^{14}C-DDT DYNAMICS IN A COLD WATER MODEL FOOD CHAIN

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ABSTRACT

The uptake, elimination, and metabolism of ^{14}C-DDT in a maple leaf-scud-rainbow trout food chain was investigated using gas chromatography, liquid scintillation, and thin-layer chromatography-autoradiography. In this modular food chain, both scud and rainbow trout accumulated more DDT from the water than from food. After adjusting for growth in the trout, scud and trout showed little elimination of residue after exposure ceased. DDT was metabolized to DDE by both organisms. The results of this study will be a baseline for comparison of the dynamics of other compounds in this model.

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TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

The proliferation of synthetic chemicals has, for a time, out-stripped the responsibility of their use. Because of new legislation such as the Toxic Substances Control Act, we are now entering a time when the benefits of each chemical must be weighed against its hazards before it is used. Unfortunately we often have too little information on the non-target organism and other environmental hazards that a compound may present. The need for these data has created interest in developing methods not only to evaluate the hazard, but to predict it.

One promising tool for predicting the movement and persistence of a residue in aquatic animals is the model food chain. This approach to residue dynamics separates trophic level exposures so that a chemical residue may be traced from water into an organism or from one organism to another. Accumulation, metabolism and elimination of a chemical may then be studied in each organism.

This model food chain is composed of three segments:

Detritus	——	Scavenger	——	Predator
(Maple leaves)		(Scud)		(Trout)
(*Acer* spp.)		(*Gammarus pseudolimnaeus*)		(*Salmo gairdneri*)

It represents a commonly occurring food chain association in cold water environments (12 C). The basic concepts of this model and a similar warm water model are the design of Johnson and Schoettger (1975).

The purpose of this study is first to determine if the leaf-scud-trout cold water model is workable in a laboratory situation; second, to provide a baseline of uptake, elimination and metabolism information; and finally, to provide some of the information necessary to fully evaluate the reproducibility of this model.

Chapter II

REVIEW OF LITERATURE

General

Over the past 20 years several hundred thousand synthetic chemical compounds have been added to our environment (Johnson 1973). Of these, pesticides have caused more concern than any other group of chemicals due to their widespread use in large quantities. In 1974 alone, U. S. manufacturers produced 1,417 million pounds of synthetic organic pesticides (Fowler and Mahan 1976) containing over 175 different active ingredients (Sanders 1975). Insecticides, fumigants, and rodenticides composed slightly under half (650 million pounds) of the total production.

Contributing to the concern over these chemicals is the transport of pesticides through meteorological and biological cycles (Woodwell 1967). Major factors involved in pesticide transport can be more specifically identified as volatilization, chemical and photodecomposition, adsorption, dilution, uptake by plants, co-distillation and mechanical movements including rain, leaching, erosion and runoff (Haan 1971, Hurtig 1972, Pionke and Chesters 1973, Ware et al. 1975). The mode of application often gives pesticides a good start in their environmental travels.

For instance, as much as 50% of the DDT sprayed over forests remains aloft until it travels some distance (Woodwell et al. 1971, Gibney 1974). Ware et al. (1972a, 1972b) found that 15-50% of methoxychlor sprayed 5 to 6 feet above fields was lost to drift or volatilization. Akesson et al. (1972) measured as little as 40% of field-sprayed material deposited within 1,000 feet of the target.

All of these transport mechanisms contribute to the continuous exposure of the aquatic environment to many synthetic chemicals, especially pesticides, even though they were not directly applied to the water. Monitoring programs on the lower Mississippi River found aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor, and toxaphene present in the water at concentrations in the ng/l (pptr) range with individual concentrations fluctuating by a factor of 10 (Brodtmann 1976).

Biocides in the aquatic environment even in minute quantities may be concentrated 100,000 times their water concentration by uptake into an organism and/or passage of this residue to higher trophic levels (Metcalf et al. 1971). This is the basic concept of bioconcentration in food chains. The ability of algae, invertebrates, and fishes to metabolize or change the form of pesticides and eliminate

or rid themselves of pesticide residues by several means is documented by many workers and is well discussed by Kenaga (1972a, 1972b), Johnson (1973), Agarwal and Gupta (1974), Rudd and Herman (1972), McKim et al. (1974, 1975), and Whitacre et al. (1972).

The potential dangers of bioconcentration in aquatic organisms reach beyond the possibility of immediate mortality. Pesticide-induced changes in ecological communities (Cairns et al. 1972, Walker 1971) and the adverse effects on individual organisms in the community are well documented. DDT has been shown to affect behavior (Baily 1972, Davy et al. 1973, Dill and Saunders 1974, Weis and Weis 1974), learning and discriminating ability (McNicholl and McKay 1974, 1975a, 1975b), ability to withstand stress (Macek 1968, MacDonald 1971), selection of temperature (Javaid 1972, Miller and Oglive 1975), osmoregulation (Leadem et al. 1974, Zeeman and Waggoner 1972), and other physiological functions (Grant and Schoettger 1972), and fin regeneration (Weis and Weis 1975) in fishes. High pesticide residue in fish eggs may cause abnormality or mortality in fish (Burdick et al. 1964, 1972, Allison et al. 1963, Johnson and Pecor 1969, Dacre and Scott 1971, Smith and Cole 1973). Biocide residues in fishes have been shown to affect the storage and elimination of other residues (Macek et al. 1970, Mayer et al. 1972).

Clearly there is a need to assess the potential accumulation of chemical residues by fish from small concentrations in the water. Field studies for detecting residues and residue movements are often long, expensive and difficult. Likewise, research necessary to establish the biological hazard of each residue is also expensive. However, a laboratory model food chain capable of predicting possible movement and persistence of chemical residues in the environment could be a valuable tool in protecting fishery resources.

Food Chain Approaches

There are several different approaches to model food chains. Perhaps the most sophisticated approach is the conceptual or methematical model of residue dynamics in and between organisms. Gillett et al. (1974) wrote an extensive work on conceptual modeling that includes many environmental cycles besides food chain activity. Fagerstrom and Asell (1973) worked with a chironomid-roach-pike food chain from which a mathematical description and computer simulation were calculated. Norstrom et al. (1976) derived a model which successfully predicted PCB and methyl-mercury residues in yellow perch from the Ottawa River. Conover and Francis (1973), Aoyama and Inoue (1973), and Sodergren (1973) derived mathematical models from hydraulic-type systems where one compartment was used to

culture members of each trophic level with a water exchange between compartments. All of these studies share a dependence upon physiological rate factors such as efficiency of food assimilation and oxygen uptake. The value of these models as tools for future use relies heavily on the accurate determination of these rate factors.

A second approach to food chain modeling is that of the microcosm or "slice of the environment" type study. Usually one dose of a chemical is administered to a container in which representatives of various trophic levels have been placed. Often this type of study will contain a terrestrial phase.

Metcalf et al. (1971) designed a microcosm study which is in wide use today (Booth et al. 1973, Coats et al. 1974, Isensee and Jones 1975, Insensee et al. 1973, Lu et al. 1975, Metcalf et al. 1973a, 1973b, 1975, Sanborn and Yu 1973, Yu et al. 1974, 1975a, 1975b, 1975c, Yu and Sanborn 1975). This microcosm used sorghum and salt marsh caterpillers for a terrestrial phase and daphnids, mosquito larvae, snails and mosquito fish for the aquatic food chain. Plankton, diatoms, and other agents are introduced to the small sterile aquarium with a spike of old aquarium water. This basic system is sometimes modified to include other aquatic organisms, such as crabs, or to omit the terrestrial phase entirely.

Kanazawa et al. (1975) used an aquatic microecosystem containing a soil-gravel layer with channel catfish, crayfish, snails, daphnids, algae, and duckweed components. The overall concept and operation were similar to Metcalf's system.

Perhaps the greatest advantages that microcosm studies offer are the small, inexpensive laboratory facilities required and the quantity of metabolism-degradation information often produced. Because the microcosm is usually a static system, the probability of detecting small quantities of metabolites or degradation products is enhanced. However, it is difficult or impossible with this approach to separate water from food uptake or elimination functions. A separate water exposure for an individual trophic level is sometimes used to avoid this problem (Metcalf et al. 1973a). This increases the data obtained and allows an expanded interpretation of results but is actually a deviation from the "slice of the environment" concept toward a segmented or compartmentalized system, the third type of model food chain.

The complete compartmental food chain gives each consumer trophic level a separate water exposure, a separate food exposure, and a combination of both. It is then possible to analyze for differences between food chain contribution to bioconcentration and direct water uptake. In a fragmented form, simple exposure of one organism to

one type of dose, either in water (Derr and Zabik 1974, Johnson et al. 1971, Murphy 1970, 1971, Murphy and Murphy 1971, Neudorf and Khan 1975, Sanborn et al. 1975, Sanders et al. 1973, Sanders and Chandler 1972, Sodergren and Svensson 1973), or orally (Buhler and Shanks 1970, Grzenda et al. 1970, Macek et al. 1970, Macek 1968, Young et al. 1971) or to both (Allison et al. 1963, Argyle et al. 1973, Macek and Korn 1970, Gaurino et al. 1974) represents the compartmental type study. These segmented exposures are often intended only to determine a chemical's effect on one organism, but the residue or metabolism information generated is often similar to that from each section of a compartmental model.

A fully segmented algae-daphnid-guppy food chain was used by Reinert (1972) to investigate the dynamics of dieldrin. He found that organisms accumulated amounts of dieldrin directly proportional to the concentration in the water and that daphnids and guppies accumulated more residue from water than from food. No metabolism-degradation analysis was done.

Johnson and Schoettger (1975) have developed and are evaluating a more complete compartmental food chain for warm-water conditions. This yeast-daphnid-bluegill food chain is currently receiving extensive evaluation with a variety of compounds. By selection of organisms, this

model, like that of Reinert (1972) is designed to operate in warm water (20 C or over).

This literature review found no model food chains specifically designed for cold water.

All food chain models offer comparative information that is useful. However, as a predictive tool, their accuracy will be greatly increased by the development of mechanisms or models which can predict the interactive effects of sediment-water-microbes and geochemical-geophysical cycles. The knowledge of form or forms in which a potential contaminant may exist is essential. Work in these areas has been done by Cairns et al. (1972), Haney and Lipsey (1973), Johnson and Kennedy (1973), Kuhr et al. (1974), Matsumura et al. (1970), Oloffs et al. (1973), Pionke and Chesters (1973), and Zoro et al. (1974).

Food Chain Components

The cold water model food chain presented here used rainbow trout (Salmo gairdneri) as a predator. Rainbow trout have a wide distribution among waters where temperatures are at least periodically below 13 C (MacCrimmon 1971). They are easily cultured and maintained in a laboratory and are economically important.

Scud (Gammarus pseudolimnaeus), has long been recognized as an important or dominant food item for trout populations. Gammarids have a wide geographic distribution (Holsinger 1972), and grow and reproduce at cold water

temperatures (Smith 1973, Pflieger 1971a). A review of 50 papers by Pflieger (1971a) and concurrent feeding studies (Pflieger 1971b, 1972, 1973) is probably the most complete information on amphipod crustaceans as prey. In Pflieger's study, rainbow trout showed good condition and weight gain when fed frozen scud at a rate of 4 to 7% (wet weight/wet weight) per day. Gammarids are often used as a "natural" food in laboratory diet studies on trout (Horak 1974, Pyle 1964).

Scud are also used in laboratory testing of pollutants (Arthur 1970, Arthur and Leonard 1970, Arthur and Eaton 1971, Sanders et al. 1973, Ahmad 1969). Costa (1966, 1967a, 1967b) concluded Gammarus pulex was very sensitive to water quality (dissolved oxygen, temperature, pH, carbon dioxide tension) and the presence of toxicants in the water.

