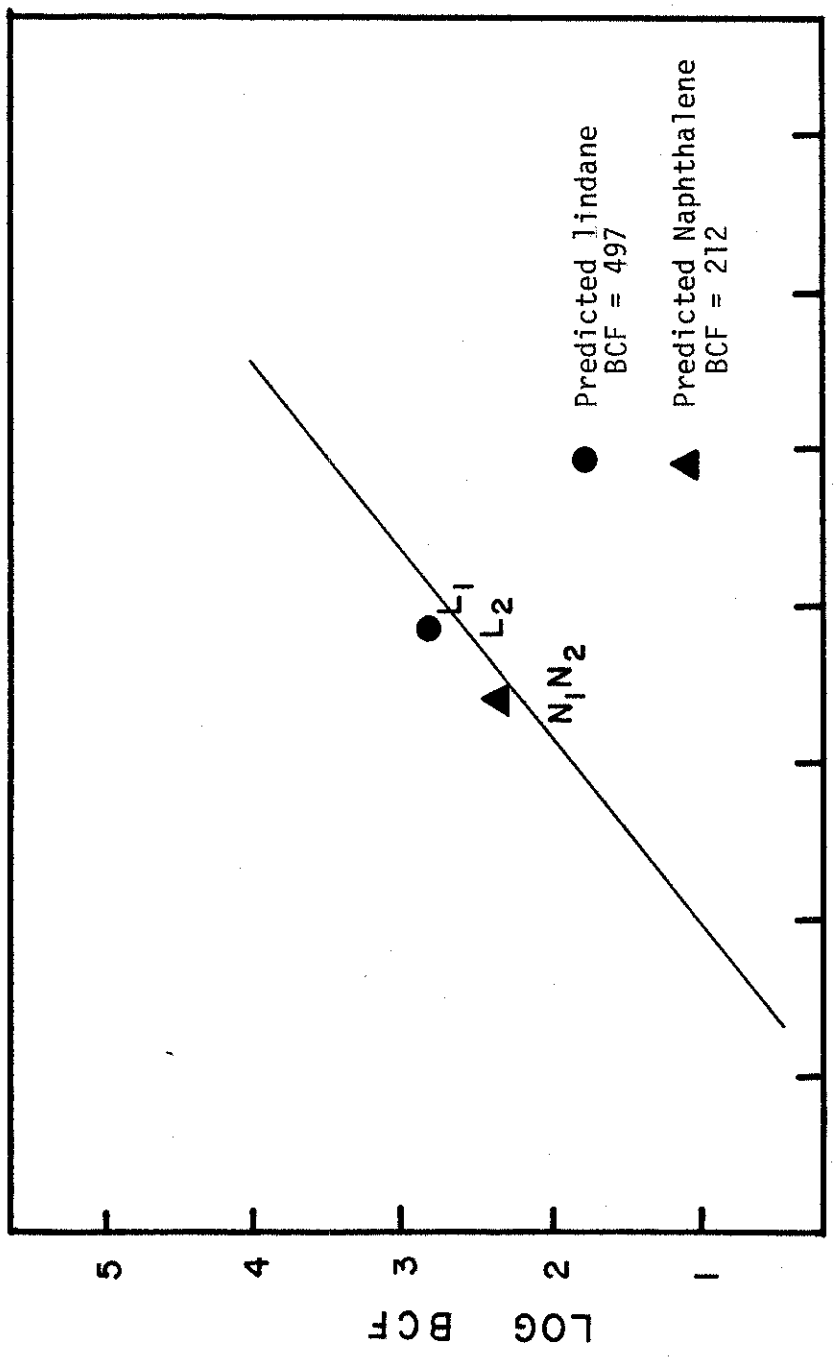

Figure 3-8. Correlation between observed vs. predicted values for log BCF and log P. N_1, N_2, L_1 and L_2 are the experimentally derived log BCF for lindane and naphthalene. Also shown are the predicted values from the equation for log BCF based on log P.

