STUDIES ON ADSORPTION AND MICROBIAL DEGRADATION OF THE PESTICIDES ISOPROPYL N-(3-CHLOROPHENYL) CARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID IN AQUEOUS SOLUTIONS

Thesis by

Henry Gerard Schwartz, Jr.

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1966

(Submitted November 12, 1965)

ACKNOWLEDGMENTS

The author would like to express his appreciation for the encouragement and guidance offered by Dr. William R. Samples throughout the course of this research. The writer is especially indebted to Dr. James J. Morgan and Dr. Jack E. McKee for their helpful criticism of this manuscript.

The radioactivity counting equipment was made available through the courtesy of Dr. Justine Garvey. The writer gratefully acknowledges Dr. Garvey's suggestions pertaining to sample preparations.

To all of the above and to Dr. Henry Borsook, Dr. Sheldon K. Friedlander, Dr. Andrew L. Gram, and Dr. Wheeler J. North, the author expresses his gratitude for reviewing this dissertation.

The graduate study culminated by this dissertation was supported by a Training Grant from the U.S. Public Health Service. Certain chemicals used in the research were provided by the Pittsburgh Plate Glass Company. Their assistance in this regard is sincerely appreciated.

For her assistance in preparing this manuscript and for her perseverance during the past three years, the author is forever indebted to his wife, Sally.

ABSTRACT

The adsorption and microbial degradation in aqueous solution of two selected pesticides, isopropyl N-(3-chlorophenyl) carbamate (CIPC) and 2,4-dichlorophenoxyacetic acid (2,4-D), were studied to better understand the influence of these processes on the persistence of pesticides in natural waters. Based on the experimental results, certain inferences have been made concerning natural water systems. Radioactive tracer techniques were utilized to follow the adsorption and degradation of the two pesticides. Additional information about the disappearance of the phenyl carbon atoms from CIPC adaptation cultures was obtained from ultraviolet spectra.

2,4-D was found strongly resistant to microbial degradation in a liquid medium of mineral salts. Less than 40 percent of the acetic acid portion of the molecule disappeared from solution in six months, even in the presence of additional sources of organic carbon. CIPC was degraded much more rapidly than 2,4-D. The isopropyl segment of the CIPC molecule was completely metabolized. Metabolism of the carbon atoms in the ring structure ceased after 40 to 60 percent of the atoms had disappeared from solution. A partial metabolic pathway for the degradation of CIPC is proposed.

The clay minerals, illite, kaolinite, and montmorillonite, suspended in dilute pesticide solutions adsorbed very little 2,4-D or CIPC. CIPC was adsorbed extensively from water solutions with powdered activated carbon. The equilibrium for the adsorption of CIPC on activated carbon was represented closely by a Freundlich isotherm. The adsorption appeared to involve physical or weak chemical bonding.

There was some indication that the adsorption of CIPC on activated carbon in a well-stirred system was diffusion controlled.

The addition of activated carbon decreased the overall rate of degradation of CIPC. It is proposed that the decomposition of CIPC in the presence of powdered activated carbon occurs principally in the aqueous phase or at the interface between the liquid and the exterior surface of the activated carbon. As CIPC is degraded in solution, additional quantities desorb. The adsorption equilibrium of the activated carbon-CIPC system is influenced by the presence of bacterial cells and metabolic intermediates.

TABLE OF CONTENTS

TITLE

TABLE OF CONTENTS (Cont'd)

TABLE OF CONTENTS (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF FIGURES (Cont'd)

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF TABLES (Cont'd)

CHAPTER 1

INTRODUCTION

Man has used poisons for many centuries to kill his own and other forms of life. Until the last two or three decades, however, the only poisons available were those obtained from nature. With the advent of modern, organic chemical synthesis, man has learned how to create a wide spectrum of complex compounds possessing toxic properties. Synthetic organic pesticides, the progenies of this chemical revolution, comprise a variety of insecticides, herbicides, fungicides, rodenticides, and many other substances toxic to specific plants and animals.

Prior to World War II, the principal economic poisons were either inorganic compounds such as those containing arsenic or naturally occurring organic substances typified by rotenone. The discovery of the insecticidal properties of dichloro-diphenyl-trichloroethane (DDT) and the herbicidal nature of 2,4-dichlorophenoxyacetic acid (2,4-D) in the early 1940's ushered in the era of the synthetic organic pesticides.

Since that time the number, variety, complexity and total production of synthetic pesticides have grown at an amazing rate. From 1947 to 1963, the production of synthetic organic pesticides increased more than five-fold to approximately 750 million pounds annually (1). It is to be expected that the production and sale of these chemicals will continue to expand at a rapid rate. Improved agricultural yields, eradication of disease vectors, and control of nuisance organisms provide an immense market for these compounds.

Without doubt, the production of the present chemicals will continue and new exotic substances will be developed.

Many of the pesticides currently being employed are resistant to degradation by chemical and biological agents. It is not surprising, therefore, that small amounts of these compounds have been isolated from many phases of the environment, including water supplies. By their very nature and purpose, synthetic organic pesticides are strong poisons to one or more living species. As such, their presence even in minute quantities in a water supply creates a potential hazard to man's health and well-being. Although pollution of water by pesticides has not resulted in any known cases of human poisoning, many instances of fish kills attributable to pesticide contamination have been reported in the literature.

The recent massive fish mortalities in the lower Mississippi River point out the urgent need for basic research into the fate of pesticides in water. At present, very little knowledge is available concerning the physical, chemical, and biological interactions between pesticides and the aqueous environment. It is the general purpose of this investigation to ascertain the fundamental factors influencing the ultimate fate of selected organic pesticides in a water environment. In particular, the research concerns the effects of microbial activity and adsorption processes on the degradation and removal of two herbicides from water. Specific objectives of the research are described in Section 2.4, Chapter 2.

CHAPTER 2

PESTICIDES IN THE ENVIRONMENT

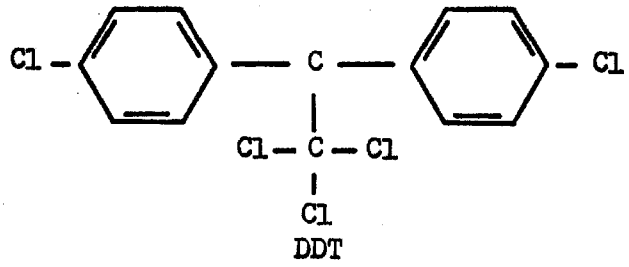
THEIR CHARACTERISTICS AND BEHAVIOR

2.1 Pesticide Chemistry and Toxicity

Many chemical compounds are employed as pesticides. In 1960 more than 200 synthetic organic pesticides were being marketed under thousands of brand names (2)(3). These compounds are often classified into two primary groups, the chlorinated hydrocarbon and the organic phosphorus pesticides, as well as a number of smaller categories, notably the carbamate poisons.

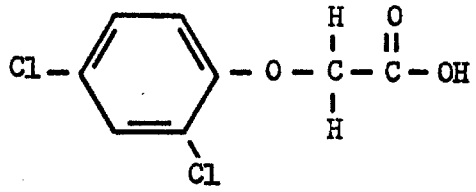
The largest volume of pesticides produced and sold comprises the chlorinated hydrocarbons. These are compounds composed of hydrogen, carbon, and chlorine atoms. Among the numerous economic poisons embraced by this category are the insecticides DDT, benzene hexachloride (BHC), aldrin, and dieldrin. Also included in this broad classification are compounds such as 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). In the strictest sense, the latter pesticides are not pure chlorinated hydrocarbons because they also contain oxygen atoms. The molecular structures of DDT and 2,4-D are shown in Figure 2-1.

The second major category is that of the organic phosphorus pesticides. This group is distinguished by the presence of a thiophosphorus linkage in the molecular structure. Malathion, chlorothion, and parathion are insecticides typical of this group of compounds. The molecular structure of parathion is presented in Figure 2-1.



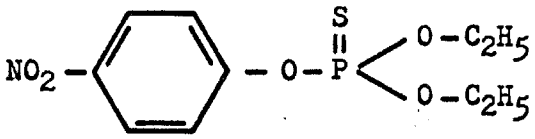
DDT

Dichloro-diphenyl-trichloroethane



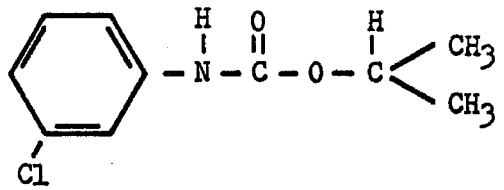
2,4-D

2,4-Dichlorophenoxyacetic acid



Parathion

0,0-diethyl-0-p-nitrophenyl phosphorothioate



CIPC

Isopropyl N-(3-chlorophenyl) carbamate

Figure 2-1. Molecular structure of typical synthetic organic pesticides(3).

Among the categories representing lesser production and sales volume, the carbamates and thiocarbamates are of particular interest in this research. These compounds are typified by the $-N-\overset{O}{\overset{\|}{C}}-O-$ or $-N-\overset{S}{\overset{\|}{C}}-S-$ linkage respectively. The molecular structure of isopropyl N-(3-chlorophenyl) carbamate (CIPC), a widely used herbicide, is shown in Figure 2-1.

The chemical behavior of pesticides in each of the three categories mentioned above varies widely. Certain generalizations can be made for each group, but it should be emphasized that these statements may not be valid for a particular compound. Typically, the chlorinated hydrocarbons have very low solubility in water, often less than 0.2 milligrams per liter, and they are relatively stable to chemical and biological attack. On the other hand, the organic phosphorus and carbamate pesticides are generally quite soluble in water. The organic phosphorus compounds often are easily hydrolyzed with the notable exception of parathion while the carbamates are somewhat more stable to chemical action. A more detailed analysis of the chemistry of these compounds can be obtained from texts by Frear (4), DeOng (5), Gunther and Blinn (6), Metcalf (7), and Shepard (8).

The nature of the job for which synthetic pesticides are employed demands that these substances be chemical poisons. Unfortunately their toxicological effects generally are not limited to the living species for which they are intended. Certain other forms of life may be susceptible.

Numerous experiments have been conducted to determine the acute and chronic toxicities as well as the carcinogenic effects of these pesticides on various animate species. Much of the work has dealt with the acute toxicities of various compounds for fish and test animals, principally rats. The values for toxicities and solubilities shown in Table 2-1 represent a compilation of data from Henderson, Tarzwell, Pickering, Surber, and Lemke (9)(10)(11)(12)(13); Frear (4); Durham (14); Fitzhugh (15); McKee and Wolf (16); and manufacturers literature (17).

Results obtained from animal toxicity studies do not always reflect the toxicity of a pesticide to man. For instance, the toxicity of BHC to rats is 1000 milligrams per kilogram, as shown in Table 2-1, but a fatal case of accidental poisoning to a small child occurred at a dosage of 180 milligrams per kilogram (18). A few controlled experiments on the toxicity of pesticides to humans have been performed, but most of the data on the effect of pesticides on man have been accumulated from reported instances of accidental poisonings. Much of this information is found in the Clinical Handbook on Economic Poisons (18).

2.2 The Soil Environment

The largest quantity of pesticides is used for crop dusting and spraying. Some of these chemicals reach the intended crops but most of them are deposited on the soil. On either the crops or the soil they may be washed by subsequent rainfall or irrigation into the nearest watercourse, or they may percolate through the soil into the

Table 2-1

The Toxicity of Selected Synthetic Organic Pesticides to Fish and Mammals

Compound	Fathead Minnows 96-hour TLm mg/l*	Rats Oral LD_{50} mg/kg**	Solubility in water mg/l at 25°C
Endrin	0.0013	5-45	0.1
Toxaphene	0.0051	69-90	-
Dieldrin	0.016	46-60	0.1
Aldrin	0.028	39-67	0.1
DDT	0.034	113	0.1
Lindane	0.056	88-125	10
BHC	2.0	1000	5-10
Para-oxon	0.33	3-3.5	-
Parathion	1.3-1.4	13	20
Chlorothion	3.0-3.2	880	40
Methyl Parathion	8.0-8.3	14-42	50
Malathion	12.5-23.0	1375	145
Guthion	0.093	13	-
CIPC	-	1500-7500	108
Endothal	320-610	-	-
Diquat	14-130	400-440	-
Dalapon	290-390	-	45,000

*TL_m= median tolerance limit; the concentration that results in 50% mortality of the test fish after 96 hours exposure.

**LD_{50}= median lethal dose from a single oral administration.

ground-water table. Whether the pesticide remains on the plant, on or near the surface of the soil, in the ground water, or in a stream or lake, its ultimate fate is a matter of concern to ecologists and environmental health engineers. In order to assess the hazards presented by a particular poison, it is necessary to know how much of the toxic material remains and how it is distributed in the environment.

A portion of a given pesticide may volatilize, but such losses to the atmosphere are minor except immediately following the spraying operation. A significant portion of the applied chemical remains as a residue on the plants and should be removed from all produce prior to its consumption. Certain pesticides, the systemic compounds in particular, are absorbed by the roots and are transmitted throughout the body of the plant. In many instances, these systemic poisons are metabolized by the plant into non-toxic end products. The physical, chemical, and biological factors that affect pesticides in the soil are similar to phenomena associated with aqueous environments. With this relationship in mind, a brief survey of the agricultural literature has been included herein.

From an agronomist's point of view, pesticides should fulfill certain requirements. If the pesticide is expected to work on organisms in or on the soil, it must remain for a period of time sufficient to accomplish its purpose. On the other hand, if the pesticide persists too long it may interfere with future crops. As a result, a considerable amount of work has been performed on the persistence of pesticide residues. This type of research, to a

large degree, has been concerned only with the extent and rate of disappearance from soil of pesticides and their toxic derivatives rather than the mechanisms involved.

It is apparent that such factors as application rate, type of soil, climatic conditions, and molecular structure affect the persistence. According to Newman et al. (19), isopropyl N-phenyl carbamate (IPC) disappears completely in two to four weeks. The addition of a chlorine atom to the molecule forms CIPC which persists for one to eight months in the soil (17). In the latter instance, CIPC residues remained in the soil for longer periods in cold weather and disappeared rapidly during the summer months. DDT was found by Jones (20) to persist longer in a sandy loam with low organic content than in a fertile black loam. With both soils, the DDT concentration showed little change during the first year, but appreciable reduction occurred during the second and third years. The percentage of DDT that disappeared from the test soils was found to be greatest with low initial concentrations of DDT in the highly organic soil.

The agricultural literature abounds with similar data for a wide variety of economic poisons. It is quite difficult to compare these findings owing to the differences in experimental techniques and environmental conditions. Alexander (21) has summarized some of these data on pesticide persistence in soil as shown in Table 2-2. A glossary of the chemical nomenclature for these compounds is contained in Appendix I. This table indicates that chlorinated hydrocarbon insecticides such as DDT, dieldrin, chlordane,

and BHC persist for years while the herbicides disappear quite rapidly.

Table 2-2

Pesticide Persistence in Soils (21)

Period of persistence					
<2 mo.	2-4 mo.	>6 mo.	>3 yr.	>5 yr.	>11 yr.
Amiben	MCPA	2,4,5-T	Dieldrin	Parathion	BHC
Amitrol	TCA	2,3,6-TBA	Toxaphene	Lead Arsenate	Chlordane
2,4-D	Monuron	Triazines	DDT		
IPC					
Dalapon					
Neburon					

The fact that a particular pesticide persists for a long period of time in a soil suggests that the compound is strongly adsorbed by the soil. Bailey and White (22) published an excellent review on the phenomena of adsorption and desorption of pesticides by soil colloids. The heavier textured and organic soils have been found to permit less leaching than the lighter textured soils such as sands and loams. This tendency was related to the relative cation-exchange capacities and surface areas of the various soil constituents. Organic matter, vermiculite, and montmorillonite possess high cation-exchange capacities, e.g. 80-400 milliequivalents per 100 grams, and large surface areas, e.g. 500-800 square meters per gram. Illite and chlorite have lower cation-exchange capacities (10-40 milliequivalents per 100 grams) and smaller surface areas (25-100

square meters per gram). Kaolinite with a cation-exchange capacity of 3-15 milliequivalents per 100 grams and a surface area of 7-30 square meters per gram resides on the low end of the spectrum. Thus, organic matter, vermiculite, and montmorillonite would be expected to have good adsorption characteristics both for pesticides of a cationic nature and those which are subject to physical adsorption. Indeed, vermiculite and montmorillonite were found to adsorb pesticides better than illite, chlorite, and kaolinite. Additional data from desorption studies indicated that pesticides adsorbed on organic matter are more strongly bound than when adsorbed on mineral constituents (22).

As might be expected, the adsorption on soil colloids is inversely related to the solubility of the pesticide. Furthermore, adsorption is dependent on pH according to Bailey and White (22). In general, the adsorption of a pesticide increases as the pH decreases. This phenomenon, in turn, reflects the degree of dissociation of the pesticide. The adsorption of 2,4-D on a hydrogen cation-exchanger, for example, was nearly twice as great at pH 2.5 as at pH 3.3. Temperature exerts an additional effect. Since most adsorption processes are exothermic and desorption is endothermic, an increase in temperature normally would reduce adsorption.

If a particular pesticide is not adsorbed by the soil and if it is resistant to chemical and biological attack, it will be leached eventually from the soil into the groundwater. For purposes of the present discussion, the compound is considered to have been

removed from the soil environment. The compounds which remain in the upper layers of soil as a result of adsorption may be subjected to chemical and biological degradation. Certain pesticides undergo chemical reactions in the soil such as the hydrolysis of most organic phosphorus compounds, the herbicide IPC (17), and the nematocide D-D (23), a dichloropropane-dichloropropene mixture. Many compounds, however, are quite stable to chemical attack and their toxic effects are gradually eliminated only by microbial action.

Much of the work in soil microbiology has dealt with the chlorophenoxyacetic acid herbicides and closely related compounds. Audus (24)(25) succeeded in isolating a bacterium of the Bacterium globiforme group which degraded 2,4-D. His initial studies indicated that 14 to 28 days were required for adaptation prior to 2,4-D degradation. 2,4-D disappeared from a soil inoculated with the adapted organisms within one day. Further studies (26) on this organism compared growth curves based on plate counts to toxicity measurements over the same time interval. After the growth curve of Bacterium globiforme had reached its plateau, the phytotoxicity of the 2,4-D remained essentially unaltered. At some later time, the toxicity rapidly dropped to zero. These experiments in liquid media of mineral salts indicated the presence of an intermediate product possessing a toxicity similar to that of 2,4-D. It was demonstrated that the addition of 2,4-D to cultures which had attained full growth, but which had not begun to detoxify the original 2,4-D, did not affect the time required for complete reduction of the phytotoxicity. Addition of 2,4-D after initial detoxication

again produced a lag period prior to being rendered harmless to plants. The latter result reflects the presence of an unstable enzyme system which had been adapted to some intermediate product.

Investigations performed with a soil system showed that once the soil acquired the ability to degrade 2,4-D it retained this capability for long periods. Coupled with the data on the adaptive nature of the Bacterium globiforme isolate, this information would indicate the presence of other soil organisms capable of degrading this herbicide. Indeed several different microorganisms have been isolated which were capable of metabolizing 2,4-D (27)(28)(29). Steenson and Walker (29) isolated three bacteria that degraded 2,4-D and that also utilized p-chlorophenoxyacetic acid. The first isolate was a strain of Flavobacterium peregrinum n.sp. which utilized p-chlorophenoxyacetic acid as well as 2,4-D. Warburg respirometer studies on this organism indicated that in its degradation of 2,4-D, eleven atoms of oxygen were required for each 2,4-D molecule. The theoretical oxygen requirement for complete biological oxidation of 2,4-D to carbon dioxide, hydrochloric acid, and water would be fifteen atoms. Measurements of the ionic chloride released during metabolism accounted for 76 percent of the chlorine originally associated with the 2,4-D. A short chain molecule, probably consisting of two or three carbon atoms and a chlorine atom, was postulated to be an end product. On the basis of other Warburg respirometer data and the principle of simultaneous adaptation, Steenson and Walker (29) eliminated p-chlorophenoxyacetic

acid, 2,4-dichlorophenol, 2-hydroxy-4-chlorophenoxyacetic acid, and 6-hydroxy-2,4-dichlorophenoxyacetic acid as possible intermediate products. The results of Fernly and Evans (30), however, indicate that the preceding findings are not necessarily true for other bacteria. Under certain conditions, they found that a pseudomonae species would yield 2,4-dichlorophenol as a metabolic intermediate in 2,4-D degradation. In addition, they isolated α-monochloromuconic acid as an intermediate.

Studies have been conducted, also, on other chlorophenoxyacetic acid compounds. Both Steenson and Walker (29) and Audus (31) found that 4-chloro-2-methylphenoxyacetic acid (MCPA) was degraded in about 70 days. Using chromatography, ultraviolet spectroscopy, and chemical titrations, Gaunt and Evans (32) were able to deduce the following metabolic pathway for MCPA with a gram-negative soil bacterium which they had isolated:

MCPA ⟶ 6-hydroxy-MCPA

⟶ 5-chloro-3-methylcatechol

⟶ α-methyl-γ-chloromuconic acid

⟶ α-methyl-γ-carboxymethylene-Δ^{α}-bulenolide

⟶ α-methylmaleyl acetate

⟶ smaller fragments ending in terminal respiratory cycle.

Investigations by Steenson and Walker (29) and Audus (31) have shown that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was degraded in a soil environment in 150 to 270 days. Metabolism of

p-chlorophenoxyacetic acid required about the same amount of time as 2,4-D (29). Respirometer studies with a species of Flavobacterium peregrinum gave evidence of 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol as intermediates in the biological breakdown of p-chlorophenoxyacetic acid (29). These same intermediates were identified by Evans and Smith (27) using a pseudomonas bacterium. Furthermore, β-chloromuconic acid was isolated from this same degradation process (33).

An excellent review of the investigations on the metabolism of phenoxyalkyl carboxylic acids, as well as some new results, was furnished by Alexander (21). Two different metabolic pathways appear to be present in the microbial decomposition of these compounds. Cultures of certain types of bacteria initially attack these pesticides by beta-oxidation of the side chain. Such a process, typical of strains of Nocardia, temporarily may yield intermediates of strong phytotoxicity. For example, the herbicide sodium 2,4-dichlorophenoxyethylsulfate is converted to 2,4-D, according to Audus (34).

The second metabolic process involves the breaking of the ether linkage by a Flavobacterium. An example of this metabolic pathway is shown in Figure 2-2 for 4-(2,4-dichlorophenoxy) butyric acid, i.e., 4-(2,4-DB) (21). Additional results substantiated this type of microbial action for other similar herbicides.

The degradation of naphthalene acetic acid, a plant growth-regulator, was investigated by Proctor (35). Again employing

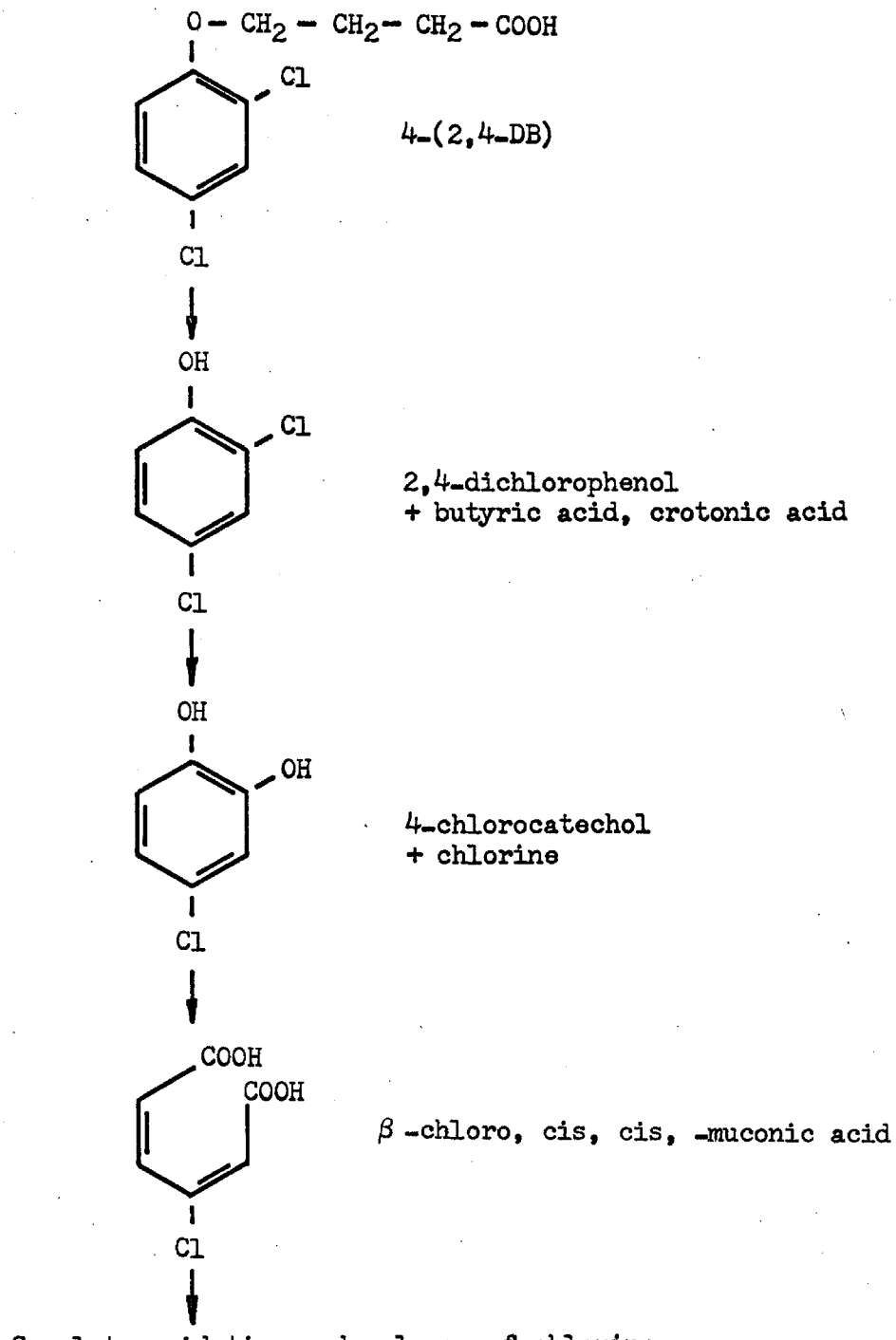


Figure 2-2. Proposed metabolic pathway in the degradation of 4-(2,4-DB) by Flavobacterium (21).

the principle of simultaneous adaptation combined with confirmatory tests using paper chromatography, the author was able to identify the metabolic intermediates. Briefly, the metabolic pathway he proposed is as follows:

α-naphthalene acetic acid ⟶ α-napthol

⟶ salicylic acid ⟶ catechol

⟶ complete respiratory cycle.

Recent investigations carried out in Germany by Korte et al. (36)(37)(38), on the drin-insecticides including aldrin, dieldrin, and telodrin, indicate that metabolism of these compounds does occur in certain fungi, mosquito larvae, and mammals. Aldrin, as had previously been known, was completely transformed into dieldrin within four months. Furthermore, it was converted into at least four other products. The diol of aldrin and the lactone of telodrin were identified. A somewhat surprising feature of the results was that, in general, the metabolites were identical for a particular compound for the fungi, mosquito larvae, and mammals.

The problem of pesticides in the soil also must be considered from the point of view of the effects that might be exerted on the microbial population by these poisons. Martin (39) studied these effects using DDT, aldrin, dieldrin, endrin, heptachlor, toxaphene, and lindane. The amounts of pesticide employed exceeded the normal application rate. In all cases, the numbers of soil bacteria and fungi were unaffected by the insecticides, and nitrification proceeded in a normal fashion. Shaw and Robinson (40) likewise found

that nitrification was unimpaired by the herbicide 2,4-D. On the other hand, 4,6-dinitro-o-cresol (DNOC) appeared to sterilize the soil partially and change the type of microflora in experiments by Briunsma (41). According to Alexander (21), the application of insecticides or herbicides to a soil, in general, does not have a profound adverse effect on the microbial community.

This brief review of the literature in the agricultural field by no means encompasses the full extent of the research on pesticides. It does serve to give insight into the complexities of the pesticide problem and to supply a foundation for research in another environment, that of water.

2.3 The Water Environment

Despite the factors in the soil that tend to render pesticides harmless, it is inevitable that limited amounts of the more stable compounds will reach water supplies. The leaching of these poisons through the soil will pollute ground waters, and runoff from treated areas will defile surface streams. Contamination by direct aerial application, whether by intent or accident, and the indiscriminate disposal of manufacturing wastes increases the probabilty of synthetic organic pesticides reaching water supplies.

Evidence that pesticides are present in surface waters has been limited until the last few years. Isolated instances of confirmed pesticide pollution have been reported in the literature (3) (42)(43). Recent refinements in analytical techniques have permitted more detailed surveys of water supplies. The most

comprehensive investigation of surface water pollution by pesticides was performed by Weaver et al. (44). Samples were taken in September, 1964 at 96 stations representing portions of the twelve major river basins in the U.S. Each sample was analyzed for the presence of the following nine chlorinated hydrocarbon pesticides: dieldrin, endrin, aldrin, DDT, DDD, DDE, heptachlor, heptachlor epoxide, and BHC. These compounds constitute a major portion of the pesticides sold in the U.S. The analytical procedure involved liquid-liquid extraction, thin-layer chromatography, and microcoulometric gas chromatography (45). This combination of techniques was sensitive to concentrations ranging from 0.002 to 0.075 micrograms per liter depending on the particular compound being identified. Dieldrin, endrin, DDT, and DDE were isolated from some samples in each of the twelve river basins. At least 50 percent of the samples from each basin contained dieldrin with decreasing occurrences of endrin, DDT, and DDE respectively. Heptachlor and aldrin were present in nine of the basins. DDD and BHC were observed at only one station while heptachlor epoxide was not found in any sample. The maximum concentrations present in any one sample were as follows:

dieldrin	0.118 micrograms per liter
endrin	0.094 micrograms per liter
DDT	0.087 micrograms per liter
DDE	0.015 micrograms per liter
DDD	0.083 micrograms per liter
aldrin	0.085 micrograms per liter

The pollution of water by pesticides has resulted in a considerable number of fish kills throughout this nation. A survey of 38 states conducted by the Public Health Service reported 382 fish kills resulting from pollution in 1962 (46). Of this total, 49 were directly attributable to pesticide contamination. Unknown pollutants caused an additional 73 fish kills. In 1955, an estimated 20 to 30 tons of fish were killed in a marsh area in St. Lucie County, Florida which had been sprayed with dieldrin to control sand fly larvae (43)(47). Ninety-one percent of the young salmon in the Miramichi River died shortly after DDT was applied to a forest in New Brunswick, Canada in 1956 (2)(47). A Maine forest was treated with DDT in 1958 resulting in a moderate fish kill (2)(42)(47). Three months later, trout were collected, analyzed, and found to contain 2.9 to 198 milligrams of DDT per kilogram. More recently, massive fish kills occurred in the lower Mississippi River (48)(49)(50)(51). Extending over a period of several years, these kills apparently resulted from the discharge of wastes from a plant near Memphis manufacturing endrin. Endrin concentrations from seven to 214 micrograms per liter were found in the river water near West Memphis. As much as 220 micrograms per liter was present in the blood of dead catfish. One unusual and, as yet, unexplained aspect of the Mississippi River incidents is the occurence of the kills near New Orleans while the pesticide source is at Memphis, several hundred miles north.

A study was performed in 1962 by Welch and Spindler (52) on the effects of DDT on fish and aquatic insects following a spraying

operation. DDT was applied at a rate of 0.5 pounds per acre to a forested area in Montana for the control of spruce budworm. Care was taken to leave a DDT-free zone one-quarter mile on either side of all streams in the area. The maximum DDT concentration was 0.020 milligrams per liter in the Little Boulder River and the presence of the pesticide was evident for seven days following spraying. Significant reductions in aquatic invertebrates occurred at some sampling points while in many areas losses were negligible. Small numbers of dead fish were recovered and contained 0.5 milligrams of DDT per kilogram of body weight.

Adverse effects on wildlife are not limited to fish. Treatment of Clear Lake in Northern California with DDD in 1949, 1954, and 1957 resulted in the death of several hundred Western Grebes (2)(47). Prior to that time Clear Lake was a favorite breeding place for these birds, but since 1957 no proliferation of this species has occurred there.

From a public-health standpoint, it is necessary to remove the pesticides from the water used for domestic supplies. Several investigations have been made on the removal of selected economic poisons from water by conventional water treatment processes. In 1945, Carollo (53) reported on the removal of DDT. At concentrations which exceed the solubility of DDT, above 0.1 milligrams per liter, coagulation followed by sedimentation produced up to 80 percent removal. The additional process of filtration increased this value to 97 percent. Only activated carbon insured complete removal of the DDT in solution.

A series of articles by Cohen et al. (54)(55)(56) contains the results of their research on the advisability of using fish poisons in water-supply reservoirs. An integral portion of this study dealt with the removal of the poisons rotenone, toxaphene, and sulfoxide. Activated carbon proved to be the most effective, single process for removing the compounds and, in addition, it removed the solvents and emulsifiers present in the commercial formulations. Chlorine and chlorine dioxide were instrumental only in the removal of rotenone. Alum coagulation was of no assistance with any of the poisons.

The removal of 2,4-D from a simulated river water was investigated previously by Schwartz (57). Various coagulants and coagulant aids proved ineffective in removing the herbicide. Once again, activated carbon was the only agent studied which satisfactorily eliminated the pesticide. Optimum removal of 2,4-D by activated carbon was accomplished at a pH of 3.0.

The most comprehensive research on the removal of pesticides from water supplies was reported by Robeck et al. (58). The six compounds studied were dieldrin, endrin, lindane, DDT, parathion, and the butoxy ethanol ester of 2,4,5-T. Actual river water from the Little Miami River was employed in these pilot-plant experiments. Chlorine and potassium permanganate had negligible effect on all of the pesticides except parathion. The latter substance was converted to the more toxic para-oxon by chlorine and potassium permanganate. Partial oxidation of the chlorinated compounds was obtained with large amounts of ozone. The series combination of coagulation,

sedimentation, and filtration removed most of the DDT; 55 percent or less of endrin, dieldrin, and 2,4,5-T; and little or no parathion and lindane. As expected, activated carbon significantly reduced the pesticide concentrations of all the compounds tested. Filter beds of activated carbon lowered the effluent pesticide concentrations to 0.01 micrograms per liter.

Buescher et al. (59) presented data on the chemical oxidation of lindane, aldrin, and dieldrin which are somewhat contrary to the previously discussed results. They found that chlorine, potassium permanganate, and ozone significantly reduced the concentration of aldrin. Dieldrin and lindane were influenced greatly by ozonation, but potassium permanganate exerted only a small effect on lindane. Aeration reduced the concentrations of all of the pesticides. The reasons for the contradictions between these two reports cannot be readily ascertained from the published information. They may lie in differences in analytical techniques, testing procedures, or the failure to identify the oxidation products. Although various chemical oxidants may well affect these economic poisons, complete oxidation to non-toxic end products does not appear likely.

Basic research on the physical, chemical, and biological phenomena associated with pesticides in water is limited. Faust and Aly (60)(61) have studied certain natural processes in surface waters relative to their effects on 2,4-D and related esters. A portion of their research dealt with the sorption of the chemicals on clay minerals, specifically kaolinite, bentonite, and illite. The data for 2,4-D, 2,4-dichlorophenol, and the isopropyl, butyl, and isooctyl

esters of 2,4-D conformed to the Freundlich expression for adsorption. The amounts sorbed on the clays, however, were quite small. For an initial concentration of 5.0 milligrams per liter, 2,4-D and 2,4-dichlorophenol removals ranged from 0.02 to 0.14 milligrams per gram of clay. Additional data showed that 2,4-D was removed best by kaolinite while the other four compounds were sorbed more efficiently by bentonite. It was suggested that the superior sorption of 2,4-D on kaolinite might reflect the numerous positive sites present on this clay as opposed to the more negative nature or higher cation-exchange capacity of illite and bentonite. The relative capacities of the clays for the other compounds appeared to be related to the effective surface areas of the clays. Experiments also indicated that 2,4-D adsorption was dependent on salt concentration and pH. Low pH values and high salt concentrations enhanced adsorption. The small amounts sorbed in all instances, however, preclude this mechanism as an effective force for removing 2,4-D from water supplies.

A series of experiments on the ultraviolet irradiation of aqueous solutions of the five compounds was reported by the same authors (61). Under high pH conditions almost total decomposition was obtained in pure solutions. The presence of gross impurities in a natural water, however, would be expected to make significant reductions improbable. Experiments were performed with the calcium and magnesium salts of 2,4-D to determine their solubilities. Solubilities ranging from 9.5 to 25×10^{-3}M virtually eliminated the possibility of precipitating 2,4-D from natural waters.

The final phase of the aforementioned research concerned the biological degradation of the 2,4-D compounds. Bottom muds from lakes contained microorganisms that were capable of degrading 2,4-D. These experiments were performed by adding mud samples to a 20 milligram per liter solution of 2,4-D. Complete breakdown of the herbicide took 65 days for a virgin mud sample and 35 days for a mud from a lake previously treated with 2,4-D. Subsequently, further additions of 2,4-D were totally degraded in 10 to 20 days. Manometric studies on the adapted microflora indicated approximately 80 percent metabolism of 2,4-D within 24 hours. These results are consistent with the adaptive nature demonstrated by soil microorganisms which metabolize 2,4-D, as discussed in Section 2.2.

Experiments on lake water produced negative results. At a concentration of three milligrams per liter, 2,4-D persisted for more than 120 days, the duration of the test. On the other hand, 2,4-dichlorophenol solutions disappeared from the lake water within 30 days. A reduction of 50 percent was evident in six days. Under unaerated and unbuffered conditions the 2,4-dichlorophenol remained for considerably longer periods. It was the conclusion of the authors that the persistence of 2,4-D in surface waters might be sufficient to necessitate its removal at water treatment plants.