Scud feed by browsing on a film of microscopic plant, animal, and organic debris covering leaves and stems of aquatic vegetation (Pennak 1953). Hargrave (1969) reported that ^{14}C labelled bacteria and algae and H^3-lignin-like compounds were ingested and assimilated with low efficiency. The detrital-omnivorous feeding habits of the scud place them in an excellent position to be exposed to pollutants by direct contact in water or by ingestion of microflora and fauna.

Maple leaves represent the detrital portion of this food chain. This choice is somewhat arbitrary, since a

variety of aquatic vegetation or detrital materials, with the known exception of oak leaves, are suitable. However, maple leaves are widely and readily available, and have been shown to be a source of biocide input to the aquatic environment (Derr 1974).

Chapter III

MATERIALS AND METHODS

General

This study was conducted at the Columbia National Fisheries Research Laboratory, U. S. Fish and Wildlife Service, Columbia, Missouri.

Acquisition and Culture

Maple Leaves

Maple leaves (*Acer* spp.) collected from Boone County, MO, were soaked in untreated water for 2 weeks prior to being used as food for the scud as recommended by Sanders (personal communication).

Scud

Scud were collected from the upper portion of Lake Taneycomo reservoir (Taney County, MO) during periods of low water. Amphipods from this area of the lake are usually *Gammarus pseudolimnaeus* (Pflieger 1971b). Collection involved the use of a rake to stir the gravel on the bottom upstream from a fine-meshed net in which floating scud were trapped. The organisms were transported to Columbia by tank truck fitted with oxygenating equipment. Mortality approaching 50% was common in scud collected in this manner.

The scud were maintained in artificial streams on maple leaves for over 2 months. The artificial streams were necessary to hold the kilogram quantities of scud required in the study. Each stream was 5.5 m long by 0.43 m wide by 0.38 m deep and was separated into 3 equal sections by wire partitions. Water was circulated with submersible pumps and was held at a constant 12 C by a water-chilling unit. Little to no mortality was observed in the artificial stream and reproduction was noted. This was taken as evidence that the organisms were in good condition. Only active and healthy scud were used.

Fish

Rainbow trout (Salmo gairdneri), supplied by the National Fish Hatcheries, U. S. Fish and Wildlife Service, were maintained in laboratory raceways at 16 C after Brauhn and Schoettger (1975). Fish that weighed over 1.0 g fed readily on whole scud. For trout less than 1.0 g it was necessary to chop the scud into pieces before feeding. The fish had an average weight of 0.8 g (range, 0.6-1.1 g) and an average total length of 4.4 cm (range, 3.8-4.9 cm) at the start of the study. Only fish in good condition were used.

Exposure System

Toxicant Metering and Exposure Containers

A flow-through diluter system modified from that of Mount and Brungs (1967), was developed for these studies. A metering device designed by McAllister et al. (1972) or a Micromedic automatic pipette was used in the diluter to control exposure concentrations. Glass aquaria with a 41 liter volume (61 cm long x 30.5 cm wide x 30.5 cm deep) set in a constant temperature water bath served as exposure containers. Oxygen-saturated well water was used for all studies and was sterilized by using an ultra-violet light (Table 1).

Biomass Loading and Water Flow

The fish-carrying capacity of the flow-through system constructed for this study was determined using a loading (g/l fish in aquaria) vs. water flow (ml/min water flow into aquaria) study. A proportional splitter box modified after Benoit and Puglisi (1975) was added to the diluter system so that the water flow could be varied from aquarium to aquarium. The amount of water received by each aquarium was recorded for 30 samples. The relative standard deviation of any one flow was found to be no greater than 5%. Height and diameter of each standpipe and length and diameter of each standpipe cover could be adjusted to obtain the desired flow. Four different flow rates were achieved

Table 1. Chemical characteristics of well water at the Columbia National Fishery Research Laboratory.[a]

Analyte	Specified sensitivity limits, mg/l	Concentration, mg/l
Ca	0.1	70
Mg	0.1	27
K	0.5	3.9
SO_4	0.01	4.4
NO_3	0.05	<0.05
NO_2	0.05	<0.036
NH_4/N	0.01	0.066
Phenol	0.001	-[b]
Cl_2	0.001	-
Cl	0.01	29
F	0.01	0.34
CN	0.005	0.006
Fe	0.01	0.014
Cu	0.001	0.0045
Zn	0.001	<0.001
Cd	0.001	<0.0005
Cr	0.01	<0.01
Pb	0.001	0.0015
Alkalinity	1.0	237
Hardness	1.0	272
pH	0.1	7.4
Temperature	$\pm$ 0.5 C	16 C

[a]Mayer, F. L., P. M. Mehrle, and W. P. Dwyer. 1975. Toxaphene effects on reproduction, growth, and mortality of brook trout. Environ. Prot. Agency (U. S.). Ecol. Res. Ser. No. EPA-600/3/75/013. 51pp.

[b]Not detectable.

from each splitter box. By using 4 of these boxes, 4 aquaria received each flow rate. Three loadings (1, 3, and 5 g of fish/l) were selected to correspond to possible use levels and an empty aquarium served as a control. Dissolved oxygen, ammonia, pH, and temperature were determined every other day.

Method of Exposure and Sampling

General

Since this food chain is a compartmental model, the ^{14}C-DDT study was actually 2 separate exposures. In each exposure ^{14}C-DDT was introduced to the exposure water in acetone solutions so that a DDT concentration of 100 ng/l (pptr) resulted. Control aquaria received only acetone.

Leaves and Scud

In the first portion of the study, scud were exposed to ^{14}C-DDT by food, water, and food + water routes for 14 days. A 7-day elimination period followed.

The flow-through apparatus was adjusted to deliver 1 liter of water every 15.7 minutes which is equivalent to 2.2 aquarium volumes per day. Two aquaria received only solvent in the water and 2 received ^{14}C-DDT at the calculated concentration of 100 ng/l (pptr). The acetone solvent did not exceed 0.2 ml/l in the aquarium water. On the first 6 sample days 1 liter of water was removed from

each aquarium and the ^{14}C-DDT was extracted for liquid scintillation analysis.

The diluter operated for 48 hours before the study was started by placing 41 g of scud into each of the 4 aquaria. The first aquarium, the control, contained no leaves and received only solvent in the water. The second aquarium contained leaves that had been presoaked in ^{14}C-DDT for 2 days but received only solvent in the water. As scud consumed the leaves in this aquarium, more presoaked leaves were added. This aquarium served as a "food" exposure. The third aquarium, the "water" exposure, received ^{14}C-DDT in the water but contained no leaves. The fourth aquarium received both treated water and leaves. Since the scud could accumulate ^{14}C-DDT from both, this constituted a "food + water" exposure.

Two 10 g samples of maple leaves exposed to ^{14}C-DDT were frozen for later extraction and analysis. One sample was removed from the aquarium, drained of excess moisture and oven dried overnight at 80 C before freezing. The second sample was washed with untreated water, then drained and oven dried before freezing. A 10 g sample of leaves which had been soaked in untreated water served as a control.

On days 1, 3, 5, 7, 10, and 14 after the start of the exposure, 5 samples of 2 scud each were removed from each exposure aquarium and 1 sample of 2 scud from the control aquarium. On the fourteenth day, scud from the "water"

exposure were frozen for later qualitative analysis of DDT and its metabolites. After the 14-day samples were taken, the scud remaining in the "water" exposure were placed in another aquarium that received only control water. On days 15, 18, and 21, 5 samples of 2 scud each were sampled from this aquarium.

Throughout the exposure, each scud sampled was blotted on a paper towel to remove excess water, weighed to the nearest mg, and then quickly processed for either liquid scintillation or frozen for later qualitative analysis.

Because of the difficulty of exposing large quantities of scud in the flow-through apparatus, a 48-hour static exposure was used to generate over 1600 g of exposed scud for fish food. This was done in 90-liter stainless steel tanks set in constant temperature water baths. The initial water concentration was 1000 ng/l (pptr). Water was changed daily during the exposure which was terminated when the ^{14}C-DDT residue in the scud approached that of the scud exposed to ^{14}C-DDT at approximately 100 ng/l (pptr) for 14 days in the flow-through system. The treated scud were immediately frozen on dry ice and stored at -30 C until used.

Fish

The second part of this study was the exposure of rainbow trout to ^{14}C-DDT. The flow-through apparatus was

adjusted so that each of 16 aquaria received 1 liter of water every 6.2 minutes which is equivalent to 5.7 aquarium volumes per day. The toxicant was introduced to the diluter system 2 days prior to placing the fish into the aquaria and was allowed to remain 1 day after the fish were removed. As in the scud study, ^{14}C-DDT was introduced to water in acetone. The acetone did not exceed a concentration of 0.03 ml/l. A 1 liter water sample was taken from the water inflow or from the aquarium to insure proper operation of the diluter and record actual water concentrations of DDT.

Fish were acclimated to the 12 C test temperature for 4 days before 49 g of the trout were placed in each aquarium to start the test. During the exposure, rainbow trout were fed 5% of their total body weight or until food was no longer consumed each day. Visual observations were taken twice each day to note any unusual conditions or mortalities in the aquaria. The fish were weighed to the nearest 0.1 g and measured to the nearest mm at the beginning and at the end of the study.

The "water" and "food + water" exposures received ^{14}C-DDT in solution while "control" and "food" exposures received only acetone in the water. The "food" and "food + water" exposures were fed ^{14}C-DDT treated scud while the "control" and "water" exposures were fed scud exposed only to acetone. Each exposure was replicated.

Fish were removed on days 7, 14, 21, and 28 after being placed in the aquaria. On each sample day, 7 fish were removed from each aquarium and blotted to remove excess water. After recording length and weight, each fish was processed for liquid scintillation analysis. On the twenty-eighth day an additional 3 fish were removed from each aquarium for qualitative analysis of the ^{14}C-DDT residue (DDT and its metabolites).

The exposure was terminated on the twenty-eighth day, and the fish were maintained in uncontaminated water and on unexposed scud for a 28-day elimination period. Five fish from each aquarium were sampled on days 35, 42, 49, and 56. On the fifty-sixth day of the study, 3 fish from each aquarium were removed for qualitative analysis of DDT residue.

Chemical Analysis

Water Quality

Every other day during the loading study and as needed in other studies, dissolved oxygen, ammonia, pH, and temperature were monitored with a YSI model 54 oxygen meter, LaMott water quality kit, a Corning model 10 pH meter, and a Brooklyn thermometer, respectively.

Preparation and Purity of ^{14}C-DDT Exposure Stock

A 50 μCi sample of ring labelled ^{14}C-DDT was obtained from Amersham Searle. The stated purity was 99% with a specific activity of 32.3 mCi/mmol or 91 μCi/mg. The purity of the ^{14}C-DDT sample was checked on a Perkin Elmer 881 gas chromatograph (GC) with a ^{63}Ni electron capture detector and a Brinkman recorder. The 1.8 m x 2 mm glass column was packed with 3% OV7 on Chromasorb WHP (80-100 mesh) and operated at 180 C. The sample was found to be over 99% DDT.

As a further check on purity, Merck 0.25 mm thin layer chromatography plates were used to cochromatogram the ^{14}C-DDT stock with unlabelled DDT, DDE, and DDD in a hexane:diethyl ether:acetic acid (200:2:2, v/v/v) solvent system (Siewierski and Helrich 1967). After developing twice, the plate was overlain with Kodak no-screen x-ray film for 1 week. The position of the spots on the resulting autoradiogram was used to identify the location of the labelled material on the chromatogram. The stock was found to be over 99% DDT. The same thin layer chromatography-autoradiography (TLC-AR) system (Johnson et al. 1971) was used throughout the study.