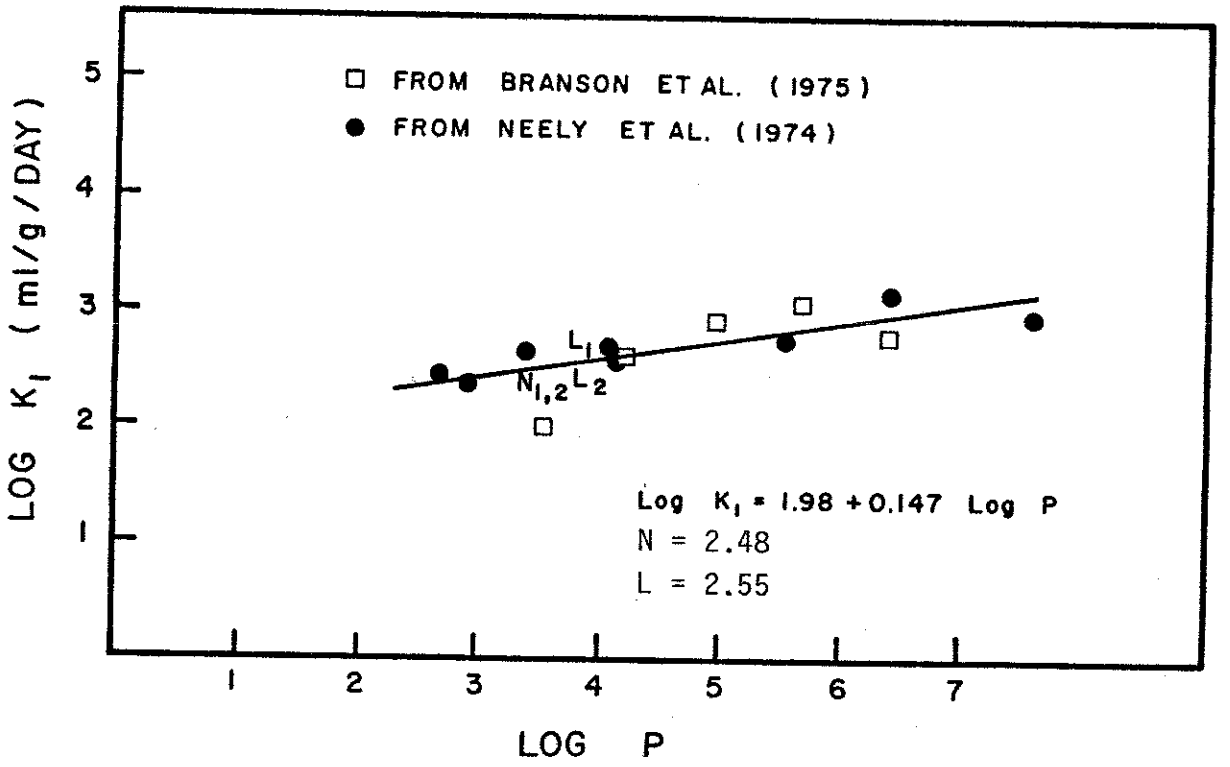

Figure 3-9. Correlation between K_1 and n-octanol-water partition coefficient (from Spacie and Hammelink (in review)). L_1, L_2, N_1 and N_2 are the experimentally derived K_1 values for lindane and naphthalene obtained during this research project. K_1 values were obtained for two concentrations (i.e., L_1 and L_2 and N_1 and N_2) for each chemical.