Chambers et al. (62)(63), examined the metabolism of a number of aromatic compounds. Widely assorted microflora were obtained from soil, compost, river mud, and sludge from a refinery waste lagoon. The microorganisms were able to degrade phenol, chlorophenols, nitrophenols, chloronitrophenols, methyl-substituted phenols, and

hydroxy phenols. Using phenol-adapted bacteria, manometric studies were performed on 104 aromatic compounds. The results showed that the addition of a nitro or chloro group to a phenol ring greatly increased its biological resistance. Dichlorophenols and dichloromethylphenols were more refractory than the respective monochlorocompounds. The benzene compounds followed the same general pattern. For example, aniline was readily metabolized while p-nitroaniline was quite resistant. The complete lists of degradable and resistant compounds are given in the publications.

In a series of articles, Bogan, Okey, and Vargas (64)(65)(66) attempted to ascertain the mechanisms involved in the microbial metabolism of pesticides. The initial phase of this work involved the attempted assimilation of a wide variety of pesticides by an unacclimated, activated sludge. It was established that most of the chlorinated hydrocarbon pesticides, including DDT, aldrin, dieldrin, endrin, and lindane, were not degraded by the microorganisms. The same finding was valid for the organic phosphorus compounds parathion, methyl parathion, malathion, tetraethyl pyrophosphate (TEPP), and diazinon. Aminotriazole also resisted degradation. Among the substances demonstrating partial breakdown were ziram, zineb, chlordane, heptachlor, and 2,4,5-T. Substantial metabolism was indicated for ferbam, maneb, 2,4-D and thanite.

Other experiments on the biologically resistant pesticides and their unsubstituted homologs led Bogan et al. to certain conclusions concerning the effect of molecular structure on persistence. If there were six or less carbon atoms between the position of the

chlorine substitution and the terminal carbon atom, microbial metabolism was adversely affected. In the case of chlorinated cyclic compounds, the presence of three or more chlorine atoms on the ring impaired the microbial degradation. This conclusion is essentially in agreement with Alexander (21) who concluded that the presence of a chlorine atom at the meta position (position 3 or 5) in the aromatic portion of a pesticide prohibited any substantial metabolism. Bogan and Okey (65)(66) hypothesized that the observed inhibition was related to the electronic effects produced in the ring by chlorine atoms. According to this hypothesis, chlorine reduces the electron density at the enzymatic reaction sites and, in effect, is competing with the enzymes for electrons.

The resistance of DDT was attributed to its steric configuration. In particular, the presence of a secondary carbon attached to the bridge carbon atom stopped degradation. Inclusion of phosphorus atoms in the molecular structure also prevented metabolism. The organic phosphorus compounds, therefore, are of a refractory nature. Most of the latter compounds hydrolyze to innocuous products in two to three days. An important exception is parathion which has a half-life of 120 days under neutral conditions.

Weber and Morris (67)(68)(69) have investigated the adsorption of alkylbenzenesulfonates and other adsorbates on activated carbon. The particle sizes of activated carbon selected for the study were: (1) passing a U.S. Standard Sieve number 50 and retained on a number 60 yielding a mean diameter of 0.273 millimeters and; (2) passing a number 100 and retained on a number 140 sieve giving a mean

diameter of 0.126 millimeters. Generally equilibrium was established only after a period of several weeks for these high-molecular-weight compounds. The adsorption rates were found to vary as the reciprocal of the diameter of adsorbent particle and the square root of the solute concentration. Other factors influencing the reaction rates were molecular size, structural configuration, and pH. Adsorption rates decreased with increasing molecular size and degree of branching in the side chains. Low pH values aided adsorption perhaps owing to changes in the carbon surfaces. Among the many compounds studied was DDT.

The authors concluded that the data on adsorption rates were consistent with the hypothesis of intraparticle transport acting as the rate-controlling step. Weber and Rumer (70) expanded upon this hypothesis. Previously, the equilibrium values for alkyl-benzenesulfonates had been shown to follow Langmuir's expression for monolayer, isothermal adsorption (69). This information combined with Fick's law of diffusion enabled the authors to calculate the diffusivity of the various compounds. The values were in the range of 3.2 to 8.3 x 10^{-7} square centimeters per second for alkyl groups of 10 carbons or more. The information contained in these reports on adsorption does not directly pertain, generally, to pesticides. It does represent fundamental research into the adsorption of organic substances from water and, therefore, it is important background material for the study of pesticides.

Pesticide research related to the water supply and water pollution fields is sparse. There is a great need for continuing research into the phenomena associated with pesticides in water. It is essential that the mechanisms of adsorption, the chemical oxidative and hydrolysis reactions, and the biological metabolism of this class of compounds be elucidated. Until such knowledge is obtained, a proper evaluation of the hazards created in water supplies cannot be made nor controls initiated.

2.4 Research Objectives

It has been the purpose of this research to investigate the effects of adsorption and microbial metabolism in an aqueous environment upon selected synthetic organic pesticides. Isopropyl-N-(3-chlorophenyl) carbamate (CIPC) and 2,4-dichlorophenoxyacetic acid (2,4-D) were chosen for this study. The specific objectives were as follows:

1. To ascertain the overall microbial degradation rates of CIPC and 2,4-D;
2. To study the microbial metabolism of CIPC;
3. To evaluate the adsorptive capacities of selected clay minerals for CIPC and 2,4-D;
4. To investigate the adsorption of CIPC on activated carbon;
5. To investigate the influence of activated carbon on the degradation of CIPC.

This information, it is hoped, will lead to a better understanding of the fate of these compounds in water supplies and assist in the evaluation of other pesticides.

CHAPTER 3

EXPERIMENTAL METHODS AND MATERIALS

This chapter contains a discussion of all the materials, analytical techniques, and experimental procedures used in the research. Insofar as possible, materials and techniques unique to one experimental series are included in the appropriate section on experimental procedures.

3.1 Chemical Reagents

The concentrations of pesticides used in these studies ranged from 0.1 to 10.0 milligrams per liter. In order to obtain accurate analyses and to trace the behavior of the pesticides, radioactively labeled 2,4-D and CIPC were utilized. Non-radioactive pesticides were used to dilute the radioactive species. These chemicals as well as others employed in this investigation are listed below.

2,4-Dichlorophenoxyacetic-2-C-^{14} acid - New England Nuclear Corporation, Number NEC-24; total weight, 2.6 milligrams; specific activity, 4.24 millicuries per millimole; total activity, 0.05 millicuries.

2,4-Dichlorophenoxyacetic acid - Eastman Organic Chemicals, Number 5532.

Isopropyl-C^{14}-N(3-chlorophenyl) carbamate - Pittsburgh Plate Glass Company, Chemical Division, Number IP-10; total weight, 19.4 milligrams; specific activity, 1.13 millicuries per millimole; total activity, 0.1 millicuries.

Isopropyl-C^{14}-N(3-chlorophenyl) carbamate - Pittsburgh Plate Glass Company, Number IP-14; total weight, 19.8 milligrams; specific activity, 1.13 millicuries per millimole; total activity, 0.1 millicuries.

Isopropyl N(3-chlorophenyl-C^{14}) carbamate - Pittsburgh Plate Glass Company, Number R-10; total weight, 17.8 milligrams; specific activity, 1.27 millicuries per millimole; total activity, 0.1 millicuries.

Isopropyl N(3-chlorophenyl-C^{14}) carbamate - Pittsburgh Plate Glass Company, Number R-14; total weight, 16.8 milligrams; specific activity, 1.27 millicuries per millimole; total activity, 0.1 millicuries.

Isopropyl-N(3-chlorophenyl) carbamate - Pittsburgh Plate Glass Company, 99 percent purity.

Sodium carbonate-C^{14} - International Chemical and Nuclear Corporation, Number 1-2060; specific activity, 58.0 millicuries per millimole; total activity, 0.50 millicuries.

Nutrient broth - Difco Laboratories, dehydrated.

Vitamin B-12 - Nutritional Biochemical Corporation, Number 2285.

p-Dioxane - Eastman Organic Chemicals, Number 2144.

Naphthalene - Eastman Organic Chemicals, Number 168, recrystallized.

Dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene) - Packard Instrument Company, Inc., Number 6002090, scintillation grade.

PPO (2,5-diphenyloxazole) - Packard Instrument Company, Inc., Number 6002023, scintillation grade.

Cab-O-sil - Cabot Corporation, Boston, Massachusetts.

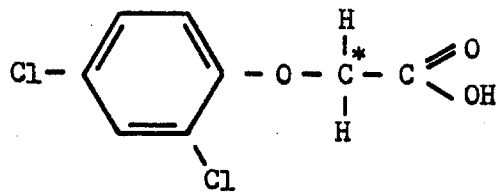
The positions of the carbon-14 atoms in the radioactive compounds used in this research are denoted by an asterisk in Figure 3-1. At least 95 percent of the radioactive molecules in the ring-labeled CIPC samples contain only one carbon-14 atom. Virtually all the labeled molecules contain two carbon-14 atoms at most. The effect of the isotope on the experimental results can be assumed to be negligible (71). With respect to all the ring-labeled CIPC molecules, the carbon-14 atoms are uniformly distributed in the ring structure, as indicated in Figure 3-1.

3.2 Sampling Procedures

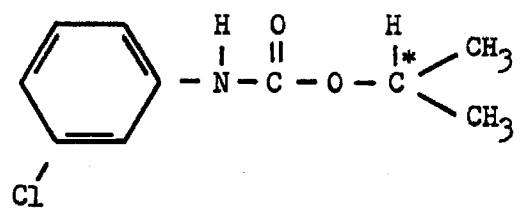
During the course of the various experiments it was necessary to distinguish between, and to measure the radioactivity associated with, the solid and liquid phases of the systems. The two techniques employed to separate the phases prior to analysis are described herein.

3.2.1 Centrifugation

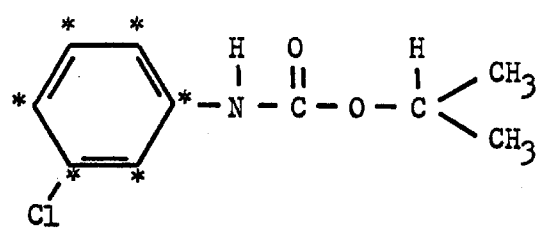
In the experiments involving biological growths and those concerning adsorption on clay minerals, the phase separation was accomplished by centrifuging the samples. Ten milliliter samples were transferred by pipette into 17.5 by 102 millimeter Pyrex tubes. These samples were placed in a Servall Angle Centrifuge, Type SPX, manufactured by Ivan Servall, Inc. Centrifugation proceeded for 15 minutes for the biological material and two minutes for the clay mineral



2,4-Dichlorophenoxyacetic-2-C^{14} acid



Isopropyl-C^{14}-N-(3-chlorophenyl) carbamate
Samples IP-10 and IP-14



Isopropyl-N-(3-chlorophenyl-C^{14}) carbamate
Samples R-10 and R-14

Figure 3-1. Location of carbon-14 atoms (denoted by asterisks) in the molecular structures of 2,4-D and CIPC.

studies. The speed of the centrifuge was approximately 2800 revolution per minute. Up to 15 samples could be accomodated at one time.

After centrifugation, one-milliliter portions of the supernatant were transferred to liquid scintillation glass vials. These vials, with a capacity of 20 milliliters, were obtained from the Packard Instrument Company, Inc., Number 6001008. Subsequent to measurements of the radioactivity, the vials were placed in a -60°C deep-freeze for storage.

The cellular material remaining at the bottom of the centrifuge tubes in certain of the biological experiments was washed ten times with distilled water. After each washing, the cells were centrifuged and the supernatant discarded. Following the final washing, the cells were filtered onto Millipore HA membranes of 0.45 micron pore size. These filters were then fixed to aluminum planchets for radioactivity measurements.

The principal source of error in the centrifugation technique was associated with the accuracy of the transfer procedure. Pyrex Number 7100, one-milliliter pipettes were employed for the final transfer to the sample vials. These pipettes were accurate to within $\pm$ 0.006 milliliters or $\pm$ 0.6 percent. This value represents the total error involved in obtaining the supernatant samples. The ten-milliliter pipette transfer prior to centrifugation does not affect the accuracy. With respect to the supernatant sample, this transfer was merely a preparatory step in obtaining a clarified solution suitable for sampling. The error arising in the initial transfer, however, does affect the results for the cellular material collected in the

bottom of the centrifuge tube. It was estimated that this ten-milliliter transfer was accurate to within ± 0.5 milliliters or ± 5 percent.

3.2.2 Filtration Through Porous Immersion Tubes

The centrifugation technique described above necessitated a 20 to 30 minute time lag between the removal of samples from the bulk suspensions and the final transfer to the scintillation vials. In the adsorption studies with activated carbon, this time delay was very important. To avoid such delays another procedure was adopted.

Sample volumes of 16.0 ± 0.5 milliliters were taken from the bulk suspension by submerging appropriate test tubes in the suspension. These volumes, containing activated carbon and the solution, were transferred to 50-milliliter beakers. A one-inch diameter, porous stainless-steel immersion tube purchased from the Ace Glass Company, Number 2003, was placed in each beaker. These immersion tubes were of two types, 5 micron and 20 micron pore sizes. Ten-milliliter volumes were drawn through the immersion tubes into bacteriological pipettes. The activated carbon remained on the surface of the porous disc or in the beaker. Finally, a one-milliliter sample of the filtrate was transferred to the scintillation vials for analysis. The elapsed time during the actual phase separation was about ten seconds.

The error discussed in Section 3.2.1 for the final one-milliliter transfer also applies to this technique. As stated previously, the transfer was 1.000 ± 0.006 milliliters. Errors arising in the

procurement of the 16.0 ± 0.5 milliliter initial samples affect only certain of the calculations.

3.3 Collection of Carbon Dioxide

For the experiments on the degradation of CIPC adsorbed on activated carbon, a measure of the amount of radioactive carbon dioxide evolved was desired. To accomplish this measurement it was necessary to collect the carbon dioxide in a closed system. An uncapped, glass scintillation vial containing two milliliters of barium hydroxide was suspended above each test suspension in a small brass basket specially constructed for this purpose. The basket was held in position by means of thin strips of brass looped over the mouth of the test container, a 300-milliliter Erlenmeyer flask. An ordinary rubber stopper sealed the flask. Periodically the vial was removed for analysis and a new vial inserted in the basket.

The barium hydroxide contained in the glass vials reacted with the evolved carbon dioxide to form barium carbonate, a highly insoluble compound. Even in a solid state barium hydroxide reacts with atmospheric carbon dioxide to form the carbonate. In preparing the barium hydroxide, it was therefore necessary to pass a concentrated solution of the hydroxide-carbonate mixture through ashless filter paper. The purified solution of barium hydroxide was placed in a stoppered vessel. Reagent-grade toluene, immiscible with water, was placed above the hydroxide solution to prevent contamination. The barium hydroxide thus prepared was standardized against a secondary standard of sulfuric acid and found to be 0.160M.

3.4 Liquid Scintillation Counting Procedure

The liquid samples obtained from the separations described in Section 3.2 were analyzed for radioactivity with a Nuclear-Chicago Corporation, Series 720, Liquid Scintillation System. In principle, this technique involves bringing an appropriate sample into intimate contact with scintillator molecules contained in a counting solution. The scintillator molecules emit light pulses when struck by beta particles or other radiation. In turn, the light pulses are recorded by ultra-sensitive photocells and a measure of the radioactivity results.

For this research, the counting solution was composed of the following constituents:

0.005 grams	Dimethyl POPOP
0.4 grams	PPO
12 grams	Naphthalene
100 milliliters	p-Dioxane.

This solution was prepared immediately before each series of measurements. The one-milliliter samples described previously were dissolved in 15 milliliters of the counting solution.

The vials were placed in the liquid scintillation system. Each vial was counted four times for a period of ten minutes for each cycle. The first cycle was disregarded to eliminate any effects arising from extraneous fluorescence. The three remaining values, corrected for the background counting rate, were averaged. Background rates were based on the counts obtained for one-milliliter portions of

distilled water dissolved in the same counting solution. Normally, this rate was between 38 and 40 counts per minute.

Counting efficiencies were determined for the particular compound being studied. Standard solutions were prepared for each pesticide and samples of these solutions were analyzed. The efficiencies were calculated on the basis of the values of the specific activities and total weight furnished by the suppliers. The average counting efficiencies are presented in Table 3-1.

Table 3-1

Liquid Scintillation Counting Efficiencies

Compound	Sample Number	Average Percent Efficiency
2,4-D	-	48.7
CIPC	IP-10	66.8
CIPC	IP-14	67.7
CIPC	R-10	64.9
CIPC	R-14	67.5

The barium carbonate samples discussed in Section 3.3 also were analyzed with liquid scintillation techniques. In order to keep the insoluble particles of barium carbonate suspended in the counting fluor, it was necessary to add a thixotropic gelling agent, Cab-o-sil, to the counting fluor. For these analyses the composition of the fluor was as follows:

0.07 grams	Dimethyl POPOP
0.7 grams	PPO

10.0 grams	Naphthalene
4.0 grams	Cab-o-sil
100 milliliters	p-Dioxane

Fifteen milliliters of the above solution were employed for each two-milliliter sample.

Sodium carbonate labeled with carbon-14 was utilized to obtain the counting efficiencies. Standard solutions of sodium carbonate were prepared in 0.160M barium hydroxide. An average counting efficiency of 56.0 percent was obtained. This value was not materially affected by the amount of barium carbonate present.

The statistics of radioactivity counting are well established. A detailed discussion of these statistics is given by Friedlander and Kennedy (71). For this discussion, it will suffice to state the equation for the net counting rate:

$$R_n = \left(\frac{M}{t_m} - \frac{M_b}{t_b}\right) \pm \sqrt{\sigma_m^2 + \sigma_b^2}$$

where R_n is the net counting rate in counts per minute; M is the number of counts for the sample; M_b is the total number of counts from background; t_m is the counting time in minutes for the sample; t_b is the counting time in minutes for background; σ_m is the standard deviation for the sample which equals $\sqrt{M} \div t_m$; and σ_b is the standard deviation for background which equals $\sqrt{M_b} \div t_b$.

As previously mentioned, the liquid scintillation samples were counted three times for a total of 30 minutes. Initially the counting rate was about 3000 counts per minute. As degradation or adsorption proceeded the counting rate for the samples diminished.

Presented in Table 3-2 are the standard deviations calculated for several counting rates ranging from 100 to 3000 counts per minute.

Table 3-2

Standard Deviations for Several Counting Rates

Based on a 30 Minute Counting Period

Gross cpm	Background cpm	Net cpm	Standard Deviation, cpm	Standard Deviation, Percent
100	40	60	2.1	3.5
500	40	460	4.2	0.9
1000	40	960	5.8	0.6
1500	40	1460	7.1	0.5
2000	40	1960	8.1	0.4
2500	40	2460	9.1	0.4
3000	40	2960	10.0	0.3

The standard error is shown in this table to be less than one percent for net counting rates above about 460 counts per minute.

During the course of the experimental work, measurements were performed on duplicate samples to check the accuracy of the sampling and liquid scintillation counting procedures. Twenty-four sets of duplicate samples produced an average deviation of 0.42 percent with a standard deviation of 0.37 percent. It was concluded from these tests that either of the sampling techniques discussed in Section 3.2 followed by liquid scintillation analysis provided very accurate measurements.

3.5 Solid Sample Analysis

As described in Section 3.2.1, cellular material from the biological study was deposited on one-inch-diameter aluminum planchets. These planchets were placed in a Nuclear-Chicago low-background Geiger counter. This unit consisted of a Model C-110B sample changer and a Model 181A decade counter. When initially preparing the counter for operation, it was necessary to determine the Geiger region for the voltage applied to the detector. The Geiger region represents the range of applied voltage over which the counting rate is relatively independent of this voltage. In order to determine this region, a standard carbon-14 source of 22,000 disintegrations per minute was placed in the counting chamber. The voltage was varied in discrete intervals and the counting rate for each voltage was calculated. From these data, the Geiger region was found to lie between 1340 and 1500 volts. In this region the variation in the counting rate was less than 70 counts per minute per 100 volts. A value of 1360 volts was selected for the operation of the counter.

To establish the background counting rate, a plain planchet was placed in the counting chamber and the counts were recorded. Employing the same relationship presented in Section 3.4 the background rate was found to be 2.7 $\pm$ 0.1 counts per minute. No attempt was made to establish the efficiency of the counter for the radioactivity associated with the cellular material, inasmuch as no accurate standard for carbon-14 in cellular material was available. Counting efficiencies were determined for several carbon-14 labeled compounds and these efficiencies were; counting standard-21.7 percent; 2,4-D-16.0 percent;

and sodium carbonate in barium hydroxide-3.7 percent. The carbon-14 associated with the bacterial cells would be expected to have a very low counting efficiency.

3.6 Experimental Procedures for the Microbial Metabolism Studies

This segment of the research was initiated to investigate the degradation of 2,4-D and CIPC in an aqueous environment. A description of the procedures employed in these experiments is presented in the following paragraphs.

3.6.1 Adaptation Cultures

The major portion of the information on the metabolism of the two herbicides was obtained from a succession of what will henceforth be termed adaptation stages. The term adaptation is used here only in its general connotation referring to the microbial cultures employed for the biological degradation studies. Initially, a mixed microflora was placed in contact with a small amount of the particular herbicide being studied in a liquid medium containing a large amount of supplemental organic carbon. In successive adaptation stages, the concentration of pesticide was increased while the amount of supplemental carbon was decreased. Each adaptation stage, with the exception of the first stage, was inoculated with organisms from the previous stage.

For 2,4-D, each adaptation stage consisted of several flasks, two of which contained the herbicide, supplemental carbon, and microorganisms. Of the remaining vessels, two contained the pesticide and microorganisms with no supplemental carbon; one was dosed with the pesticide and supplemental carbon, but devoid of microorganisms; one

was composed of supplemental carbon alone in the presence of microorganisms; and one or two flasks contained only the pesticide. All of the test vessels contained a liquid medium of mineral salts. Distilled water obtained from the Keck Laboratory system was added to bring all of the cultures to the same initial volume. Each such adaptation series was designed to determine the effects of supplemental carbon on the degradation rate, the losses resulting from evaporation and adsorption on glass, and the effect of supplemental carbon on the background counting rate.

The adaptation stages for CIPC were similar in nature. In essence two series, each identical in composition to those described for 2,4-D, were prepared for each adaptation stage. One contained the isopropyl-labeled CIPC and the other contained the ring-labeled CIPC. These two series were run concurrently to assess the rate of degradation of two portions of the molecule.

For these studies, the bulk of the test volume was composed of a liquid medium of mineral salts. This medium was similar to that of Gray and Thornton (72) and is presented in Table 3-3. The stock solutions were prepared with reagent grade chemicals. Along with the distilled water, they were sterilized in an autoclave. The autoclave was operated at a pressure of 15 pounds per square inch and a temperature of 248^{o}F for a minimum of 20 minutes. To prevent chemical reactions and subsequent precipitation, the aforementioned components were mixed after sterilization and the potassium phosphate was added last. The pH of the medium was 7.6. Volumes of 190 milliliters were transferred to 300-milliliter Erlenmeyer flasks.

Table 3-3

Composition of Mineral Salts Medium

Constituent	Concentration of Stock Solution	Milliliters Added to One Liter of Distilled Water
HCl	0.5N	2.0
K_2HPO_4	50%	2.0
NaCl	5%	2.0
$CaCl_2$	2.5%	2.0
$MgSO_4$	5%	2.0
$(NH_4)_2SO_4$	25%	4.0
Vitamin B-12	0.005 μg/ml	2.0

Stock solutions of the radioactive pesticides were prepared. For the first stage of adaptation, the 2,4-D was dissolved in acetone, the isopropyl-labeled CIPC in water, and the ring-labeled CIPC in 95 percent ethanol. These solutions had radioactivity concentrations of 0.1 millicuries per liter. One-milliliter volumes, containing 0.1 microcuries, were added to the liquid media in the flasks. Chemically this addition produced a concentration of about 0.09 milligrams of CIPC per liter and 0.005 milligrams of 2,4-D per liter. A quantity of non-radioactive 2,4-D was then added to bring the total 2,4-D concentration up to 0.1 milligrams per liter. The first-stage adaptation series for CIPC required no additional pesticide.

Nutrient broth was used as a source of supplemental carbon for the experiments. A stock solution of 20 grams per liter was prepared and sterilized prior to its addition to the cultures. For the first stage, ten milliliters were added per flask. Based on a final volume of 205 milliliters per culture, the nutrient broth concentration was 975 milligrams per liter.

As sources of microorganisms for the first adaptation stage, samples were obtained from the activated sludge unit and the effluent from the primary sedimentation basin of the Whittier Narrows Water Reclamation Plant. In addition, samples of the flow and slime from the Dominguez Channel in Los Angeles were utilized. The Dominguez Channel contains the waste effluents from several oil refineries. It was hoped that this source would contain microorganisms adapted to petroleum or petrochemical substances. One-milliliter quantities from each of the sources provided, it was anticipated, a widely assorted microflora for the initial adaptation stage.

All of the glassware utilized in the transfer procedures as well as the culture vessels were sterilized at 356°F in a Freas Model 124 oven. The flasks were fitted with sterilized gauze stoppers to prevent contamination.

After the cultures were inoculated, the flasks were placed on a Gyrotory Shaker, Model G25, manufactured by the New Brunswick Scientific Company. This unit was operated at room temperature. Periodically, the shaker was turned off and samples were taken from the cultures for analysis. The sampling and analytical techniques employed were described in Section 3.2.1.

Once significant or complete degradation became evident a second adaptation stage was initiated. The procedure followed for this stage and the subsequent stages was essentially the same as described previously for the first stage. The microorganisms for this second stage were obtained from the cultures of the first-stage adaptation to the appropriate pesticide. This inoculum consisted of one-milliliter volumes from a flask containing the pesticide plus supplemental carbon and from a culture containing only the pesticide. In succeeding stages of adaptation, the inoculum was obtained from the preceding stage.

For the second stage and subsequent ones, the quantity of radioactivity was increased to 0.5 microcuries per flask for the 2,4-D and 0.4 microcuries for the CIPC. This increase produced better resolution and accuracy. In addition, the total amount of pesticide, including the non-radioactive form, was increased and the nutrient broth concentration decreased. The concentrations of pesticide and nutrient broth employed for all of the adaptation stages is given in Table 3-4.

3.6.2 Ultraviolet Spectroscopic Analysis

An ultraviolet analysis was performed on extracts from the fifth stage of adaptation to CIPC. This analysis was designed to measure qualitatively the presence or absence of the phenyl ring after significant degradation had occurred.

According to Shelley and Umberger (73), phenol demonstrated a peak in ultraviolet region at a wavelength of 271 millimicrons.

Table 3-4

Concentrations of Pesticide and Nutrient Broth

for the Adaptation Stages

Adaptation Stage Number	Pesticide	Concentration in mg/l	
		Pesticide	Nutrient Broth
1	2,4-D	0.1	975
2	2,4-D	1.0	792
1	CIPC	0.1	975
2	CIPC	1.0	786
3	CIPC	5.4	597
4	CIPC	5.4	400
5	CIPC	5.4	200
6	CIPC	5.4	100

Ortho-, meta-, and para-chlorophenol in 0.1N NaOH produced two maxima at about 237 to 244 and 292 to 298 millimicrons in a study by Smith, Spencer, and Williams (74). In these three instances the major peak was that at the 237 to 244 millimicron wave length. Parke and Williams (75) showed that 2,4-dichlorophenol also demonstrated two peaks. One occurred at 244 and the other at 304 millimicrons in a 0.1N NaOH solution. The molar extinction coefficients were 9500 and 3400 liters mole^{-1} centimeter^{-1} respectively. These values are similar to those calculated for ortho-, meta-, and para-chlorophenol (74). Meta-chloroaniline in absolute ethanol demonstrated maxima at 242 and 295 millimicrons according to Bilbo and Wyman (76).

The molecular structure of CIPC contains a monochlorophenyl ring. This portion of the pesticide resembles closely meta-chloroaniline. Based on the literature cited above, this structure should and indeed did demonstrate the ultraviolet spectrum for a chlorophenol. Stock solutions of CIPC prepared in chloroform were analyzed with a Beckman Model DK-2 Ratio Recording Spectrophotometer. Two maxima were evident, one at 241 millimicrons and the other at 278 millimicrons. Calculations yielded molar extinction coefficients of about 13,000 and 940 liters $mole^{-1}$ $centimeter^{-1}$ respectively. The standards and all samples were analyzed in fused silica cuvettes with a one-centimeter path length.

Direct ultraviolet analysis of the adaptation culture proved to be impossible. Even after clarification by centrifugation the solution contained substances that interferred with the spectrum and masked the CIPC. In order to eliminate this interference a chloroform extraction was performed. Approximately 40-milliliter volumes from the cultures were placed in centrifuge tubes. Following centrifugation, 24 milliliters of the supernatant were transferred to a separatory funnel. A one-step extraction with reagent grade chloroform was then performed. The chloroform extract was then subjected to ultraviolet analysis. This procedure eliminated most of the interference from the biological samples.

3.7 Experimental Procedures for the Adsorption Studies

The adsorption of CIPC and 2,4-D on various adsorbents was investigated in these experiments. Activated carbon, illite,

kaolinte, and montmorillonite were the adsorbents studied. A description of the procedures and materials used in this study is contained in the following paragraphs.

3.7.1 General Procedures

The experiments were normally conducted in one-liter beakers containing 800 milliliters of test solution. The beakers were coated with Siliclad, a silicone concentrate manufactured by Clay-Adams Inc., to prevent retention of the adsorbents on the walls of the containers. To distilled water maintained at a desired pH value were added the pesticide and the adsorbent being tested. This mixture was placed on a multiple stirring apparatus manufactured by Phipps and Bird, Inc. With the six monel paddles on this unit, six solutions could be stirred simultaneously. For all of the experiments, a stirring rate of 100 revolutions per minute was used. In order to prevent evaporation, Saran-Wrap was employed to cover the test containers. Openings were cut in the Saran-Wrap to permit removal of the paddles. Periodically, samples were taken and analyzed for the amount of pesticide remaining in solution. For those experiments with clay minerals, the phase separation was by centrifugation as described in Section 3.2.1. Porous immersion tubes, discussed in Section 3.2.2, were utilized to separate activated carbon from the bulk solution. Analysis of the supernatant was accomplished by liquid scintillation counting techniques, Section 3.4. The entire experimental and sampling procedure was conducted in a room of

constant temperature and controlled humidity. With one exception, all experiments were conducted at 20°C.

A series of experiments to determine the total capacity of the activated carbon for CIPC differed slightly from the experiments described above. In this instance, the test vessels were two-liter beakers and initially contained 1800 milliliters of solution. Once equilibrium had been closely approached, about 24 hours, more CIPC was added. Several such additions of CIPC were made over a period of one week.

Under the conditions described in this section, it was possible to vary several parameters and to study the subsequent effects. Experiments at pH 6.9 were performed for varying amounts of adsorbent and adsorbate. Activated-carbon concentrations were varied from 25 to 250 milligrams per liter in the presence of 5.0 to 10.0 milligrams of CIPC per liter. In another series, montmorillonite was varied from 50 to 800 milligrams per liter for a CIPC concentration of 0.4 milligrams per liter. The adsorption of 2,4-D on 800 milligrams per liter of montmorillonite was also studied.

Experiments on all the adsorbents were made at three pH values, 4.8, 6.9, and 9.2. For these studies the absorbate and adsorbent concentrations were held constant. Finally, the effect of temperature was investigated. One experiment was performed on the adsorption of CIPC on carbon at a temperature of 37.5°C. For all of the conditions described above and in conjunction with the normal experiments, blanks were included to eliminate any effects arising from evaporation or adsorption on the walls of the beakers.

3.7.2 Activated Carbon

Nuchar C 190 powdered activated carbon was used as the adsorbent for an extensive study with CIPC. This carbon was produced by the West Virginia Pulp and Paper Company. Prior to use, the activated carbon was sieved in the dry state. Following this operation, the carbon passing a number 120 U.S. Standard Sieve and retained on a number 140 was subjected to wet sieving with distilled water. This operation was performed by passing distilled water through the carbon retained on the number 140 sieve. When the wash water showed no evidence of further fine material, the wet sieving operation was halted. The carbon remaining on the sieve was dried for 24 hours at 200°C in a Thelco Model 18 oven. After cooling in a dessicator, this carbon was used to prepare a 10-gram per liter stock suspension for use in the experiments. Use of a stock suspension insured maximum wetting of the carbon prior to its addition to the test vessels.

A microscopic examination was made of the activated carbon obtained above in order to determine the particle size distribution. The measurements were made with a Porton eyepiece graticule. This eyepiece enables the viewer to measure particles as small as 0.001 millimeters in diameter. A total of 390 particles were measured. The diameter of the median particle or D_{50} value was 1.65 microns with a geometric standard deviation of 2.27.

3.7.3 Clay Minerals

Three clay minerals, viz. illite, kaolinite, and montmorillonite, were selected for these studies. They were obtained from

Ward's Natural Science Establishment, Inc. These clay minerals were among a wide variety of such substances carefully selected and analyzed by the American Petroleum Institute. The samples selected for these investigations were kaolinte 9a from Mesa Alta, New Mexico, montmorillonite 23 from Chambers, Arizona, and illite 36 from Morris, Illinois. A great deal of information on these minerals is given in the API Project 49 reports (77). Pertinent data from these publications are presented in Table 3-5.

Table 3-5

Information on Clay Minerals (77)

Characteristic	Kaolinite	Montmorillonite	Illite
pH	6.32	7.47	-
Base exchange capacity-meq./100g	6.8	147.5	24.0
Size composition in %.			
> 1/256 mm	50.8	85.9	26.6
1/256-1/512 mm	6.6	11.6	16.6
1/512-1/1024 mm	5.4	4.4	19.3
1/1024-1/2048 mm	3.6	2.4	3.2
< 1/2048 mm	33.6	0.2	34.3
Percent impurities	5	4	10

Prior to use, the clay minerals were dried for 48 hours in a 140°C oven. Quantities for the experiments were weighed out on an analytical balance. These amounts were then added in the dry state to the test solutions.

3.7.4 Chemical Solutions

Radioactively labeled CIPC and 2,4-D were used in the adsorption studies. The radioactivity concentration for all the experiments with CIPC was 2.0 microcuries per liter. Stock solutions of non-radioactive CIPC were employed to bring the total chemical concentrations up to the desired levels. One experimental series was performed on the adsorption of 2,4-D on montmorillonite. For this series, 2.5 microcuries per liter were used yielding a chemical concentration of 0.13 milligrams per liter. No additional 2,4-D was employed.

Three chemical solutions were utilized to maintain the experimental systems at specified pH levels. At a concentration of 0.0061M in the test solution, monobasic sodium phosphate produced a pH of 4.8. A combination of 0.0038M monobasic and 0.0024M dibasic sodium phosphate gave a pH of 6.9. To obtain a pH of 9.2, 0.0062M sodium borate was employed. The stock solutions were prepared with reagent grade chemicals. A Beckman Model M, pH meter was used for all pH determinations.

In order to eliminate any trace organics that might be present, the distilled water from the Keck Laboratory system was redistilled. This second distillation was performed in an all-glass system. Small amounts of sodium hydroxide and potassium permanganate were added to the water prior to distillation to insure the removal of the organic matter. The twice-distilled water was stored in a large carboy until its use. All of the adsorption experiments were conducted with this water.

3.8 Experimental Procedures for the Studies on the Degradation of Pesticides in the Presence of an Adsorbent

The experimental procedures employed for this section of the research were essentially the same as those used for the microbial metabolism studies. Only CIPC was utilized for these investigations, however.

As previously, 300-milliliter Erlenmeyer flasks were selected as the test vessels. Equal volumes, 140 milliliters, of the liquid medium of mineral salts described in Section 3.6.1 were added to each of 13 flasks. Varying amounts of CIPC were added to the media. Ten of the flasks contained isopropyl-labeled CIPC and the remaining three contained ring-labeled CIPC. To seven of the vessels were added activated carbon. A description of the activated carbon was presented in Section 3.7.2. Distilled water was added to bring all the suspensions up to the same volume. These suspensions were placed on the Gyrotory Shaker and were agitated for about 60 hours. In so doing, adsorption equilibrium was established prior to the inoculation of the cultures. At this point, eleven of the flasks were inoculated with microorganisms obtained from the sixth-stage adaptation of CIPC. The total volume of all the vessels was then 151 milliliters. A summary of the constituents of the 13 flasks is presented in Table 3-6.

The vials for the collection of carbon dioxide described in Section 3.3 were suspended above the cultures in flasks 1,2,6,7,10,11, and 12. These vials normally were replaced every 12 to 24 hours

Table 3-6

Composition of the Systems Used to Study the Influence of Activated Carbon on the Degradation of CIPC

Flask Number	Radioactive Label	Concentration of CIPC		Activated Carbon	Presence of Microorganisms
		µc/l	mg/l	mg/l	
1	Isopropyl	7.9	5.0	0	Yes
2	Isopropyl	7.9	5.0	0	Yes
3	Isopropyl	7.9	3.0	0	Yes
4	Isopropyl	5.3	1.1	0	Yes
5	Isopropyl	2.6	0.5	0	Yes
6	Isopropyl	7.9	5.0	24.8	Yes
7	Isopropyl	7.9	5.0	24.8	Yes
8	Isopropyl	7.9	5.0	12.4	Yes
9	Isopropyl	7.9	5.0	12.4	Yes
10	Isopropyl	7.9	5.0	24.8	No
11	Ring	7.9	5.0	0	Yes
12	Ring	7.9	5.0	24.8	Yes
13	Ring	7.9	5.0	24.8	No

during the experiment. Measurements of the radioactive carbon dioxide collected in the vials were performed as discussed in Section 3.4.

Supernatant samples from the suspensions were obtained by centrifugation, (see Section 3.2.1). Such samples were taken periodically and analyzed with the aid of the liquid scintillation counter (Section 3.4).

CHAPTER 4

THE MICROBIAL DEGRADATION OF THE HERBICIDES 2,4-D AND CIPC

The persistence of pesticides in an aqueous environment is probably dependent on a number of natural processes. It is to be expected that biological decomposition is a significant factor in the ultimate fate of these compounds. At present, there is very little known about the microbial degradation of synthetic organic pesticides in water. This portion of the research was initiated to study the microbial degradation of 2,4-D and CIPC in an aqueous environment. The results and a discussion of these studies are contained in this chapter.