After dilution to a working volume with acetone, the stock was used to prepare triplicate samples for liquid scintillation counting. Fifty μl were pipetted into each vial, then scintillating solutions (Rodgers and Stalling 1972) were added and mixed. After storage overnight in the dark, each vial was counted 3 times to 5% error by a Beckman liquid scintillation counter. Using the conversion

of 2.22×10^6 dpm/mCi, the specific activity of the ^{14}C-DDT sample with this instrument was found to be 37.4 mCi/mmol. Dilutions of the ^{14}C-DDT stock with unlabelled DDT were used for all exposures. The specific activity of these dilutions ranged from 3.95 to 10.0 cpm/ng.

Water-quantitative Residue Analysis

One-liter water samples were used to determine water concentrations of radiolabelled material with liquid scintillation. Each sample was placed in a separatory funnel with 10 ml of isooctane, shaken for 3 minutes, then allowed to set for at least 10 hours to separate. Seven milliliters of isooctane were pipetted from the separatory funnel into a liquid scintillation vial with the scintillation solutions. The extraction efficiency for 9 replicate spiked samples was 87%.

After extraction, the water samples were counted in the liquid scintillator at least 3 times until 5% error was reached. The sequence for calculating concentrations in water from water samples was: Average raw counts (cpm) - Background (cpm) ÷ Quench correction factor ÷ Specific activity (cpm/ng) ÷ 0.7 (7/10 of extractant used for sample) ÷ Extraction efficiency = Water concentration (ng/l).

Background counts on the Beckman scintillation counter were approximately 40 cpm in the water and organism samples. For purposes of this study, the assumption that the labelled

material in a sample must have at least 40 cpm (total 80 cpm/sample) to be reliable was made. In all tests the specific activity of the ^{14}C-DDT was not less than 3.95 cpm/ng. This allows a minimum detection limit of 10 ng of DDT in any one sample.

Water-qualitative Residue Analysis

Two-liter samples of water from the trout study were extracted with methylene chloride in a separatory funnel for GC analysis of the ^{14}C-DDT residues. After agitation for 30 minutes, the methylene chloride was allowed to separate from the water. The extractant was then removed from the separatory funnel and evaporated to a smaller volume for analysis.

Leaves-quantitative and Qualitative Analysis

The samples of detrital material were ground with 4 times their weight of Na_2SO_4 and column extracted using 5% diethyl ether in petroleum ether. After separation in a florsil column (Hesselberg and Johnson 1972) the samples were analyzed using gas chromatography. A sample spiked with 1 ng of DDT was extracted with 53% efficiency.

Scud and Fish-quantitative Analysis

Radiometric samples of scud and rainbow trout were prepared according to Johnson et al. (1971) using Triton X-100 in Toluene (2:3 v/v) as a tissue solubilizer and

Beckman Fluoralloy dry mix dissolved in toluene as a scintillation fluor (Rodgers and Stalling 1972). All samples were counted at least 3 times until 5% error was reached. The sequence for calculating the total body residue (TBR) for organism samples was: Average raw counts (cpm) - Background (cpm) ÷ Quench correction factor ÷ Specific activity (cpm/ng) ÷ Sample wet weight (mg) = Total body residue (ug/g, w/w)

As a check on the accuracy of the homogenization technique of sample preparation for liquid scintillation, 5 samples of exposed scud and 5 samples of exposed rainbow trout were prepared for scintillation by using a Packard Tri-carb autocombustion apparatus. The combustion technique "burns" the organism samples and traps the liberated $^{14}CO_2$ in solutions suitable for liquid scintillation counting. Total body residues compared between the 2 methods of preparation were found not significantly different ($P < .10$).

Scud and Fish-qualitative Analysis

For use with the TLC-AR analysis, samples of exposed scud were ground with 4 times their weight of Na_2SO_4 then column extracted with 5% diethyl ether in petroleum ether. Spiked control samples were extracted with 100% efficiency. A small portion of this lipid-containing extractant was dried overnight at 80 C to gravimetrically estimate lipid content in the scud. The major portion of the extractant was

prepared for TLC-AR by separating the lipid material and the pesticide with a florsil column extraction (Hesselberg and Johnson 1972).

Trout samples were prepared for TLC-AR in the same manner except that gel permeation chromatography (Stalling et al. 1972) was substituted for the florsil extraction step.

Quench Correction for Liquid Scintillation

Since the presence of some materials can interfere with detection in a liquid scintillation sample, definition of permissible amounts of homogenized rainbow trout, scud, and isooctane (2,2,4-trimethyl pentane) was done by constructing quench correction curves. Whole scud and whole trout were separately homogenized using Triton X-100 as a solubilizer. Scintillation vials containing different weights of each organism were prepared in triplicate. Each vial was mixed with the scintillation solution and a known spike of ^{14}C-DDT. As a control, triplicate vials containing only the ^{14}C-DDT spike and scintillation solutions were prepared. Counts for the 3 vials of each weight of each organism were averaged and, after correction for background, were expressed as a percentage of the control counts.

The quench correction curves (Figs. 1, 2, 3, Tables 2, 3, 4) permitted observation of the following quantities of each material and its corresponding quench.

Fig. 1. Quench curve for liquid scintillation samples containing isooctane.

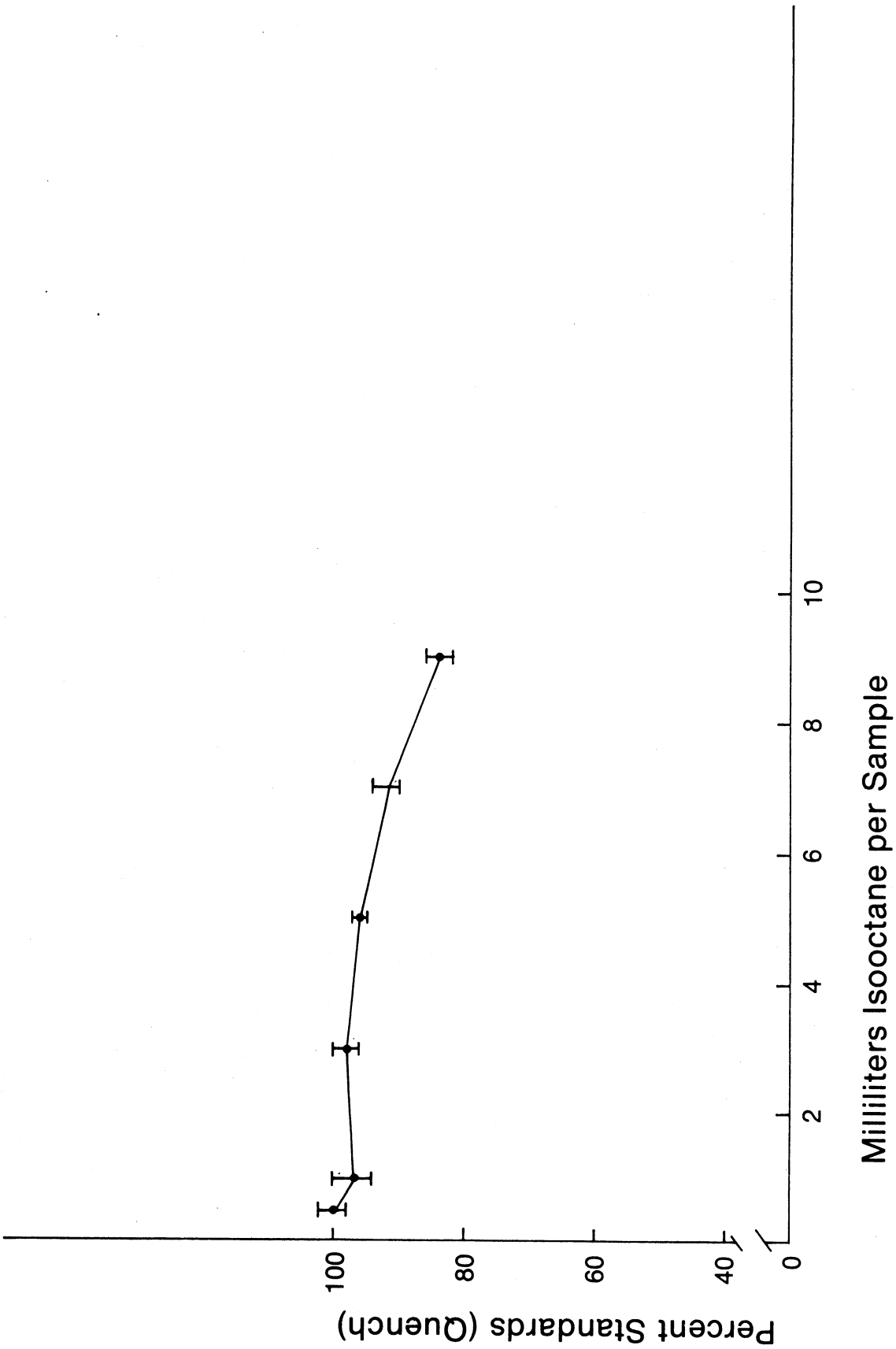

Percent Standards (Quench)
100
80
60
40
0
2
4
6
8
10
Milliliters Isooctane per Sample

Fig. 2. Quench curve for liquid scintillation samples containing homogenized scud.

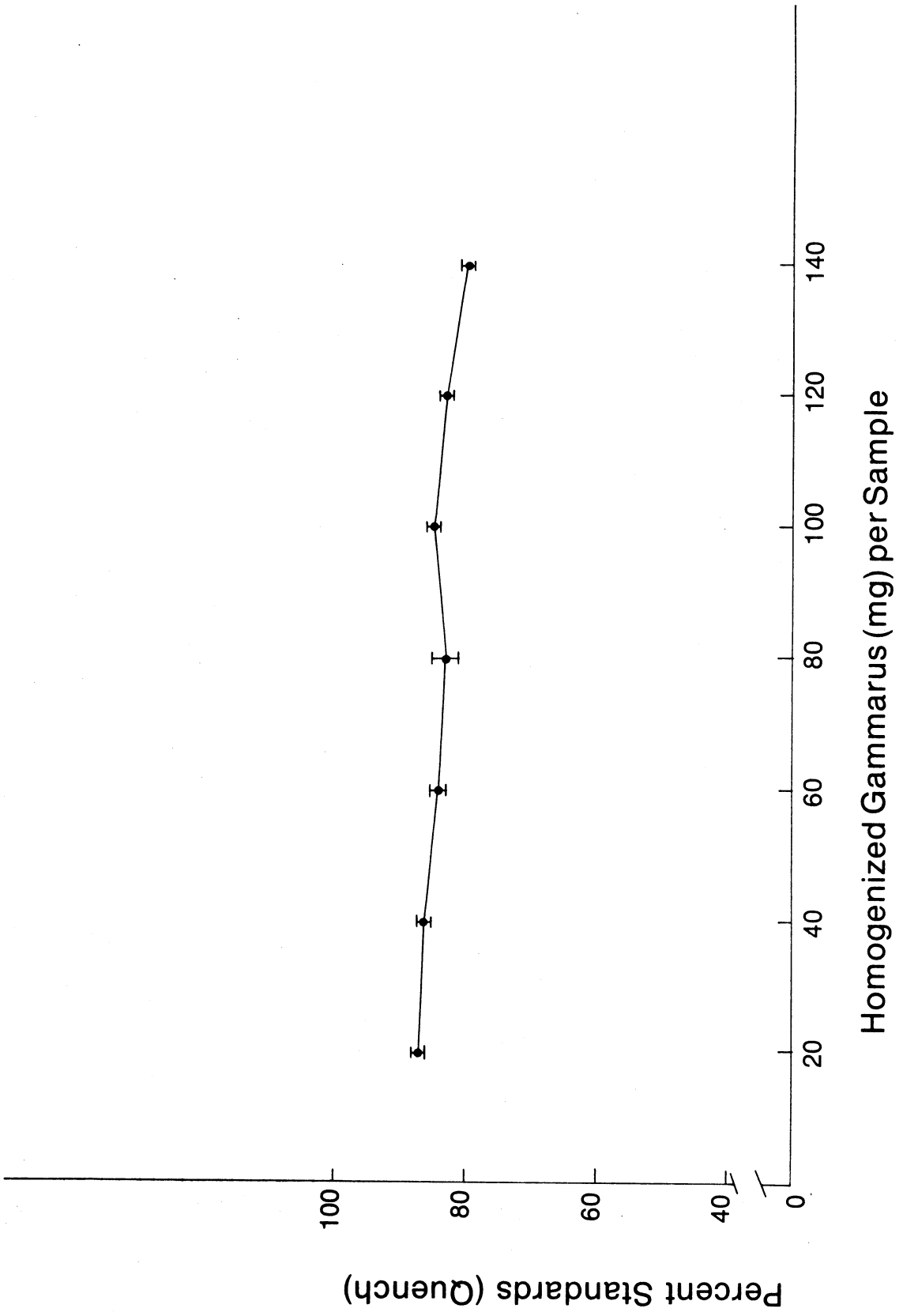

Percent Standards (Quench)
100
80
60
40
0
20
40
60
80
100
120
140
Homogenized Gammarus (mg) per Sample

Fig. 3. Quench curve for liquid scintillation samples containing homogenized rainbow trout.