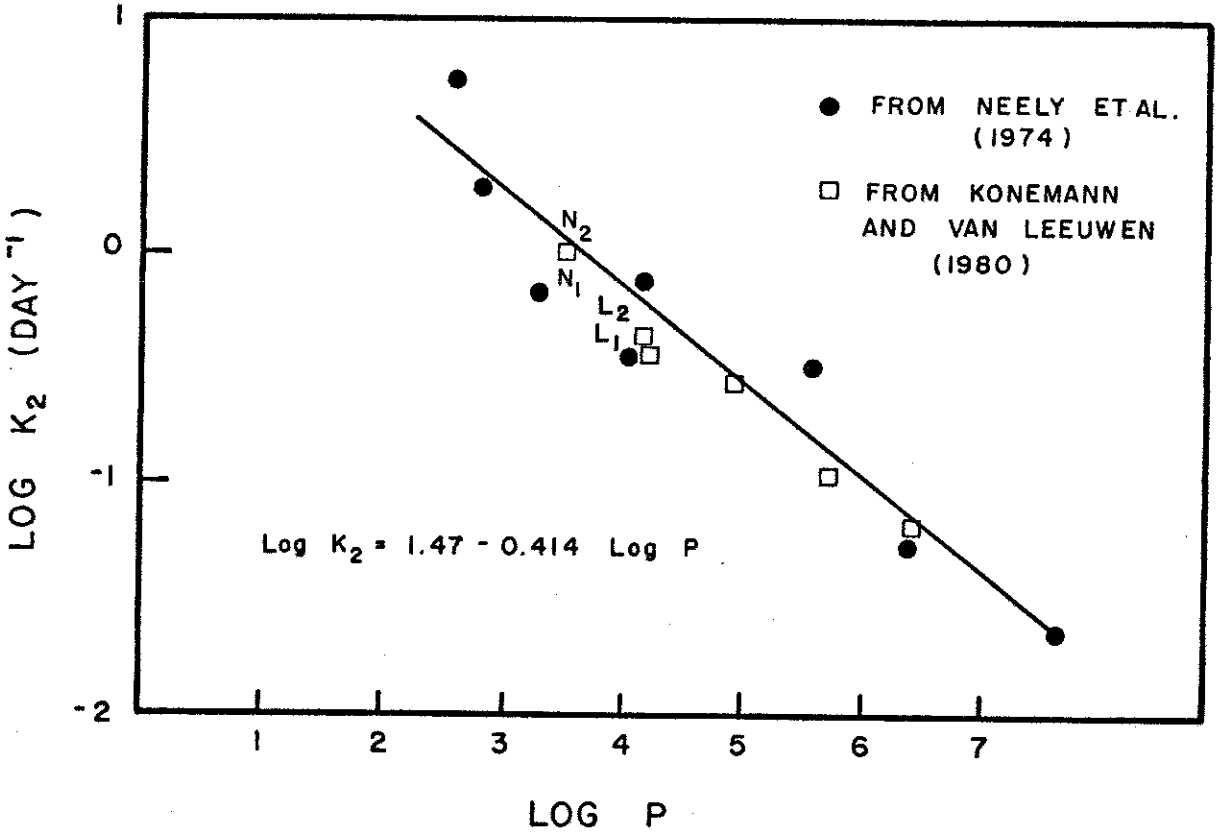

Figure 3-10. Correlation between K_2 and n-octanol-water partition coefficient (from Spacie and Hammelink (in review). L_1, L_2, N_1 and N_2 are the experimentally derived K_2 values for lindane and naphthalene obtained during this research project. K_2 values were obtained for two concentrations (i.e., L_1 and L_2 and N_1 and N_2) for each chemical.

The widest scatter around the regression line occurred for chemicals with log P of less than 2.5. They suggest chemicals having low lipid solubility and relatively high water solubility have less predictability of BCF.

Neely et al. (12) reported correlations between log P and log BCF for several chemicals and found close agreement between the experimental and calculated BCFs. An equation of the straight line of best fit was determined and used to predict the bioconcentration of other chemicals from their n-octanol water partition coefficient. Neely et al. suggested that chemicals having large standard deviation from the mean of the regression line not be used in prediction based on partition coefficient. A tested example is DDT with a log P of 5.23.

Results of studies on brominated biphenyls by Zitko (18) also suggest some non-linearity between log P and log BCF. He reported that non-linear correlations are likely especially for high melting point solids and low molecular weight substances like the brominated biphenyls.

From the literature, it appears that bioconcentration of chemicals with partition coefficients (log P) of 2 to about 5 are very predictable. This implies that BCF, K_1 and K_2 can be predicted from the n-octanol water partition coefficient (log P) for chemicals having log P in the range of 2 to 5. Beyond this range partitioning alone may not be a good predictor of BCF. Spacie and Hemmelink (15) superimposed on the regression line a sigmoid curve (Figure 3-11)