4.1 Results

4.1.1 Adaptation Cultures for 2,4-D

For the first adaptation stage, the 2,4-D and nutrient broth concentrations were 0.1 and 970 milligrams per liter respectively. Acetone was used as a solvent carrier for 2,4-D and was present in the systems at a concentration of 0.1M. Samples were taken periodically for 77 days. The results from this adaptation series are presented in Table 4-1. Duplicate samples were taken at the 58.8-day time period. One set was handled in the routine manner. The duplicate set was acidified with HCl prior to centrifuging the samples. Any radioactive carbon-14 dioxide was driven off in this manner prior to the liquid scintillation analysis. Only system 2 demonstrated a significant difference between the duplicate samples as shown in Table 4-1.

Table 4-1

First Stage Adaptation to 2,4-D

Characteristic	System Number				
	1	2	3	4	5
Initial 2,4-D, mg/l	0.100	0.100	0.100	0.100	0.100
Nutrient broth, mg/l	970	970	0	0	970
Inoculated	Yes	Yes	Yes	Yes	No[a]
2,4-D remaining, percent (b)					
14.8 days	101.3	88.8	90.5	87.9	95.4
33.8 days	84.0	86.2	83.5	84.5	91.5
58.8 days	87.4	90.3	85.6	84.3	96.8
58.8 days (c)	86.3	74.6	81.5	79.1	97.3
77.6 days	78.9	81.3	72.7	64.3	90.2
2,4-D remaining, micromole liter^{-1}					
0.0 days	0.45	0.45	0.45	0.45	0.45
14.8 days	0.46	0.40	0.41	0.40	0.43
33.8 days	0.38	0.39	0.38	0.38	0.41
58.8 days	0.40	0.41	0.39	0.38	0.44
58.8 days (c)	0.39	0.34	0.37	0.36	0.44
77.6 days	0.36	0.37	0.33	0.29	0.41

(a) Visible evidence of contamination prior to 14-day sample.

(b) 2,4-D measurements based on presence of the number two carbon atom in the acetic acid group.

(c) Acidified with HCl prior to analysis

The values given in this table were based on the counting rates of the samples relative to the rate for a sterile system containing only 2,4-D. This system was run concurrently with the rest of the series. The maximum reduction in radioactivity occurred in system 4 where 64.3 percent of the number-2-carbon atoms of the acetic acid groups remained after 78 days. Henceforth, the quantity of the number-2-carbon atoms disappearing will be referred to as the amount of 2,4-D removed.

The second adaptation stage for 2,4-D initially contained 1.0 milligrams of 2,4-D per liter and 792 milligrams of nutrient broth per liter. The carrier solvent for the 2,4-D was ethyl alcohol. Concentrations of the ethyl alcohol in the experimental systems were 0.08M. The inoculum consisted of one-milliliter volumes taken from systems 1 and 3 of the first series. This inoculum was obtained during the sixty-eighth day of the first stage adaptation. The second stage adaptation series was begun at this time and continued for 175 days. Data obtained from this series are given in Table 4-2. The values for the amounts of 2,4-D remaining were again computed on the basis of values obtained for systems containing only 2,4-D without microorganisms. For the second adaptation series, two such systems were used. Although system 15 was initially sterile, visible evidence of contamination was noticed after one week. At this time the previously clear solution became turbid. This contamination resulted in the disappearance of 2,4-D evident in Table 4-2. The largest reduction in the amount of 2,4-D in solution was in system 15. About 23 percent of the 2,4-D disappeared in 175 days. In the same length of

Table 4-2

Second Stage Adaptation to 2,4-D

Characteristic	System Number				
	11	12	13	14	15
Initial 2,4-D, mg/l	1.0	1.0	1.0	1.0	1.0
Nutrient broth, mg/l	792	792	0	0	792
Inoculated	Yes	Yes	Yes	Yes	No[a]
2,4-D remaining, percent (b)					
4.8 days	94.3	95.4	86.4	95.6	98.2
11.8 days	89.8	90.0	94.4	96.8	78.7
18.7 days	92.6	96.8	94.8	97.4	89.0
24.7 days	90.2	92.0	93.2	94.6	87.4
43.8 days	88.8	91.2	91.8	94.6	85.8
78.0 days	88.0	90.0	91.6	91.0	80.9
175.8 days	82.2	85.6	87.6	89.1	76.6
2,4-D remaining, micromole liter^{-1} (b)					
0.0 days	4.52	4.52	4.52	4.52	4.52
4.8 days	4.26	4.31	3.91	4.32	4.44
11.8 days	4.06	4.07	4.27	4.38	3.56
18.7 days	4.19	4.38	4.28	4.40	4.02
24.7 days	4.08	4.16	4.21	4.28	3.95
43.8 days	4.01	4.12	4.15	4.28	3.88
78.0 days	3.98	4.07	4.14	4.11	3.66
175.8 days	3.72	3.87	3.96	4.03	3.46

(a) Visible evidence of contamination prior to 11-day samples.

(b) 2,4-D measurements based on presence of the number-2-carbon atom in the acetic acid group.

time, 11 to 18 percent reductions were observed in the remaining four biological systems.

No further adaptation cultures were prepared for the 2,4-D. The information obtained from the two cultures was sufficient for this study, as explained in Section 4.2.1.

4.1.2 Adaptation Cultures for CIPC

Each adaptation stage for CIPC consisted of two sets of samples. One set contained isopropyl-labeled CIPC and the other ring-labeled CIPC. In all other respects the two series were identical. With respect to the pesticide, both systems were assumed to undergo similar chemical and biological reactions. It was therefore possible to trace simultaneously the degradation of both the isopropyl group and the ring segment of the CIPC molecules. For brevity, the radioactively labeled forms will be indicated as CIPC-I for the isopropyl carbon-14 and CIPC-R for the carbon-14 ring.

The first adaptation stage for CIPC contained 0.095 milligrams per liter CIPC-I or 0.090 milligrams per liter CIPC-R depending on the system. Nutrient broth was added to specified flasks at a concentration of 975 milligrams per liter. The inoculum was obtained from sewage and industrial wastes. Tables 4-3 and 4-4 contain the data from this adaptation stage.

Almost all of the isopropyl carbon disappeared from systems 101 and 102 containing supplemental carbon. About 67 percent reduction was realized in the inoculated cultures without the added carbon source (systems 103 and 104). The initially sterile cultures

Table 4-3

First Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number					
	101	102	103	104	105	107
Initial CIPC-I, mg/l	0.095	0.095	0.095	0.095	0.095	0.095
Nutrient broth, mg/l	975	975	0	0	975	0
Inoculated	Yes	Yes	Yes	Yes	No*	No*
Isopropyl carbon atoms remaining, percent						
32.2 days	3.4	7.5	49.5	49.2	74.6	58.1
45.0 days	3.6	7.2	38.3	35.8	27.6	19.8
45.0 days**	3.6	7.2	36.2	34.6	24.6	19.5
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}						
0.0 days	0.445	0.445	0.445	0.445	0.445	0.445
32.2 days	0.015	0.033	0.220	0.219	0.332	0.258
45.0 days	0.016	0.032	0.170	0.159	0.123	0.088
45.0 days**	0.016	0.032	0.161	0.154	0.109	0.087

*Visible evidence of contamination prior to 32-day samples

**Acidified with HCl prior to analysis

Table 4-4

First Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number					
	201	202	203	204	205	207
Initial CIPC-R, mg/l	0.090	0.090	0.090	0.090	0.090	0.090
Nutrient broth, mg/l	975	975	0	0	975	0
Inoculated	Yes	Yes	Yes	Yes	No*	No*
Initial counting rate, cpm	32.8	41.0	43.3	46.6	21.3	33.1
Ring carbon atoms remaining, percent						
32.2 days	32.3	26.8	56.2	50.1	28.9	84.9
45.0 days	30.3	25.5	50.8	44.8	27.2	62.8
45.0 days**	30.6	23.0	49.2	44.9	25.9	58.9
Ring carbon atoms remaining, micromole liter^{-1}						
0.0 days	2.53	2.53	2.53	2.53	2.53	2.53
32.2 days	0.82	0.68	1.42	1.27	0.73	2.15
45.0 days	0.77	0.65	1.29	1.13	0.69	1.59
45.0 days**	0.77	0.58	1.24	1.14	0.66	1.49

* Visible evidence of contamination prior to 32-day samples

**Acidified with HCl prior to analysis

became contaminated resulting in reductions of 75 and 80 percent for systems 105 and 107, respectively.

The ring portion of the CIPC molecule was more resistant to degradation. As was true for the isopropyl group, the disappearance of the ring-carbon atoms was greatest in the presence of supplemental carbon where the average reduction in ring-carbon concentration was 72 percent. Only system 205 demonstrated an equivalent removal. The latter as well as system 207 became turbid during the early portion of the experiment indicating bacterial contamination. Systems 203 and 204 containing no supplemental carbon demonstrated 50 to 55 percent reductions in ring-carbon concentrations.

As used in this dissertation, the term supplemental carbon refers to the nutrient broth added to certain of the systems. Ethyl alcohol was also present in the systems. The low solubility of CIPC in water necessitated the use of ethyl alcohol as a solvent for the stock solutions. The concentration of ethyl alcohol in the first adaptation stage was 0.004M for the CIPC-R systems. No ethyl alcohol was contained in the CIPC-I cultures. For the remaining adaptation stages, the ethyl alcohol concentration in the experimental systems ranged from 0.01M to 0.1 M.

The 45-day samples were run in duplicate in the same manner described for 2,4-D. As shown in Tables 4-3 and 4-4, only a small difference was observed between the amounts of carbon-14 in the normal and acidified samples.

Using one-milliliter portions from systems 101 and 103 of the first stage of adaptation to CIPC the second stage was initiated.

This inoculum was obtained after 47 days of operation of the first stage. The concentration of pesticide for the second series of cultures was 1.0 milligrams per liter and the nutrient broth concentration was approximately 785 milligrams per liter. The CIPC-I cultures were analyzed over a period of 28.6 days and the CIPC-R cultures for 34.7 days. Data obtained from the second adaptation stage are presented in Tables 4-5 and 4-6. These same data are presented graphically in Figures 4-1 and 4-2. The values for the amount of CIPC remaining were based on the observed counting rates relative to the initial counting rate for each system. This procedure was used for all of the remaining adaptation stages.

Essentially complete degradation of the isopropyl group was observed within eight days for systems 111 and 112 containing supplemental carbon. The absence of nutrient broth resulted in reduced amounts of degradation in systems 113 and 114. Forty-nine to fifty-seven percent of the isopropyl group in the latter two systems remained after 29 days. The effect of supplemental carbon on the degradation of the ring structure was not noticeable. The ring-carbon atoms in systems 211 and 212 containing nutrient broth disappeared at a rate similar to that for systems 213 and 214 containing no additional carbon.

The systems that initially were devoid of microorganisms, systems 115, 117, and 118 for CIPC-I and 215, 217, and 218 for CIPC-R, demonstrated at least partial metabolism of the CIPC during the course of the experiment. In each instance, the reduction in pesticide concentration was accompanied by turbidity in the systems.

Table 4-5

Second Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number						
	111	112	113	114	115	117	118
Initial CIPC-I, mg/l	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Nutrient broth, mg/l	785	785	0	0	785	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	2904	2930	2963	2948	2959	2959	2925
Isopropyl carbon atoms remaining, percent							
2.0 days	42.0	46.4	98.9	95.8	98.3	99.4	100.8
7.8 days	2.5	2.8	93.1	92.7	90.9	100.1	100.1
10.0 days	2.1	2.2	90.7	89.8	91.1	97.5	98.9
14.7 days	1.7	2.2	83.9	79.4	86.8	95.5	96.5
21.8 days	1.6	1.7	71.8	62.1	84.7	91.5	92.8
28.6 days	1.4	1.5	56.7	48.7	81.1	89.1	89.0
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	4.63	4.63	4.63	4.63	4.63	4.63	4.63
2.0 days	1.94	2.15	4.58	4.44	4.55	4.60	4.67
7.8 days	0.12	0.13	4.31	4.29	4.21	4.63	4.63
10.0 days	0.10	0.10	4.20	4.16	4.22	4.51	4.58
14.7 days	0.08	0.10	3.88	3.68	4.02	4.42	4.47
21.8 days	0.07	0.08	3.32	2.88	3.92	4.24	4.30
28.6 days	0.06	0.07	2.63	2.25	3.75	4.13	4.12

*Visible evidence of contamination prior to 7-day sample.

**Visible evidence of contamination prior to 10-day samples.

Table 4-6

Second Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number						
	211	212	213	214	215	217	218
Initial CIPC-R, mg/l	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Nutrient broth, mg/l	787	787	0	0	787	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No*	No*
Initial counting rate, cpm	2953	2929	2989	2947	2979	2956	2970
Ring carbon atoms remaining, percent							
2.0 days	93.8	94.3	98.1	99.1	98.9	99.5	98.1
7.8 days	75.4	66.0	80.3	78.4	90.2	89.4	88.2
10.0 days	74.0	64.6	78.2	74.4	91.6	89.4	88.1
14.7 days	70.4	63.6	72.2	67.9	89.4	88.3	88.1
21.8 days	66.4	60.7	65.3	62.5	89.0	88.6	87.9
28.6 days	61.9	55.3	61.2	58.3	87.7	87.0	86.9
34.7 days	58.5	52.9	59.6	57.6	87.3	88.0	88.5
Ring carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	27.0	27.0	27.0	27.0	27.0	27.0	27.0
2.0 days	25.3	25.5	26.5	26.8	26.7	26.9	26.5
7.8 days	20.4	17.8	21.7	21.2	24.4	24.1	23.8
10.0 days	20.0	17.4	21.1	20.1	24.7	24.1	23.8
14.7 days	19.0	17.2	19.5	18.3	24.1	23.8	23.8
21.8 days	17.9	16.4	17.6	16.9	24.0	23.9	23.7
28.6 days	16.7	14.9	16.5	15.7	23.7	23.5	23.5
34.7 days	15.8	14.3	16.1	15.6	23.6	23.8	23.9

*Visible evidence of contamination prior to 7-day samples.

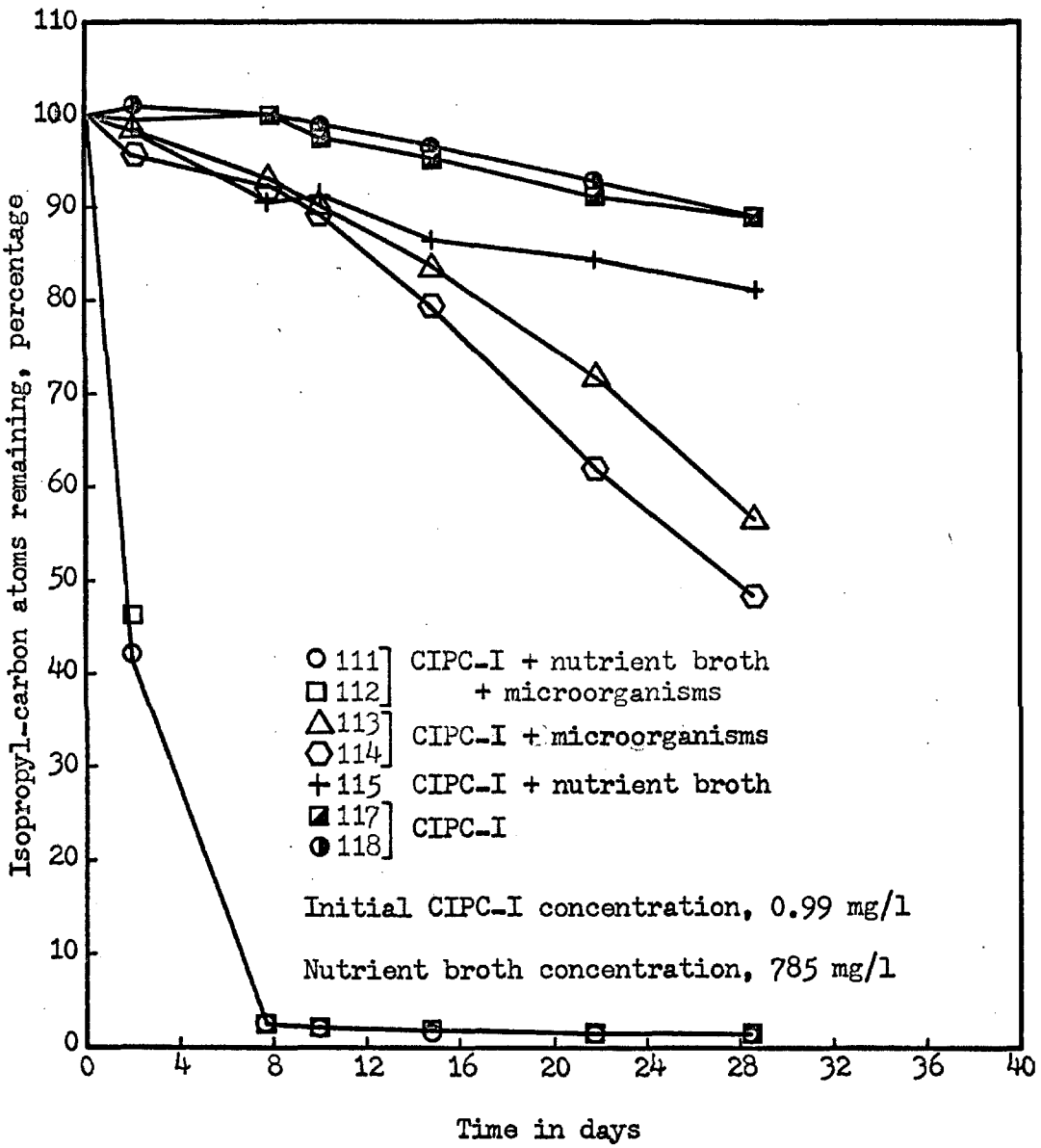


Figure 4-1. Second stage adaptation to isopropyl-labeled CIPC.

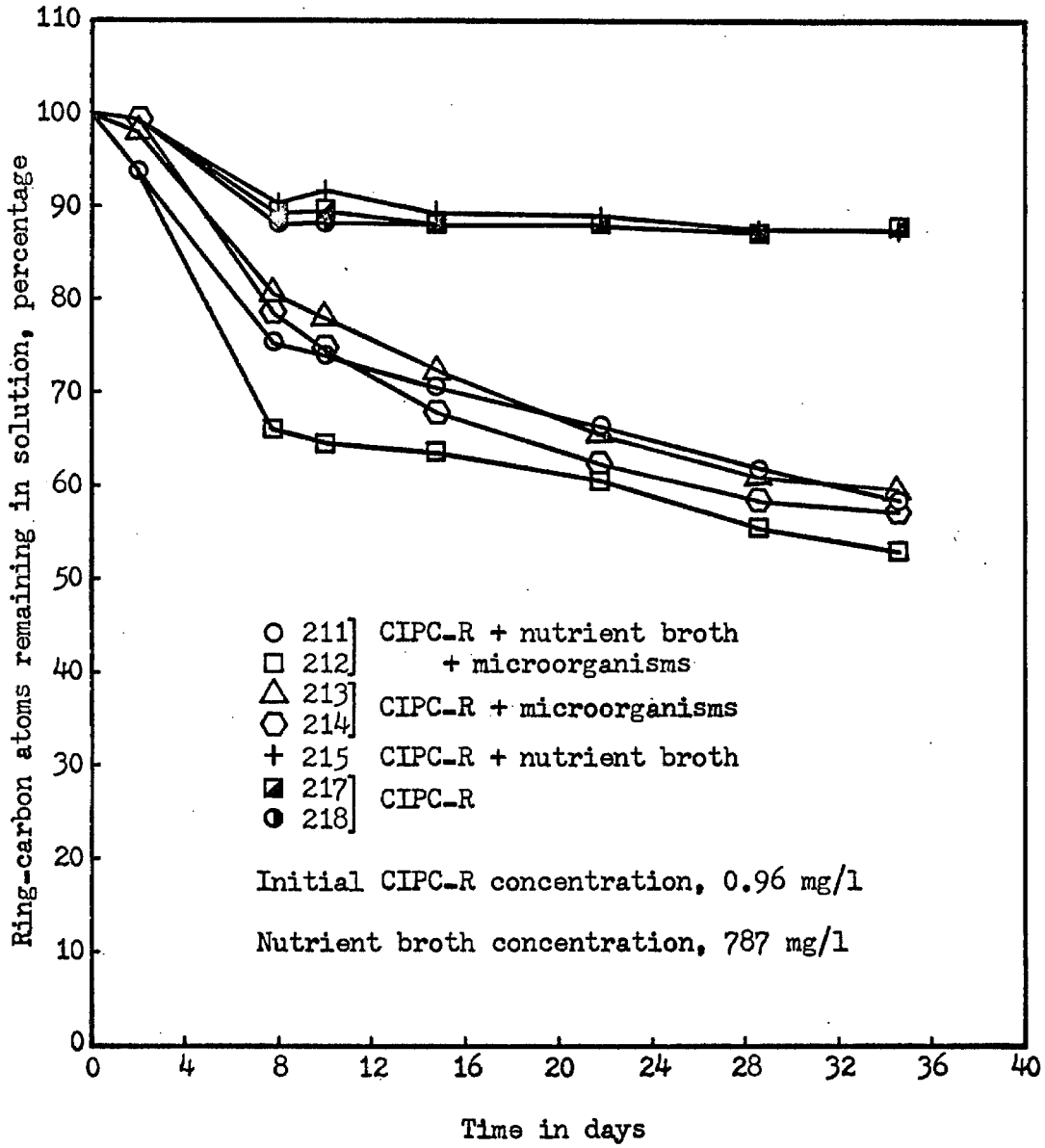


Figure 4-2. Second stage adaptation to ring-labeled CIPC.

Such turbidity, as previously mentioned, indicated bacterial contamination.

For the third adaptation stage, the CIPC concentration was increased to 5.4 milligrams per liter. The nutrient broth concentration employed for this series was 597 milligrams per liter. Once again, one-milliliter portions from the previous adaptation cultures were used as the sources of microorganisms. In this case, the inoculum was obtained from systems 111 and 113 during the twenty-ninth day of the second adaptation stage. The third stage extended for 24 days for CIPC-I and 58 days for CIPC-R. The information collected in this adaptation stage is given in Tables 4-7 and 4-8 and graphically represented in Figures 4-3 and 4-4.

Generally the systems in this third adaptation stage behaved in a manner similar to those in the second stage. From 95 to 97 percent of the isopropyl groups were removed from solution in six days in the presence of nutrient broth (systems 121 and 122). In the absence of supplemental carbon (systems 123 and 124), only 53 to 57 percent of the isopropyl group disappeared from solution in 24 days. System 125, initially sterile, became contaminated during the second day. Within six days, 85 percent of the isopropyl groups had vanished from this solution. As is evident in Figure 4-3, the elimination of isopropyl group in system 125 was comparable to that in systems 121 and 122 that had been inoculated from previous cultures. The control systems 127 and 128 became contaminated after the first week. The reduction in the isopropyl group for these systems was 45 to 52 percent after 24 days.

Table 4-7

Third Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number						
	121	122	123	124	125	127	128
Initial CIPC-I, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	597	597	0	0	597	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	3054	3065	3066	3020	3025	3049	3071
Isopropyl carbon atoms remaining, percent							
1.1 days	94.8	95.2	100.5	100.8	99.2	100.1	100.4
2.2 days	75.0	80.8	99.3	96.4	96.6	100.7	100.0
3.1 days	69.4	78.3	97.7	90.1	89.1	98.7	99.4
6.1 days	3.0	5.2	82.1	79.5	14.6	99.9	99.3
24.1 days	2.3	2.6	47.3	43.0	3.5	48.4	54.9
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	25.3	25.3	25.3	25.3	25.3	25.3	25.3
1.1 days	24.0	24.1	25.4	25.5	25.1	25.3	25.4
2.2 days	19.0	20.4	25.1	24.4	24.4	25.5	25.3
3.1 days	17.6	19.8	24.7	22.8	22.5	25.0	25.1
6.1 days	0.8	1.3	20.8	20.1	3.7	25.3	25.1
24.1 days	0.6	0.7	12.0	10.9	0.9	12.2	13.9

*Visible evidence of contamination prior to second-day sample.

**Visible evidence of contamination prior to 24-day samples.

Table 4-8

Third Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number						
	221	222	223	224	225	227	228
Initial CIPC-R, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	597	597	0	0	597	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	3086	3062	3071	3074	3117	3017	3092
Ring carbon atoms remaining, percent							
3.1 days	89.5	90.6	97.0	88.4	86.6	100.6	97.0
6.1 days	61.5	84.2	83.7	78.2	73.0	100.9	97.7
24.1 days	38.7	61.6	60.3	65.4	69.8	82.7	81.3
58.3 days	34.3	44.6	40.9	51.1	68.4	81.7	76.8
Ring carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	152	152	152	152	152	152	152
3.1 days	136	138	147	134	132	153	147
6.1 days	93	128	127	119	111	153	149
24.1 days	59	94	92	99	106	126	124
58.3 days	52	68	62	78	104	124	117

*Visible evidence of contamination prior to 3-day sample.

**Visible evidence of contamination prior to 24-day samples.

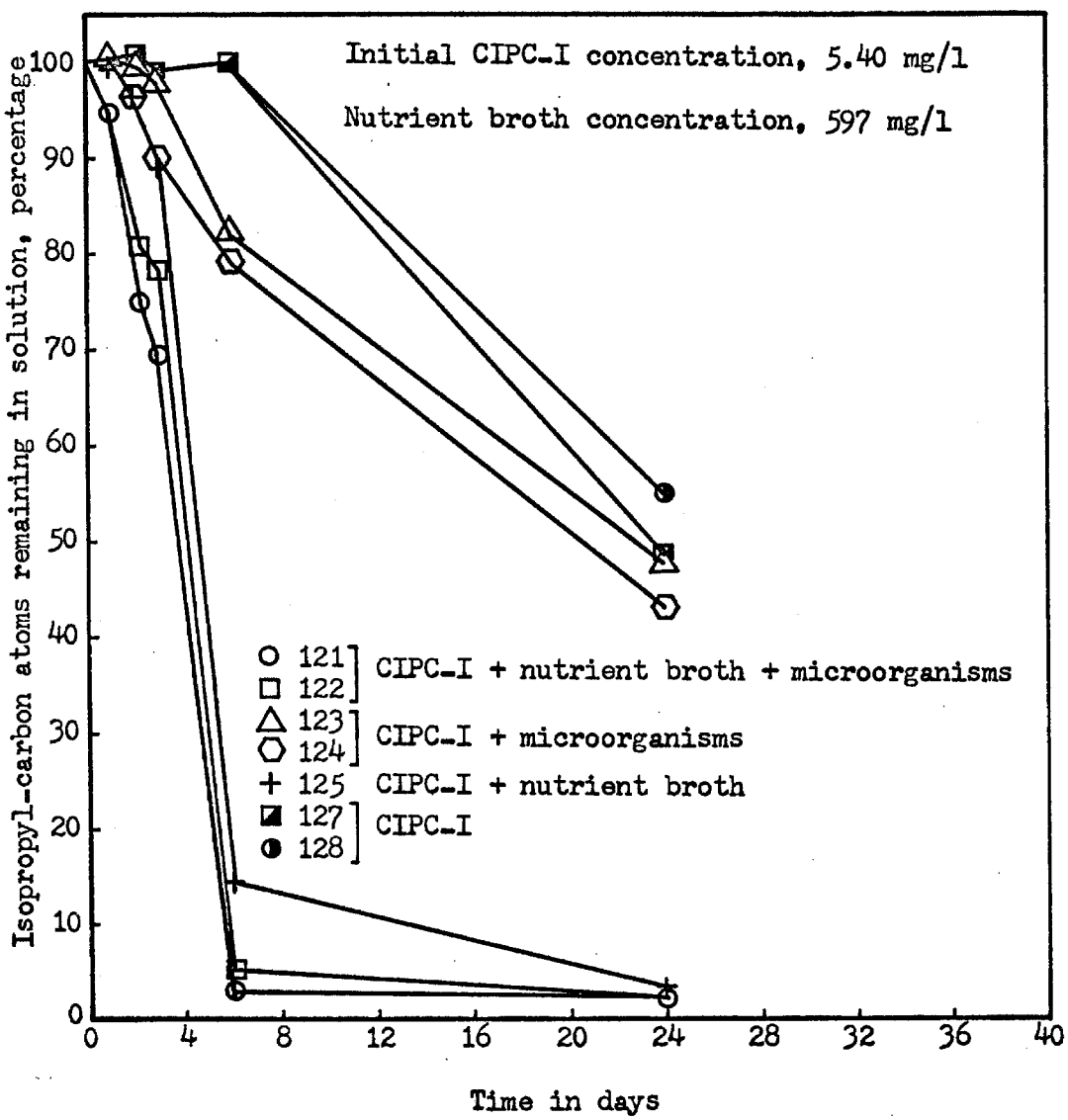


Figure 4-3. Third stage adaptation to isopropyl-labeled CIPC.

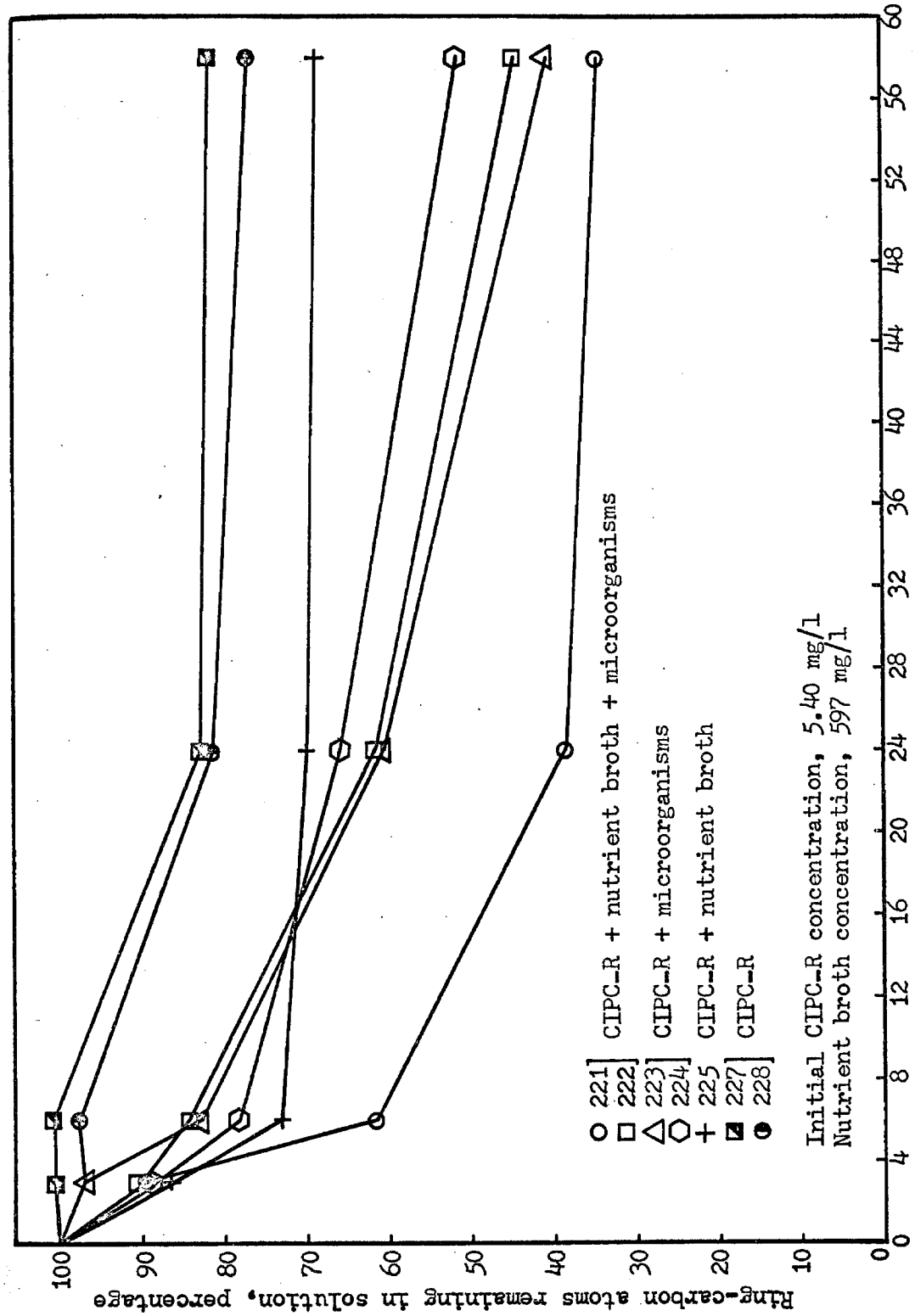


Figure 4-4. Third stage adaptation to ring-labeled CIPC.

Degradation of the ring portion of the CIPC molecule again appeared to be independent of the presence of supplemental carbon. From 35 to 51 percent of the carbon-14 label remained after 58 days in systems 221, 222, 223, and 224. Systems 225, 227, and 228, initially sterile, gave evidence of lesser amounts of degradation after contamination.

The fourth adaptation stage was initiated after the thirty-sixth day of the third adaptation cultures. For this series, the inoculum was obtained from systems 121, 123, and 221 of the previous stage. The pesticide concentration was maintained at 5.4 milligrams per liter, but the nutrient broth concentration was reduced to 400 milligrams per liter. Analyses were performed over a period of 20 days for the CIPC-I cultures and 44 days for the CIPC-R cultures. The results of this study are given in Tables 4-9 and 4-10. Figures 4-5 and 4-6 present the information in graphical form.

In the third adaptation stage, the removal of 95 percent of the isopropyl group from solution required six days. Systems 131 and 132 in the fourth stage, containing nutrient broth, needed 20 days to accomplish the same percentage reduction. The rate of disappearance of the isopropyl segment in systems 133 and 134 devoid of supplemental carbon decreased markedly after five days. Approximately 66 percent of the carbon-14 labeled atoms remained after 20 days of culturing. The initially sterile systems 135, 137, and 138 showed no evidence of contamination for about 11 days.

Remarkable consistency was observed for the CIPC-R systems. All of the inoculated systems, numbers 231 to 234, demonstrated

Table 4-9

Fourth Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number						
	131	132	133	134	135	137	138
Initial CIPC-I, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	400	400	0	0	400	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	3020	3026	3014	3014	3013	3021	3042
Isopropyl carbon atoms remaining, percent							
5.1 days	62.5	63.8	68.3	67.7	99.1	100.9	99.0
8.0 days	54.2	59.7	70.3	71.2	98.9	99.6	100.3
11.0 days	11.8	16.7	70.7	70.4	96.8	100.0	98.4
20.1 days	3.6	3.6	65.8	65.7	73.0	94.3	86.7
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	25.3	25.3	25.3	25.3	25.3	25.3	25.3
5.1 days	15.8	16.1	17.3	17.1	25.1	25.5	25.0
8.0 days	13.7	15.1	17.8	18.0	25.0	25.2	25.4
11.0 days	3.0	4.2	17.9	17.8	24.5	25.3	24.9
20.1 days	0.9	0.9	16.6	16.6	18.5	23.9	21.9

*Visible evidence of contamination prior to 11-day sample.

**Visible evidence of contamination prior to 20-day samples.

Table 4-10

Fourth Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number						
	231	232	233	234	235	237	238
Initial CIPC-R, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	402	402	0	0	402	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	3260	3091	3077	3077	3031	3061	3068
Ring carbon atoms remaining, percent							
5.1 days	67.9	73.1	65.3	68.0	100.1	99.6	98.4
8.0 days	66.3	69.4	65.1	66.6	99.2	99.4	97.8
11.0 days	67.1	71.6	66.4	67.6	97.8	98.5	98.7
20.1 days	65.2	69.4	65.2	67.7	95.9	97.6	98.0
44.1 days	55.7	60.0	63.7	64.9	80.9	95.9	95.9
Ring carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	152	152	152	152	152	152	152
5.1 days	103	111	99	103	152	151	150
8.0 days	101	105	99	101	151	151	149
11.0 days	102	109	101	103	149	150	150
20.1 days	99	105	99	103	146	148	149
44.1 days	85	91	97	99	123	146	146

*Visible evidence of contamination prior to 11-day sample.

**Visible evidence of contamination prior to 44-day samples.

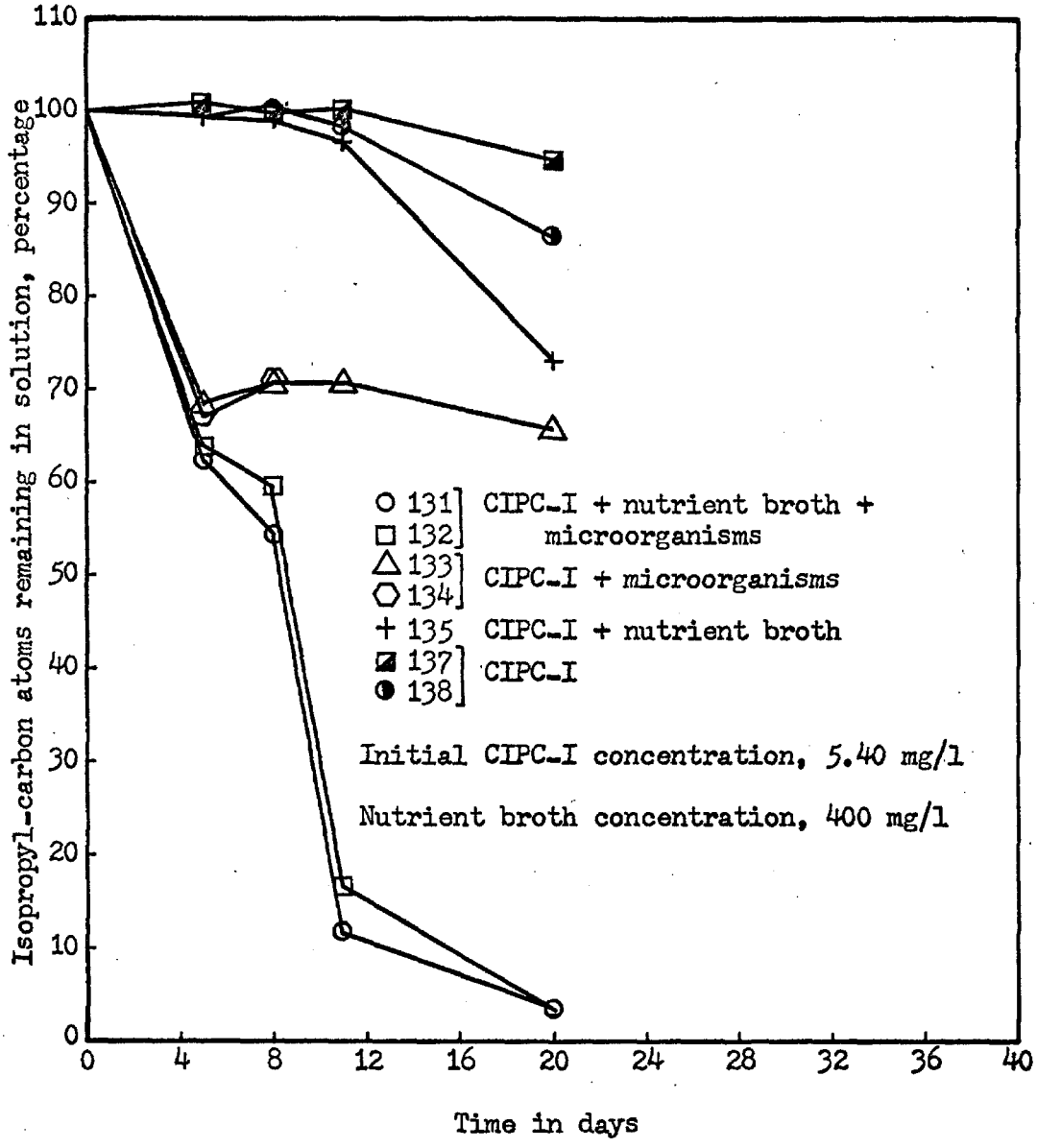


Figure 4-5. Fourth stage adaptation to isopropyl-labeled CIPC.