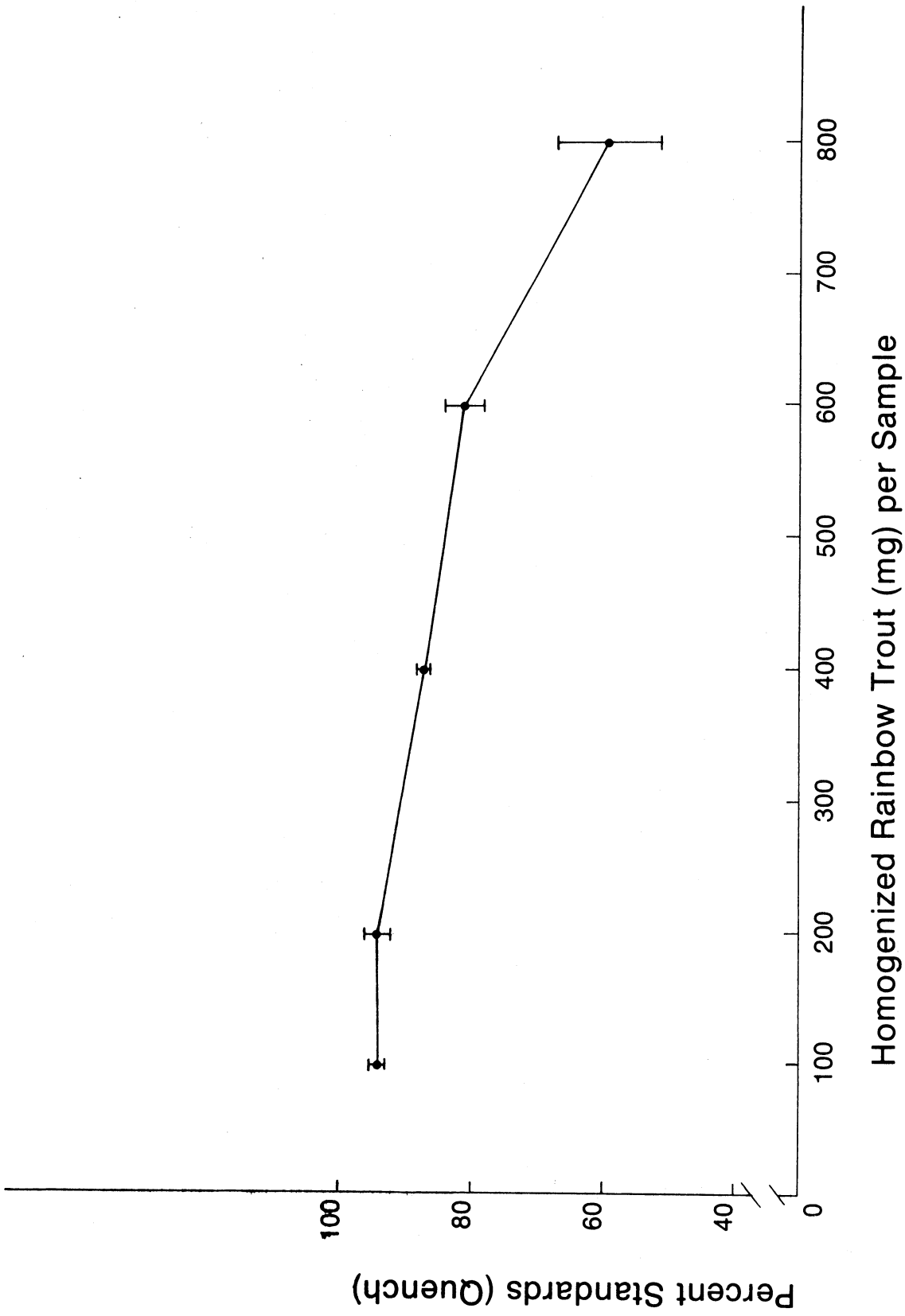

Percent Standards (Quench)
100
80
60
40
0
100
200
300
400
500
600
700
800
Homogenized Rainbow Trout (mg) per Sample

Table 2. Average CPM, standard error, and percent standard (quench correction factor) for triplicate liquid scintillation samples containing isooctane.

Isooctane (ml)	Average CPM	Standard error	Percent standard
0	461	9.0	Standard
0.5	461	9.0	100
1	445	11.5	97
3	451	7.0	98
5	444	3.0	96
7	424	9.9	92
9	385	5.2	84

Table 3. Average CPM, standard error, and percent standard (quench correction factor) for triplicate liquid scintillation samples containing homogenized gammarus.

Gammarus (mg)	Average CPM	Standard error	Percent standard
0	524	4.4	Standard
20	455	2.3	87
40	451	3.8	86
60	439	5.7	84
80	432	9.0	83
100	445	6.2	85
120	434	4.2	83
140	416	2.0	80

Table 4. Average CPM, standard error, and percent standard (quench correction factor) for triplicate liquid scintillation samples containing homogenized rainbow trout.

Rainbow trout (mg)	Average CPM	Standard error	Percent standard
0	1017	6.2	Standard
100	959	7.7	94
200	955	15.0	94
400	886	8.2	87
600	826	34.7	81
800	606	83.0	59

Material	Sample contents	Correction factor
Homogenized scud	140 mg	0.80
Homogenized rainbow trout	600 mg	0.81
Isooctane	9 ml	0.84

These amounts were considered the maximum allowable in a liquid scintillation sample since an acceptable quench correction factor of not less than 0.80 was arbitrarily set. A scud or water sample could be processed as 1 liquid scintillation sample since their ordinary weight or volume was less than 140 mg or 9 ml. The rainbow trout had to be divided into several samples since they averaged 800 mg at the beginning of the study.

Statistical Analysis

The data were analyzed using a 2 x 2 factorial analysis of variance for the weight and length of the fish and a 2-way analysis of variance for comparison of the TBR between treatments. A least significant difference (LSD) test was used to compare means. Reproducibility was evaluated using the coefficient of variation and an intraclass correlation. The data from the loading vs. flow study were analyzed using a multiple linear regression on the resulting oxygen and ammonia data on both initial and final loading values. Snedecor and Cochran (1967) was the reference used for all statistical analyses.

Chapter IV

RESULTS AND DISCUSSION

Pre-exposure Residues

The residues (μg/g, ppm) of DDT and its metabolites, DDE and DDD, the percent lipid in the study components before exposure were:

Residue	Maple leaves	Scud	Rainbow trout
DDT	ND	0.004	ND
DDE	ND	0.003	0.058
DDD	ND	0.003	ND
Percent lipid		0.7%	2.0%

One composite 10 g sample of leaves, one composite 10 g sample of scud, and one composite 30 g sample of trout were used to generate this residue information. The maple leaves had no detectable (ND) DDT, DDE or DDD residues, and rainbow trout had no detectable residues of DDT or DDD. The minimum detection limit was 0.0005 μg/g (ppm). This residue information can serve as an index of previous exposure. Since DDT residues are correlated with percent lipid (Earnest and Benville 1971), the lipid content of organisms is a useful point of comparison for later studies. These data indicate Lake Tancycomo to be a source for low residue scud. This would be expected since no intensive agriculture or urbanization exists in this area.

Detrital Compartment

The results (μg/g, ppm) of the GC analysis of the maple leaves were:

Residue	Rinsed	Unrinsed	Control
DDT	1.84	1.79	0
DDE	0.06	0.06	0

Based on one spiked sample, the recovery was 53%. Because of the low sample number and recovery percentage, the accuracy of these data are somewhat suspect. However, the data do indicate that the ^{14}C-DDT is available to the scud through the maple leaves. The residue in the water rinsed sample being nearly identical to the non-rinsed sample indicates that the ^{14}C-DDT is strongly adsorbed to the leaf surface. These data and the fact that scud readily feed on soaked maple leaves recommend this detrital material for use in this type of model food chain.

Scud Compartment

Total Body Residue

^{14}C-DDT dynamics in scud were characterized by rapid uptake during the 14-day exposure without evidence of a plateau (Fig. 4, Table 5). Scud definitely assimilate DDT from ingested materials and the contribution from the food source represented 29% of the TBR shown in the "food + water" exposure. The elimination phase reflects little or

Fig. 4. Total body residue (µg/g, ppm) and standard error for scud exposed to ^{14}C-DDT by three routes.

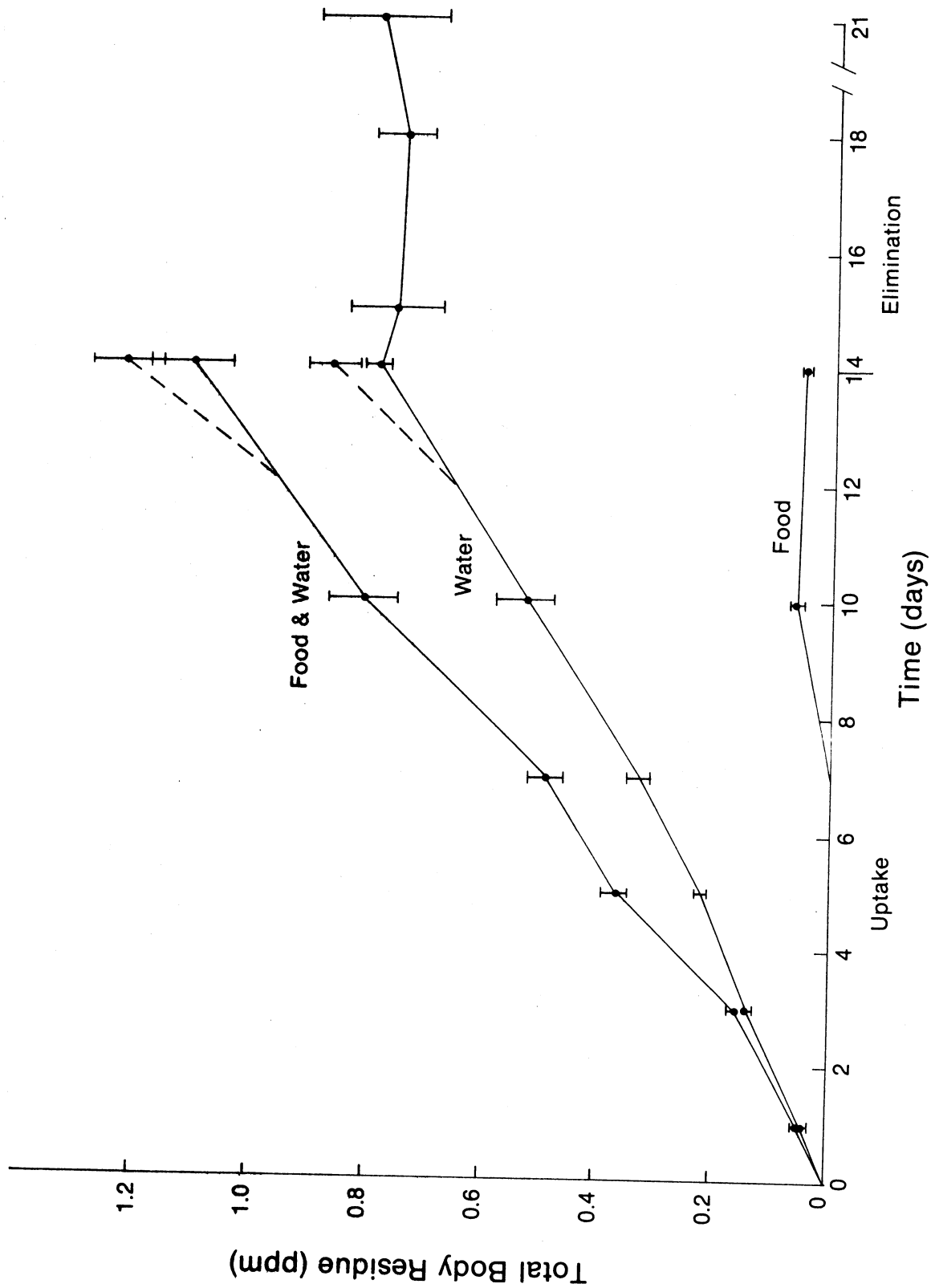

Total Body Residue (ppm)
1.2
1.0
0.8
0.6
0.4
0.2
0
Food & Water
Water
Food
0
2
4
6
8
10
12
14
16
18
21
Uptake
Elimination
Time (days)