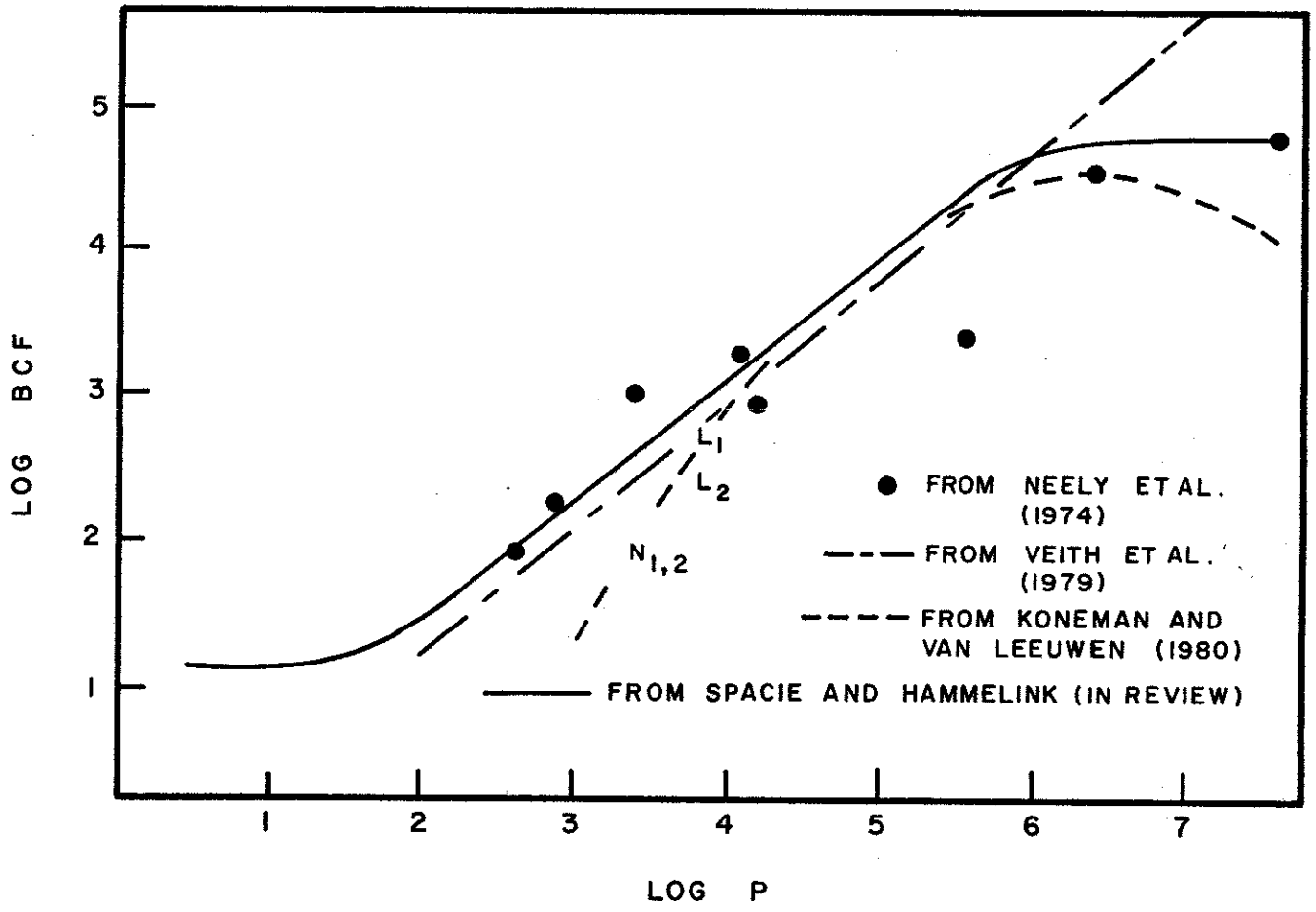

Figure 3-11. Spacie and Hemmelink's (in review) proposed sigmoid relationship between BCF for whole fish and n-octanol-water partition coefficient. L_1, L_2 and N_1 and N_2 are the experimentally derived BCF values for lindane and naphthalene obtained during this research project. BCF values were obtained for two concentrations (i.e., L_1 and L_2 and N_1 and N_2) for each chemical.

representing a drug transport model. This model predicts linearity in the region of log P = 2 to 5 where uptake is governed by partitioning and has an inflection at the lower end for small molecular weight substances where diffusion occurs through membrane pores. Their model also includes an upper inflection where log P no longer controls uptake but water solubility does and diffusion is at the boundary layer between water and membrane. The drug transport model includes several independent properties that influence uptake of chemicals into membranes. These include partition coefficient, water solubility, molecular weight and membrane permeability. Spacie and Hammelink suggested that a model of this type may be more helpful in predictions for a wider variety of chemicals.

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CHAPTER IV

SUMMARY

Bioconcentration of chemicals by fish is one fate of a chemical in aquatic ecosystem. The extent to which a chemical bioconcentrates may be important to the fish and the consumers of the fish.