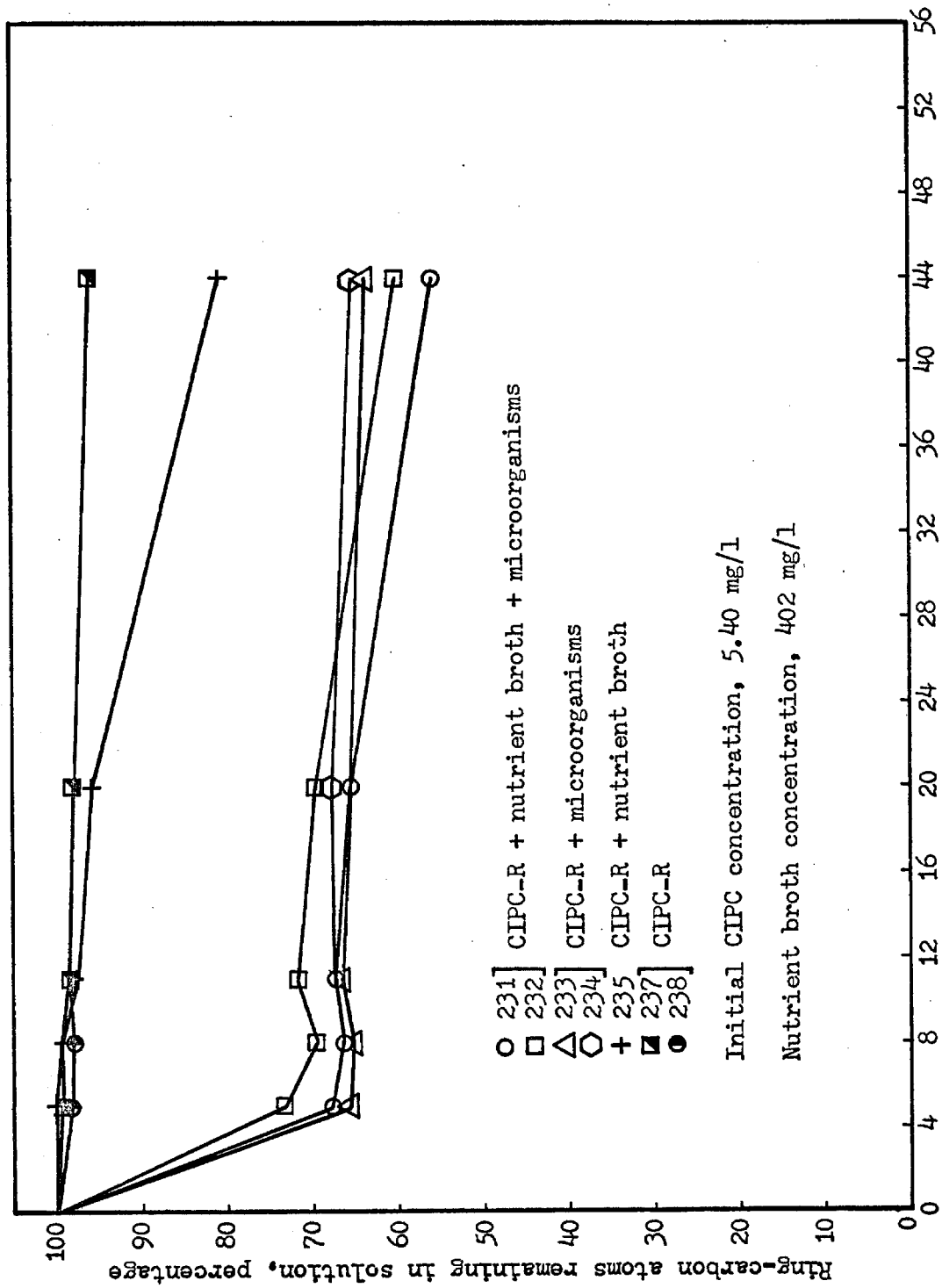


Figure 4-6. Fourth stage adaptation to ring-labeled CIPC.

equivalent removals of the ring-labeled carbon atoms from solution. After 44 days, 55 to 65 percent of the carbon in the ring remained in solution.

During the twenty-third day of operation, systems 131 and 133 provided the inoculum for the fifth stage of adaptation to CIPC. Again the concentration of herbicide was 5.4 milligrams per liter. The nutrient broth concentration was decreased to 200 milligrams per liter. Analyses of this new series proceeded for 21 days for the CIPC-I cultures and 98 days for the CIPC-R systems. In Tables 4-11 and 4-12 and Figures 4-7 and 4-8, the data obtained during this series are presented.

The results obtained from the fifth set of cultures were quite similar to the results of the fourth adaptation stage. Ninety-five to ninety-six percent of the isopropyl group disappeared in 21 days in the presence of supplemental carbon (systems 141 and 142). About 50 percent of the isopropyl group remained when no supplemental source was available (systems 143 and 144). Contamination of the initially sterile systems 145, 147, and 148 produced 13 to 17 percent radioactivity reductions after 21 days.

Breakdown of the ring portion of the molecule was again incomplete. Even after 98 days, 39 to 52 percent of the ring carbon atoms remained. System 248, initially sterile, lost 42 percent of the labeled atoms after contamination. The remaining initially sterile systems, numbers 245 and 247, gave no visible evidence of contamination throughout the adaptation period.

Table 4-11

Fifth Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number						
	141	142	143	144	145	147	148
Initial CIPC-I, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	200	200	0	0	200	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No*	No*
Initial counting rate, cpm	3009	3056	3030	3022	2986	3037	3053
Isopropyl carbon atoms remaining, percent							
3.0 days	79.3	76.2	73.6	70.9	99.5	98.2	98.4
7.8 days	58.7	59.6	66.5	66.0	98.1	97.7	98.4
15.0 days	21.6	20.2	61.9	59.8	81.9	96.5	92.4
21.0 days	5.5	3.9	49.4	48.8	82.6	86.6	86.6
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	25.3	25.3	25.3	25.3	25.3	25.3	25.3
3.0 days	20.1	19.3	18.6	17.9	25.2	24.8	24.9
7.8 days	14.9	15.1	16.8	16.7	24.8	24.7	24.9
15.0 days	5.5	5.1	15.7	15.1	20.7	24.4	23.4
21.0 days	1.4	1.0	12.5	12.3	20.9	21.9	21.9

*Visible evidence of contamination prior to 15-day samples.

Table 4-12

Fifth Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number						
	241	242	243	244	245	247	248
Initial CIPC-R, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	200	200	0	0	200	0	0
Inoculated	Yes	Yes	Yes	Yes	No	No	No*
Initial counting rate, cpm	3065	3380	3075	3068	2990	3046	3073
Ring carbon atoms remaining, percent							
3.0 days	77.5	69.8	69.9	71.7	98.5	98.2	97.9
7.8 days	67.0	59.5	63.2	63.2	99.0	97.3	97.0
15.0 days	65.2	57.4	64.5	63.7	97.0	96.4	96.2
21.0 days	66.5	58.1	64.9	63.6	96.9	96.4	95.6
36.0 days	63.2	58.1	64.7	63.3	98.7	96.2	67.0
42.2 days	58.7	52.4	65.3	61.2	98.4	95.9	51.2
52.9 days	56.4	49.9	62.8	56.5	98.1	96.7	49.0
75.2 days	52.1	46.6	57.7	44.4	94.2	94.2	55.0
97.9 days	50.5	44.8	52.4	39.2	95.2	94.0	57.7
Ring carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	152	152	152	152	152	152	152
3.0 days	118	106	106	109	150	149	149
7.8 days	102	90	96	96	150	148	147
15.0 days	99	87	98	97	147	147	146

Table 4-12(cont'd)

Characteristic	System Number						
	241	242	243	244	245	247	248
Ring carbon atoms remaining, (cont'd)							
21.0 days	101	88	99	97	147	147	145
36.0 days	96	88	98	96	150	146	102
42.2 days	89	80	99	93	150	146	78
52.9 days	86	76	95	86	149	147	74
75.2 days	79	71	88	67	143	143	84
97.9 days	77	68	80	60	145	143	88

*Visible evidence of contamination prior to 36-day sample.

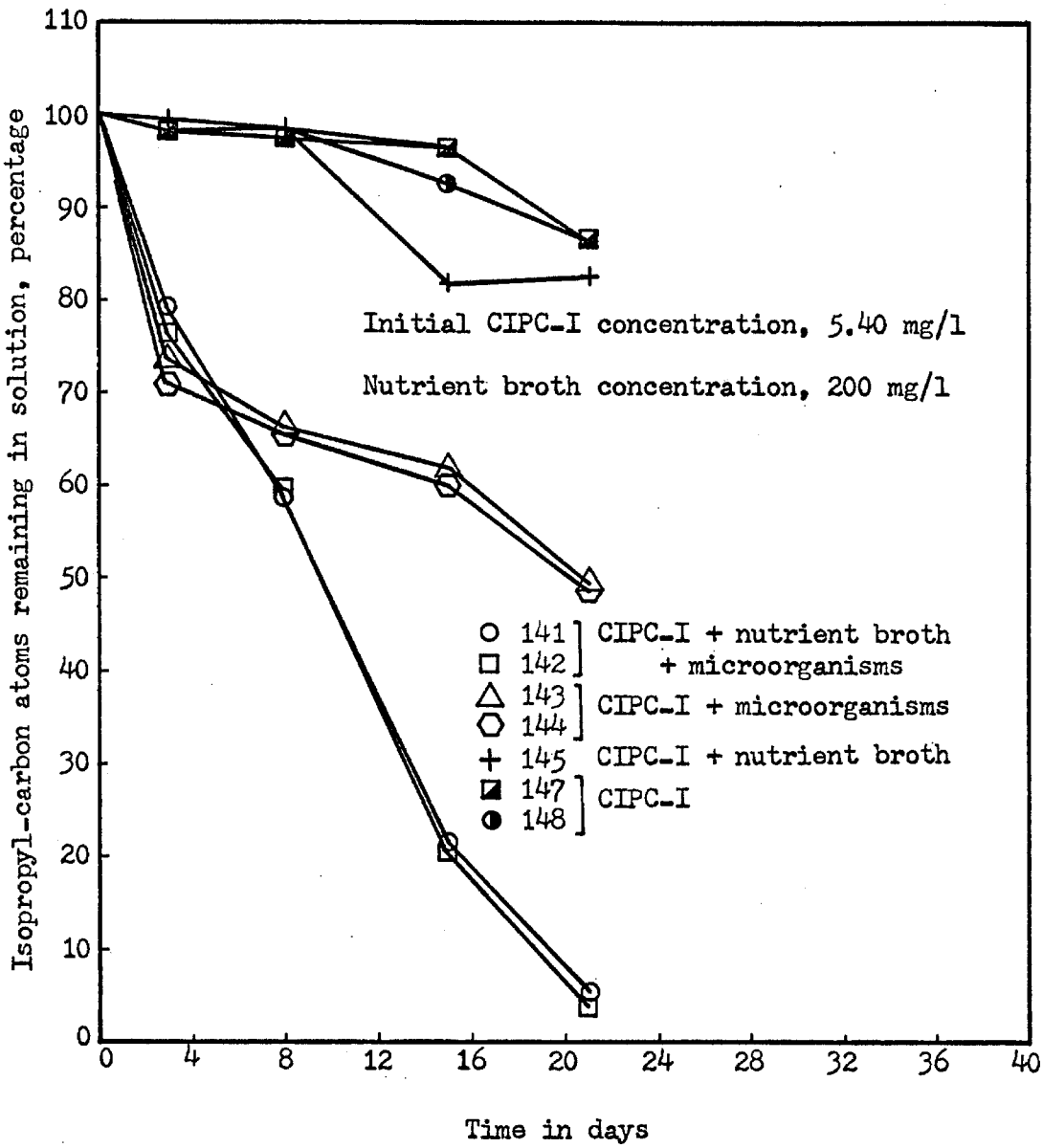


Figure 4-7. Fifth stage adaptation to isopropyl-labeled CIPC.

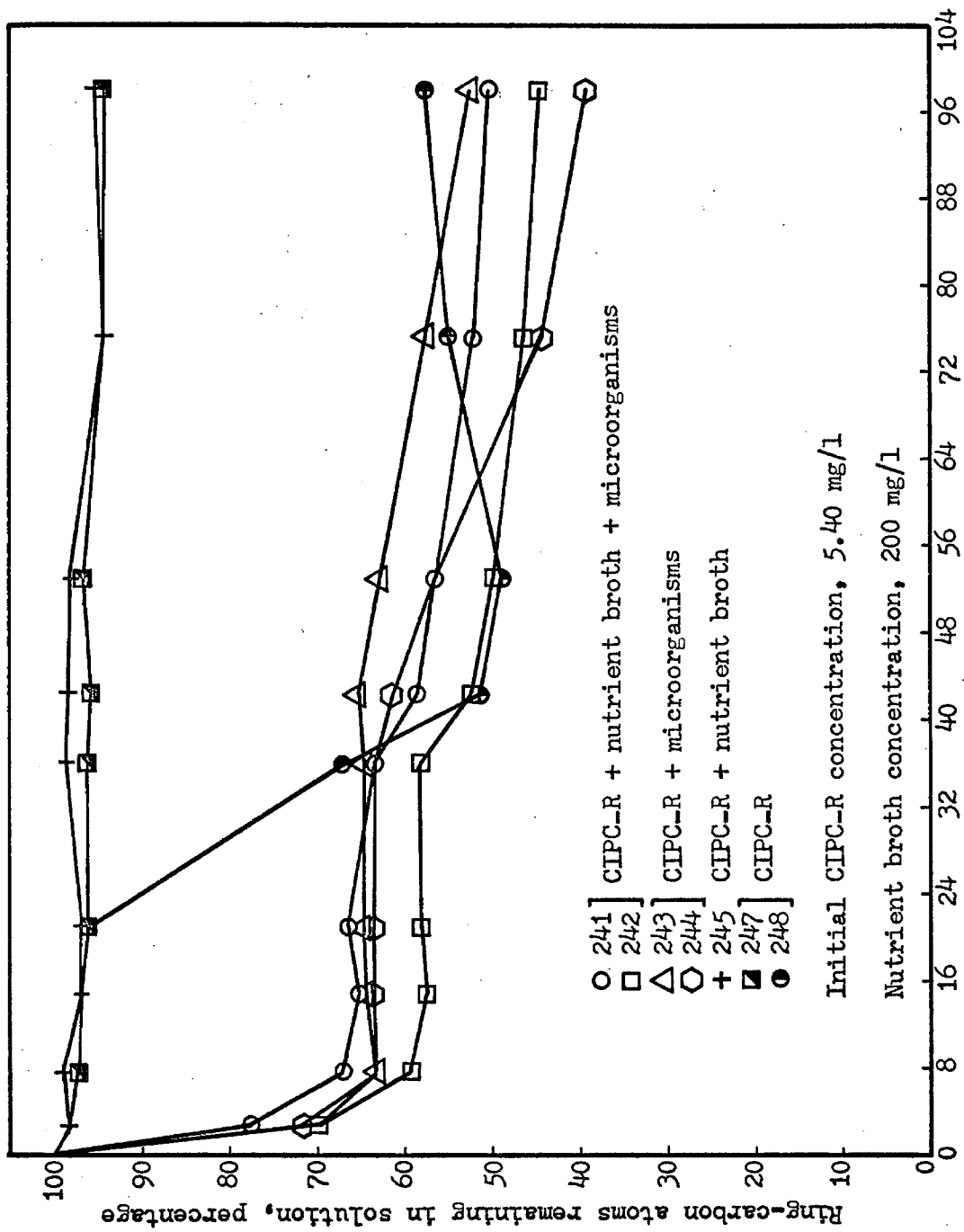


Figure 4-8. Fifth stage adaptation to ring-labeled CIPC.

The sixth and final adaptation stage was begun during the twenty-second day of the fifth stage. Systems 141 and 143 provided the inoculum for the sixth stage. The concentration of nutrient broth was reduced to 100 milligrams per liter while the CIPC concentration was maintained at 5.4 milligrams per liter. Both the CIPC-I and CIPC-R cultures were analyzed at various intervals over a period of 76 days. The data from these cultures are tabulated in Tables 4-13 and 4-14 and graphically depicted in Figures 4-9 and 4-10.

One marked change was observed in the CIPC-I systems. In all of the inoculated systems, numbers 151 to 154, the isopropyl groups disappeared from solution at about the same rate. This series was the first instance where the presence or absence of nutrient broth did not affect the disappearance of the isopropyl carbon atoms from solution. Approximately 95 percent of the isopropyl groups were removed in 20 days. The previous adaptation stage produced similar values for those systems containing nutrient broth.

Degradation of the ring portion of the molecule proceeded in the same manner as observed for previous adaptation stages. After 76 days, 46 to 55 percent of the carbon atoms in the ring remained. Again, no effect was observed with the addition of nutrient broth. The sterile systems 257 and 258 gave no visible evidence of contamination during the entire adaptation period for the sixth stage. Only two percent of the ring-labeled atoms in these systems disappeared in the first 41 days of the sixth stage.

At the conclusion of the sixth stage of adaptation to CIPC, this portion of the research was terminated. A considerable amount

Table 4-13

Sixth Stage Adaptation to Isopropyl-Labeled CIPC

Characteristic	System Number						
	151	152	153	154	155	157	158
Initial CIPC-I, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	100	100	0	0	100	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No**	No**
Initial counting rate, cpm	2992	3001	3003	2987	3036	2993	3024
Isopropyl carbon atoms remaining, percent							
1.7 days	91.7	91.9	92.5	94.0	97.5	99.8	99.1
8.8 days	45.5	47.1	55.2	53.4	96.6	99.5	97.8
13.8 days	18.6	31.8	30.2	26.1	90.4	101.1	97.4
20.0 days	7.0	4.8	6.2	4.2	88.4	88.7	86.8
30.5 days	4.8	2.8	3.8	2.9	85.0	84.4	78.0
41.0 days	4.3	-	3.7	3.4	81.5	84.9	76.0
53.0 days	3.1	-	2.8	2.4	76.5	80.8	73.6
75.7 days	2.6	-	2.8	2.4	69.7	75.0	71.6
Isopropyl carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	25.3	25.3	25.3	25.3	25.3	25.3	25.3
1.7 days	23.2	23.3	23.4	23.8	24.7	25.2	25.1
8.8 days	11.5	11.9	14.0	13.5	24.4	25.2	24.8
13.8 days	4.7	8.0	7.6	6.6	22.9	25.6	24.6

Table 4-13(cont'd)

Characteristic	System Number						
	151	152	153	154	155	157	158
Isopropyl carbon atoms remaining (cont'd)							
20.0 days	1.8	1.2	1.6	1.1	22.4	22.4	22.0
30.5 days	1.2	0.7	1.0	0.7	21.5	21.4	19.7
41.0 days	1.1	-	0.9	0.9	20.6	21.5	19.2
53.0 days	0.8	-	0.7	0.6	19.4	20.4	18.6
75.7 days	0.7	-	0.7	0.6	17.6	19.0	18.1

*Visible evidence of contamination prior to 13-day sample.

**Visible evidence of contamination prior to 20-day samples.

Table 4-14

Sixth Stage Adaptation to Ring-Labeled CIPC

Characteristic	System Number						
	251	252	253	254	255	257	258
Initial CIPC-R, mg/l	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Nutrient broth, mg/l	100	100	0	0	100	0	0
Inoculated	Yes	Yes	Yes	Yes	No*	No	No
Initial counting rate, cpm	3095	3165	3165	3129	3076	3181	3142
Ring carbon atoms remaining, percent							
1.7 days	92.2	90.2	92.3	91.9	98.5	99.1	99.4
8.8 days	74.2	78.1	68.4	81.5	90.8	98.1	99.1
13.8 days	72.3	75.8	74.7	83.9	91.2	98.8	98.3
20.0 days	73.3	71.6	70.7	68.8	89.7	98.1	98.4
30.5 days	66.4	65.3	65.4	62.6	89.5	98.0	97.8
41.0 days	57.4	57.1	57.2	55.2	88.3	97.7	98.0
53.0 days	51.9	52.0	55.2	50.0	86.8	94.5	96.0
75.7 days	47.3	47.0	54.7	46.3	86.7	93.7	95.1
Ring carbon atoms remaining, mole liter^{-1}x10^{-6}							
0.0 days	152	152	152	152	152	152	152
1.7 days	140	137	140	140	150	151	151
8.8 days	113	119	104	124	138	149	151
13.8 days	110	115	114	128	139	150	149

Table 4-14(cont'd)

Characteristic	System Number						
	251	252	253	254	255	257	258
Ring carbon atoms remaining, (cont'd)							
20.0 days	111	109	107	105	136	149	150
30.5 days	101	99	99	95	136	149	149
41.0 days	87	87	87	84	134	149	149
53.0 days	79	79	84	76	132	144	146
75.7 days	72	71	83	70	132	142	145

*Visible evidence of contamination prior to 8-day sample.

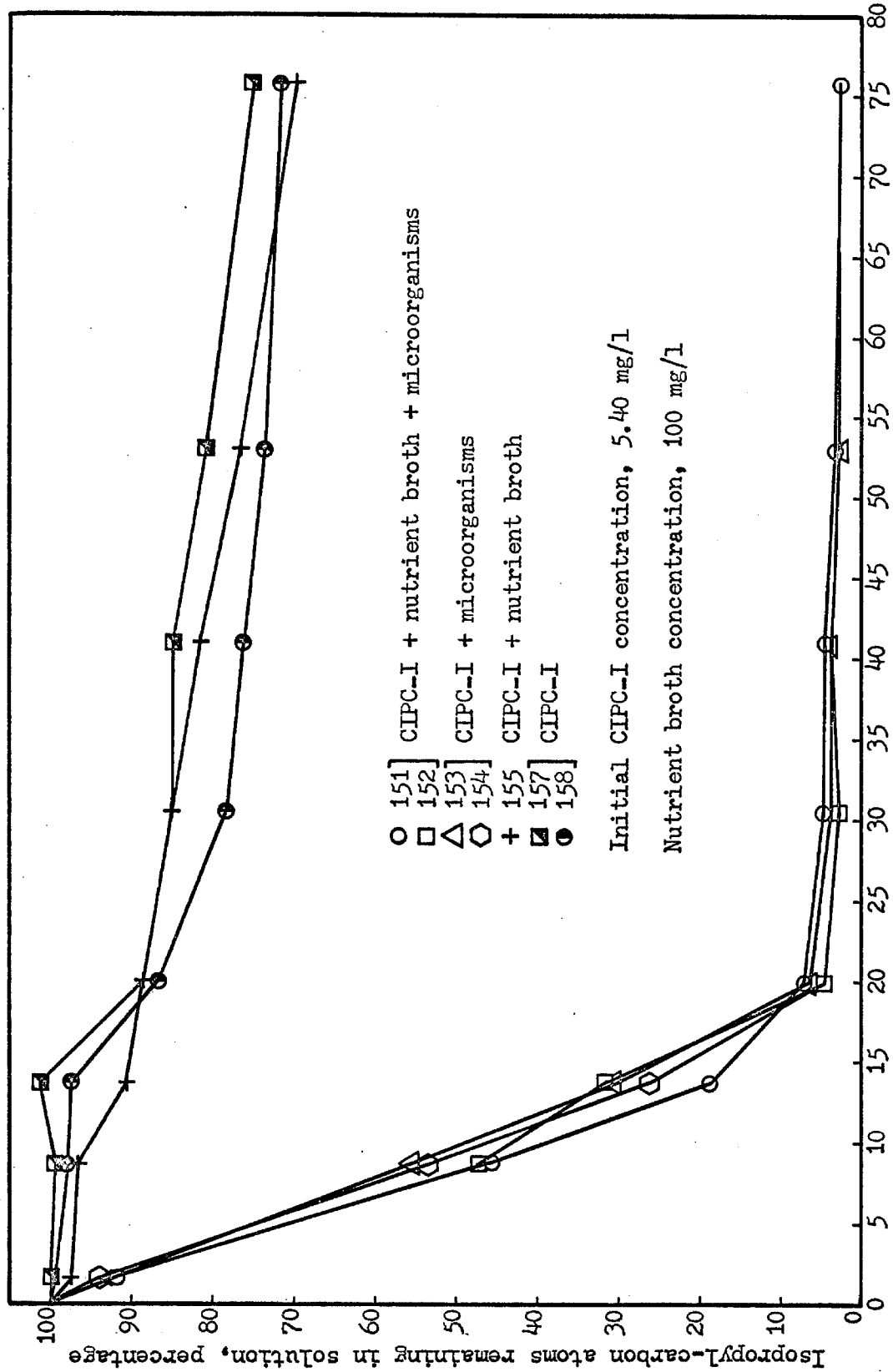


Figure 4-9. Sixth stage adaptation to isopropyl-labeled CIPC.

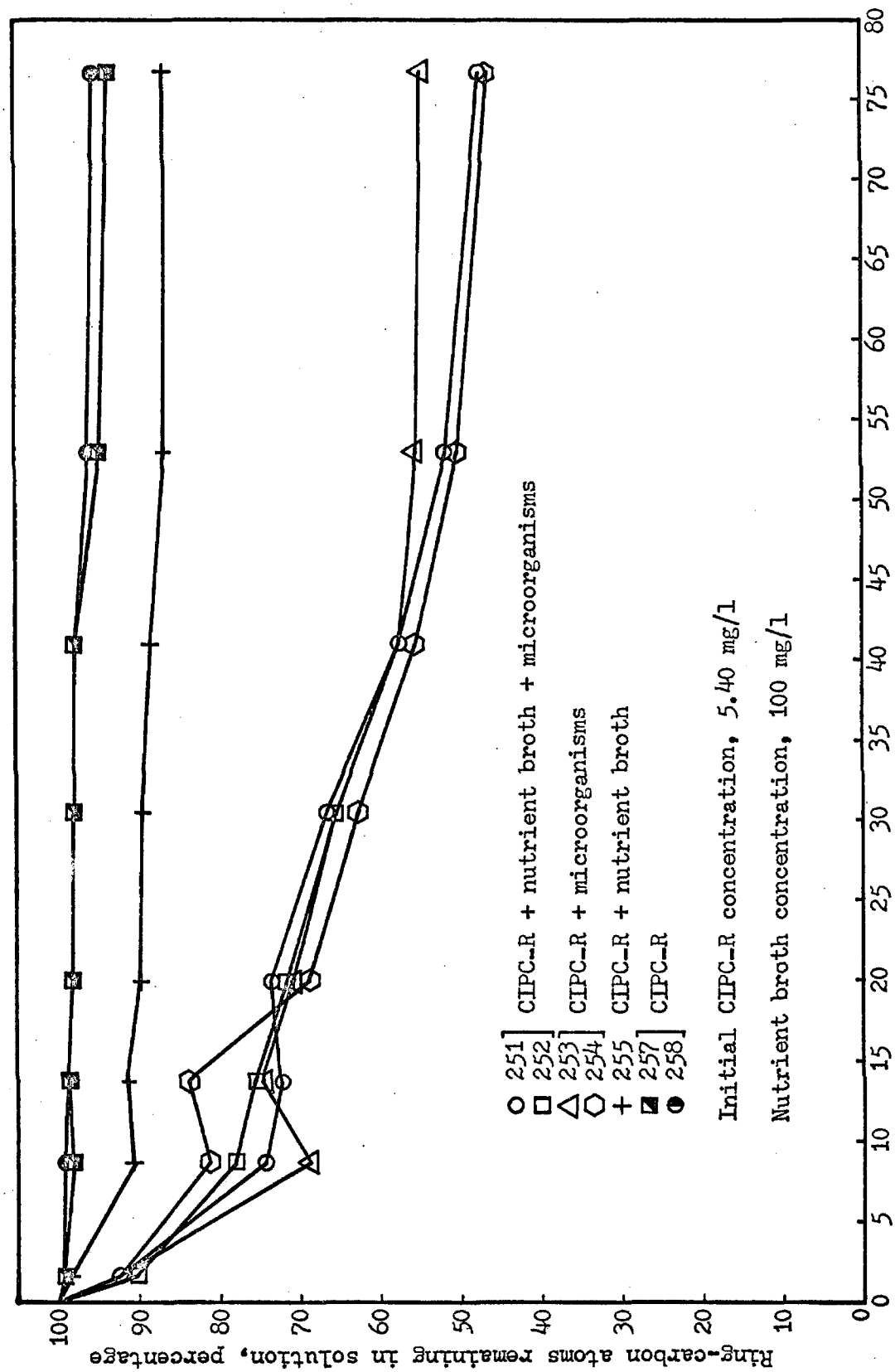


Figure 4-10. Sixth stage adaptation to ring-labeled CIPC.

of information was obtained during the eight months of operation of the adaptation cultures. A discussion of the significance of these results is contained in Section 4.2.2.

4.1.3 Ultraviolet Spectroscopy

It was necessary to explain the significance of the results with the ring-labeled CIPC. Based on the information obtained from the adaptation cultures it was hypothesized that the phenyl rings were only partially degraded. In order to test this hypothesis, ultraviolet spectra were obtained for most of the systems in the fifth stage of adaptation to CIPC-R.

To obtain a sample satisfactory for ultraviolet analysis, a chloroform extraction technique was employed. The extractions and analysis were performed on systems 241 to 248 during the ninety-eighth day of the fifth adaptation stage. In Figure 4-11 are shown the ultraviolet spectra for standard solutions of CIPC in chloroform. The spectra for the extracted samples are presented in Figure 4-12.

It was apparent from the ultraviolet spectra that the phenyl ring was still present in solution in systems 245 and 247. These two systems remained sterile during the fifth stage of adaptation. As shown in Table 4-12, Section 4.1.2, 94 to 95 percent of the labeled carbon atoms in the aromatic rings were present at the time of the ultraviolet analysis. Based on the values obtained from the radioactivity measurements, the amount of CIPC expected in the chloroform extracts was about 12.2 milligrams per liter. Using the percentage transmittance values at 241 millimicrons, the concentrations of

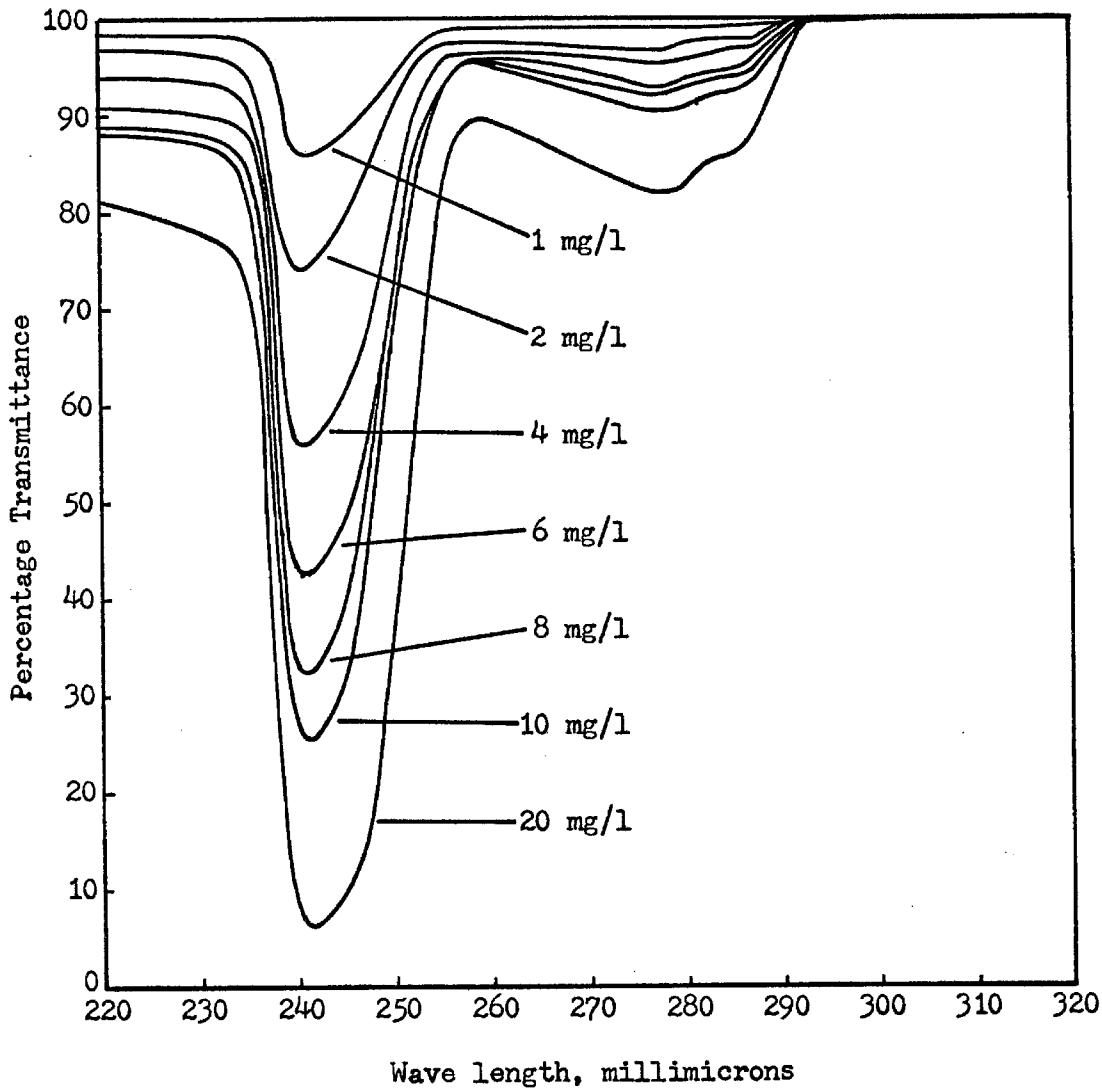


Figure 4-11. Ultraviolet spectra of standard solutions of CIPC in chloroform.

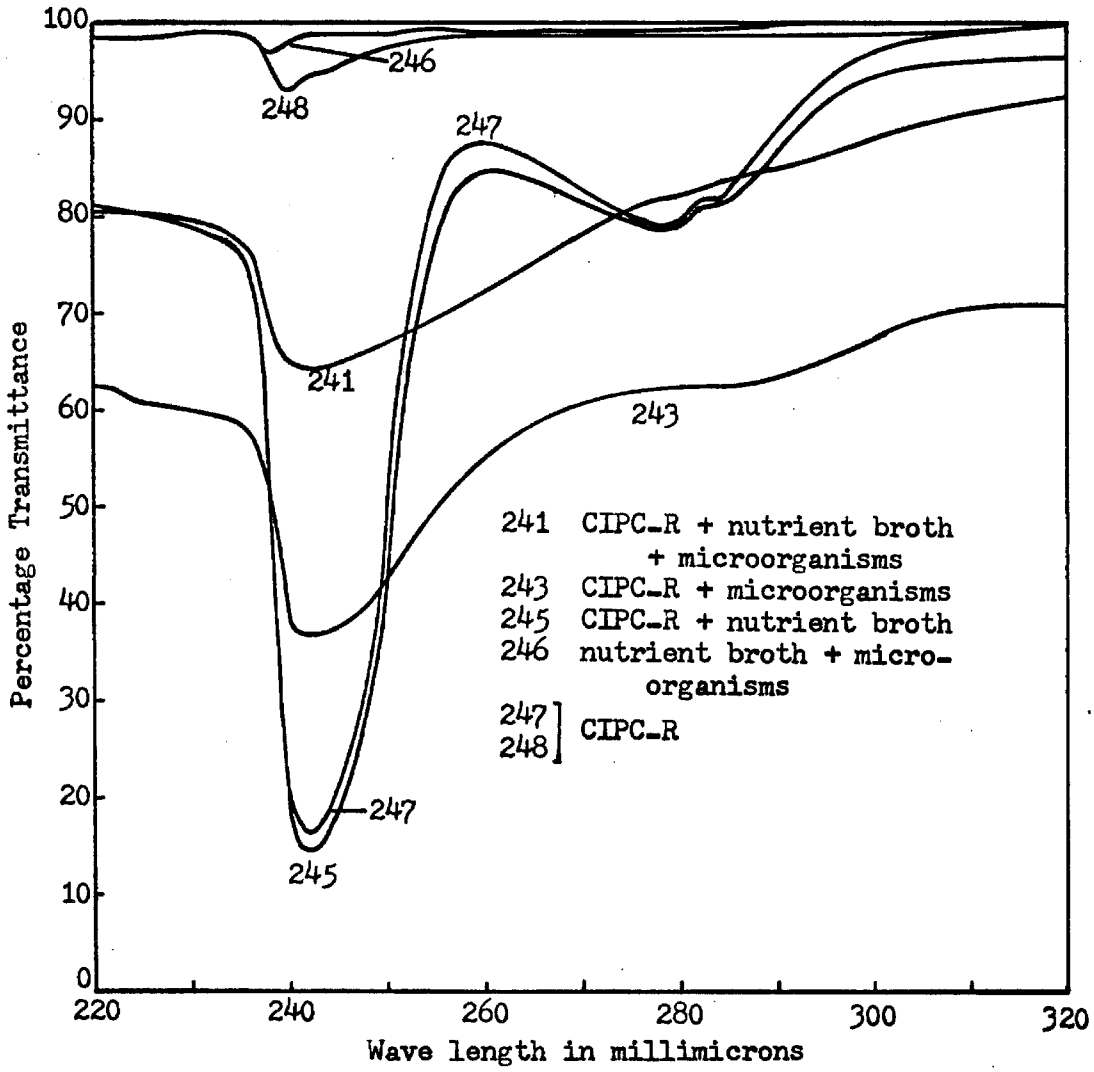


Figure 4-12. Ultraviolet spectra of chloroform extracts from the fifth adaptation stage.