Table 5. Mean total body residue (μg/g, ppm) and standard error for scud exposed to ^{14}C-DDT.

Exposure	Sample day								
	1	3	5	7	10	14	15	18	21
Food	ND*	ND*	ND*	ND*	0.06* (0.01)	0.05* (0.01)			
Water	0.04 (0.01)	0.14 (0.01)	0.22* (0.01)	0.33* (0.02)	0.53* (0.05)	0.79* (0.02)	0.76 (0.08)	0.75 (0.05)	0.79 (0.11)
Food + water	0.05 (0.01)	0.16 (0.01)	0.37* (0.02)	0.49* (0.03)	0.81* (0.06)	1.11* (0.07)			
"Voided" water						0.87[1] (0.05)			
"Voided" food + water						1.22[1] (0.06)			

* Significant difference ($P > .05$) within each sample day.

[1] No significant difference ($P < .05$) between water and "voided" water; food + water and "voided" food + water.

ND not detectable. Treated as 0 residue in calculation.

no reduction in DDT residues at the end of the 7-day elimination period.

The "water" exposure produced approximately 16 times the TBR produced by the "food" exposure. The difference between "water" and "food + water" exposures was greater than the magnitude of the TBR for "food" exposure only. However, the methods used to generate a "food only" exposure could have led to a lower TBR. The leaves soaked in ^{14}C-DDT were placed into the "food only" aquarium which received untreated water. This might have led to a loss of ^{14}C-DDT to the water in amounts too small to have been detected by liquid scintillation. Certainly no more DDT residue could be accumulated by the leaves in the "food only" exposure as the study progressed but the leaves in the "food + water" exposure had a constant source of DDT input and an opportunity to adsorb greater amounts of the chemical.

The broken lines that appear on "food + water" and "water" exposures at day 12 and extend to day 14 represent a small number of scud placed in polyethylene buckets (covered on the bottom with fine mesh screen) which were suspended in the aquaria. The scud held in the buckets in the "food + water" exposure could not reach the food material on the bottom of the aquarium. This was an attempt to determine how much ^{14}C-DDT the scud from this exposure contained in their intestine when sampled for

liquid scintillation. The scud in the bucket were in this manner "voided" for 48 hours. Scud in the "water" exposure were placed in a similar bucket to serve as a check on the effect, if any, of the bucket. The "voiding" scud in each bucket showed an insignificantly ($P < .05$) higher TBR than the scud free in their respective aquarium. The results give little information on the contribution of the contents in the intestine of scud to the TBR estimates. However, this illustrates the variety of sources of variation in uptake data. In this case a slight change in the exposure container produced a uniform slight increase in the TBR of DDT in scud.

Metabolism

Scud displayed remarkable consistency in their conversion of DDT to DDE (Table 6). No differences in conversion were produced by the different exposures. This information compares well with Johnson et al. (1971) who found that Gammarus fasciatus exposed to DDT for 3 days contained 79% DDT and 21% DDE.

Zoro et al. (1974) found that iron porphyrins can convert DDT to DDD under Anaerobic conditions, and therefore, some tissues that may not be able to metabolize DDT while living may do so after dying. All spiked, extracted control samples showed some evidence of minor percentages of DDT conversion. The findings of Zoro possibly explain this conversion in spiked control samples and may induce a small amount of error in percent conversion of DDT to DDE and DDD.

Table 6. Percent total labelled ^{14}C-DDT (by TLC) in scud after 14 days of exposure.

Metabolite	Control		Food		Water		Food + water	
DDT	95[1]	0	88	88	88	88	88	88
DDE	5	0	12	12	12	12	12	12
DDD	0	0	0	0	0	0	0	0
Unknowns	0	0	0	0	0	0	0	0

[1] Control sample spiked with ^{14}C-DDT then extracted.

DDT Concentration in Water

The concentration of DDT in the aquaria during this exposure was much less at the 24-hour sample than later in the study (Table 7). This indicated that the scud could have temporarily depressed the water concentration of DDT when first placed in the Aquaria, and one water sample per day per aquarium may not be adequate for reliable information on ^{14}C-DDT concentrations.

Fish Compartment

Total Body Residue

The trout nearly doubled their weight in the 56 days that they were fed scud at 5% per day (wet weight/wet weight basis). Fish were observed to be in excellent condition throughout the exposure. No significant differences ($P<.05$) existed in the average weights of fishes sampled between aquaria in any one sample day.

The rainbow trout uptake and elimination study (Fig. 5, Table 8) showed rapid uptake of DDT and apparent elimination trends with no evidence of a plateau being reached. The curve displays the magnitude of the difference between food and water uptake. Clearly, when trout are exposed to DDT at 0.65 µg/g (ppm) in their food and to DDT at about 100 ng/l (pptr) in the water, the uptake of DDT from water is about 10 times greater than that from food. This results in a much smaller difference in the TBR between "water" and "food + water" exposures than

Table 7. Water concentration (ng/l, pptr) of ^{14}C-DDT in aquaria during scud exposure (one sample per day per aquarium).

Exposure	1	3	5	7	10	14
Control	0	0	0	0	0	0
Food	0	0	0	0	0	0
Water	20	80	87	87	90	80
Food + water	20	80	101	73	81	117

Fig. 5. Total body residue (µg/g, ppm) for each exposure type and for each replicate for rainbow trout exposed to ^{14}C-DDT.

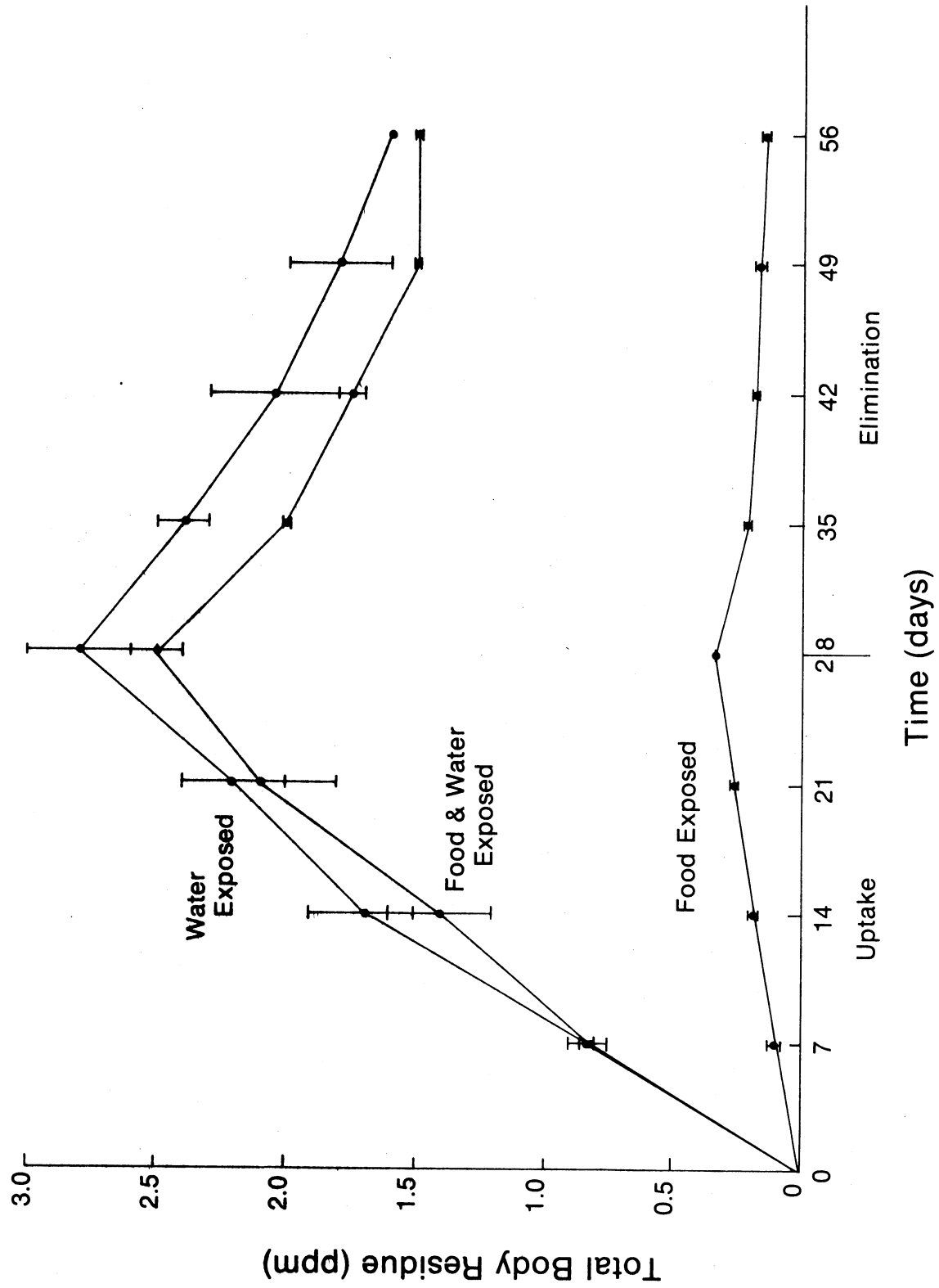

Total Body Residue (ppm)
3.0
2.5
2.0
1.5
1.0
0.5
0
Water Exposed
Food & Water Exposed
Food Exposed
0
7
14
21
28
35
42
49
56
Uptake
Elimination
Time (days)

Table 8. Mean total body residue for each exposure type (μg/g, ppm) and for each replicate for rainbow trout exposed to ^{14}C-DDT.