The bioconcentration of chemicals is the result of the uptake and elimination of the chemical by the fish. The rates of these processes can be determined by bioconcentration tests using the plateau approach or the accelerated kinetic approach. These tests are designed to determine the maximum measured concentration of the chemical in the fish as the result of continued exposure at a constant concentration of the chemical. From these tests an uptake rate constant, K_1, depuration rate constant, K_2, and bioconcentration factor, BCF, can be determined.

The correlation between the n-octanol-water partition coefficient, log P, and K_1, K_2 and BCF make it possible to predict these rates. Consequently, bioconcentration experiments can be optimized. While bioconcentration of chemicals with partition coefficients (log P) of 2 to 6 is very predictable, beyond this range it has limitations. A drug transport model has been developed for use, and it may lead to better prediction of a wider range of chemicals.

Fish Uptake-Depuration Rate Constants

Based on the data obtained in this study, there was no difference in the uptake rate kinetics between naphthalene and lindane. For both chemicals, uptake was very rapid being completed in less than one-half day. Exposure concentration did not have an effect on the uptake rate of the chemicals lindane and naphthalene, each with two exposure concentrations had similar rate constants. In fact, when plotted with currently reported data, it appears that the uptake rate constants for all the chemicals are similar.

The depuration rate constants for the two chemicals were markedly different. Naphthalene depuration was completed in less than one-half day, whereas, lindane depuration required four days. The process was concentration dependent. Both lindane and naphthalene depuration rates at lower exposure concentrations were slower than those observed at the higher exposure concentration.

Bioconcentration Factors

Statistically, BCFs for lindane and naphthalene were different. They are, however, within an order of magnitude of each other and are, therefore, considered the same for bioconcentration testing. Exposure concentration did not affect the BCF of naphthalene. However, in the case of lindane, the lower exposure concentration (3 µg/L, nominally) the BCF was greater than the BCF for the higher exposure concentration, (30 µg/L, nominally). Because of this it is difficult to determine the dependency or independency of the lindane BCF and exposure concentrations without further testing.

Lipid Normalized BCF vs. Non-Lipid Normalized BCF

In the naphthalene tests the lipid normalized values were more variable than the non-lipid normalized values. For lindane, however, the lipid normalized values were less variable than the non-lipid normalized values. Perhaps there would be greater utility in lipid-normalized data if there were less variation in organism size and if chemicals of greater lipophilicity were investigated.

Predicted vs. Experimentally Derived Values

A comparison of the uptake rate constants, depuration rate constants and BCFs to those predicted by the n-octanol-water partition coefficient (log P) showed good agreement in all cases. When the observed values in this study were plotted with data reported in literature, there were no obvious deviations from the predictive model.

Table 4-1 summarizes the conclusions of this research.

TABLE 4-1

SUMMARY OF CONCLUSIONS

Hypotheses	Conclusions
1) No difference in uptake rate constants between lindane and naphthalene.	There was no difference in uptake rate constants for the two chemicals.
2) No difference in depuration rate constants between lindane and naphthalene.	There was a difference in depuration rate constants between the test chemicals.
3) Exposure concentrations make no difference in both kinetics for lindane and naphthalene.	Exposure concentration makes no difference in uptake kinetics but depuration kinetics were concentration dependent.
4) No difference in BCFs for lindane and naphthalene.	BCFs were statistically difference for the test chemicals. They are, however, in the same order of magnitude and considered the same for bioconcentration testing.
5) Exposure concentration makes no difference in BCF.	Exposure made no difference in naphthalene BCF. For lindane the 20 g/L concentration BCF was statistically different than the BCF for 200 g/L.
6) No difference between lipid normalized values vs. non-lipid normalized values.	There was no difference in lipid normalized and non-lipid normalized values.
7) No difference between experimentally derived values vs. predicted values.	Predictions of BCF, K_1 and K_2 based on log P were very similar to the experimentally derived values.