CIPC were estimated to be 13.7 and 12.8 milligrams per liter for systems 245 and 247 respectively.

The spectra for systems 241 and 243 were quite similar. Both systems produced peaks at about 242 millimicrons. This peak corresponded to one of the peaks for CIPC. It should be noted, however, that the shapes of the spectra in this region differed markedly from the shape of the CIPC wave. The waves for systems 241 and 243 were much broader than the CIPC wave. In addition, the secondary peak at 278 millimicrons was not in evidence for these systems.

System 246 contained nutrient broth and microorganisms, but no pesticide. Liquid scintillation analyses on sample 246 indicated a counting rate of less than five counts per minute above background throughout the fifth adaptation stage. Even though this system contained no CIPC, a slight peak was noticeable at 238 millimicrons.

System 248 was initially sterile, but became contaminated after three weeks of adaptation. Subsequently, 43 percent of the carbon atoms in the aromatic rings were degraded. The only distinguishable peak in the spectrum for system 248 occurred at 240 millimicrons. At that wavelength the percentage transmittance was 93.0 percent.

4.2 Discussion

4.2.1 Degradation of 2,4-D

2,4-D has been found to undergo relatively rapid decomposition in the soil, being degraded within one to two months (22)(24)(25). Faust and Aly (61) found that 2,4-D was metabolized in bottom muds from lakes within 65 days (Section 2.3). The same authors,

however, indicated that no breakdown of the pesticide occurred in lake water over a period of 120 days. The data from the present study indicated that a portion of the 2,4-D molecule was partially degraded by bacteria in a dilute salts medium.

The microbial breakdown of 2,4-D proceeded at a slow rate in both adaptation cultures. In the first stage, initially containing 0.1 milligrams of 2,4-D per liter, 64 to 81 percent of the labeled carbon atoms remained after 78 days as shown in Table 4-1, Section 4.1.1. After the same time interval, 81 to 92 percent of the initial 2,4-D concentration of 1.0 milligrams per liter remained in the second stage of adaptation (Table 4-2, Section 4.1.1). An additional 97 days produced total removals ranging from 11 to 23 percent. The results indicate that 2,4-D is not subject to extensive degradation by the microbial population in a dilute salts media. The relative rate of degradation of 2,4-D was slightly faster in the first adaptation stage than in the second stage. Bacterial growth in the systems containing nutrient broth was very vigorous in both adaptation stages. Although visibly less active, bacterial growth was still substantial in the systems devoid of supplemental carbon.

It is to be noted that the 2,4-D determinations in this research were based on the number-2-carbon atoms of the acetic acid moiety. The results of Faust and Aly (61) were based on the ultraviolet analysis of the aromatic ring. From the work by Okey and Bogan (66)(67), it is to be anticipated that the chlorinated ring structure would be more resistant to biological attack than the acetic acid group. The literature (61) indicates that no destruction of the

aromatic segment of 2,4-D was evident in a lake water while the present study shows that limited degradation of the acetic acid group may occur in a similar environment. These two studies, therefore, appear to be consistent.

The data from the first adaptation stage indicate that supplemental carbon has no positive effect on the rate or total amount of degradation. On the contrary, those systems devoid of nutrient broth demonstrated slightly greater amounts of degradation. In the second adaptation stage, this tendency was reversed. A small increase in degradation was evident for the systems that contained supplemental carbon. The differences, however, were small and it can be concluded that the presence of a supplemental carbon source did not materially affect the microbial metabolism of 2,4-D.

In water environments, the metabolism of 2,4-D has been shown to be much slower than in soil systems. The present studies were conducted in a liquid medium of mineral salts. This medium was designed to provide optimum conditions for bacterial growth. The essential nutrients for bacterial growth as well as a large supply of supplemental carbon were present. From a chemical standpoint, this liquid media would be as suitable for microbial activity as most soil environments. The major difference between the two environmental systems would appear to be the solid matter. Indeed, according to Mitchell (78) the presence of solid surfaces may greatly enhance the metabolism of a particular substrate. The effect of the surfaces may be traced to the tendency for both the bacteria and the substrate to accumulate at the solid surfaces. In essence, the surfaces are a

means of more readily supplying nutrients to the organisms. The enhanced rate of degradation of organic compounds in well-seeded soil systems is not limited to 2,4-D. Many organic compounds are metabolized more rapidly in soils than in water environments. For example, alkyl benzene sulfonates strongly resist breakdown in natural water supplies. McMichael and McKee (79) have recently shown that ABS can be readily degraded in intermittant percolation beds.

In summary, the degradation of 2,4-D was quite limited. Based on these studies, it does not appear that 2,4-D would be removed from a contaminated water supply by microbial activity.

4.2.2 Degradation of CIPC

With the aid of isopropyl- and ring-labeled CIPC, it was possible to trace the behavior of two distinct portions of the herbicide molecule. In the ensuing paragraphs, the results of the dual adaptation cultures and their significance are discussed.

In each of the six adaptation stages, significant quantities of both the isopropyl and ring portions of the molecule disappeared. Before attributing this disappearance to microbial degradation, it is necessary to eliminate other possible explanations. As previously discussed in Section 3.6.1, each adaptation series contained sterile systems that were analyzed in the same manner as the inoculated cultures. The purpose of these systems was to distinguish between the losses of CIPC resulting from biological action and losses arising from physical and chemical processes. In particular, any losses resulting from adsorption on the glass walls of the flasks,

volatilization, or chemical breakdown would have been evident in the sterile systems. Although the exact cause would not have been established, an over-all measure of such losses would have resulted. At no time during the adaptation procedures was there evidence of significant losses resulting from physical or chemical processes. During the course of every adaptation stage, one or more of the initially sterile systems demonstrated substantial losses of CIPC. In every instance, however, such reductions in CIPC concentration were accompanied by turbidity in the solutions. Such turbidity was attributed to contamination of the samples during the sampling procedure. All of the systems initially devoid of microorganisms in the isopropyl-labeled CIPC series eventually became contaminated. Certain of these systems did remain sterile for one to two weeks. Systems 157 and 158 in the fifth adaptation stage remained sterile for 14 days. At this time 97.4 to 101.1 percent of the isopropyl-carbon atoms were still present. A number of systems in the ring-labeled CIPC adaptation stages did not become contaminated during the experimental period. This feature was true with systems 237 and 238 of the fourth adaptation stage, systems 245 and 247 of the fifth stage, and systems 257 and 258 of the sixth stage. Even after 98 days, only six percent or less of the ring-carbon atoms had disappeared from systems 245 and 247. Based on these results it was concluded that CIPC losses arising from pure physical or chemical actions were negligible for both the isopropyl and ring moieties. The same conclusion was reached for the 2,4-D systems.

To eliminate any effects on the background counting rate resulting from the presence of nutrient broth and microorganisms, each adaptation series contained an additional control. This control consisted of a system comprising nutrient broth and microorganisms in the liquid media of mineral salts, but without the pesticide. Radioactivity measurements of samples from these systems showed virtually no change in the counting rate as opposed to the normal blanks of distilled water. Any errors resulting from bacterial or nutrient broth interferences, therefore, were eliminated.

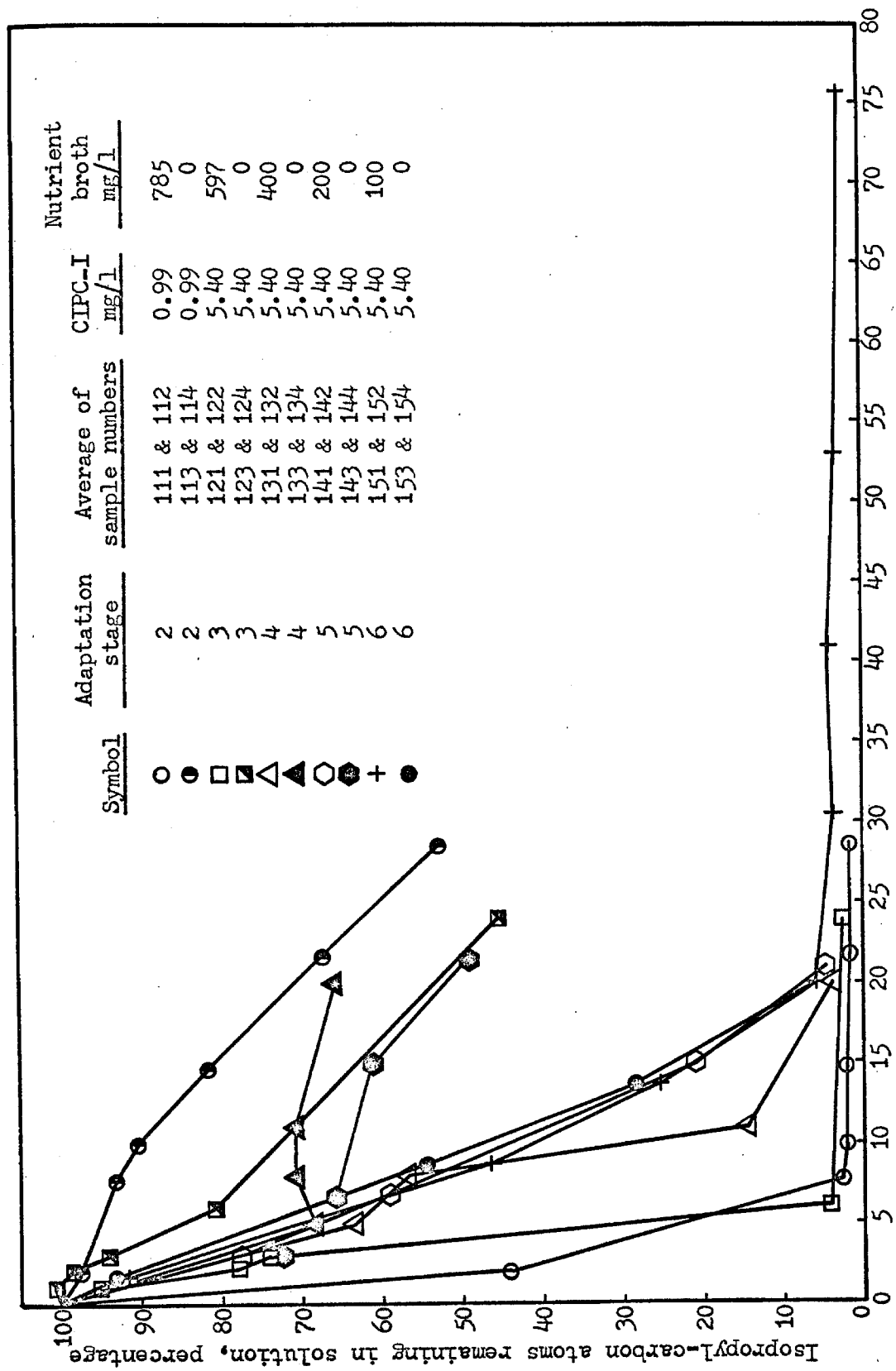
Another possible explanation for the disappearance of CIPC was adsorption of the pesticide on the cellular material. If adsorption of the entire molecule had occurred the relative reduction in the concentration of radioactivity in solution would have been the same for both the CIPC-I and CIPC-R systems. In every adaptation stage, the removal of the isopropyl group greatly exceeded that of the ring portion for those systems containing nutrient broth. Furthermore, if such adsorption occurred it would be dependent upon the concentration of microorganisms. The last five adaptation stages for CIPC-R demonstrated complete independence of culture concentrations. Reductions in the concentrations of ring-carbon atoms were the same both for those systems containing nutrient broth and for those without supplemental carbon. The former evidenced the presence of much higher microbial concentrations. This same independence was evident in the sixth adaptation stage for the isopropyl group, as shown previously in Figure 4-9, Section 4.1.2. These data, therefore, clearly show that any significant disappearance of the herbicide

cannot be attributed to the adsorption on bacterial cells of the entire molecule or of either of the two labeled portions.

It can be concluded with reasonable certainty that the observed reductions in pesticide concentration in the adaptation cultures were a direct result of microbial metabolism. The remainder of this chapter will be devoted to a discussion of the biological degradation of CIPC.

In all of the adaptation cultures, the maximum percentage degradation of the isopropyl groups greatly exceeded the percentage degradation of the ring portions. The data from the inoculated CIPC-I cultures are graphically summarized in Figure 4-13. This figure shows that at least 94 percent of the isopropyl moiety was removed within about 20 days in the presence of supplemental carbon. In adaptation stages two and three, 95 percent degradation was realized in the first eight days.

Degradation of the isopropyl groups proceeded much more slowly in the cultures devoid of nutrient broth, with the exception of the sixth adaptation stage. In the systems containing nutrient broth, there was a definite tendency for the rates of degradation of the isopropyl portion, expressed in percentages, to decrease with decreasing ratios of nutrient broth to pesticide. This fact is discernible from the curves in Figures 4-13. With successive adaptation stages the ratio of nutrient broth to CIPC-I decreased. Likewise, the percentage rates of reduction in the number of isopropyl-carbon atoms in solution tended to decrease with successive adaptation cultures.



Symbol	Adaptation stage	Average of sample numbers	CIPC-I mg/l	Nutrient broth mg/l
○	2	111 & 112	0.99	785
●	2	113 & 114	0.99	0
□	3	121 & 122	5.40	597
▣	3	123 & 124	5.40	0
△	4	131 & 132	5.40	400
▲	4	133 & 134	5.40	0
⬡	5	141 & 142	5.40	200
⬢	5	143 & 144	5.40	0
+	6	151 & 152	5.40	100
●	6	153 & 154	5.40	0

Figure 4-13. Degradation of isopropyl-labeled CIPC. Comparison of adaptation stages.

The increased rate at which the isopropyl segment of the CIPC molecule is metabolized in the presence of nutrient broth can be explained in several ways. The observed effect may be a direct result of the size of the microbial population. That is, the rate of degradation is a function of the number of microorganisms present. Microbial growth in the nutrient broth systems greatly exceeded that in the corresponding systems without nutrient broth. The results also could reflect the presence of a constituent in the nutrient broth necessary for the rapid development of microorganisms capable of metabolizing the isopropyl segment.

Finally, the results may be related to the formation of induced enzyme systems. One of the necessary conditions for the formation of induced enzymes is the presence of an energy source (80). This source may be a second substrate, endogenous material, or sometimes the inducer substrate itself, once some of the enzyme has been synthesized. Nutrient broth would provide an abundant source of energy for the synthesis of induced enzymes that may be necessary for the metabolism of the isopropyl-carbon atoms. Ethanol, present in relatively small amounts in all the experimental systems, furnishes another source of energy.

At this point, the distinction should be made between the terms adaptation and induced enzyme formation. Adaptation refers to changes that occur in an organism that make the organism more suitable for survival and growth in its environment. Induced enzyme formation is one of several specific ways an organism can adapt to its surroundings. Detailed discussions of adaptation can be found in

the texts by Umbreit (81), Fruton and Simmonds (82), and Dixon and Webb (80).

During the sixth adaptation stage, degradation of the isopropyl portions of the CIPC molecules was no longer dependent upon the presence of nutrient broth. Systems 153 and 154, containing no nutrient broth, metabolized the isopropyl groups as rapidly as did systems 151 and 152 that contained 100 milligrams per liter of nutrient broth. In all four of these samples, degradation of the isopropyl segment proceeded at a rate almost identical to the rate for the nutrient-broth systems in the fifth stage. These results indicate the presence in the sixth adaptation stage of some microorganism capable of rapidly metabolizing the isopropyl-carbon atoms even in the absence of nutrient broth. The existence of such an organism in the systems may be a result of contamination or the evolution of a mutant bacterial strain.

Before examining the results from the CIPC-R systems a brief discussion concerning the microbial degradation of aromatic compounds will be presented. The metabolic pathways involved in the decomposition of relatively simple phenyl compounds is quite consistent. Certain strains of Vibrio and Pseudomonas have been shown to degrade phenol and benzoic acid in the following manner (82):

benzoic acid and phenol ⟶ catechol

⟶ cis, cis muconic acid ⟶ γ-carboxymethyl Δ^{α}-butenolide

⟶ β-ketoadipic acid ⟶ succinic acid + acetic acid

A similar pathway was evidenced for the oxidation of p-cresol and p-hydroxybenzoic acid using the same organisms:

p-cresol ⟶ p-hydroxybenzoic acid

⟶ protocatechuic acid ⟶ β -carboxymuconic acid

⟶ β -ketoadipic acid ⟶ smaller fragments

Stanier et al. (83)(84) showed that P. fluorescens degraded p-benzoic acid by the same pathway. Hayaishi et al. (85) isolated pyrocatechase from Pseudomonas cells grown on anthranilate. The purified enzyme was able to catalyze oxidative cleavage of catechol forming cis, cis muconic acid. Previously cited results (Section 2.2) indicate that the chlorophenoxyalkanoic acid pesticides are degraded in the same manner by soil microorganisms. Metabolism of the alkanoic acid side chain may be initiated by beta oxidation or by cleavage at the ether linkage (Figure 2-2, Section 2.2) depending on the type of microorganism.

A somewhat unusual metabolic pathway has been observed for the degradation of the amino acid tyrosine by strains of Vibrio and Pseudomonas (80)(82). Initially, the amino group is removed followed by the migration of the side chain to the adjacent carbon atom:

tyrosine ⟶ p-hydroxyphenylpyruvic acid

⟶ 2,5-dihydroxyphenylpyruvic acid

⟶ homogentisic acid ⟶ maleylacetoacetate

⟶ fumarylacetoacetate ⟶ fumaric acid + acetoacetic acid

In light of the preceding discussion, the molecular structure of CIPC can be examined with respect to its possible mode of degradation. CIPC contains a chlorine atom in the meta-position. According to Alexander (21), the presence of a chlorine atom in the meta-position renders the phenyl structure quite resistant to microbial

decomposition. The effect of chlorine atoms on the degradation of phenyl compounds is much less pronounced when the substitution is on either the ortho- or para-carbon atom. On this basis, the phenyl ring of CIPC would be expected to resist microbial degradation.

The biodegradability of CIPC conceivably might be altered by the metabolism of the side chain. For instance, the addition of a hydroxyl group on the carbon atom in the para-position relative to the chlorine substitution and the replacement of the amino segment by another hydroxyl group would yield 4-chlorocatechol. The chlorine atom would then be located in the para-position rendering the phenyl structure degradable. Indeed, 4-chlorocatechol has been shown to be a metabolic intermediate in the degradation of several chlorophenoxy-alkanoic acid pesticides (21)(27)(29). Cleavage of the phenyl group, however, may not lead to complete metabolism of the ring-carbon atoms. As mentioned in Sections 2.2 and 2.3, a short chain, chlorinated hydrocarbon may remain as an end product following ring cleavage and partial metabolism of the ring-carbon atoms.

It might be anticipated, therefore, that certain metabolic changes may occur in the molecular structure of CIPC which would lead to ring cleavage. A short chain, chlorinated hydrocarbon might remain as a final end product. In the absence of such changes in the molecular structure, the 3-chlorophenyl ring would be expected to resist microbial metabolism.

The adaptation cultures for ring-labeled CIPC produced remarkably consistent results. In no instance was metabolism of the ring-carbon atoms complete. The amounts of ring-carbon atoms

remaining for the four inoculated cultures are summarized in Table 4-15. These values represent the results of the final measurements at the termination of each adaptation stage.

The total amounts and rates of degradation of the ring structure did not depend upon the presence of supplemental carbon, with the exception of the first stage of adaptation. After 45 days of the first adaptation series, 25 to 30 percent of the ring portion remained in systems 201 and 202 containing nutrient broth (Table 4-4, Section 4.1.2). At the same time, 45 to 51 percent of the ring atoms were still present in systems 203 and 204 devoid of supplemental carbon. In all of the subsequent stages, the presence of nutrient broth had no effect on the metabolism of the ring segment. Furthermore, there was no tendency for the rate or total amount of degradation to change with successive adaptation cultures.

As can be seen in Figures 4-2, 4-4, 4-6, 4-8, and 4-10 in Section 4.1.2, the shapes of the degradation curves were quite similar. Most of the metabolism occurred in the first eight days of each adaptation series. After this time, the curves leveled off and only small changes were observed for the remainder of each cultivation period. In essence, the microbial metabolism of the ring atoms had ceased after about 50 percent of the labeled atoms had disappeared.

To obtain additional information on the metabolism of the phenyl ring, an ultraviolet analysis of certain systems was performed. Following a chloroform extraction, the ultraviolet spectra of samples from systems 241, 243, 245, 246, 247, and 248 from the fifth adaptation stage were obtained. These analyses were performed during the

Table 4-15

Amounts of CIPC-R Remaining in the Inoculated Systems

at the Termination of Each Adaptation Stage

Adaptation Stage	System Numbers	Elapsed Time, Days	CIPC-R Remaining, mg/l		CIPC-R Remaining, percent		
			Maximum	Minimum	Maximum	Minimum	Average
1	201-204	45.00	0.046	0.023	50.8	25.5	37.8
2	211-214	34.67	0.57	0.51	59.6	52.9	57.2
3	221-224	58.31	2.76	1.85	51.1	34.3	42.7
4	231-234	44.08	3.50	3.01	64.9	55.7	61.8
5	241-244	97.94	2.83	2.12	52.4	39.2	46.7
6	251-254	75.69	2.95	2.50	54.7	46.3	48.8

ninety-eighth day of adaptation. At that time the following amounts of ring-carbon atoms remained in solution:

System Number	Ring Carbon Atoms in Solution	
	percent	micromole liter^{-1}
241	50.5	77
243	52.4	80
245	95.2	145
247	94.0	143
248	57.7	88

Previous analyses of standard CIPC solutions showed that this compound produced the typical ultraviolet spectrum of a chlorophenol (Figure 4-11, Section 4.1.2). In other words, the ultraviolet spectrum of CIPC is characteristic of the ring portion of the molecule. As previously discussed, cleavage of the phenyl ring yields a muconic acid as an intermediate in the degradation of simple aromatic compounds. Muconic acid compounds have strong ultraviolet adsorption spectra. β-carboxymuconic acid, for example, has a peak at 255 millimicrons with a molar extinction coefficient of about 8200 liter mole^{-1} centimeter^{-1}(84). Subsequent microbial transformation of this compound to β-ketoadipic acid eliminates any substantial ultraviolet spectrum. Likewise, the smaller metabolic fragments do not produce ultraviolet spectra in the region from 220 and 300 millimicrons. Cleavage of the phenyl ring of CIPC with the subsequent metabolism of some of the ring carbon atoms, therefore, would eliminate any ultraviolet spectrum.

The ultraviolet spectra of systems 245 and 247 (Figure 4-12, Section 4.1.2) produced the typical CIPC or chlorophenol spectra. These systems remained sterile throughout the experimental period and contained 94 to 95 percent of the initial CIPC concentration. The fact that samples from these two systems produced the anticipated spectra verified the transfer of CIPC to the chloroform phase by the extraction procedure. Indeed, estimates of the amount of CIPC in solution based on the standard solutions of CIPC agreed within about 10 percent with the values calculated on the basis of the radioactivity measurements.

System 246 contained nutrient broth and microorganisms, but was devoid of CIPC. Only a very small peak at 238 millimicrons was observed in the ultraviolet spectrum of this system. Possible interferring substances associated with the nutrient broth or the microorganisms, therefore, were eliminated by the chloroform extraction procedure.

Systems 241 and 243 did not show a distinct peak at 278 millimicrons. In system 243, however, the spectrum tended to level off in this region. Both systems produced rather large peaks at 242 millimicrons. The wave form for these peaks differed from the standard CIPC spectrum. In particular, the wave for systems 241 and 243 was broader and shorter than the CIPC wave. This peak cannot be attributed to interferences arising from the nutrient broth or the microbial population as evidenced by the spectrum of system 246. It may be concluded that the observed spectra of systems 241 and 243 represents some metabolic product. The wave form may indicate metabolic changes in the side chain of CIPC.

At the time of the chloroform extractions, the percentages of ring-carbon atoms remaining in solution in systems 241 and 243 were 50.5 and 52.4, respectively. If it is assumed that the peak at 242 millimicrons is the CIPC peak, then the percentage of intact phenyl rings may be estimated. On this basis, 25 and 54 percent of the phenyl rings remained in solution in systems 241 and 243. The assumption that the calibration curve for standard solutions of CIPC can be applied to the microbial systems may not be a valid one. In view of the complex nature of the microbial systems, no significance can be attached to the quantitative differences between the spectra of systems 241 and 243. Nonetheless, an approximate measure of the number of phenyl rings in solution can be made. A substantial fraction of the ring-carbon atoms in solution would appear to be associated with intact phenyl rings.

The ultraviolet spectra of system 248 indicated almost complete loss of the ring character. This system produced only a small peak at 239 millimicrons. System 248 was initially sterile, but became contaminated. When the ultraviolet analysis was performed, 57.7 percent of the ring-carbon atoms remained in solution. It is apparent from the ultraviolet spectrum that most of these ring-carbon atoms must be associated with acyclic, metabolic end products.

The data obtained from the adaptation stages combined with the results from the ultraviolet analyses suggest two different microbial systems. In the first system, all six carbon atoms in the ring portions of 40 to 60 percent of the CIPC molecules are metabolized. The remaining ring-carbon atoms constitute intact phenyl rings. The

ultraviolet spectra of systems 241 and 243 seemed to be in accord with this interpretation of the adaptation data. A significant proportion of the ring-carbon atoms in solution apparently remained in the phenyl structure. The question must be raised as to why only some of the phenyl groups of CIPC are metabolized. Conceivably, a preliminary metabolic step converts some of the rings into less resistant arrangements. The formation of 4-chlorocatechol from some of the CIPC molecules, as mentioned previously, might leave the ring structure more susceptible to biological oxidation. The biodegradability of the phenyl structure may be related to the way in which the amino side chain is metabolized.

In the second system, the phenyl groups of every CIPC molecule are cleaved with the subsequent metabolism of two to four of the ring-carbon atoms. As in the first system, 40 to 60 percent of the ring-carbon atoms are removed from solution. A short chain chlorinated hydrocarbon remains as a biologically resistant end product. Under these circumstances, the phenyl character of the system would disappear. System 248 would appear to be consistent with this type of metabolic pathway. The ultraviolet analysis of system 248 showed that the phenyl groups had disappeared almost entirely in spite of the presence of 57.7 percent of the ring-carbon atoms in solution.

A comparison of the initial rates of degradation of the ring- and isopropyl-carbon atoms yields certain information regarding the mode of biological attack. Summarized in Table 4-16 are the data from the first few days of each adaptation stage. In adaptation stages two and three, the relative degradation of the ring segment lagged

Table 4-16

Amounts of CIPC-I and CIPC-R Remaining During the Early Periods of Each Adaptation Stage

Adaptation Stage	Elapsed Time Days	Percent Remaining in Nutrient-Broth Cultures		Percent Remaining in Cultures Without Nutrient Broth	
		CIPC-I	CIPC-R	CIPC-I	CIPC-R
2	2.00	42.0-46.4	93.8-94.3	95.8-98.9	98.1-99.1
	7.75	2.5- 2.8	66.0-75.4	92.7-93.1	78.4-80.3
3	3.08	69.4-78.3	89.5-90.6	90.1-97.7	88.4-97.0
	6.10	3.0- 5.2	61.5-84.2	79.5-82.1	78.2-83.7
4	5.08	62.5-63.8	67.9-73.1	67.7-68.3	65.3-68.0
	8.02	54.2-59.7	66.3-69.4	70.3-71.2	65.1-66.6
	10.96	11.8-16.7	67.1-71.6	70.4-70.7	66.4-67.6
5	2.96	76.2-79.3	69.8-77.5	70.9-73.6	69.9-71.7
	7.83	58.7-59.6	59.5-67.0	66.0-66.5	63.2-63.2
	15.02	20.2-21.6	57.4-65.2	59.8-61.9	63.7-64.5
6	1.67	91.7-91.9	90.2-92.2	92.5-94.0	91.9-92.3
	8.77	45.5-47.1	74.2-78.1	53.4-55.2	68.4-81.5
	13.75	18.6-31.0	72.3-75.8	26.1-30.2	74.7-83.9

considerably behind that of the isopropyl moiety in the initial periods for those samples containing supplemental carbon. For the remaining adaptation cultures, the initial percentage degradation rates for the ring structure and the isopropyl portion were comparable. The nutrient-broth cultures in the fifth stage degraded the ring portion faster than the isopropyl group.

The second series of values presented in Table 4-16 represents the percentage degradation of the ring- and isopropyl-carbon atoms in those cultures devoid of nutrient broth. Over the first eight days of every adaptation series, with the exception of the sixth stage, the rates of degradation for the ring structure were faster than the rates of destruction of the isopropyl segment. In some instances, the relative ring degradation greatly exceeded that of the isopropyl moiety.

The results summarized in Table 4-16 clearly indicated that the degradation of the ring segment proceeded at least as fast as the degradation of the isopropyl group. These data suggest the possibility that the ring structure was partially degraded before the isopropyl-carbon atom was metabolized. Perhaps the CIPC molecule is bisected at the amino nitrogen atom or the ester oxygen atom. Degradation of the isopropyl and ring portions could then proceed independently.

The studies on the degradation of CIPC can now be summarized. Breakdown of the CIPC molecules was definitely ascribable to biological action. The addition of nutrient broth to the microbial systems greatly increased the rate at which the isopropyl-carbon atoms were

metabolized. Nutrient broth had no effect on the rate of degradation of the phenyl group.

Metabolism of the isopropyl-carbon atoms proceeds quite rapidly in the presence of nutrient broth. Ninety-five percent of these atoms were utilized by the organisms within 20 days. With respect to the ring segment, two different microbial systems apparently were observed. In one case, only some of the ring structures were cleaved. Following cleavage, the ring-carbon atoms were completely metabolized. A large fraction of the CIPC molecules apparently resisted ring cleavage for several months. The fact that some of the rings undergo cleavage may be a result of changes in the structure of these molecules such as a conversion from CIPC to 4-chlorocatechol. In a second system containing contaminant organisms, all of the phenyl groups were cleaved. Subsequently, two to four of the ring-carbon atoms were metabolized leaving a short chain molecule possibly composed of two to four carbon atoms and a chlorine atom. This end product resisted further degradation for at least three months. The isolation and identification of the specific metabolic intermediates and end products were beyond the scope of this research.

Under the biological conditions that might occur in natural water supplies, intact CIPC molecules may persist for weeks or months. The metabolic intermediates and end products would be anticipated to remain for many months.

CHAPTER 5

THE ADSORPTION OF 2,4-D AND CIPC ON ACTIVATED CARBON AND CLAY MINERALS

The second major portion of this research concerned the adsorption of 2,4-D and CIPC on activated carbon and several clay minerals. Activated carbon was selected because of its widespread use in water treatment plants and the clay minerals were chosen for their resemblance to the particulate matter in rivers. It was the intent of this investigation to ascertain the abilities of these substances to remove pesticides from water. The results of these studies and a discussion of their significance are contained in this chapter.

5.1 Results

5.1.1 Adsorption on Clay Minerals

Illite, kaolinite, and montmorillonite were the clay minerals employed in these investigations. The physical and chemical characteristics of these minerals were previously presented in Table 3-5, Section 3.7.3. In all of the adsorption experiments with the clays, the phase separation prior to analysis was accomplished by centrifugation. The experiments were all conducted at 20°C.

The most extensive set of experiments was performed on the adsorption of CIPC on montmorillonite. Initial pesticide concentrations of 0.40 milligrams per liter were employed. Montmorillonite concentrations ranged from 100 to 800 milligrams per liter. For the clay concentrations of 800 milligrams per liter, the initial pH values of the solutions were 4.80, 6.85, and 9.35. Solutions for the

lower montmorillonite concentrations were buffered at pH 6.85. Isopropyl-labeled CIPC was employed to trace the adsorption. The results of these experiments are presented in Table 5-1. During the course of this set of tests, a small loss in liquid volume was observed. This loss was attributed to evaporation of the water with no loss of pesticide. Since the surface area and temperature were constant, the loss to evaporation varied linearly with time. On this basis the data were corrected for the evaporation loss. As indicated, the corrected values for percent reductions in CIPC concentration are given in Table 5-1. After 47 hours the corrected value differed by only 1.6 percent from the measured value.

One additional source of error became evident during the activated carbon experiments. In experimental systems without activated carbon, about five percent of the CIPC disappeared from solution within 24 hours. Possible explanations for this loss are presented in the next section. With respect to the clay mineral systems, the observed reductions in the concentrations of CIPC may not be wholly attributable to adsorption on the minerals, but may be due in part to some other phenomena.

It is evident from Table 5-1 that very little CIPC disappeared from solution. After 47 hours a maximum of 4.0 percent of the CIPC had been removed in those systems containing 400 and 800 milligrams of montmorillonite per liter. Comparable values were obtained for the other systems.

Two other series of experiments were performed on the adsorption of CIPC on clay minerals. In one series, illite was

Table 5-1

Adsorption of CIPC on Montmorillonite at 20°C

Characteristic	System Number						
	G1	G2	G3	G4	G5	J1	J2
Initial CIPC-I, mg/l	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Montmorillonite, mg/l	50	100	200	400	800	800	800
Initial pH	6.85	6.85	6.85	6.85	6.85	4.80	9.35
Final pH	6.85	6.85	6.85	6.85	6.85	6.10	9.10
Initial counting rate, cpm	3106	3086	3072	3109	3106	3118	3124
CIPC-I remaining, percent*							
1 hour	99.8	101.0	100.4	99.6	100.9	99.3	99.9
2 hours	100.3	101.0	99.6	100.5	99.8	98.9	99.1
4 hours	100.2	100.3	100.8	99.0	100.1	98.6	98.0
8 hours	97.3	98.1	99.6	96.5	96.6	--	--
9 hours	--	--	--	--	--	98.8	97.9
20 hours	96.8	98.0	97.9	96.9	96.6	--	--
23 hours	--	--	--	--	--	97.2	96.6
47 hours	96.5	96.8	96.8	96.0	96.0	--	--
CIPC-I remaining, mg/l*							
1 hour	0.40	0.40	0.40	0.40	0.40	0.40	0.40
2 hour	0.40	0.40	0.40	0.40	0.40	0.40	0.40
4 hours	0.40	0.40	0.40	0.40	0.40	0.39	0.39

Table 5-1 (cont'd)

Characteristic	System Number						
	G1	G2	G3	G4	G5	J1	J2
CIPC-I remaining, mg/l* (cont'd)							
8 hours	0.39	0.39	0.40	0.39	0.39	--	--
9 hours	--	--	--	--	--	0.40	0.39
20 hours	0.39	0.39	0.39	0.39	0.39	--	--
23 hours	--	--	--	--	--	0.39	0.39
47 hours	0.39	0.39	0.39	0.38	0.38	--	--

*Adjusted for evaporation loss.

employed as the adsorbent and in the other, kaolinite was used. In both instances the adsorption was examined at three pH values, 4.80, 6.85, and 9.35. The clay concentration was 800 milligrams per liter for all of these tests. Table 5-2 contains the data from both experimental series. As with the montmorillonite series, only a small reduction in pesticide concentration was observed. The maximum decrease in CIPC concentration after 21-hour was 4.0 percent for the illite sample at pH 6.8.

The final series involved the adsorption of 2,4-D on montmorillonite. Again the adsorption was measured for solutions with initial pH values of 4.80, 6.85, and 9.35. The montmorillonite concentration was 800 milligrams per liter. Results from these experiments are presented in Table 5-3. After 23 hours, less than one percent of the 2,4-D had been removed. This experimental series concluded the studies on clay minerals.

5.1.2 Adsorption on Activated Carbon

The studies with activated carbon were confined to CIPC. Previous studies have been made on the adsorption of 2,4-D on activated carbon (57). The characteristics of the adsorbent and the experimental procedures for these investigations were discussed in Section 3.7. During this portion of the research, the effects of pH, temperature, adsorbate concentration, and adsorbent concentration on the adsorption of CIPC on activated carbon were examined.

It was desirable to select adsorbate and adsorbent concentrations such that approximately 50 percent of the CIPC would remain

Table 5-2

Adsorption of CIPC on Illite and Kaolinite at 20°C

Characteristic	System Number					
	H1	H2	H3	H4	H5	H6
Initial CIPC-I, mg/l	0.40	0.40	0.40	0.40	0.40	0.40
Clay mineral, type	illite	illite	illite	kaolinite	kaolinite	kaolinite
Clay mineral, mg/l	800	800	800	800	800	800
Initial pH	6.85	4.80	9.35	6.85	4.80	9.35
Final pH	6.75	5.30	9.00	6.75	5.30	9.00
Initial counting rate, cpm	3119	3103	3064	3142	3092	3106
CIPC-I remaining, percent*						
1 hour	97.0	97.4	98.1	98.7	99.6	98.6
2 hours	97.4	96.8	99.1	99.0	99.2	98.1
3 hours	96.4	98.4	99.1	98.4	99.2	98.9
6 hours	98.1	98.6	98.2	97.7	99.6	97.5
9 hours	95.8	97.1	98.3	97.7	98.0	97.8
21 hours	96.0	96.3	97.3	96.7	96.4	97.0
CIPC-I remaining, mg/l*						
1 hour	0.39	0.39	0.39	0.39	0.40	0.39
2 hours	0.39	0.39	0.40	0.40	0.40	0.39

Table 5-2 (cont'd)

Characteristic	System Number					
	H1	H2	H3	H4	H5	H6
CIPC-I remaining, mg/l* (cont'd)						
3 hours	0.39	0.39	0.40	0.39	0.40	0.40
6 hours	0.39	0.39	0.39	0.39	0.40	0.39
9 hours	0.38	0.39	0.39	0.39	0.39	0.39
21 hours	0.38	0.39	0.39	0.39	0.39	0.39

*Adjusted for evaporation loss.