	Exposure				Elimination			
	Sample Day							
Exposure	7	14	21	28	35	42	49	56
Food	0.10*	0.18*	0.26*	0.28*	0.22*	0.19*	0.17*	0.15*
	(0.09-0.12)	(0.17-0.19)	(0.25-0.26)	(0.28-0.28)	(0.22-0.23)	(0.18-0.20)	(0.15-0.18)	(0.14-0.15)
Food +	0.82	1.4*	2.1	2.5*	2.0*	1.7*	1.5*	1.5
water	(0.76-0.89)	(1.2 -1.6)	(1.8 -2.4)	(2.4 -2.6)	(2.0 -2.0)	(1.7 -1.8)	(1.5 -1.5)	(1.4 -1.7)
Water	0.83	1.7*	2.2	2.8*	2.4*	2.1*	1.8*	1.6
	(0.80-0.85)	(1.5 -1.9)	(2.0 -2.4)	(2.5 -3.0)	(2.3 -2.5)	(1.8 -2.3)	(1.6 -2.0)	(1.6 -1.6)

* Indicates significant difference ($P < .05$) within each sample day.

between "water" and "food" exposures. In this case the apparent inversion in the TBR values between "water" and "food + water" is probably due to differences in the water concentration of DDT in these exposures (Table 9).

The much greater residue produced by "water" exposure as compared to "food" exposure does not in itself imply that the greatest hazard comes from direct water uptake. The different routes of exposure could result in differential storage or metabolite formation and therefore a changed potential for detrimental effects on the organism. Biological activity of a residue as well as quantity, must determine the relative importance of each pathway since the concentration at the site of action is the most important consideration toxicologically (Mayer, personal communication).

Reinert and Bergman (1974) found that DDT-R (DDT, DDE, and DDD) residues in Great Lakes salmonids varied with size, life stage, tissue and location of collection. However, DDT-R residues were in the 2.2 to 18 µg/g (ppm) range. The 28-day residues for trout in this study in "water" and "food + water" exposures were 2.4 to 3.0 µg/g (ppm). This indicates that the magnitude of uptake found in this study is within the range of that observed in the environment.

Macek et al. (1970) found that rainbow trout retained 20 to 24% of DDT ingested from a dry pelleted ration. The feeding of each individual fish in the cold water model study was not controlled, but using the measured food

Table 9. Mean water concentration (ng/l, pptr) and range of ^{14}C-DDT in aquaria and in inflow during rainbow trout exposure.

Exposure	Sample day									
	-1	0	1	2	5	7	14	21	28	29
Food + water aquaria	100 (96-105)	104 (95-112)	69 (63-74)	69 (62- 76)	80 (74- 86)	81 (73- 88)	76 (68- 83)	83 (79- 87)	95 (93- 96)	107 (106-108)
Inflow					110 (97-123)	109 (100-117)	106 (97-114)	123 (120-126)	121 (120-122)	120 (119-120)
Water aquaria	142 (135-149)	141 (133-149)	94 (89-99)	93 (85-101)	109 (102-115)	112 (95-129)	95 (87-103)	83 (82- 84)	81 (78- 84)	101 (95-106)
Inflow					147 (134-159)	149 (137-161)	146 (133-159)	119 (116-121)	113 (110-116)	114 (112-116)

residue of 0.65 µg/g (ppm) and the feeding rate of 5% per day, the resulting TBR at 28 days represents approximately 40% of the calculated DDT dosage. The higher percentage of ^{14}C-DDT retention in this study might be attributed to the difference in food received by the trout. A natural food item may be assimilated with greater efficiency than an artificial food, as has been proposed by Horak (1974).

The elimination portion of this study, based on TBR (Fig. 5), indicated a reduction in the DDT residue that was accumulated during the exposure. The reduction in TBR for the 28-day period was about 28%. However, the average weight of the fish increased about 24% during this time period. This comparison indicates that the total residue eliminated during the 28 days was much less than Fig. 10 seems to indicate and that much of the decline in TBR is due to the dilution effect of fish growth.

Metabolism

No differences existed in metabolites formed by rainbow trout in the different exposures (Table 10). However, the overall average DDT (80%) and DDE (18%) at day 28 showed definite changes (DDT 65% and DDE 35%) by the end of the elimination period on day 56. This increased conversion of DDT to DDE was probably due to the effect of time since the fish were changing the DDT to the more persistent DDE residue. Zinck and Addison (1975) found

Table 10. Percent total labelled ^{14}C-DDT (by TLC) in rainbow trout after 28 days of exposure and 28 days of elimination.

Metabolite	Control	Food		Water		Food + water	
		Day 28, Exposure					
DDT	94[1]	0	78	80	78	81	79	83
DDE	1	0	19	20	16	18	19	15
DDD	5	0	3	0	6	1	2	2
Unknowns	0	0	0	0	0	0	0	0
		Day 28, Elimination					
DDT	98[1]	0	68	70	69	63	63	60
DDE	1	0	32	30	31	37	37	39
DDD	1	0	0	0	0	0	0	1
Unknowns	0	0	0	0	0	0	0	0

[1] Control sample spiked with ^{14}C-DDT then extracted.

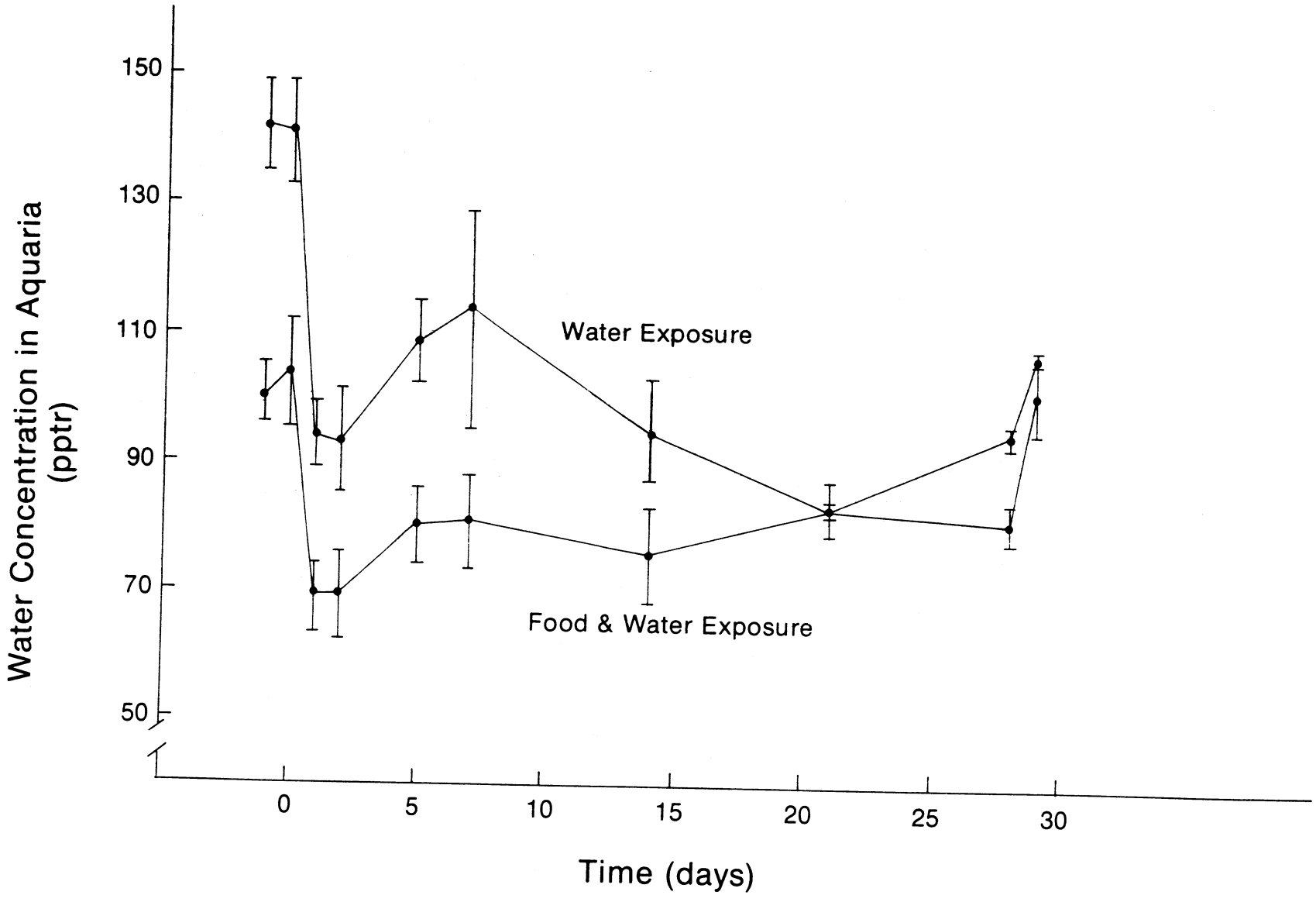
that at 10 C, brook trout converted 13% of the intramuscularly-injected DDT to DDE after 16 weeks. Reinert et al. (1974) found that rainbow trout exposed to p,p'-DDT in bath contained two residues, p,p'-DDT and p,p'-DDE. The p,p'-DDT composed 88-93% of the total DDT residue. The percentage of DDT residue at day 28 in this study (80%) compares well with Reinert's study after one considers that this exposure was 4 weeks and Reinert's data were for fish exposed 12 weeks.

DDT Concentration in Water

The water concentration of DDT was determined one day before introduction of the fish and one day after removal of all fish. The sharp drop of the water concentration on day 1 and sharp recovery on day 29 (Fig. 6) showed that the fish depressed the water concentration of DDT by about 33% in both "water" and "food + water" exposures in the first 24 hours even though each aquarium received a water flow of 5.7 aquarium volumes per day. This indicates that rapid uptake of ^{14}C-DDT by fish occurred throughout the study. One would expect this to possibly continue until a plateau residue level was reached in the fish.

The GC analysis of DDT in water in the exposure tanks was determined to be 60 ng/l (pptr) with no detectable DDE and DDD residues, and compared well with the 81 ng/l (pptr) measured by liquid scintillation. Both analyses were based

Fig. 6. Mean water concentration (ng/l, pptr) and range of ^{14}C-DDT in aquaria during rainbow trout exposure.


Water Concentration in Aquaria (pptr)
150
130
110
90
70
50
Water Exposure
Food & Water Exposure
0
5
10
15
20
25
30
Time (days)

on two samples. The GC results confirm that the labelled material in solution existed mostly as DDT and was not appreciably degraded in the exposure tank. This information would be especially important for an exposure with a chemical that was readily degraded in water. The water uptake of a degradation product would not then be interpreted as uptake of the parent material.

Model Requirements and Evaluation for Fish

Biomass Loading and Water Flow

The study of fish loading and water flow provided the following multiple linear regression equations and their regression coefficients.

Ammonia

Using initial loading: (0.77)

$$Y = 0.1249 - 0.0004 \text{ (flow)} + 0.1524 \text{ (loading)} - 0.0004 \text{ (flow x loading)}$$

Using final loading: (0.72)

$$Y = 0.1120 - 0.0005 \text{ (flow)} + 0.1226 \text{ (loading)} - 0.0004 \text{ (flow x loading)}$$

Oxygen

Using initial loading: (0.80)

$$Y = 7.1563 - 0.0050 \text{ (flow)} - 1.1565 \text{ (loading)} + 0.0007 \text{ (flow x loading)}$$

Using final loading: (0.88)

$$Y = 7.3777 + 0.0036 \text{ (flow)} = 0.1354 \text{ (loading)} + 0.0022 \text{ (flow x loading)}$$

where

flow = ml/min water inflow to aquaria

loading = g/l fish in aquaria

ammonia and oxygen in mg/l

These equations, described by Figs. 7, 8, 9, 10, Table 11 allow an investigator to calculate the quantity of radioactive material necessary to expose the mass of trout required for his study. By setting an acceptable minimum oxygen and/or maximum ammonia concentration, the equations will estimate the water flow required. An estimate of required labelled material may be calculated by using the water flow data, the toxicant concentration, and the duration of the study.