APPENDIX

TABLE 1

CHEMICAL CHARACTERIZATION OF WATER FROM THE IAS AQUATIC TOXICOLOGY LABORATORY WELL

Parameter	Filtered	Unfiltered
pH	8.7	8.7
Alkalinity (mg/l)	465	475
Acidity (mg/l)	0	0
Hardness (mg/l)	4	4
Chlorides (mg/l)	13	15
Sulphates SO_4-S (mg/l)	90	90
Orthophosphate PO_4-P (mg/l)	0.027	0.027
Total phosphate-PO_4-P (mg/l)	0.029	0.029
Ammonia NH_3-N (mg/l)	0.15	0.14
Nitrate NO_3-N (mg/l)	0.101	0.141
Total Dissolved Solids (mg/l)	728	824
Total Suspended Solids (mg/l)	18	18
Turbidity (NTU)	2	4
Apparent Color (color units)	5	5
True Color (color units)	5	5
Total Organic Carbon (mg/l)	---	---

TABLE 2

GENERAL CONDITIONS FOR BIOCONCENTRATION TESTS

Specific Test Condition	Naphthalene	Lindane
Species[1]	Bluegill	Bluegill
Fish Weight	$\bar{X}$ = 11.3 gm	$\bar{X}$ = 4.9 gm
Number of fish per test chamber	40	36
Number of sample times	9	9
Number of fish per time	4 per tank	4 per tank
C_w[2] (high)	98.8 - 139.7	26.5 - 33.1
C_w (low)	11.7 - 11.3	2.62 - 3.04
Uptake time	4 days	6 days
Depuration time	7 days	12 days
Flow rate per fish weight	1.9 ℓ/g/day	5 ℓ/g/day
Fish fed	No	During depuration
Water samples	5 per tank	9 per tank
Fish samples	63 + 9 controls	54 + 9 controls
C_f[3] mg/kg	Individual fish analyzed	Individual fish analyzed
Lipids	Each fish	Each fish
Analysis Method	GC - FID	GC - EC

[1]Whole fish analyzed

[2]Range of concentration of chemical in test chambers

[3]Concentration of chemical in fish

TABLE 3

SAMPLING SCHEDULE FOR LINDANE BIOCONCENTRATION TESTS

	Day of Experiment	Fish Sampling Agenda	Water Temp.	Number of Fish Sampled Per Tank	Total Number Of Fish Analyzed 3µg/l Exposure	Total Number Of Fish Analyzed 30µg/l Exposure	Total Number Of Fish Analyzed Controls
Uptake	0	0	23°C	0	0	0	4
	1	1	23	4	3	3	1
	2	2	22	4	3	3	1
	3	-	22	0	0	0	0
	4	3	23	4	3	3	1
	5	-	23	0	0	0	0
	6	4	23	4	3	3	1
Depuration	7	5	23°C	4	3	3	1
	8	-	22	0	0	0	0
	9	6	22	4	3	3	1
	10	-	22	0	0	0	0
	11	-	22	0	0	0	0
	12	-	22	0	0	0	0
	13	7	22	4	3	3	1
	14	-	20	0	0	0	0
	15	-	20	0	0	0	0
	16	8	20	4	3	3	1
	17	-	20	0	0	0	0
	18	9	18	4	3	3	1

TABLE 4

SAMPLING SCHEDULE FOR NAPHTHALENE BIOCONCENTRATION TESTS

	Day of Experiment	Fish Sampling Agenda	Water Temp.	Number Of Fish Sampled Per Tank	Total Number Of Fish Analyzed 20µg/l Exposure	Total Number Of Fish Analyzed 200µg/l Exposure	Total Number Of Fish Analyzed Controls
Uptake	0	0	28°C	0	0	0	4
	1	1	28	4	4	4	1
	2	2	28	4	3	4	1
	3	3	28	4	3	4	1
	4	4	28	4	4	4	1
Depuration	5	5	27°C	4	3	4	1
	6	6	27	4	3	4	1
	7	-	27		0	0	0
	8	7	27	4	3	4	1
	9	-	27		0	0	0
	10	8	28	4	2	3	1
	11	9	28	4	0	2	1

TABLE 5

EFFICIENCY OF RECOVERY OF TEST CHEMICALS, NAPHTHALENE AND LINDANE, FROM FISH TISSUE. OTHER FISH SAMPLES HAD BEEN PREVIOUSLY EXTRACTED FOR 15 HOURS AND NO CHEMICAL WAS DETECTED BY GC ANALYSIS[a]

Chemical	Spike Concentration (mg/kg)	Chemical Concentration in Fish (mg/kg)	Present Recovery
Naphthalene	1.0	1.35	> 100
	1.0	1.15	> 100
	4.0	4.02	> 100
	4.0	3.60	90
Lindane	2.0	1.47	73.6
	0.2	0.19	93.2

[a]Determination of the efficiency of the method used to extract the chemical residue from fish tissue was accomplished by spiking macerated fish tissue with known concentrations of naphthalene and lindane. These samples were then carried through the extraction method (soxhlet extraction, florisil column, condensation and analysis on GC). From these data, an extraction efficiency of 97.5 for naphthalene and 83.4 for lindane were derived and used in all subsequent calculations.