Table 5-3

Adsorption of 2,4-D on Montmorillonite at 20°C

Characteristic	System Number		
	J3	J4	J5
Initial 2,4-D mg/l	0.13	0.13	0.13
Montmorillonite, mg/l	800	800	800
Initial pH	6.85	4.80	9.35
Final pH	6.85	5.85	9.10
Initial counting rate, cpm	2632	2613	2625
2,4-D remaining, percent*			
1 hour	99.7	99.7	99.8
2 hours	100.3	99.3	99.4
4 hours	99.9	99.9	100.2
9 hours	98.2	99.6	99.4
23 hours	99.1	99.8	99.9
2,4-D remaining, mg/l*			
1 to 23 hours	0.13	0.13	0.13

*Adjusted for evaporation loss.

in solution at equilibrium. The first experimental series was conducted at a CIPC concentration of 5.0 milligrams per liter for activated carbon dosages of 100 and 250 milligrams per liter. The stirring rate for all of the adsorption studies was 100 revolutions per minute. Table 5-4 contains the data collected from these experiments. The variation in pesticide concentration with time is graphically presented in Figure 5-1. System 1 containing no activated carbon produced erratic results during the first four hours. These results were considered erroneous and were disregarded. The remaining blank (system 4) demonstrated a gradual loss of pesticide during the test period. After 22 hours, there was a six-percent decrease in the counting rate. Such a decrease was typical of all the adsorption experiments. Moreover, there was a small loss in volume during the duration of the experiments. This loss was attributed to evaporation.

It was necessary to correct for the effects of the above changes in the test solutions in order to calculate the true amounts of CIPC adsorbed on the carbon. The evaporation loss was directly proportional to the elapsed time since the surface area was constant. The non-carbon loss of CIPC evident in Figure 5-1 was assumed to be proportional to time and concentration. This type of relationship represents a number of possible mechanisms for CIPC losses. Diffusion-controlled adsorption to the glass walls or stirring paddles, or any number of first-order reactions involving the CIPC would yield such relationships. By first correcting the data for evaporation losses and then accounting for the non-carbon CIPC loss, the

Table 5-4

Adsorption of CIPC on Activated Carbon, 100 and 250 Milligrams Per Liter, at pH 6.9 and 20°C

Characteristic	System Number					
	1	2	3	4	5	6
Initial CIPC, mg/l	5.00	5.00	5.00	5.00	5.00	5.00
Activated carbon, mg/l	0	250	250	0	100	100
Initial counting rate, cpm	3129	3130	3153	3323	3320	3326
CIPC remaining in solution, mg/l						
0.25 hours	5.28	0.72	1.00	4.95	2.62	3.16
0.50 hours	5.63	0.16	0.26	5.11	1.48	1.94
1.00 hours	6.38	0.10	0.11	4.99	0.72	1.11
2.00 hours	5.16	0.05	0.08	4.96	0.26	0.50
4.00 hours	5.56	0.05	0.04	4.99	0.15	0.26
8.00 hours	4.98	0.01	0.04	4.88	0.09	0.16
22.00 hours	4.98	0.03	0.05	4.70	0.02	0.10
CIPC adsorbed, mg. per mg. carbon						
0.25 hours	--	0.0171	0.0160	--	0.0237	0.0184
0.50 hours	--	0.0194	0.0189	--	0.0351	0.0306
1.00 hours	--	0.0196	0.0196	--	0.0427	0.0388
2.00 hours	--	0.0197	0.0197	--	0.0472	0.0449
4.00 hours	--	0.0198	0.0198	--	0.0484	0.0473
8.00 hours	--	0.0199	0.0198	--	0.0490	0.0484
22.00 hours	--	0.0199	0.0198	--	0.0496	0.0488

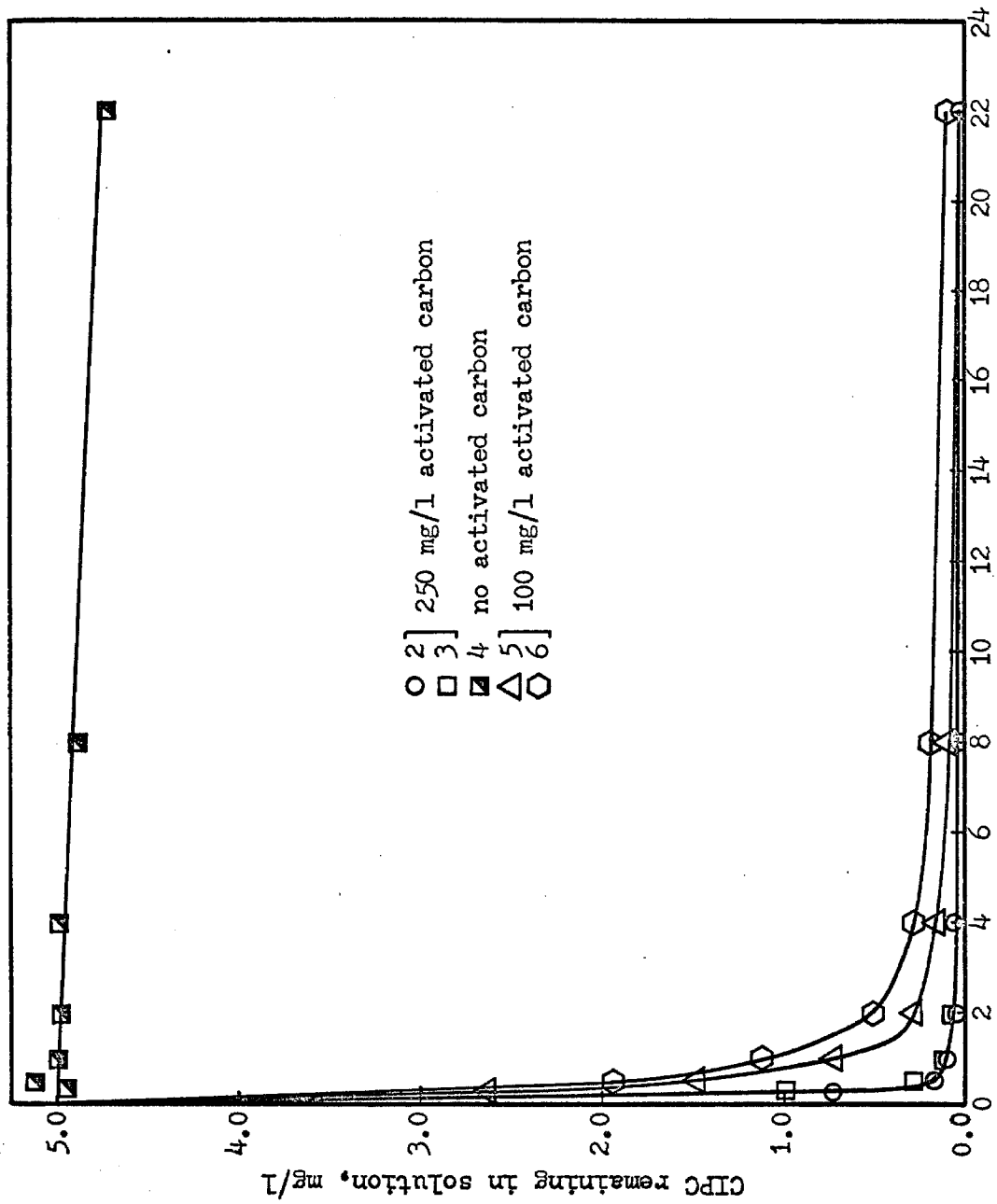


Figure 5-1. Adsorption of CIPC on activated carbon (100 and 250 mg/l) at pH 6.9 and 20°C.

true amounts of CIPC absorbed were calculated. These values are given in Table 5-4 and are graphically shown in Figure 5-2.

For the adsorbate and adsorbent concentrations used in these experiments, more than 95 percent of the CIPC was adsorbed on the carbon within four hours. Calculations based upon data such as these are extremely sensitive to relatively small analytical errors. Much of this sensitivity would be eliminated if a larger percentage of the CIPC remained in solution at equilibrium. A second experimental series was performed at pH 6.9 and 20°C to obtain more suitable adsorbate and adsorbent concentrations. The initial CIPC concentrations were raised to 10.0 milligrams per liter and the activated carbon concentrations were reduced to 25 and 50 milligrams per liter. Table 5-5 and Figure 5-3 and 5-4 contain the data obtained from these experiments.

The data from the three experiments with 50 milligrams of activated carbon per liter were in close agreement. Approximately 95 percent of the CIPC was adsorbed on the carbon in 24 hours. Some variations were noted between the two suspensions containing 25 milligrams of carbon per liter. After 24 hours, the difference in the amounts of CIPC remaining in solution was about one milligram per liter. Much of this difference was attributed to unequal concentrations of activated carbon. Retention of the carbon on the walls of the beakers, to the stirring paddles, to the initial transfer pipette, and to the sampling test tubes would account for certain adsorbent concentration differences. The initial adsorbate and adsorbent

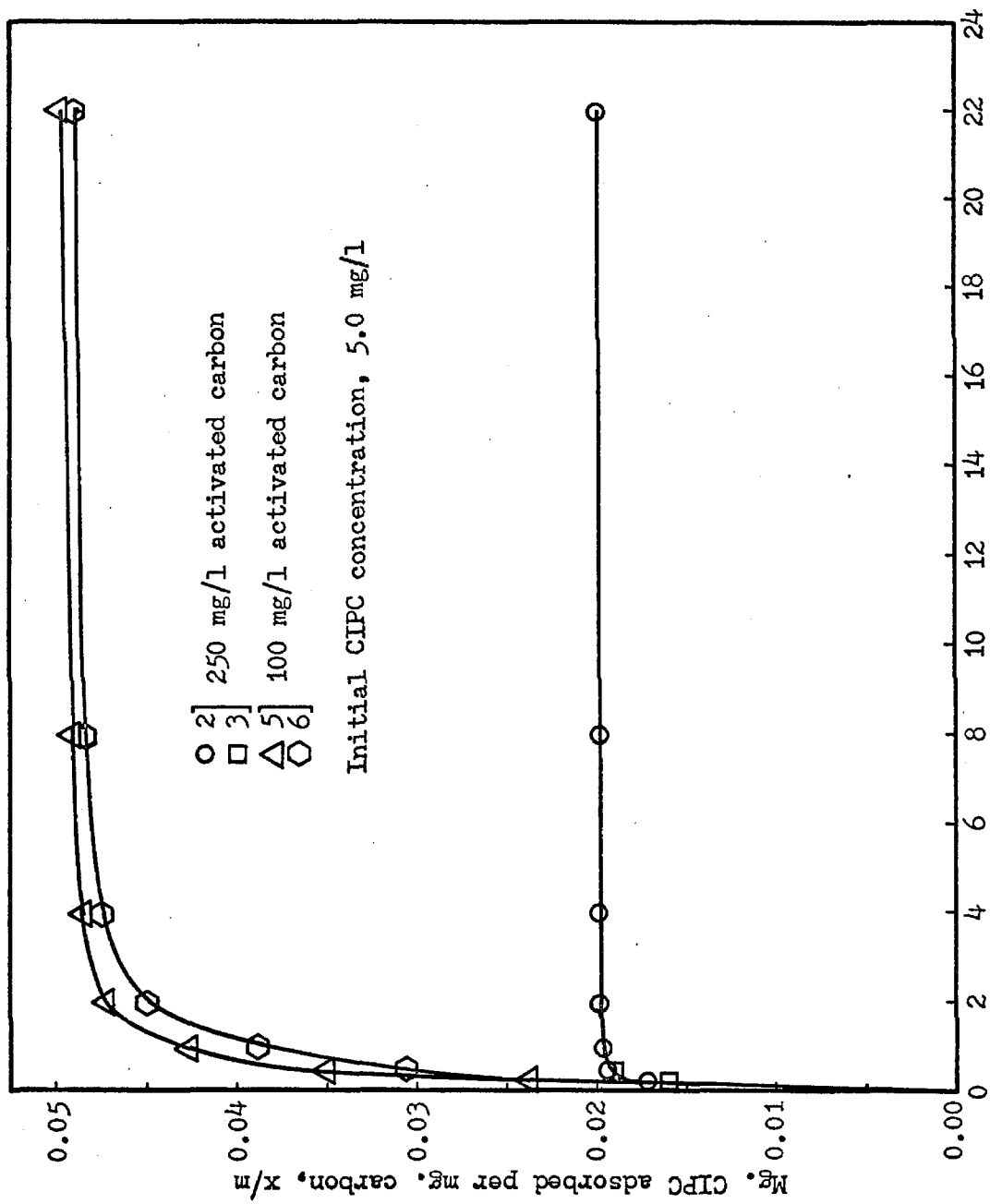


Figure 5-2. CIPC adsorbed per unit weight of activated carbon (100 and 250 mg/l) at pH 6.9 and 20°C.

Table 5-5

Adsorption of CIPC on Activated Carbon, 25 and 50 Milligrams Per Liter, at pH 6.9 and 20°C

Characteristic	System Number					
	1	2	3	4	5	6
Initial CIPC, mg/l	10.00	10.00	10.00	10.00	10.00	10.00
Activated carbon, mg/l	0	50	50	25	25	50
Initial counting rate, cpm	3206	3214	3190	3256	3202	3259
CIPC remaining in solution, mg/l						
0.25 hours	9.77	6.98	7.13	8.22	8.63	6.92
0.50 hours	9.89	5.63	5.98	7.34	7.95	5.23
1.00 hours	9.73	3.90	4.26	6.39	7.07	3.52
2.00 hours	9.84	2.16	2.19	5.09	6.16	1.64
4.00 hours	9.73	1.40	1.14	4.36	5.54	0.89
8.00 hours	9.54	0.90	0.61	4.08	5.26	0.53
24.00 hours	9.32	0.68	0.40	3.80	4.82	0.35
CIPC adsorbed, mg. per mg. carbon						
0.25 hours	--	0.060	0.057	0.071	0.055	0.062
0.50 hours	--	0.087	0.080	0.106	0.082	0.095
1.00 hours	--	0.122	0.114	0.144	0.116	0.129
2.00 hours	--	0.158	0.156	0.195	0.152	0.167
4.00 hours	--	0.171	0.176	0.223	0.175	0.182
8.00 hours	--	0.181	0.187	0.232	0.184	0.189
24.00 hours	--	0.185	0.191	0.236	0.192	0.192

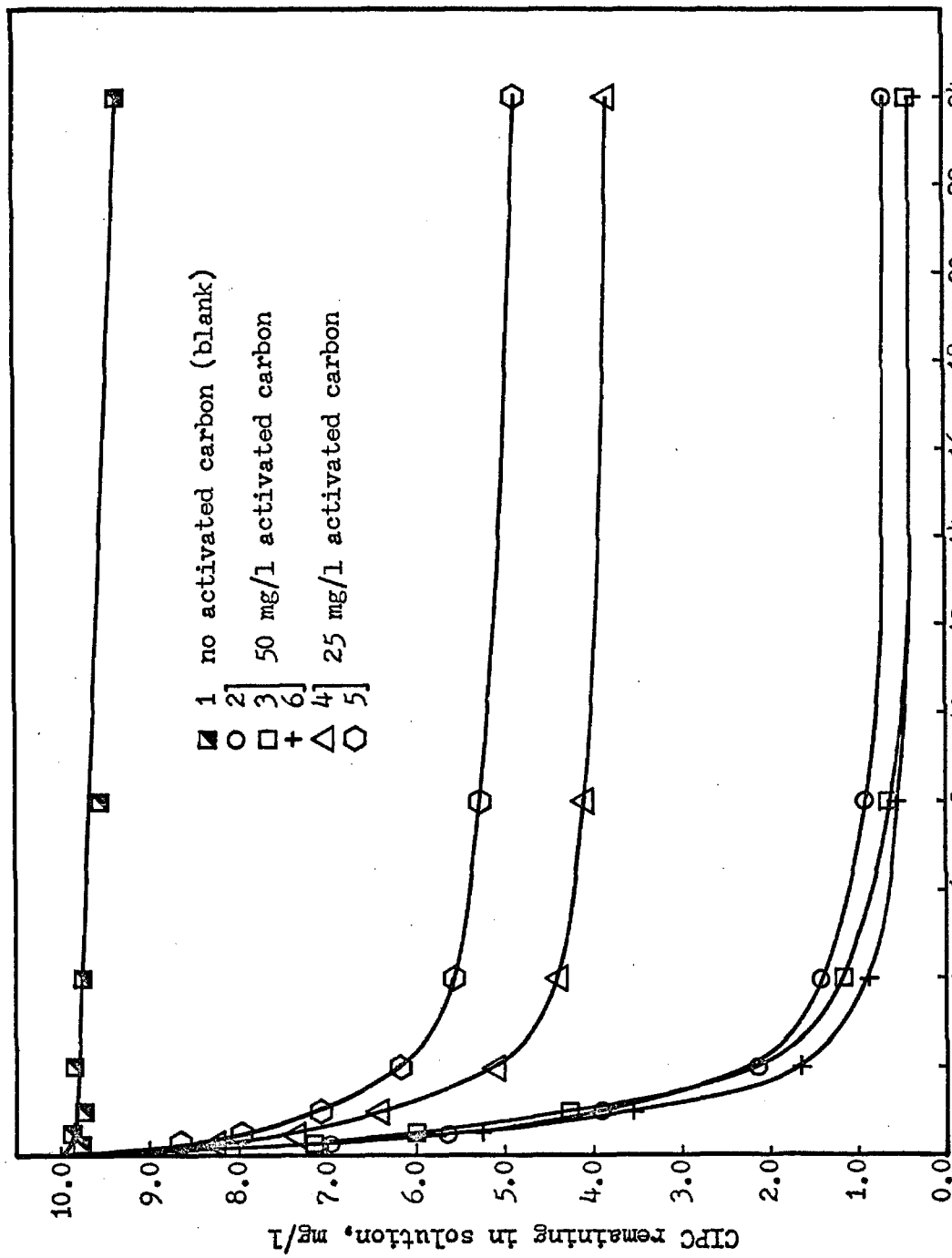


Figure 5-3. Adsorption of CIPC on activated carbon (25 and 50 mg/l) at pH 6.9 and 20°C.

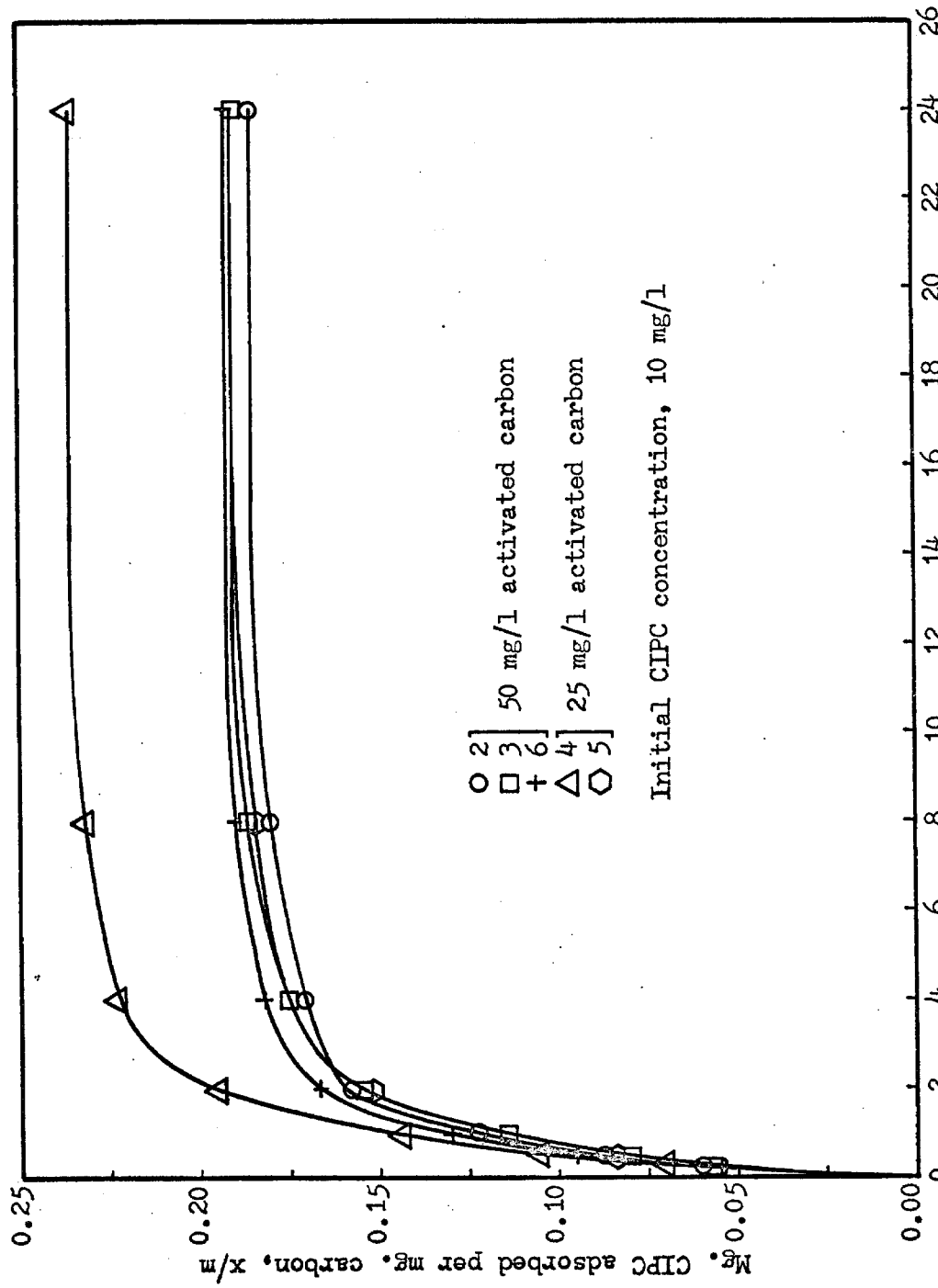


Figure 5-4. CIPC adsorbed per unit weight of activated carbon (25 and 50 mg/l) at pH 6.9 and 20°C.

concentrations used in these experiments were selected for the remaining investigations.

A separate study was initiated to measure the adsorption capacity of the activated carbon for CIPC. The procedure for these experiments differed slightly from the previous tests. Absorbent and initial adsorbate concentrations remained at 25 or 50 and 10 milligrams per liter respectively, but the initial volume was increased to 1800 milliliters. Additional quantities of CIPC were added to the suspensions after 24 hours. This procedure was repeated several times and the adsorption observed over a period of about five days. The data acquired during this period are presented in Table 5-6 and Figures 5-5 and 5-6.

During the first 72 hours of these experiments, the ratio of radioactive CIPC to non-radioactive CIPC was kept constant. In other words, the ratio of labeled CIPC to plain CIPC for the 24- and 48-hour additions was the same as that for the initial pesticide dosage. Only non-radioactive CIPC, however, was used for the 73-hour addition. The absence of additional radioactive CIPC decreased the overall ratio of radioactive to non-radioactive CIPC. Table 5-6 contains two sets of values for the 73- to 128-hour time interval. The first set of values, denoted by a single asterisk, was based on the assumption that the radioactive CIPC on the carbon and in solution was completely redistributed in a very short time. That is, the radioactive molecules were redistributed so as to provide a new ratio of radioactive CIPC to non-radioactive CIPC that was uniform throughout the solution and the carbon. Assuming that the radioactive CIPC

Table 5-6

Studies on the Adsorption Capacity of Activated Carbon for CIPC at pH 6.9 and 20°C

Characteristic	System Number			
	1	2	3	4
Activated carbon	25	25	50	50
Initial counting rate	3415	3465	3421	3442
CIPC remaining in solution, mg/l				
0.00 hours	10.00	10.00	10.00	10.00
0.25 hours	8.19	8.23	7.78	7.47
0.50 hours	8.32	7.91	7.10	6.85
1.00 hours	7.47	6.99	5.54	5.48
2.00 hours	6.44	6.15	3.60	3.74
4.00 hours	5.77	4.97	1.90	2.34
8.00 hours	5.63	4.84	0.98	1.22
23.25 hours	5.16	4.36	0.40	0.59
23.50 hours		CIPC added		
23.50 hours	14.80	13.92	9.98	10.17
23.75 hours	14.61	13.67	8.34	9.16
24.00 hours	14.39	13.40	7.89	8.64
24.50 hours	14.25	13.64	7.46	8.27
25.50 hours	14.12	13.35	7.16	7.93
27.50 hours	14.22	13.29	7.08	7.85
31.50 hours	14.19	13.27	6.97	7.66
48.25 hours	13.87	12.97	6.64	7.23

Table 5-6 (cont'd)

Characteristic	System Number			
	1	2	3	4
CIPC remaining in solution, (cont'd)				
48.50 hours		CIPC added		
48.50 hours	24.18	23.37	17.08	17.67
48.75 hours	23.70	22.88	16.07	16.93
49.00 hours	23.74	22.90	15.78	16.82
49.50 hours	23.82	22.64	15.64	16.65
50.50 hours	23.43	22.81	15.56	16.42
52.50 hours	23.72	22.88	15.35	16.47
56.50 hours	23.65	22.67	15.35	16.10
72.00 hours	22.75	22.25	14.88	15.81
73.00 hours		CIPC added		
73.00 hours*	33.89	33.37	26.01	26.95
75.00 hours*	31.75	30.23	21.98	22.53
81.00 hours*	31.25	30.07	22.65	23.31
97.00 hours*	31.01	30.10	22.37	22.83
128.00 hours*	31.11	30.33	22.68	23.18
73.00 hours**	33.89	33.37	26.01	26.95
75.00 hours**	35.08	33.84	28.95	28.92
81.00 hours**	34.50	33.67	29.83	29.94
97.00 hours**	34.26	33.67	29.44	29.29
128.00 hours**	34.37	33.94	29.83	29.78

Table 5-6 (cont'd)

Characteristic	System Number			
	1	2	3	4
CIPC adsorbed, mg. per mg. carbon				
0.00 hours	0	0	0	0
0.25 hours	0.072	0.071	0.044	0.051
0.50 hours	0.067	0.084	0.046	0.063
1.00 hours	0.102	0.120	0.089	0.090
2.00 hours	0.142	0.154	0.128	0.125
4.00 hours	0.169	0.202	0.162	0.153
8.00 hours	0.176	0.208	0.181	0.176
23.25 hours	0.197	0.228	0.192	0.188
23.50 hours		CIPC added		
23.50 hours	0.197	0.228	0.192	0.188
23.75 hours	0.204	0.238	0.225	0.209
24.00 hours	0.213	0.249	0.234	0.219
24.50 hours	0.219	0.240	0.243	0.226
25.50 hours	0.225	0.251	0.249	0.233
27.50 hours	0.221	0.254	0.251	0.235
31.50 hours	0.225	0.257	0.253	0.239
48.25 hours	0.242	0.274	0.261	0.249
48.50 hours		CIPC added		
48.50 hours	0.242	0.274	0.261	0.249
48.75 hours	0.262	0.294	0.281	0.264
49.00 hours	0.260	0.293	0.286	0.266

Table 5-6 (cont'd)

Characteristic	System Number			
	1	2	3	4
CIPC adsorbed (cont'd)				
49.50 hours	0.257	0.304	0.289	0.270
50.50 hours	0.274	0.298	0.291	0.274
52.50 hours	0.264	0.297	0.296	0.274
56.50 hours	0.270	0.308	0.297	0.282
72.00 hours	0.313	0.334	0.309	0.291
73.00 hours		CIPC added		
73.00 hours*	0.313	0.334	0.309	0.291
75.00 hours*	0.400	0.460	0.390	0.379
81.00 hours*	0.428	0.474	0.379	0.366
97.00 hours*	0.453	0.488	0.390	0.382
128.00 hours*	0.474	0.511	0.392	0.383
73.00 hours**	0.313	0.334	0.309	0.291
75.00 hours**	0.268	0.318	0.251	0.252
81.00 hours**	0.297	0.331	0.236	0.234
97.00 hours**	0.326	0.348	0.252	0.255
128.00 hours**	0.355	0.372	0.261	0.262

*Assuming complete redistribution or radioactive CIPC.

**Assuming no redistribution of adsorbed radioactive CIPC.

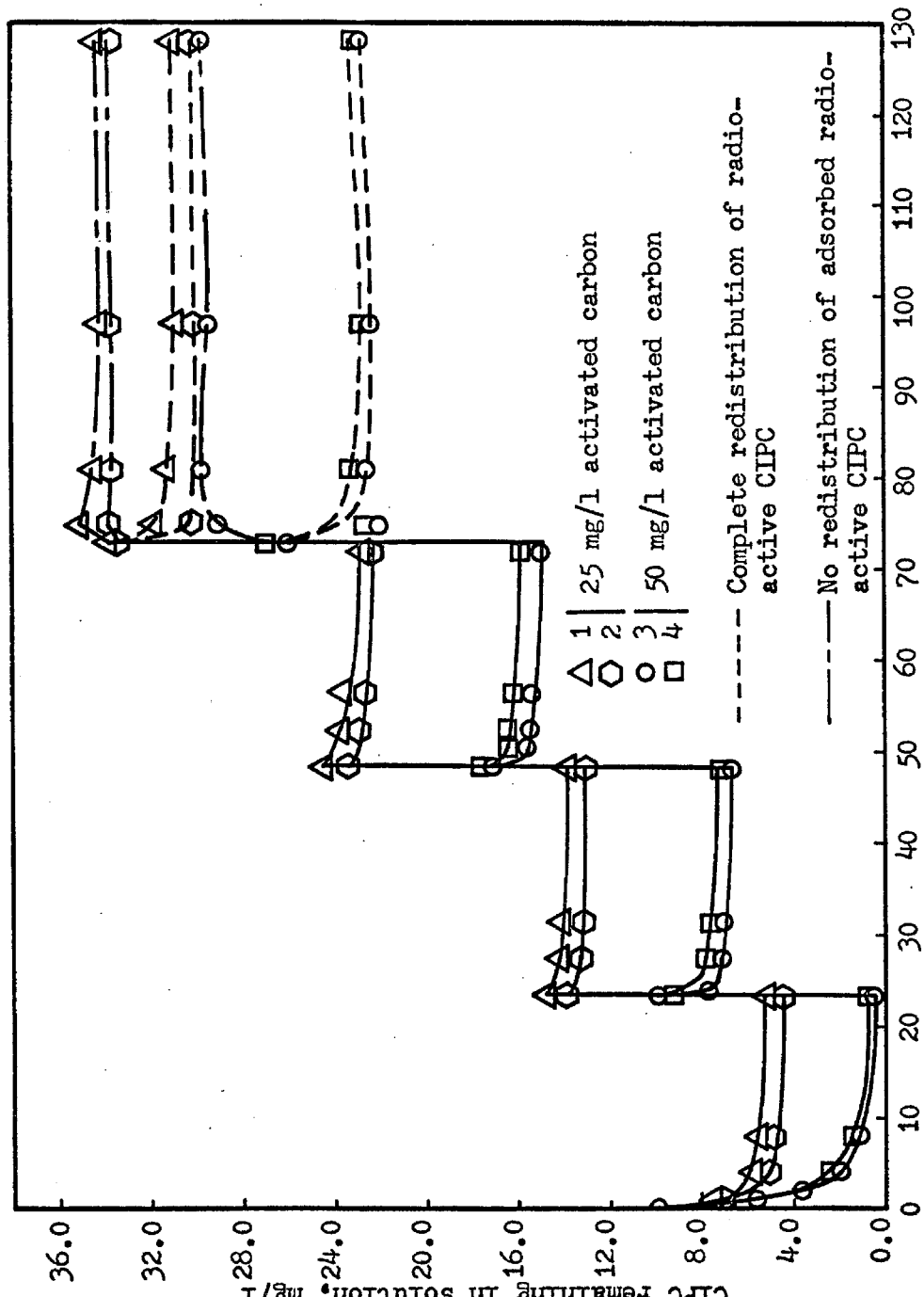


Figure 5-5. Adsorption capacity studies on the CIPC-activated carbon system at pH 6.9 and 20°C.

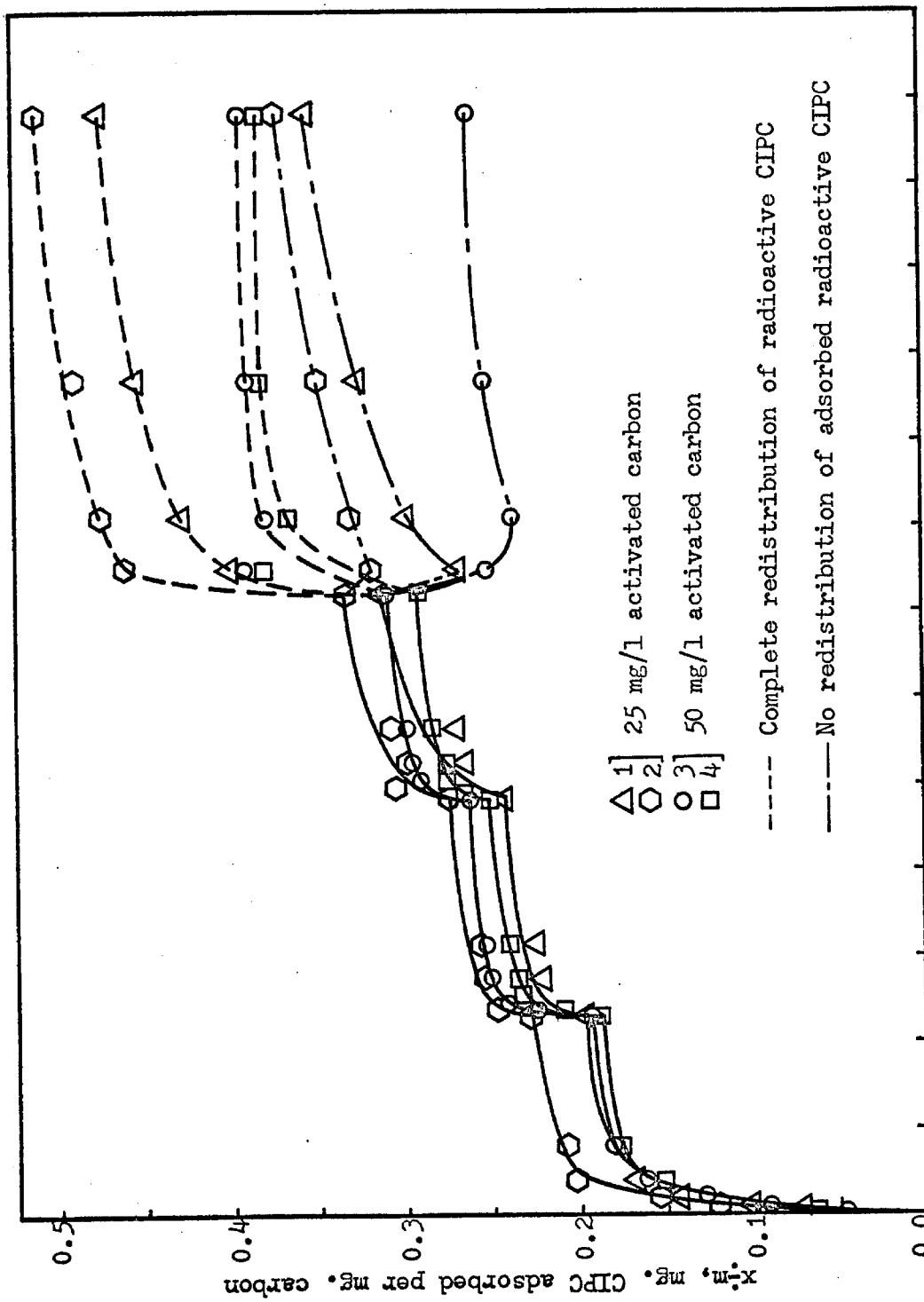


Figure 5-6. Adsorption capacity studies. Milligrams CIPC adsorbed per unit weight of activated carbon at pH 6.9 and 20°C.

adsorbed on the carbon was irreversibly attached and that redistribution of the radioactivity occurred only in the solution phase, a second set of values was calculated. These two interpretations represent the maximum and minimum conditions of radioactivity redistribution that might be expected. The two extremes are plotted in Figures 5-5 and 5-6.

Studies were also conducted on the effects of pH on the adsorption process. A solution initially at a pH of 4.85 was used for the first series. The results from these experiments are compiled in Table 5-7 and are graphically depicted in Figure 5-7. During the experiments, the pH of the suspensions rose slightly to 5.3. Generally, the adsorption data from this series of experiments were very similar to the data obtained at pH 6.85. Once again, duplicate systems demonstrated some differences. For activated carbon concentrations of 25 milligrams per liter, the adsorption of CIPC in duplicate systems differed by about one milligram per liter. At 50 milligrams of carbon per liter the difference was 0.5 milligrams per liter.

Another series of tests was performed at a pH of 9.30 and a temperature of 20°C. Sodium borate was employed to maintain these suspensions at pH 9.3. The data obtained from these experiments are presented in Table 5-8 and Figure 5-8. Duplicate systems agreed quite closely in these experiments. As with the other experiments, equilibrium was essentially reached after 20 to 24 hours.