By using the recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) on acceptable oxygen and ammonia concentrations, this study defined working biomass levels of 1.5 g/l or 61.5 g trout per 41 liter aquarium. This loading level was defined on the basis of the oxygen equations since it was the most restrictive of the two when using the Committee's recommendations that dissolved oxygen be not less than 60% saturation and un-ionized ammonia not exceed 20 µg/l (ppb). In using these data, it must be remembered that the equations were derived for trout held at 12 C for 30 days. Changing species, time, and/or temperature can introduce a large source of error in the calculations. Equations for both initial loadings (day 0) and final loadings (day 31) were determined. Initial loading equations will be most useful in a study where fish are periodically sampled, and final loading data are more appropriate for studies where

Fig. 7. Multiple linear regression for oxygen using final biomass loading.

$y = 7.3777 + 0.0036\ (\text{flow}) - 1.1354\ (\text{loading}) + 0.0022\ (\text{flow x loading})$

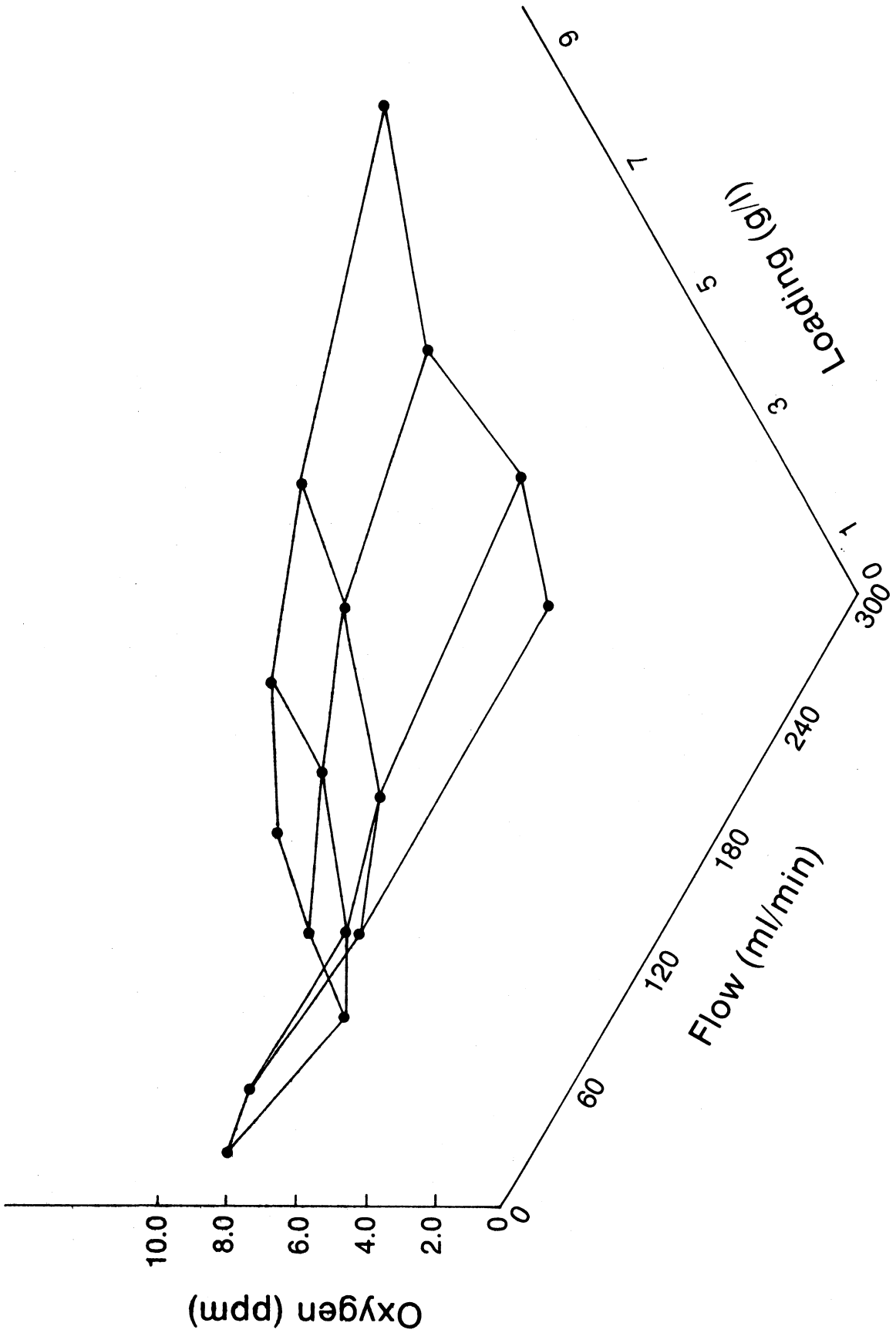

Oxygen (ppm)
10.0
8.0
6.0
4.0
2.0
0
Flow (ml/min)
0
60
120
180
240
300
Loading (g/l)
0
1
3
5
7
9

Fig. 8. Multiple linear regression for oxygen using initial biomass loading.

$$y = 7.1563 - 0.0050 \text{ (flow)} - 1.1565 \text{ (loading)} + 0.0007 \text{ (flow x loading)}$$

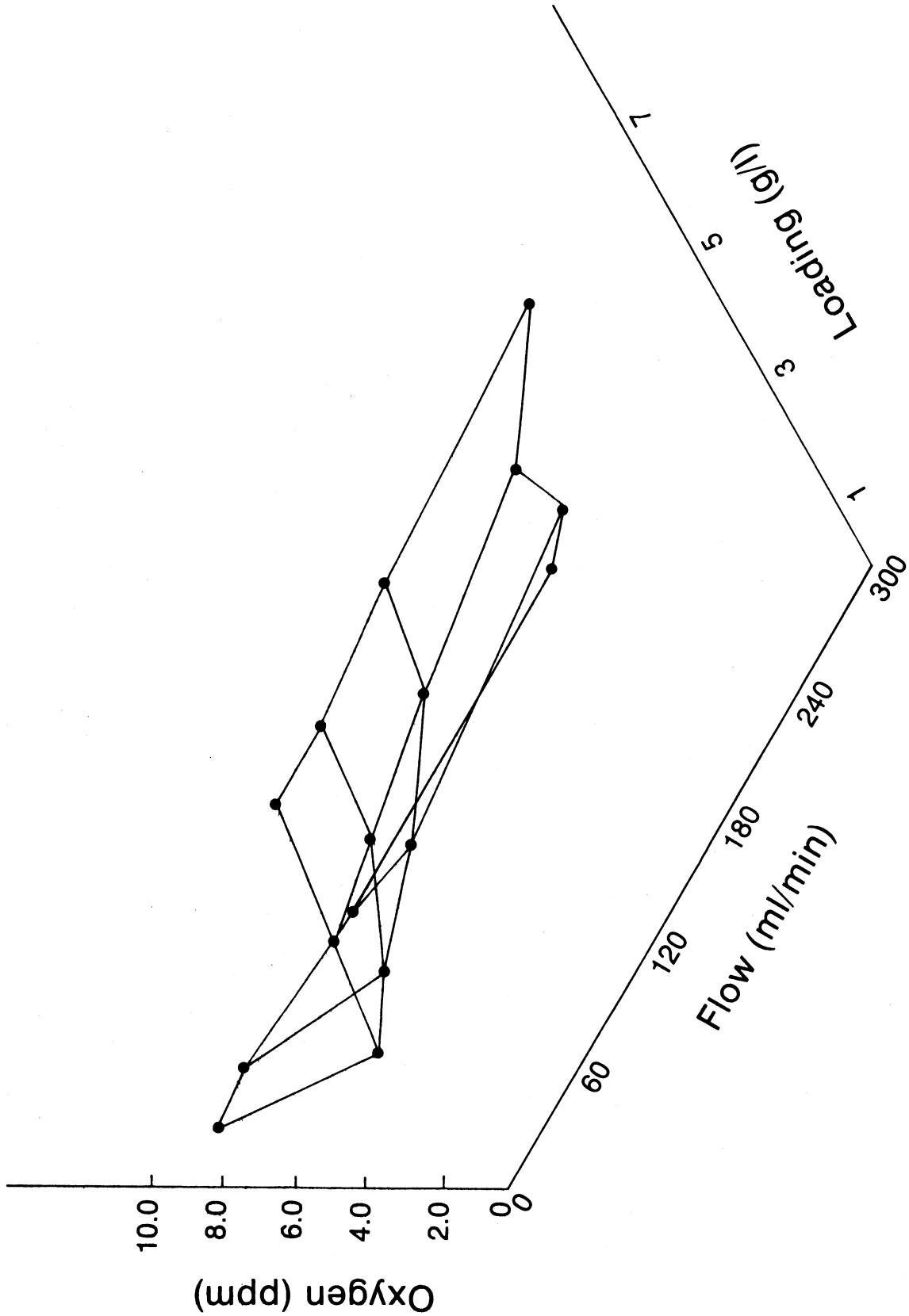

Oxygen (ppm)
10.0
8.0
6.0
4.0
2.0
0.0
Flow (ml/min)
60
120
180
240
300
Loading (g/l)
1
3
5
7

Fig. 9. Multiple linear regression for ammonia using final biomass loading.

$y = 0.1120 - 0.0005\ (\text{flow}) + 0.1226\ (\text{loading}) - 0.0004\ (\text{flow x loading})$

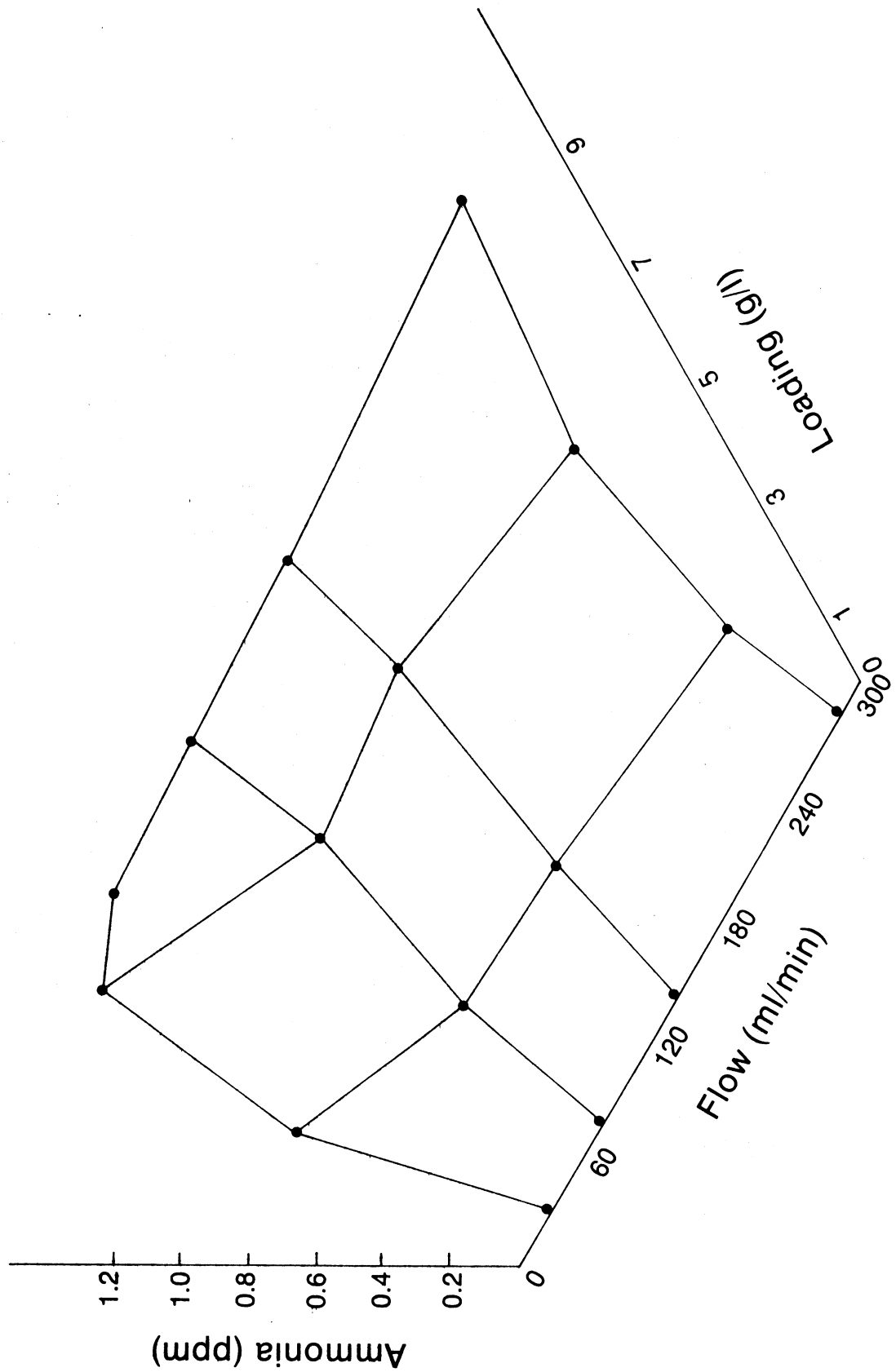

Ammonia (ppm)
0.2
0.4
0.6
0.8
1.0
1.2
0
60
120
180
240
300
Flow (ml/min)
1
3
5
7
9
Loading (g/l)