TABLE 6

DATA FOR FISH BIOCONCENTRATION EXPERIMENT WITH LINDANE

CHEM	E_DOSE	A_DOSE	DAY	FISH_NO	FISH_WT	LIPID	FISHCONC
L	0	0.00	0	1	.	.	0.001
L	0	0.00	1	1	2.18	0.080	0.001
L	0	0.00	2	1	8.05	0.728	0.001
L	0	0.00	4	1	3.07	0.072	0.001
L	0	0.00	6	1	6.00	0.232	0.001
L	0	0.00	7	1	4.55	0.128	0.001
L	0	0.00	9	1	7.24	0.128	0.001
L	0	0.00	13	1	7.85	0.180	0.001
L	0	0.00	16	1	7.80	0.220	0.001
L	0	0.00	18	1	7.87	0.284	0.001
L	3	2.62	0	0	.	.	0.001
L	3	3.03	1	1	9.37	0.292	0.937
L	3	3.03	1	2	6.95	0.060	0.955
L	3	3.03	1	3	7.91	0.260	1.244
L	3	3.04	2	1	4.29	0.484	3.038
L	3	3.04	2	2	8.67	0.248	1.319
L	3	3.04	2	3	3.83	0.116	1.354
L	3	2.40	4	1	6.19	0.116	0.752
L	3	2.40	4	2	3.40	0.080	0.743
L	3	2.40	4	3	2.26	0.024	0.294
L	3	2.62	6	1	3.31	0.072	1.366
L	3	2.62	6	2	6.23	0.140	1.046
L	3	2.62	6	3	5.63	0.132	1.205
L	3	0.00	7	1	5.26	0.044	0.657
L	3	0.00	7	2	3.70	0.112	0.413
L	3	0.00	7	3	2.40	0.112	1.108
L	3	0.00	9	1	3.08	0.104	0.259
L	3	0.00	9	2	3.37	0.204	0.237
L	3	0.00	9	3	7.13	0.264	0.448
L	3	0.00	13	1	5.94	0.236	0.090
L	3	0.00	13	2	5.11	0.136	0.016
L	3	0.00	13	3	8.70	0.248	0.081
L	3	0.00	16	1	4.43	0.112	0.018
L	3	0.00	16	2	3.18	0.060	0.021
L	3	0.00	16	3	11.20	0.216	0.007
L	3	0.00	18	1	3.94	0.056	0.010
L	3	0.00	18	2	3.80	0.064	0.039
L	3	0.00	18	3	2.16	0.032	0.018
L	30	29.77	0	0	.	.	0.001
L	30	30.30	1	1	1.20	0.028	12.524
L	30	30.30	1	2	3.02	0.344	0.304
L	30	30.30	1	4	4.40	0.052	2.594
L	30	31.90	2	1	8.86	0.112	3.648
L	30	31.90	2	2	4.79	0.124	8.524
L	30	31.90	2	3	2.55	0.088	17.316
L	30	31.40	4	2	4.40	0.148	11.486
L	30	31.40	4	3	5.10	0.116	5.013
L	30	31.40	4	4	4.41	0.100	4.946
L	30	32.67	6	1	7.23	0.132	4.985
L	30	32.67	6	2	4.43	0.044	3.333
L	30	32.67	6	3	6.00	0.136	5.231
L	30	0.00	7	1	6.34	0.188	4.888
L	30	0.00	7	2	4.45	0.100	1.225
L	30	0	7	4	6.32	0.128	1.383
L	30	0	9	1	4.19	0.136	1.873
L	30	0	9	2	3.99	0.400	5.167
L	30	0	9	3	3.06	0.018	0.565
L	30	0	13	1	4.50	0.068	0.006
L	30	0	13	2	2.04	0.072	0.267
L	30	0	13	3	9.10	0.140	0.031
L	30	0	16	1	3.32	0.025	0.124
L	30	0	16	2	4.40	0.116	0.024
L	30	0	16	3	4.88	0.136	0.031
L	30	0	18	1	5.09	0.056	0.003
L	30	0	18	2	3.10	0.040	0.008
L	30	0	18	3	2.02	0.052	0.013