The final series of adsorption experiments was conducted at the elevated temperature of 37°C and a pH of 6.9. Table 5-9 and Figure 5-9 contain the data from these tests. Comparison with the

Table 5-7

Adsorption of CIPC on Activated Carbon

at pH 4.85 and 20°C

Characteristic	System Number				
	1	2	3	4	5
Initial CIPC, mg/l	10.00	10.00	10.00	10.00	10.00
Activated carbon, mg/l	0	50	50	25	25
Initial pH	4.85	4.85	4.85	4.85	4.85
Final pH	5.3	5.3	5.3	5.3	5.3
Initial counting rate, cpm	3178	3159	3271	3216	3194
CIPC remaining in solution, mg/l					
0.25 hours	9.92	7.84	7.29	8.73	8.44
0.50 hours	10.06	6.39	5.94	7.84	7.62
1.00 hours	10.03	4.73	4.08	6.98	6.42
2.00 hours	9.91	2.66	2.39	5.73	5.06
4.00 hours	9.75	1.73	0.99	5.40	4.22
8.00 hours	9.82	1.28	0.55	5.12	3.95
20.00 hours	9.59	1.03	0.41	4.90	3.78
CIPC adsorbed, mg. per mg. carbon					
0.25 hours	--	0.043	0.054	0.051	0.062
0.50 hours	--	0.072	0.081	0.086	0.095
1.00 hours	--	0.105	0.118	0.120	0.142
2.00 hours	--	0.146	0.152	0.170	0.197

Table 5-7 (cont'd)

Characteristic	System Number				
	1	2	3	4	5
CIPC adsorbed, mg. per mg. carbon (cont'd)					
4.00 hours	--	0.165	0.180	0.182	0.230
8.00 hours	--	0.174	0.188	0.192	0.239
20.00 hours	--	0.178	0.191	0.195	0.242

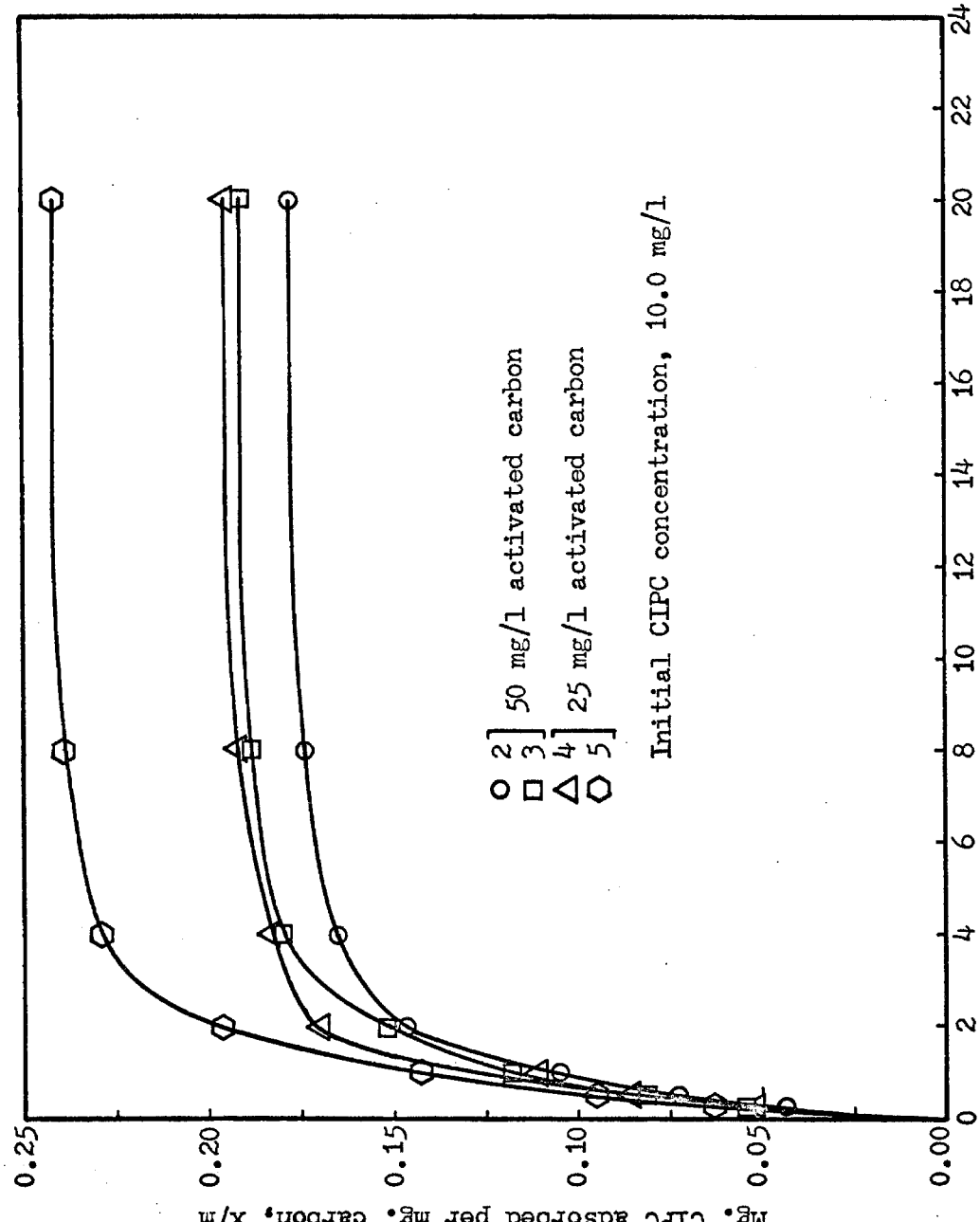


Figure 5-7. Milligrams of CIPC adsorbed per milligram of activated carbon at pH 4.8-5.3 and 20°C.

Table 5-8

Adsorption of CIPC on Activated Carbon

at pH 9.30 and 20°C

Characteristic	System Number					
	1	2	3	4	5	6
Initial CIPC, mg/l	10.00	10.00	10.00	10.00	10.00	10.00
Activated carbon, mg/l	0	50	50	25	25	25
Initial pH	9.30	9.30	9.30	9.30	9.30	9.30
Final pH	9.00	8.60	8.60	8.85	8.75	9.00
Initial counting rate, cpm	3214	3203	3176	3213	3213	3184
CIPC remaining in solution, mg/l						
0.25 hours	9.89	7.11	7.38	8.42	8.34	8.46
0.50 hours	9.86	5.24	6.01	7.71	7.46	7.64
1.00 hours	9.88	3.59	4.42	6.37	6.21	6.24
2.00 hours	9.69	1.84	2.66	5.19	5.02	4.99
4.00 hours	9.79	0.76	1.30	4.54	4.18	4.08
8.00 hours	9.72	0.47	0.80	4.37	3.91	3.77
22.00 hours	9.51	0.30	0.60	4.10	3.65	3.57
CIPC adsorbed, mg. per mg. carbon						
0.25 hours	--	0.058	0.052	0.063	0.066	0.062
0.50 hours	--	0.095	0.080	0.091	0.101	0.094
1.00 hours	--	0.128	0.111	0.144	0.151	0.150

Table 5-8 (cont'd)

Characteristic	System Number					
	1	2	3	4	5	6
CIPC adsorbed, mg. per mg. carbon (cont'd)						
2.00 hours	--	0.163	0.146	0.191	0.198	0.199
4.00 hours	--	0.184	0.173	0.216	0.230	0.234
8.00 hours	--	0.190	0.183	0.222	0.240	0.246
22.00 hours	--	0.193	0.187	0.227	0.245	0.249

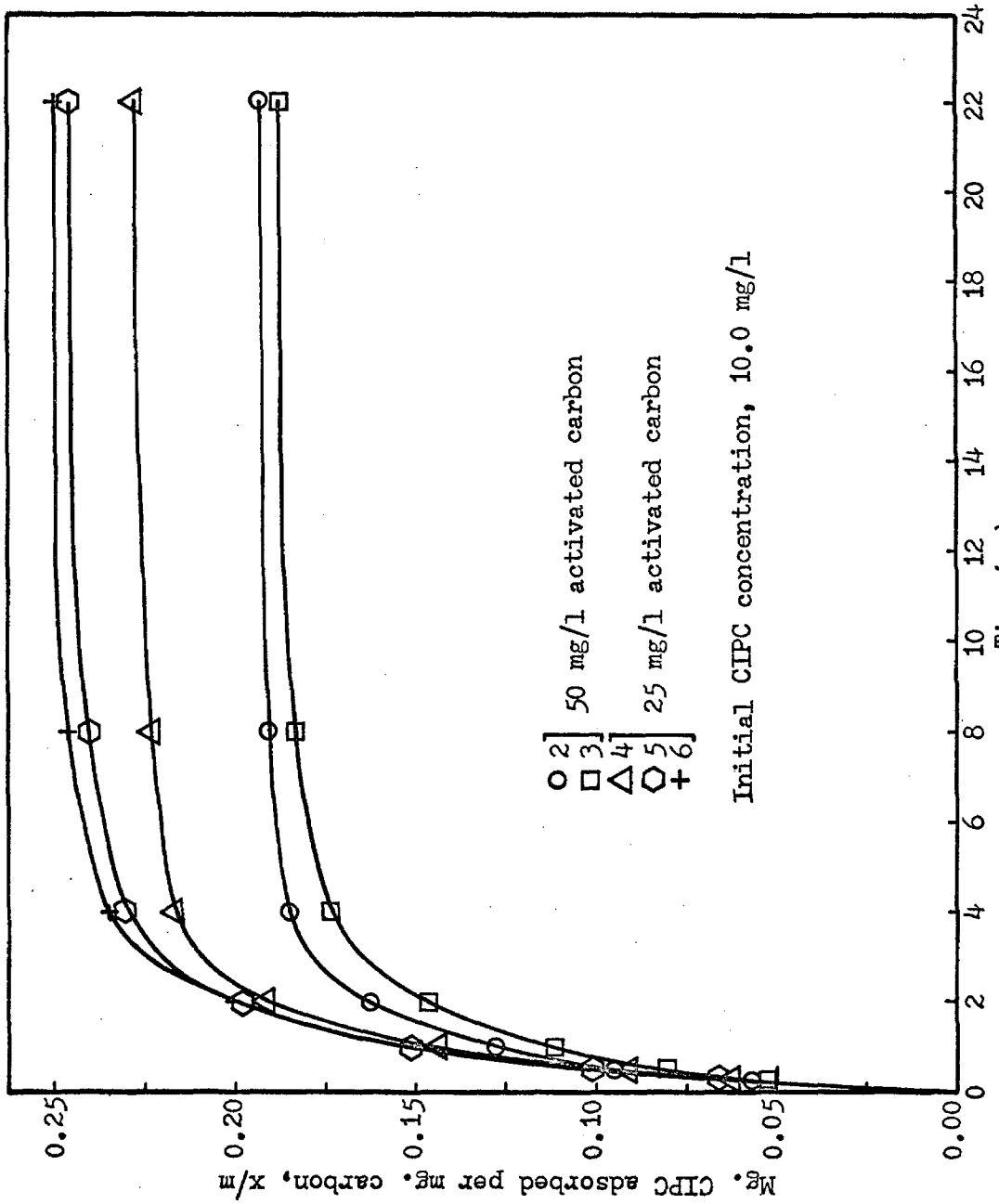


Figure 5-8. Milligrams of CIPC adsorbed per milligram of activated carbon at pH 8.6-9.3 and 20°C.

Table 5-9

Adsorption of CIPC on Activated Carbon

at pH 6.9 and 37°C

Characteristic	System Number				
	1	2	3	4	5
Initial CIPC, mg/l	10.00	10.00	10.00	10.00	10.00
Activated carbon, mg/l	50	50	25	25	0
Initial pH	6.85	6.85	6.85	6.85	6.85
Final pH	6.85	6.85	6.85	6.85	6.85
Initial counting rate, cpm	3078	3094	3066	3050	3054
CIPC remaining in solution, mg/l					
0.25 hours	6.10	6.67	8.26	8.38	9.93
0.52 hours	4.69	4.84	7.53	7.46	9.84
1.00 hours	2.58	2.58	6.36	6.00	9.85
2.00 hours	1.09	1.12	5.72	5.27	9.74
4.00 hours	0.66	0.56	5.45	5.02	9.66
8.00 hours	0.45	0.37	5.27	4.73	9.18
21.00 hours	0.39	0.29	4.91	4.33	8.29
CIPC adsorbed, mg. per mg. carbon					
0.25 hours	0.077	0.066	0.069	0.064	--
0.52 hours	0.105	0.102	0.097	0.100	--
1.00 hours	0.147	0.147	0.143	0.157	--

Table 5-9 (cont'd)

Characteristic	System Number				
	1	2	3	4	5
CIPC adsorbed, mg. per mg. carbon (cont'd)					
2.00 hours	0.177	0.176	0.166	0.184	--
4.00 hours	0.185	0.187	0.173	0.190	--
8.00 hours	0.189	0.190	0.173	0.195	--
21.00 hours	0.189	0.191	0.164	0.194	--

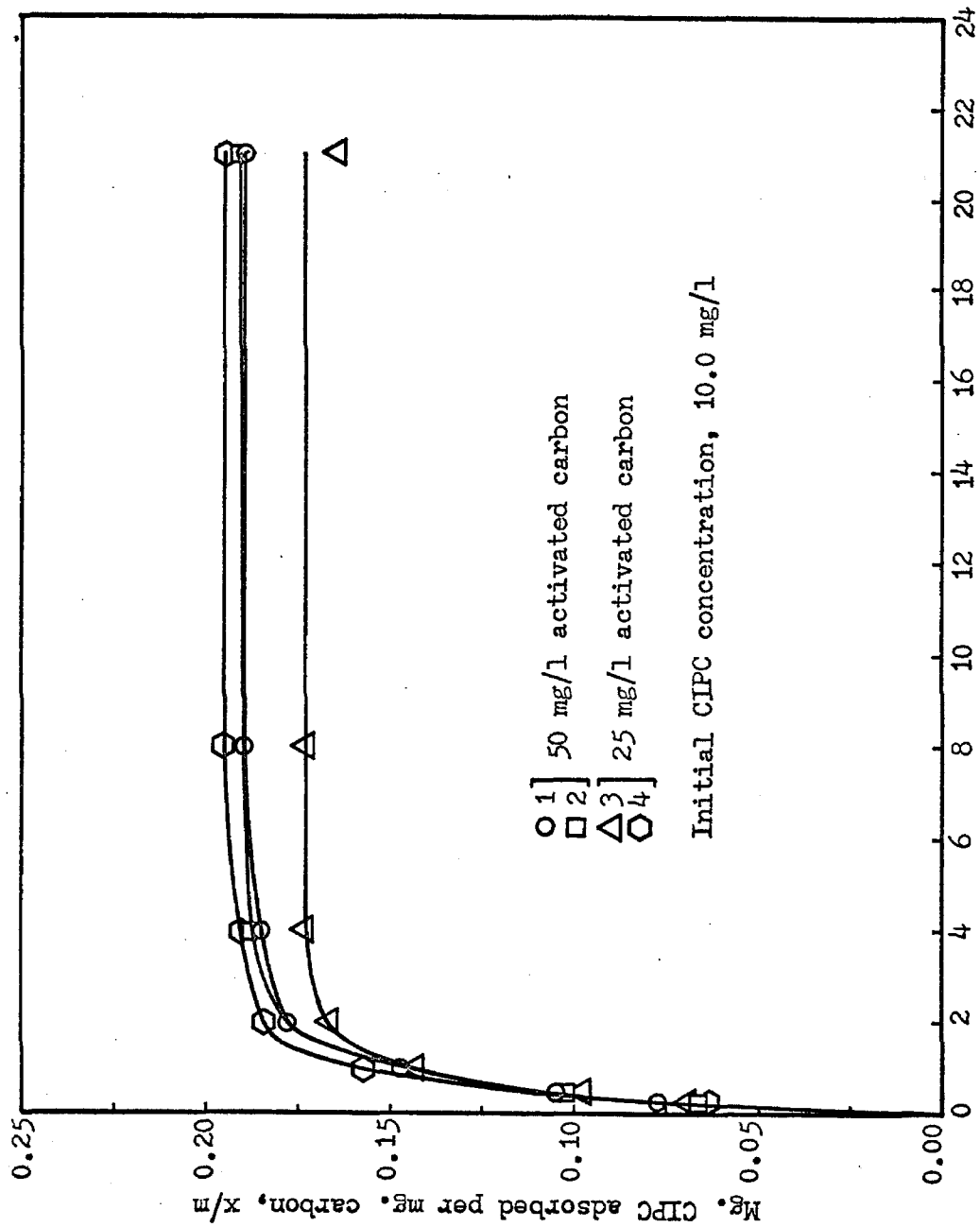


Figure 5-9. Milligrams of CIPC adsorbed per milligram of activated carbon at pH 6.9 and 37°C.

other experiments indicated that the total amount of adsorption at 37°C was somewhat less than at 20°C.

5.2 Discussion

5.2.1 Adsorption on Clay Minerals

These studies were undertaken to determine the abilities of inorganic suspended solids in natural water supplies to remove pesticides from solution. The inorganic particulate matter employed in these investigations (illite, kaolinite, and montmorillonite) represented a wide spectrum of clay minerals. Significant quantities of related substances are found in many natural river and stream systems. If pesticides were adsorbed on such matter in large quantities, their subsequent removal at water treatment plants might be facilitated. Coagulation, sedimentation, and filtration would remove the particulate matter from the water.

The results of the experiments with CIPC were presented in Tables 5-1 and 5-2, Section 5.1.1. Substantial pesticide removal was not observed for any of the clay minerals under any experimental condition. Almost uniformly, the maximum removals ranged from 3.0 to 4.0 percent. The reductions in the concentration of CIPC, however, may not have been ascribable to adsorption on the clay minerals. In the systems without any adsorbent in the activated carbon studies, CIPC losses ranged from four to six percent in 24 hours. Adsorption of CIPC on the experimental equipment or volatilization of the CIPC may account for most, if not all, of the observed CIPC losses. The

amount of CIPC that disappeared from solution does represent the maximum amount that might be attributed to adsorption.

The disappearance of 2,4-D from solution in the montmorillonite systems was very slight. After 23 hours, 99.1 to 99.9 percent of the 2,4-D remained at the three pH values. The data obtained were in agreement with the results of Aly and Faust (61). They studied the adsorption of 2,4-D on bentonite. In their experiments, the 2,4-D concentrations were 3.7 to 21.0 milligrams per liter and the bentonite concentrations were approximately 25 grams per liter. At these concentrations, the removal of 2,4-D followed Freundlich's expression. Extrapolation of their data down to a 2,4-D concentration of 0.13 milligrams per liter gives an expected removal of only 0.002 milligrams per gram of clay or 1.5 percent.

In conclusion, the results of these investigations indicated that, at most, only a small percentage of any 2,4-D or CIPC present in a natural waterway will be removed by the inorganic particulate matter. The 800 milligram per liter clay concentration is representative of a river with a high suspended-solids content. As an example, the average suspended-solids content of the Missouri River near St. Louis, Missouri in 1960 was 782 milligrams per liter (57). At such solids concentrations, the pesticide removals were so small as to render obscure any relationships existing between the removals and factors such as pH and cation exchange capacity.

5.2.2 Adsorption on Activated Carbon

Activated carbon is widely used in water treatment plants for the elimination of objectionable tastes and odors. The great affinity of activated carbon for chlorine, hydrogen sulfide, and a multitude of organic compounds makes it a valuable tool for the purification of drinking water. Previous studies on the removal of pesticides from water supplies (Section 2.3) have indicated that adsorption on activated carbon was the most effective process for this purpose. The earlier studies encompassed a variety of chlorinated hydrocarbon and organic phosphorus pesticides. It was the intent of this portion of the research to investigate the removal of the carbamate herbicide CIPC from water with powdered activated carbon. The activated carbon-2,4-D system has been studied previously (57).

Significant quantities of CIPC were adsorbed by the carbon under a variety of experimental conditions. The results of these studies were presented in Section 5.1.2. At pH 6.9 and 20°C, initial pesticide concentrations of 5.0 milligrams per liter were reduced to less than 0.5 milligrams per liter within two hours with activated carbon dosages of 100 and 250 milligrams per liter. After 22 hours, less than 0.1 milligrams of CIPC per liter remained. Under similar conditions, 25 and 50 milligrams of adsorbent per liter in two hours removed approximately 50 and 80 percent respectively of the CIPC, initially present at a concentration of 10.0 milligrams per liter. The respective maximum reductions after 24 hours were 38 to 48 and 93 to 96 percent. It is evident from Figures 5-1, 5-2, and 5-3, Section 5.1.2, that equilibrium was essentially attained after 20 to 24 hours

for all these experiments. After four hours, further concentration reductions were quite small.

An additional series of experiments was conducted at pH 6.9 and 20°C to determine the total adsorption capacity of the activated carbon for CIPC. A comparison of the data contained in Tables 5-5 and 5-6 indicates close agreement between the first 23 hours of the capacity studies and the previously discussed experiments at the same concentrations.

Subsequent additions of CIPC resulted in further adsorption. Approximately equal amounts of CIPC were added after 23, 48, and 73 hours. Under these conditions, decreased incremental amounts of adsorption would be anticipated. With the exception of the third equilibrium period for the systems containing 25 milligrams of carbon per liter, the data fulfilled this expectation. Roughly equal amounts of CIPC were adsorbed in the experiments with 25 milligrams of carbon per liter during the second and third equilibrium periods. The fact that the second period did not demonstrate larger removals than the third may be attributed to the temporary adherence of a relatively large proportion of the carbon to the walls of the test vessels and the stirring paddles. Those systems containing 50 milligrams of carbon per liter demonstrated decreasing increments of adsorption through the third equilibrium period. The data from these systems indicated that the adsorption capacity was rapidly being approached.

The final addition of CIPC contained no radioactively labeled pesticide. Immediately after this addition, therefore, the ratio of radioactive to non-radioactive CIPC molecules in solution was

significantly decreased. The ratio of radioactive to non-radioactive species in the previous additions had been maintained at the value established initially. At the moment of the final addition, the ratio for the adsorbed CIPC molecules can be assumed to have remained at the initial value. The ratio for the adsorbed molecules, therefore, was less than that for the molecules in the bulk of solution just after the addition of CIPC. Thus, the data obtained during the 72- to 128-hour period reflect not only the progression to a new chemical equilibrium, but also any redistribution of the radioactivity that may have occurred.

The results of the final equilibrium were examined in terms of the two extremes of radioactivity redistribution. First, complete redistribution of the radioactivity was assumed to have occurred within a very short time after the CIPC addition. On this basis, it was possible to calculate the apparent amounts of adsorption presented in Table 5-6 and Figures 5-5 and 5-6. The resulting values were not consistent with the previous equilibrium periods. This assumption led to much larger increases in the amount of CIPC adsorbed than in the previous equilibrium interval. Furthermore, all of the systems demonstrated increasing radioactivity levels in the bulk solution between the 97- and 128-hour analyses. Such an increase indicated that while chemical equilibrium probably had been obtained, redistribution of the radioactivity was not yet complete.

The other extreme was examined by assuming that no redistribution of radioactivity occurred between the solid and liquid phases. In essence, the adsorption of CIPC was assumed to be irreversible.

The evaluation of the data with respect to the above assumption gave rise to the apparent initial desorption of CIPC. Obviously this apparent desorption is inconsistent with the assumption of adsorption irreversibility as well as the accepted phenomena of adsorption.

It was concluded from these two interpretations of the data that redistribution of the radioactive CIPC molecules between the solid and liquid phases did take place. This redistribution, however, occurred rather slowly and was not complete even after 55 hours. The increased levels of radioactivity in the bulk solution gave conclusive evidence of the reversibility of this particular adsorption process. Such reversibility is typical of physical adsorption as opposed to chemisorption. Further discussion of this aspect is contained later in this section.

Using the data from all of the studies conducted at pH 6.9 and 20°C, Freundlich and Langmuir adsorption isotherms were constructed. The conditions that existed after 24 hours were assumed to represent equilibrium. While this assumption may not be strictly valid, certainly equilibrium conditions were closely approached after 24 hours of contact.

The Freundlich equation for adsorption is:

$$\frac{x}{m} = kC^{1/n}$$

where x is the mass of the substance adsorbed at equilibrium; m is the mass of the adsorbent; C is the concentration in solution at equilibrium; and k and n are constants. This equation is an empirical relationship that is obeyed for many adsorption systems. For such

systems, a plot of log $\frac{x}{m}$ versus log C will yield a straight line with a slope of $\frac{1}{n}$. The equilibrium data from the present studies are graphically depicted in this manner in Figure 5-10. The straight line shown in the accompanying figure was determined by a least squares statistical analysis. Although a small amount of scatter is noticeable, the experimental results obey Freundlich's equation quite well. From Figure 5-10, the values of n and k were calculated to be 9.1 and 0.20 respectively.

While the Freundlich expression is an empirical relationship, Langmuir's equation has a theoretical basis. The complete derivation of this relationship is contained in most texts on physical chemistry, e.g., Daniels and Alberty (86). This relationship is based on two principal assumptions. The first is that the adsorbate forms a monomolecular layer on the adsorbent surface. Secondly, the adsorption sites all possess the same characteristics and are equivalent in all respects. The final form of the equation as related to liquid-solid systems is:

$$\frac{x}{m} = \frac{abC}{1+aC}$$

where x is the mass adsorbed at equilibrium; m is the mass of the adsorbent; C is the concentration in solution at equilibrium; and a and b are constants.

The selection of C and $C \div x/m$ for the coordinate axes results in a linear plot of the data for systems obeying Langmuir's equation. The equilibrium data from the studies are presented in such a form in Figure 5-11. A least-squares statistical analysis of these

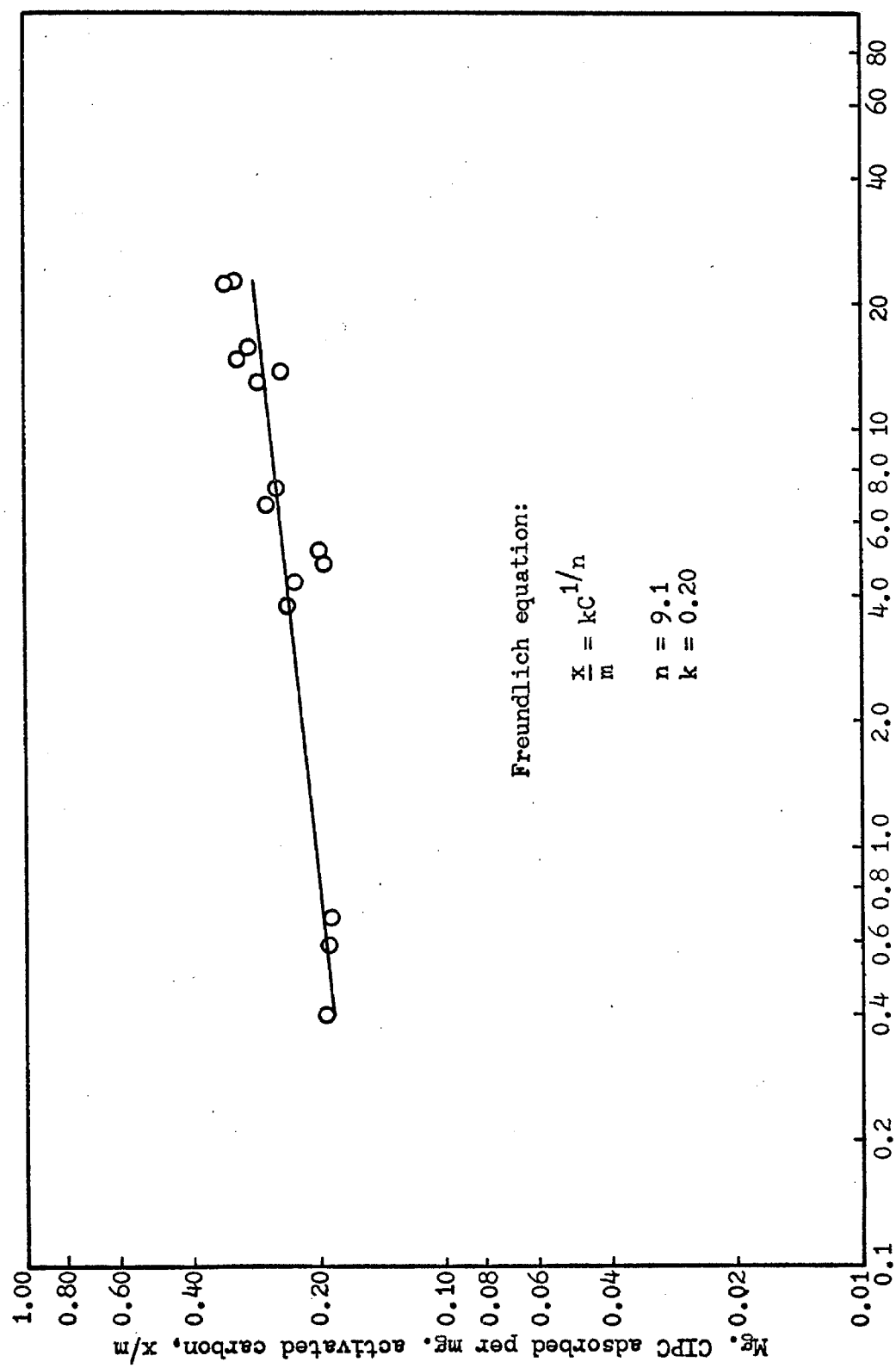


Figure 5-10. Freundlich isotherm for the adsorption of CIPC on activated carbon at pH 6.9 and 20°C.

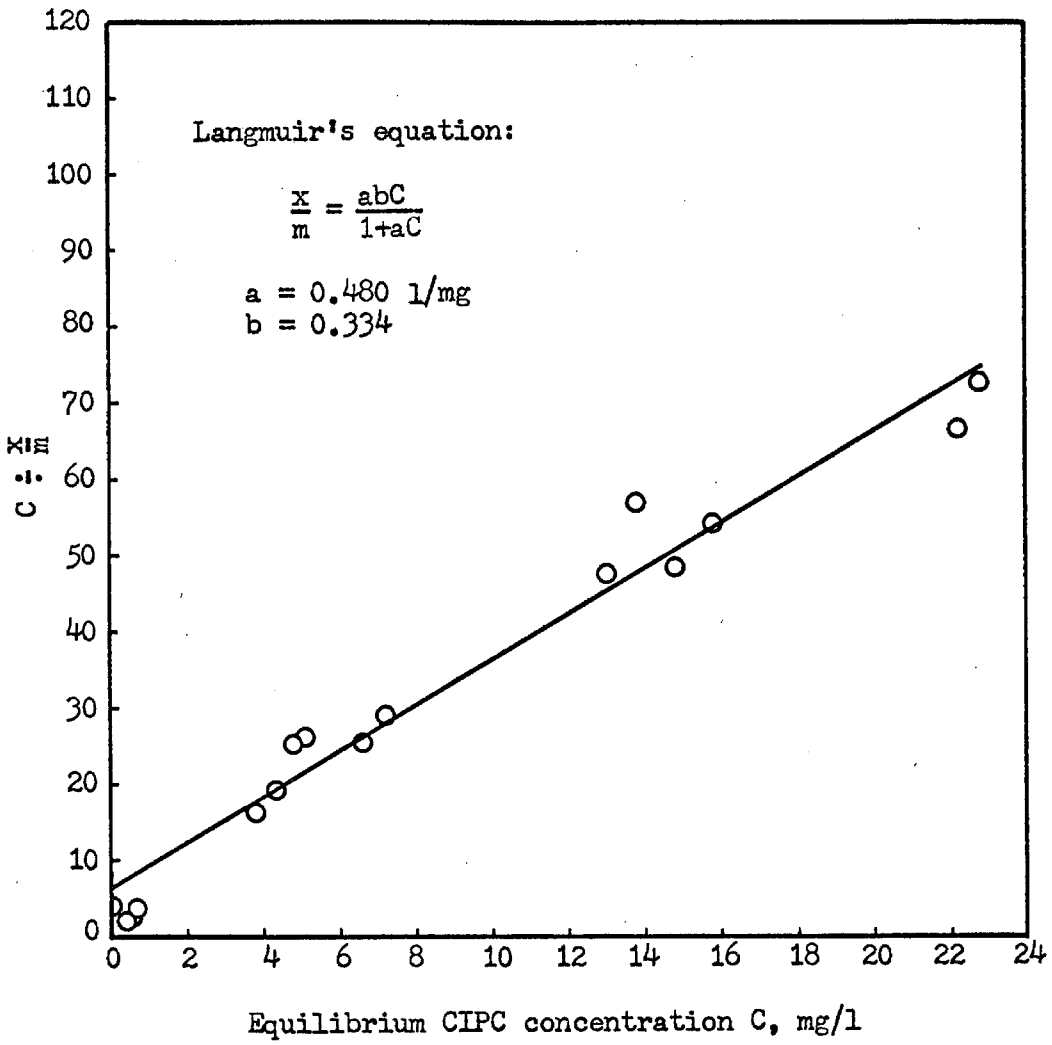


Figure 5-11. Langmuir isotherm for the adsorption of CIPC on activated carbon at pH 6.9 and 20°C.

results yielded the straight line shown in Figure 5-11. A reasonably good correlation between the data and Langmuir's expression was obtained. The values for the constants a and b were computed to be 0.480 and 0.334 respectively.

As the concentration C approaches infinity the value of $\frac{x}{m}$ approaches b. Thus, the value of b represents the theoretical capacity of the adsorbent for the particular adsorbate. A theoretical capacity of 0.334 milligrams of CIPC per milligram of activated carbon was indicated for the current investigation. This value agrees quite well with the values obtained in the actual capacity studies. Information from those investigations showed that the capacity was being approached when the $\frac{x}{m}$ values ranged from 0.291 to 0.334 after three equilibrium periods. The extrapolated data for the fourth equilibrium period indicated that the capacity might range somewhat higher.

The Freundich and Langmuir isotherms provide a convenient method for estimating the amount of activated carbon necessary to reduce the concentration of CIPC to a particular level at equilibrium. Table 5-10 contains the results of such computations for different initial concentrations of CIPC. The values presented in Table 5-10 represent equilibrium conditions attained after approximately 24 hours of contact. Previously cited results, however, indicated that equilibrium values were closely approached after four hours. Calculations based on these isotherms, therefore, may be considered approximately valid for four-hour contact periods.

Table 5-10

Amounts of Activated Carbon Required to Reduce Various CIPC Concentrations to Acceptable Levels Within 24 Hours

Initial CIPC Concentration mg/l	Desired Final CIPC Concentration mg/l	Concentration of Activated Carbon Required, mg/l	
		Freundlich Isotherm	Langmuir Isotherm
5.0	1.0	20.0	37.0
5.0	0.5	24.3	69.5
3.0	0.5	13.5	38.8
1.0	0.5	2.7	7.7
1.0	0.1	6.0	59.0
0.5	0.1	2.7	26.2

Obvious discrepancies exist between the amounts of carbon required for a given CIPC removal computed with the Freundlich equation and the same values based on the Langmuir equation. At low equilibrium concentrations the Langmuir isotherm appears to yield erroneous results. The experimental data obtained for CIPC equilibrium concentrations below one milligram per liter lie below the isotherm as shown in Figure 5-11. At these concentrations the adsorption was considerably greater than that indicated by the isotherm. While the Langmuir expression may be considered to apply to a wide range of equilibrium concentrations its validity at the low ranges for the carbon-CIPC system is questionable. The Freundlich isotherm appears

to represent the actual adsorption process more closely for low equilibrium concentrations of CIPC.

According to Steel (87), the normal quantities of activated carbon employed in water treatment plants range from 0.2 to 8.5 milligrams per liter. The maximum CIPC concentration that might be anticipated in a water supply would be considerably less than one milligram per liter. Four hours of contact with eight milligrams per liter would reduce the CIPC concentration from 1.0 down to 0.1 milligrams per liter or less based on the Freundich expression.

Previous studies on the adsorption of organic compounds on activated carbon have indicated a definite effect of pH on the reaction rates. Weber and Morris (67) found that the rate of adsorption of alkylbenzenesulfonates was faster in suspensions maintained at pH values below 4.0. Lower hydrogen-ion concentration resulted in correspondingly slower rates of adsorption. These authors attributed this pH dependency to changes in the surface characteristics of the adsorbent. Schwartz (57) indicated that low pH increased the total amount of the herbicide 2,4-D adsorbed on activated carbon. Both the alkylbenzenesulfonates and 2,4-D are of an anionic nature. Since activated carbon surfaces possess a negative charge, the electrostatic forces would tend to repulse these adsorbent and adsorbate species. Low pH values would favor the presence of the molecular species as opposed to the anionic species. At the same time, the increasing hydrogen-ion concentrations would tend to reduce the negative charge of the carbon surfaces. The net effect, therefore, would be to

reduce the electrostatic repulsive forces and, hence, to increase the rate and total amount of adsorption.

An examination of the molecular structure of CIPC indicates that there would be little tendency for it to ionize in aqueous solution. At low pH, some protonation of the amino group might be observed. The acidity constant of the acid form of CIPC is estimated to be on the order of 10^{-5}. Under strongly acid or alkaline conditions the situation is complicated by the hydrolytic conversion of CIPC to meta-chloraniline, CO_2, and isopropyl alcohol. In the pH range selected for these experiments i.e., pH 4.8 to 9.3, CIPC is not subject to hydrolytic conversion. Consequently, at pH values of 6.9 and 9.3 the compound would be expected to exist almost entirely in the molecular form as opposed to the ionic species. At pH 4.8, appreciable quantities of the protonated molecule may exist.

The results of the studies on the effects of pH on the adsorption of CIPC were presented in Section 5.1.2. For comparison, these data are reproduced in Figures 5-12 and 5-13. A time scale of $t^{\frac{1}{2}}$ was employed for these graphs. Such a coordinate system linearized the initial portions of the adsorption curves. The significance of this relationship between the amount of CIPC adsorbed and the elapsed time will be discussed in a subsequent portion of this chapter. For purposes of the present discussion, the use of the above system of coordinates provides a convenient method for comparing the initial rates of adsorption.