Fig. 10. Multiple linear regression for ammonia using initial biomass loading.

$$y = 0.1249 - 0.0004 \text{ (flow)} + 0.1524 \text{ (loading)} - 0.0004 \text{ (flow x loading)}$$

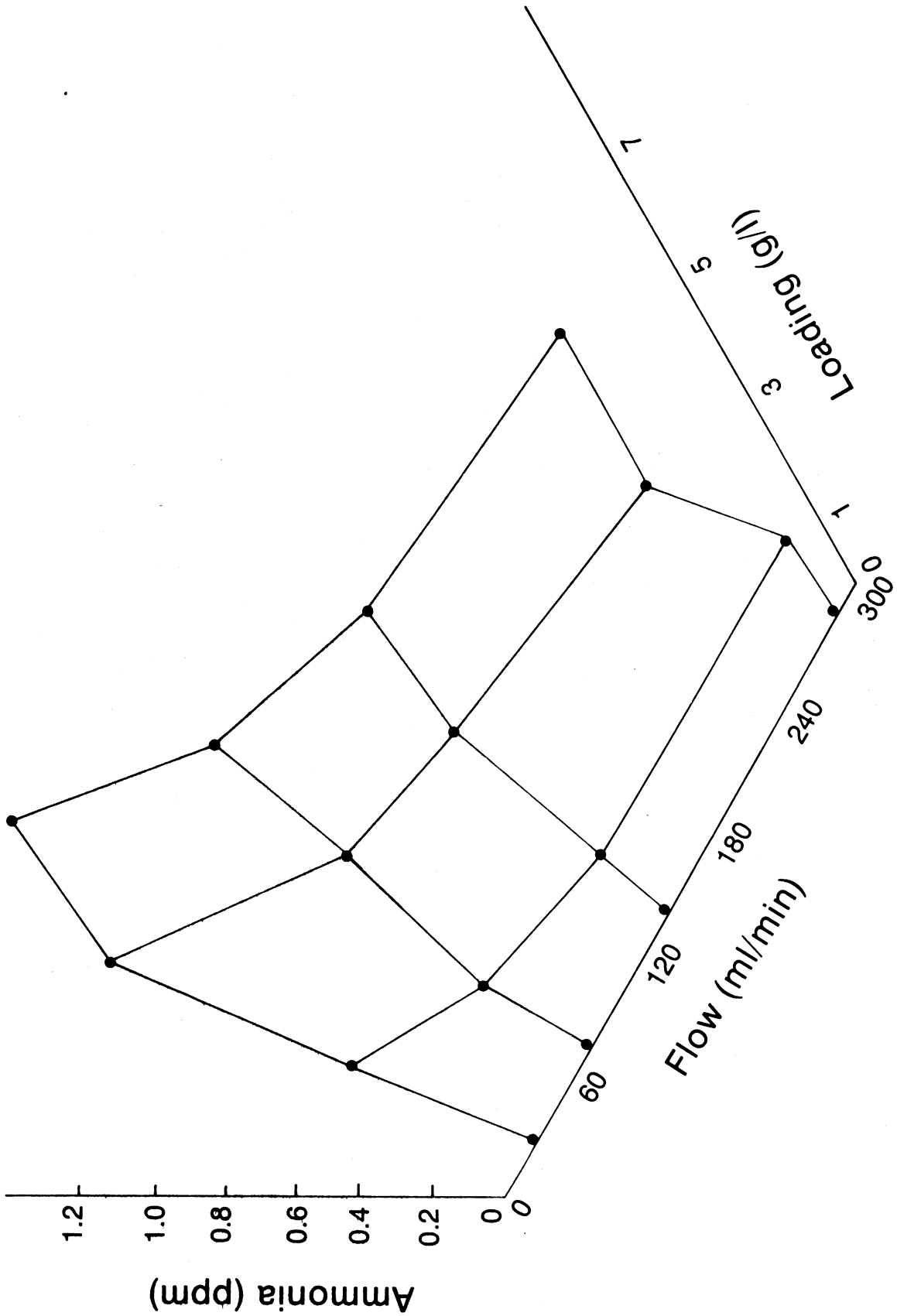

Ammonia (ppm)
0
0.2
0.4
0.6
0.8
1.0
1.2
Flow (ml/min)
60
120
180
240
300
Loading (g/l)
1
3
5
7

Table 11. Water flow, biomass and average oxygen and ammonia (29 and 31 day readings) for the rainbow trout loading vs. flow study.

Aquaria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Water flow (ml/min)	32	79	140	295	274	136	71	34	27	78	137	257	295	131	59	27
Beginning loading (g/l)	5.0	3.0	3.0	0	5.0	1.0	5.0	1.0	0	1.0	5.0	3.0	1.0	0	0	3.0
Ending loading (g/l)	5.0	4.6	5.2	0	8.8	2.2	6.3	1.8	0	2.0	7.3	5.4	2.0	0	0	3.7
Oxygen (mg/l)	2.6	3.5	4.1	8.8	3.7	6.1	2.7	3.9	8.9	5.0	3.1	5.4	7.5	8.9	9.2	2.9
Ammonia (mg/l)	0.8	0.4	0.3	0	.25	.09	.55	.45	0	.2	.35	.15	.09	0	0	.9

the population of the aquarium is not sampled until the end of the study.

Sample Size

It is important that an adequate number of samples are taken from each exposure on each sample date. The seven fish sampled in this study on the twenty-eighth day of exposure were used to calculate the required sample number to achieve a 95% confidence limit with the amount of variance present in this study (Snedecor and Cochran 1967). The statistically derived sample number was 4.6 organisms. For the amount of variation in this study the sample size of 7 was more than adequate. In future studies a reduction of sample numbers to 5 or 6 may be desirable to eliminate unnecessary work and expense. However, from preliminary research, Mayer (personal communication) has hypothesized that the sample size should be determined from each class of contaminants since variation tends to be larger with more degradable types of chemicals, especially during elimination.

Reproducibility

Two indications of reproducibility were calculated from this study. First, the coefficient of variation (relative standard deviation) for each exposure and replicate was calculated. The "food + water" exposure

clearly displayed less relative variance than did either of the other exposures.

Exposure	Trout, 28-day mean TBR (ppm)	Coefficient of variation
Food	0.28	27%
Food	0.29	22%
Water	2.51	14%
Water	3.02	16%
Food + water	2.36	9%
Food + water	2.62	8%

The second index is the intraclass correlation which identified where the greatest amount of variation exists. From this study the variations associated with each aquarium, between replicates, and due to treatments were estimated. The variation within each aquarium and between replicates was about equal in this exposure. The variation between replicates could even be reduced by equalizing the differences in exposure concentration reflected in Fig. 6, Table 6. The variation indicated by the different treatments was about 100 times greater than that within each aquarium. In this study the variation between replicates and within aquaria were minor compared to treatment effects indicating that the results of this model food chain would be highly reproducible.

Reinert et al. (1974) found that rainbow trout exposed to an average concentration of 137 ng/l (pptr) of DDT in water at 10 C for 4 weeks contained a total DDT and DDE residue of 2.3 µg/g (ppm). Even though the fish in Reinert's study weighed over 30 g, their TBR compares closely with residues in trout exposed for 4 weeks (2.4 and 3.0 µg/g, ppm) in this model food chain. This is evidence that this model food chain could be reproducible when conducted in different laboratories or by different investigators.

Reproducibility is an important factor in any laboratory procedure. This study was not intended to generate data on all of the possible sources of variation for a complete estimate of reproducibility. For instance, this exposure with one replicate did not include variations that might be induced by changing toxicants, sources of fish, or times of the year. However, the data from this study can be used to help evaluate these sources by expanded programs.

Model Design

A laboratory model food chain should have realistic components (i.e., naturally-occurring food items offered to a predator). The maple leaf-scud-rainbow trout food chain is a widely-occurring association, although in nature the detrital material may be a combination of plant and animal materials in addition to leaves. Besides realistic food

chain components, relevant concentrations of toxicant should be used. Johnson and Schoettger (1975) recommend selection of concentrations on the basis of acute toxicity, recommended use rates, or projected environmental concentrations. Acute toxicity data are often the most readily obtainable of the three, and a factor of 1/10 to 1/1000 of the LC50 value for the most sensitive food chain species used has been recommended (Johnson and Schoettger 1975). However, Mayer (personal communication) cautions against using a factor of the LC50 value because of varying slopes in mortality curves between different chemicals. Mayer (1976) also found that the magnitude of chemical uptake in fish could be greatly changed simply by exposure concentration alone.

A biological model should also provide rapid testing so that results are readily available for decision-making processes in the control of hazardous chemicals. The choice of exposure length is important since it is functional in determining the total time required for the study. Duration of the exposure for this model was 14 days for scud and 28 days for rainbow trout with an equivalent elimination period for each. In general, a longer exposure has a potentially greater chance of producing more information than a shorter one. However, both organisms can be easily maintained in aquaria for this period of time and the calculated total length of the laboratory work,

given even limited facilities and staffing, would be a maximum of 65 to 100 days per study. Yet a 14- and 28-day exposure and withdrawal for the respective organisms was long enough to establish uptake, metabolism, and elimination trends.

Finally, a good biological model must be relative to the environment and reproducible. The relativeness of this model will be reflected in how well it predicts possible movements and persistence of a chemical residue in the environment. The most severe test of reproducibility is that comparison of data between laboratories and investigators. It is not difficult to compare the trends of uptake and elimination between model food chain studies and between models and the environment, such as in Metcalf et al. (1971); however, applying a statistical test on the same to determine reproducibility and relativeness is very difficult. It may be that not enough is known about the influence of environmental variables on residues to allow an analytical comparison of model laboratory studies to chemical dynamics in natural food chains. This study should, however, provide a baseline for comparison of other compounds, and a basis for analysis of reproducibility in the future.

Chapter V

CONCLUSIONS

1. A working model food chain of leaf-scud-trout has been elaborated.

2. This model food chain is easily adaptable to laboratory conditions. The selection and association of organisms are sound and no major flaws in the design were observed. More developmental work on the detrital material would improve the model.

3. DDT displays definite trends of uptake, elimination, and metabolism in this food chain. The data recorded here provide a baseline with which other chemicals can be compared.

4. Within the limits of this study, the model has displayed adequate reproducibility to indicate potential use as a low cost, rapid, relevant laboratory tool which may be capable of predicting trends in chemical dynamics in aquatic ecosystems.

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The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

^{14}C-DDT DYNAMICS IN A COLD WATER MODEL FOOD CHAIN

presented by Michael J. Nevins

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance.

Robert A. Campbell

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