TABLE 6--CONTINUED

DATA FOR FISH BIOCONCENTRATION EXPERIMENT WITH NAPHTHALENE

CHEM	E_DOSE	A_DOSE	DAY	FISH_NO	FISH_WT	LIPID	FISHCONC
N	0	0.00	0	0	.	.	0.01
N	0	0.00	1	1	30.00	.	0.01
N	0	0.00	2	1	3.90	0.660	0.01
N	0	0.00	3	1	4.60	0.080	0.01
N	0	0.00	4	1	2.30	0.020	0.01
N	0	0.00	5	1	1.50	0.020	0.01
N	0	0.00	6	1	7.10	0.240	0.01
N	0	0.00	7	1	7.90	0.156	0.01
N	0	0.00	8	1	23.80	1.360	0.01
N	0	0.00	9	1	27.10	0.976	0.01
N	20	17.50	0	0	.	.	0.01
N	20	11.75	1	1	27.90	0.670	0.75
N	20	11.75	1	2	21.20	1.004	2.68
N	20	11.75	1	3	4.41	0.112	0.14
N	20	11.75	1	4	2.66	0.044	0.01
N	20	11.00	2	1	16.80	0.568	1.13
N	20	11.00	2	3	5.18	0.099	2.28
N	20	11.00	2	4	1.79	0.032	0.01
N	20	7.47	3	1	4.10	0.028	0.40
N	20	7.47	3	2	6.68	0.128	0.69
N	20	7.47	3	4	6.42	0.060	0.03
N	20	8.30	4	1	17.70	1.268	2.23
N	20	8.30	4	2	9.68	0.372	1.46
N	20	8.30	4	3	1.11	0.020	0.01
N	20	8.30	4	4	10.07	0.068	1.20
N	20	0.00	5	1	16.97	0.504	0.27
N	20	0.00	5	2	1.61	0.016	0.01
N	20	0.00	5	3	1.59	0.016	0.01
N	20	0.00	6	1	16.42	0.464	0.28
N	20	0.00	6	2	2.77	0.056	0.01
N	20	0.00	6	3	2.24	0.024	0.01
N	20	0.00	7	1	9.02	0.194	0.01
N	20	0.00	7	2	3.21	0.032	0.01
N	20	0.00	7	3	2.25	0.056	0.01
N	20	0.00	8	1	37.91	1.924	0.03
N	20	0.00	8	2	23.40	0.888	0.01
N	200	246.00	0	0	.	.	0.01
N	200	129.00	1	1	17.00	.	7.40
N	200	129.00	1	2	16.25	0.528	10.87
N	200	129.00	1	3	13.35	0.572	15.78
N	200	129.00	1	4	1.55	0.012	0.01
N	200	117.60	2	1	25.90	.	13.16
N	200	117.60	2	2	8.50	1.560	4.26
N	200	117.60	2	3	4.15	0.052	2.16
N	200	117.60	2	4	4.41	1.280	0.01
N	200	111.90	3	1	21.50	0.716	13.00
N	200	111.90	3	2	22.30	0.912	15.00
N	200	111.90	3	3	5.23	0.044	25.10
N	200	111.90	3	4	2.20	0.640	6.95
N	200	93.90	4	1	16.70	0.444	10.70
N	200	93.90	4	2	39.29	1.504	39.26
N	200	93.90	4	3	6.00	0.096	2.84
N	200	93.90	4	4	4.93	0.084	3.73
N	200	0	5	1	13.00	2.220	0.82
N	200	0	5	2	6.77	0.064	0.32
N	200	0	5	3	6.40	0.076	3.29
N	200	0	5	4	6.74	0.212	1.68
N	200	0	6	1	1.59	0.028	0.01
N	200	0	6	2	6.87	0.144	0.47
N	200	0	6	3	4.17	0.124	0.01
N	200	0	6	4	5.16	0.084	0.01
N	200	0	7	1	18.20	0.472	0.46
N	200	0	7	2	16.30	0.512	0.42
N	200	0	7	3	8.93	0.148	0.01
N	200	0	8	1	16.40	0.216	0.07
N	200	0	8	2	6.49	0.040	0.01
N	200	0	8	3	12.79	0.376	0.01
N	200	0	9	1	18.25	0.604	0.01
N	200	0	9	2	17.25	0.908	0.10

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