Manifest in the accompanying graphs are significant differences between duplicate systems. These differences were attributed

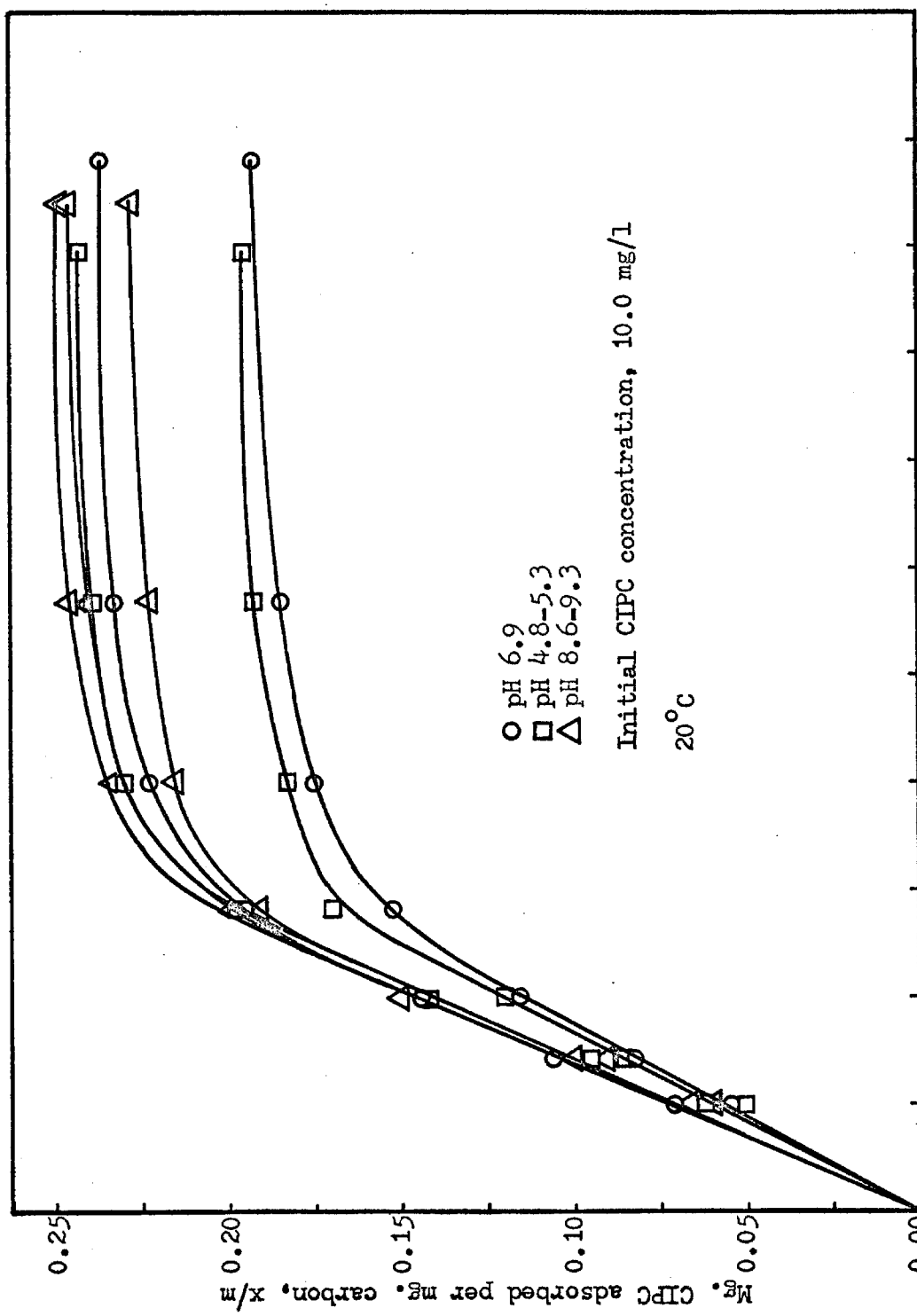


Figure 5-12. The adsorption of CIPC on 25 mg/l of activated carbon for different pH values.

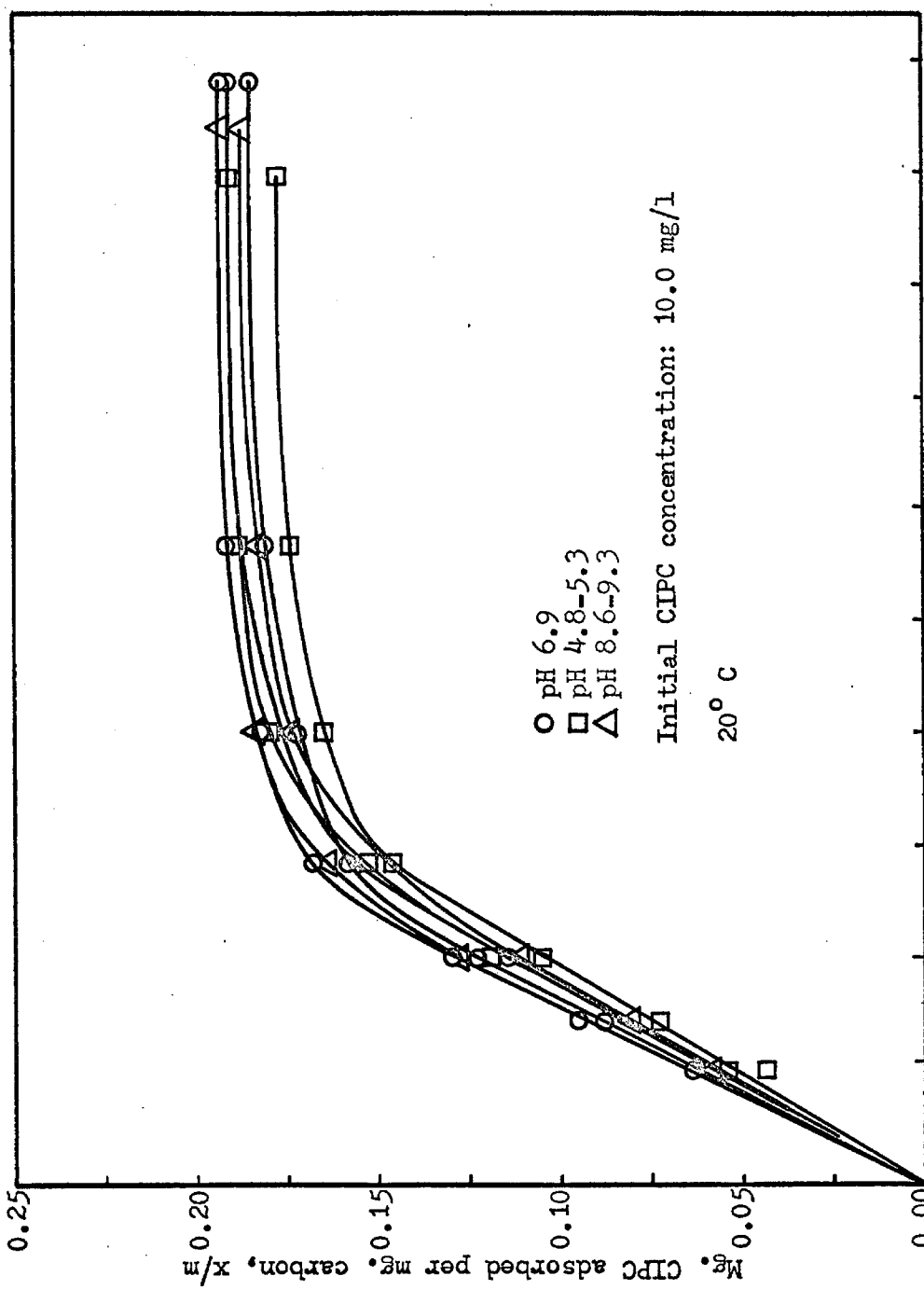


Figure 5-13. The adsorption of CIPC on 50 mg/l of activated carbon for different pH values.

previously to retention of unequal quantities of adsorbent on the surfaces of the experimental containers and apparatus. In spite of these variations, the results are of value in assessing the effects of pH on adsorption. Attention should be focused on those systems in each of the three pH ranges that showed the largest amounts of adsorption. For an activated-carbon concentration of 25 milligrams per liter (Figure 5-12) the maximum removals occurred at pH 8.6 to 9.3. At pH 4.8 to 5.3 slightly less CIPC was adsorbed. Almost identical maximum quantities of CIPC were adsorbed in each pH range for an adsorbent concentration of 50 milligrams per liter. Moreover, the initial adsorption rates did not exhibit any effects attributable to pH changes for either of the adsorbent concentrations.

Over the pH range of 4.8 to 9.3, it can be concluded that the total amount and rate of adsorption of CIPC on activated carbon remain essentially constant. Any changes that may occur in the character of the carbon surfaces, therefore, do not affect the adsorption of CIPC. The preceding statement by no means excludes the possibility that the hydrogen-ion concentration influences the nature of the adsorbent surfaces. Indeed, alterations in the electrokinetic nature of the surfaces would be anticipated. The results of this investigation indicate that the pH dependency reported in the literature for the adsorption of highly ionic compounds may not be solely ascribable to changes in the adsorbent surfaces. Rather, the observed effects of of hydrogen-ion concentration may be at least partially related to changes in the ionic character of the adsorbate.

The data from the studies at 20°C and 37°C are presented for comparison in Figures 5-14 and 5-15. Generally, the initial adsorption rates were faster at 37°C than at 20°C. On the other hand, the total amounts of CIPC adsorbed were greater for the lower temperature.

The information contained in the accompanying graphs can be used to estimate the heat of reaction and the activation energy for the adsorption of CIPC on activated carbon. The heat of reaction is related to the equilibrium constant in the following way:

$$\frac{d\ln K}{dt} = \frac{\Delta H}{RT^2}$$

where K is the equilibrium constant; T is the absolute temperature; ΔH is the heat of reaction; and R is the gas constant. The preceding expression is known as the van't Hoff equation (86). The heat of reaction, ΔH, can be assumed to be constant over small temperature ranges. Integration of the van't Hoff equation then yields:

$$\ln \frac{K_2}{K_1} = \frac{\Delta H(T_2-T_1)}{RT_1T_2}$$

In order to calculate the equilibrium constant, the following reaction model was selected:

$$P + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PC$$

where P is the pesticide; C is the activated carbon; PC is the adsorbed pesticide-carbon complex; and k_1 and k_2 are reaction rate constants. Derivation of Langmuir's expression for adsorption also involves the use of the foregoing reaction model. The equilibrium constant K can be expressed as:

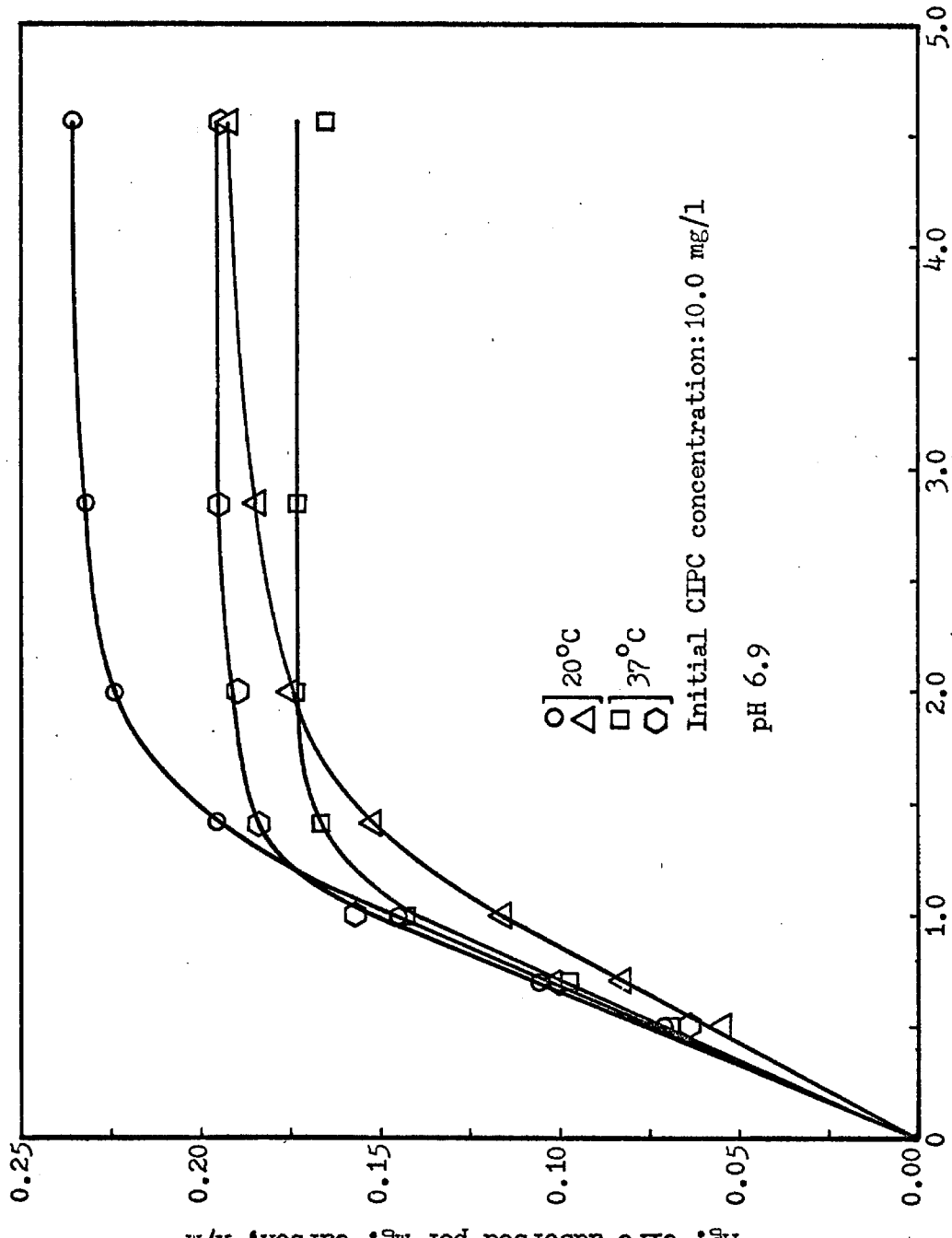


Figure 5-14. The adsorption of CIPC on 25 mg/l activated carbon at 20°C and 37°C

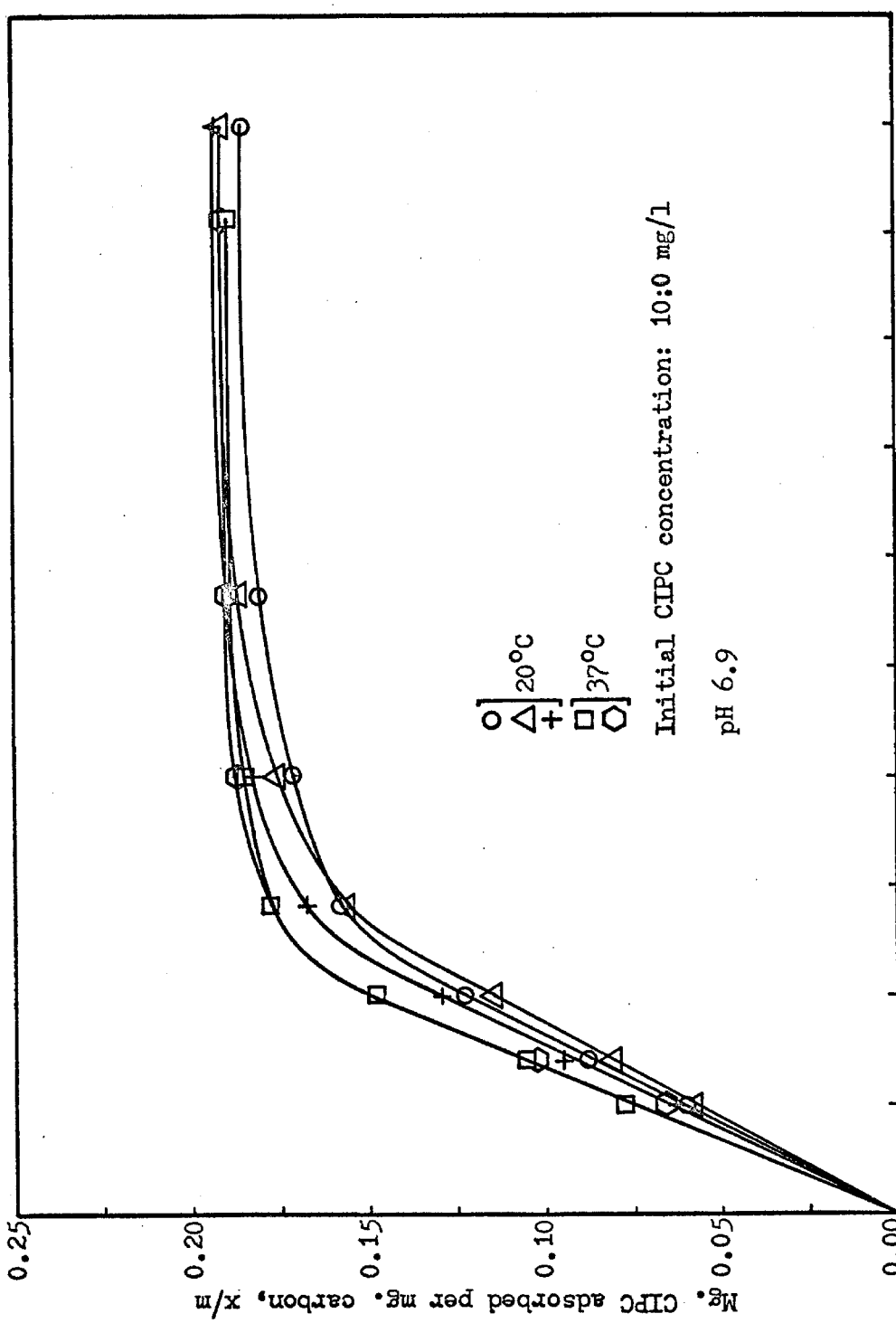


Figure 5-15. The adsorption of CIPC on 50 mg/l activated carbon at 20°C and 37°C.

$$K = \frac{(PC)}{(P_o - PC)(C_o - PC)}$$

where PC is the amount of pesticide adsorbed per unit volume; P_o is the initial pesticide concentration; and C_o is the concentration of activated carbon sites. C_o can be expressed in terms of the capacity of the carbon for the pesticide. For the activated carbon-CIPC system, the value of 0.334 milligrams of CIPC per milligram of carbon was employed. This value for the capacity was obtained from the Langmuir isotherm in Figure 5-11.

Values for the equilibrium constants for the adsorption of CIPC on carbon at 20°C and 37°C were calculated and are given in Table 5-11. For the adsorbent concentrations of 25 milligrams per liter, the heat of reaction was computed to be -6.1 kilocalories per mole based on the average values of the equilibrium constants. If instead the values for K for the samples in which the maximum adsorption occurred at each temperature were used in the calculation, the heat of reaction was found to be -7.3 kilocalories per mole. The use of the maximum adsorption data was justified because these results may be expected to represent more closely the values for systems experiencing no loss of adsorbent on the surfaces of the experimental apparatus.

Similar computations were performed utilizing the data for carbon concentrations of 50 milligrams per liter. The heat of reaction was +3.0 kilocalories per mole based on the average equilibrium constant. A value of +1.9 kilocalories per mole was obtained for the systems demonstrating maximum adsorption.

Table 5-11

Experimental Equilibrium and Initial Rate Constants for the Adsorption of CIPC on Activated Carbon at 20°C and 37°C

Temperature °C	Activated Carbon Concentration mg/l	Equilibrium Constant liter micromole^{-1}	Initial Rate Constant millimole gram^{-1} hours$^{-\frac{1}{2}}$
20	25	0.135	0.538
20	25	0.069	0.679
20	50	0.389	0.534
20	50	0.709	0.571
20	50	0.822	0.604
37	25	0.042	0.655
37	25	0.068	0.712
37	50	0.713	0.688
37	50	0.987	0.688

It is to be noted that the experiments with 25 milligrams of carbon per liter indicated an exothermic reaction process i.e., a negative value for ΔH, while the studies with 50 milligrams of adsorbent per liter indicated an endothermic process. At the higher carbon concentrations, almost all of the CIPC was removed after 20 hours. Small experimental errors under these conditions would be reflected in substantial errors in the equilibrium constants and, hence, in the values for the heat of reaction. For adsorbent concentrations of 25 milligrams per liter, similar errors produced much smaller differences in the calculated equilibrium constants. The heat of reaction for the activated carbon-CIPC system, therefore, was concluded to be on the order of -7.0 kilocalories per mole.

The heat of reaction in part reflects the energies of the bonds broken and formed during the reaction. For typical adsorption processes, the energy of the adsorption bond is of the same order of magnitude as the heat of reaction. In some instances, these two quantities may be almost identical. The heat of the reaction for the adsorption of CIPC on carbon is quite low indicating physical or weak chemical bonding. Strong chemical bonds possess energies on the order of 100 kilocalories per mole. Heats of reaction for chemisorption processes, therefore, would be considerably higher than the values indicated from these experiments. Electrostatic and hydrogen bonding are illustrative of physical adsorption. For this system, hydrogen bonding might exist between the amino hydrogen atom of the CIPC molecule and carbonyl oxygen atoms existing on the carbon surface. This type of bonding is of importance in protein chains (82)(88).

While the actual bonding mechanism cannot be established, it can be concluded that some type of physical bonding exists between the CIPC and the activated carbon.

A relationship similar to the van't Hoff equation exists between the activation energy and the rate of reaction. This expression developed by Arrhenius is as follows (86):

$$\frac{d\ln k}{dt} = \frac{\Delta H_a}{RT^2}$$

where k is the reaction rate; T is the absolute temperature; ΔH_a is the activation energy; and R is the gas constant. Integration of this equation yields a form identical to that for the van't Hoff equation:

$$\ln \frac{k_2}{k_1} = \frac{\Delta H_a(T_2-T_1)}{RT_1T_2} .$$

The linear initial portions of the adsorption curves shown in Figures 5-14 and 5-15 were used to calculate the initial rate constants shown in Table 5-11. Using the average values for these constants, the activation energy was calculated to be 1.7 kilocalories per mole.

The kinetics of the adsorption of organic compounds on activated carbon in an aqueous medium have been rather obscure. Recently Weber, Morris, and Rumer (67)(68)(69)(70) have postulated that intraparticle diffusion is the controlling step in the adsorption of certain organic substances on carbon. Attempts to correlate the experimental data from the present study with several theoretical reaction and diffusion models met with little success.

The adsorption of organic substances on activated carbon in a turbulent system involves several processes. It consists of the

transfer of molecules to the laminar boundary layer surrounding each adsorbent particle, diffusion through the boundary layer to the carbon surface, reaction or adsorption on the surface, and diffusion into the complex matrix of the particle. If the rate-controlling step is substantially slower than the other three processes, the overall rate of adsorption may be represented by the mathematical formulation for the controlling process.

It was assumed that the mass transfer to the laminar boundary layer was not a controlling step. In view of the rapid mixing of the suspension, this assumption appeared reasonable. As a first attempt to fit the data to a mathematical expression, the reaction at the carbon surface was considered. Again the second order reaction model was used:

$$P + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PC$$

The equation relating the concentrations of reactants and the rate constants is derived in many texts on physical chemistry, e.g., Daniels and Alberty (86), and will not be presented in this discussion. It will suffice to state that the experimental data conformed reasonably well to the mathematical expression for the first two hours. For the remainder of the time, no such correlation existed.

Subsequently, the experimental results were examined in light of several diffusion models. Diffusion to the carbon surface was investigated by combining Fick's first law of diffusion with the Langmuir's equation for adsorption. The Langmuir expression was used to

relate the amount of CIPC adsorbed to the surface concentration. Assuming that diffusion was the controlling step, equilibrium was essentially maintained at all times between the adsorbed CIPC and the CIPC at the carbon surface. No agreement was obtained between the derived equation and the experimental results.

The diffusion of substances from solutions of limited volume into spheres, cylinders, and planes in well-stirred systems is discussed in the text by Crank (89). The expressions for the three particle geometries were applied to the adsorption results with limited success. Fair agreement was obtained for the first 30 minutes to one hour of the experiments.

One of the most striking features of the adsorption data was the linear relationship between the amount of CIPC adsorbed and the square root of time during the initial periods of the experiments. Previously, this relationship was employed to compare initial adsorption rates. The linear dependency was followed for about the first hour of the studies as shown in Figures 5-12 to 5-15. Mathematical models for many different diffusion processes predict the same dependency for the initial periods. Among the systems demonstrating this feature are the diffusion into various geometrical bodies in stirred solutions, discussed previously, and the diffusion to a hollow sphere from a concentric surface maintained at constant concentration. Numerous examples of this relationship between the amount of material transferred to a surface and $t^{\frac{1}{2}}$ are presented in the book by Crank (89). While the experimental data did not fit any mathematical model of

adsorption kinetics examined, the adsorption curves were similar in shape to those for many diffusion controlled sorption processes.

The failure to find a relatively simple model for the adsorption process may be related, in part, to experimental errors. Near the equilibrium point, the diffusion equations and the expression for a second order reaction tend to magnify small analytical errors greatly. Of greater importance, however, is the fact that the experimental systems were not designed for kinetic studies. Rather, they were intended to and indeed did yield pertinent information concerning the ability of activated carbon to adsorb CIPC. These systems were very complex and not suitable for precise mathematical description. In order to study the adsorption kinetics properly, the experimental conditions, particularly the fluid dynamics and the particle geometry, must be well-defined.

A few summary statements are in order at this point. The herbicide CIPC can be removed from water by adsorption on activated carbon. Residual CIPC concentrations of less than 0.1 milligrams per liter for initial concentrations of one milligram per liter can be obtained with less than ten milligrams of carbon per liter. Equilibrium was reached within 24 hours and was closely approached after four hours of contact. The equilibrium conditions can be represented quite well by the Freundlich isotherm.

Little or no effect was observed with changes in hydrogen-ion concentration. This fact suggests that pH dependencies for ionic compounds may be the result of shifts in the degree of ionization as as well as changes in the surface characteristics of the adsorbent.

The heat of adsorption was calculated to be about -7.0 kilocalories per mole indicating physical or weak chemical bonding.

The experimental data did not lend themselves to precise mathematical interpretation. Diffusion to the surface or into the carbon pores and reaction at the surface remain as possible rate-controlling steps in the adsorption process.

CHAPTER 6

THE INFLUENCE OF ACTIVATED CARBON ON THE DEGRADATION OF CIPC

In the previous chapter, adsorption studies indicated that little if any CIPC or 2,4-D was adsorbed on various clay minerals. The possibility exists that other particulate substances in water supplies are capable of adsorbing these compounds. Biologically resistant organic matter such as cellulose fibers might adsorb significant quantities of pesticides. Certain amounts of pesticide may be removed in this manner from the water phase. These compounds would remain, however, as an integral part of a complex biological system. The decomposition of 2,4-D and CIPC in an aqueous environment was discussed in Chapter 4. As the final portion of the research, a study was performed to ascertain the effects of activated carbon on the degradation of CIPC. Activated carbon was selected because of its known ability to adsorb significant quantities of CIPC. Moreover, the adsorption studies with this same system in Chapter 5 provide useful background information.

6.1 Results

The experimental procedures employed for this study were essentially the same as those used in the degradation studies previously discussed in Chapter 4. One additional measurement was performed throughout the experiment. Carbon dioxide evolved during the breakdown of the pesticide was collected in barium hydroxide and analyzed for carbon-14 radioactivity. In this way, the quantities of

the isopropyl- and ring-labeled carbon atoms evolved as carbon dioxide could be determined.

Thirteen systems were used for this study. The precise composition of each system was given in Table 3-6, Section 3.8. Of the ten systems containing CIPC-I, five also contained activated carbon. The remainder held only CIPC-I in concentrations ranging from 0.5 to 5.0 milligrams per liter. The degradation at various substrate concentrations could then be compared with the metabolism of the isopropyl group in the presence of activated carbon. Two of the three CIPC-R systems contained 25 milligrams of carbon per liter. System 11 held 5.0 milligrams of CIPC-R per liter with no adsorbent.

The duration of this investigation was 50 days. During that period, samples were withdrawn for liquid scintillation analysis. Results from these measurements are presented in Tables 6-1 and 6-2 and Figures 6-1 and 6-2. In the accompanying tables and figures, the radioactivity that remained in solution was related to the amount of isopropyl- or ring-carbon atoms that were present. Systems 1 and 2, initially containing 5.0 milligrams of CIPC-I per liter but devoid of activated carbon, lost the isopropyl-carbon atoms quite rapidly for the first ten days. After 50 days, 24 to 28 percent of the radioactivity remained in solution. Initial substrate concentrations of 3.00, 1.06, and 0.53 milligrams per liter resulted in smaller percentage reductions.

All of the inoculated systems containing activated carbon demonstrated an increase in the radioactivity in solution during the first two to seven days. Thereafter, the level of radioactivity in

Table 6-1

Degradation of Isopropyl-Labeled CIPC in the Presence of Activated Carbon

Characteristic	System Number									
	1	2	3	4	5	6	7	8	9	10
Initial CIPC-I, mg/l	5.00	5.00	3.00	1.06	0.53	5.00	5.00	5.00	5.00	5.00
Activated carbon, mg/l	0	0	0	0	0	25	25	12.4	12.4	25
Inoculated	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No*
Initial counting rate, cpm	12,656	12,769	12,736	8450	4218	12,730	12,669	12,606	12,384	12,566
Isopropyl carbon atoms remaining in solution, percent										
-2.5 days[a]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.0 days[b]	99.2	99.9	99.9	97.8	98.4	9.6	9.1	52.9	48.3	9.3
1.0 days	95.5	95.6	95.9	94.4	95.3	14.8	15.2	53.8	50.5	7.6
2.0 days	88.5	89.0	94.0	91.8	93.3	17.6	22.7	55.9	52.2	9.1
3.0 days	81.2	83.3	92.3	90.6	90.8	19.9	19.0	53.8	53.7	8.5
4.0 days	75.1	79.3	90.5	89.1	89.0	20.1	21.3	54.3	51.5	11.1
5.0 days	66.3	75.2	90.0	86.3	88.0	21.5	23.4	54.8	52.8	8.2

Table 6-1(cont'd)

Characteristic	System Number									
	1	2	3	4	5	6	7	8	9	10
Isopropyl carbon atoms remaining in solution, percent (cont'd)										
7.0 days	51.8	61.2	88.6	85.1	83.8	20.1	22.0	55.8	53.4	17.5
8.0 days	48.4	55.7	87.2	83.5	84.1	20.1	21.8	54.5	52.4	14.4
9.0 days	44.1	51.4	86.2	81.3	86.1	19.8	21.7	55.4	53.1	17.0
11.0 days	44.8	45.5	85.7	79.6	83.5	20.0	21.2	56.1	51.3	19.6
14.0 days	44.3	43.0	84.4	77.6	79.9	18.4	20.8	55.7	51.1	24.1
18.1 days	42.7	43.1	83.6	72.5	77.2	19.1	19.9	56.0	51.4	27.0
25.1 days	39.6	37.4	81.5	70.3	67.2	18.0	19.2	56.5	50.4	28.1
30.1 days	36.8	33.8	81.0	66.4	65.5	16.4	17.2	57.1	51.4	28.6
50.0 days	27.7	23.8	78.1	48.8	51.8	13.4	11.6	60.0	55.1	24.2

Table 6-1(cont'd)

Characteristic	System Number									
	1	2	3	4	5	6	7	8	9	10
Isopropyl carbon atoms remaining in solution, micromole liter^{-1}										
-2.5 days[a]	23.4	23.4	14.0	4.96	2.48	23.4	23.4	23.4	23.4	23.4
0.0 days[b]	23.2	23.4	14.0	4.85	2.44	2.3	2.1	12.4	11.3	2.2
1.0 days	22.3	22.4	13.4	4.68	2.36	3.5	3.6	12.6	11.8	1.8
2.0 days	20.7	20.8	13.2	4.55	2.31	4.1	5.3	13.1	12.2	2.1
3.0 days	19.0	19.5	12.9	4.49	2.25	4.7	4.4	12.6	12.6	2.0
4.0 days	17.6	18.6	12.7	4.42	2.21	4.7	5.0	12.7	12.1	2.6
5.0 days	15.5	17.6	12.6	4.28	2.18	5.0	5.5	12.8	12.4	1.9
7.0 days	12.1	14.3	12.4	4.22	2.08	4.7	5.2	13.1	12.5	4.1
8.0 days	11.3	13.0	12.2	4.14	2.09	4.7	5.1	12.7	12.3	3.4
9.0 days	10.3	12.0	12.1	4.03	2.14	4.6	5.1	13.0	12.4	4.0
11.0 days	10.5	10.6	12.0	3.95	2.07	4.7	5.0	13.1	12.0	4.6
14.0 days	10.4	10.1	11.8	3.85	1.98	4.3	4.9	13.0	12.0	5.6

Table 6-1(cont'd)

Characteristic	System Number									
	1	2	3	4	5	6	7	8	9	10
Isopropyl carbon atoms remaining in solution, micromole liter^{-1} (cont'd)										
18.1 days	10.0	10.1	11.7	3.60	1.91	4.5	4.7	13.1	12.0	6.3
25.1 days	9.3	8.8	11.4	3.49	1.67	4.2	4.5	13.2	11.8	6.6
30.1 days	8.6	7.9	11.3	3.29	1.62	3.8	4.0	13.4	12.0	6.7
50.0 days	6.5	5.6	10.9	2.42	1.28	3.1	2.7	14.0	12.9	5.7

*Visible evidence of contamination prior to 7-day sample.

(a) Immediately preceding the addition of activated carbon.

(b) Immediately preceding the addition of microorganisms.

Table 6-2

Degradation of Ring-Labeled CIPC in the Presence of Activated Carbon

Characteristic	System Number		
	11	12	13
Initial CIPC-R, mg/l	5.00	5.00	5.00
Activated carbon, mg/l	0	25	25
Inoculated	Yes	Yes	No*
Initial counting rate, cpm	12,377	12,353	12,298
Ring carbon atoms remaining in solution, percent			
-2.5 days[a]	100.0	100.0	100.0
0.0 days[b]	94.4	21.2	52.8
1.0 days	90.8	14.6	14.0
2.0 days	86.6	18.1	13.4
3.0 days	82.4	19.4	13.8
4.0 days	78.7	22.6	13.7
5.0 days	75.5	23.1	10.6
7.0 days	67.4	22.5	12.0
8.0 days	63.7	22.1	14.1
9.0 days	58.3	22.0	16.8
11.0 days	51.5	22.1	20.6
14.0 days	52.6	21.7	23.8
18.1 days	53.9	21.4	29.1
25.1 days	54.7	22.9	37.4
30.1 days	53.4	23.8	39.6

Table 6-2(cont'd)

Characteristic	System Number		
	11	12	13
Ring carbon atoms remaining in solution, percent (cont'd)			
50.0 days	51.8	24.5	46.8
Ring carbon atoms remaining in solution micromole liter^{-1}			
-2.5 days[a]	140	140	140
0.0 days[b]	132	30	74
1.0 days	128	20	20
2.0 days	122	25	19
3.0 days	116	27	19
4.0 days	110	32	19
5.0 days	106	32	15
7.0 days	95	32	17
8.0 days	89	31	20
9.0 days	82	31	24
11.0 days	72	31	29
14.0 days	74	30	33
18.1 days	76	30	41
25.1 days	77	32	52
30.1 days	75	33	56
50.0 days	73	34	66

*Visible evidence of contamination prior to 7-day sample.
(a) Immediately preceding the addition of activated carbon.
(b) Immediately preceding the addition of microorganisms.

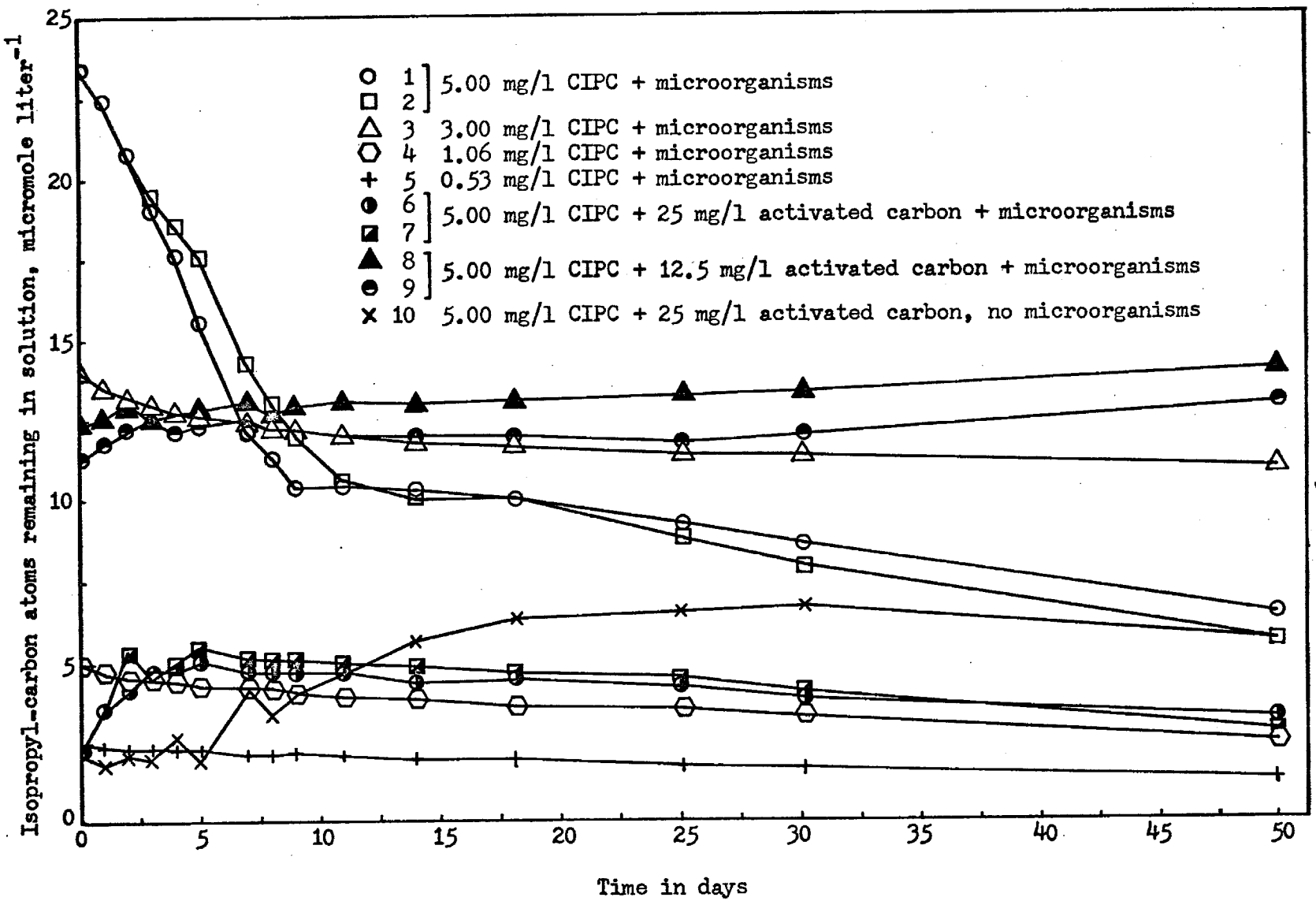


Figure 6-1. Degradation of adsorbed CIPC. Isopropyl-carbon atoms remaining in solution.

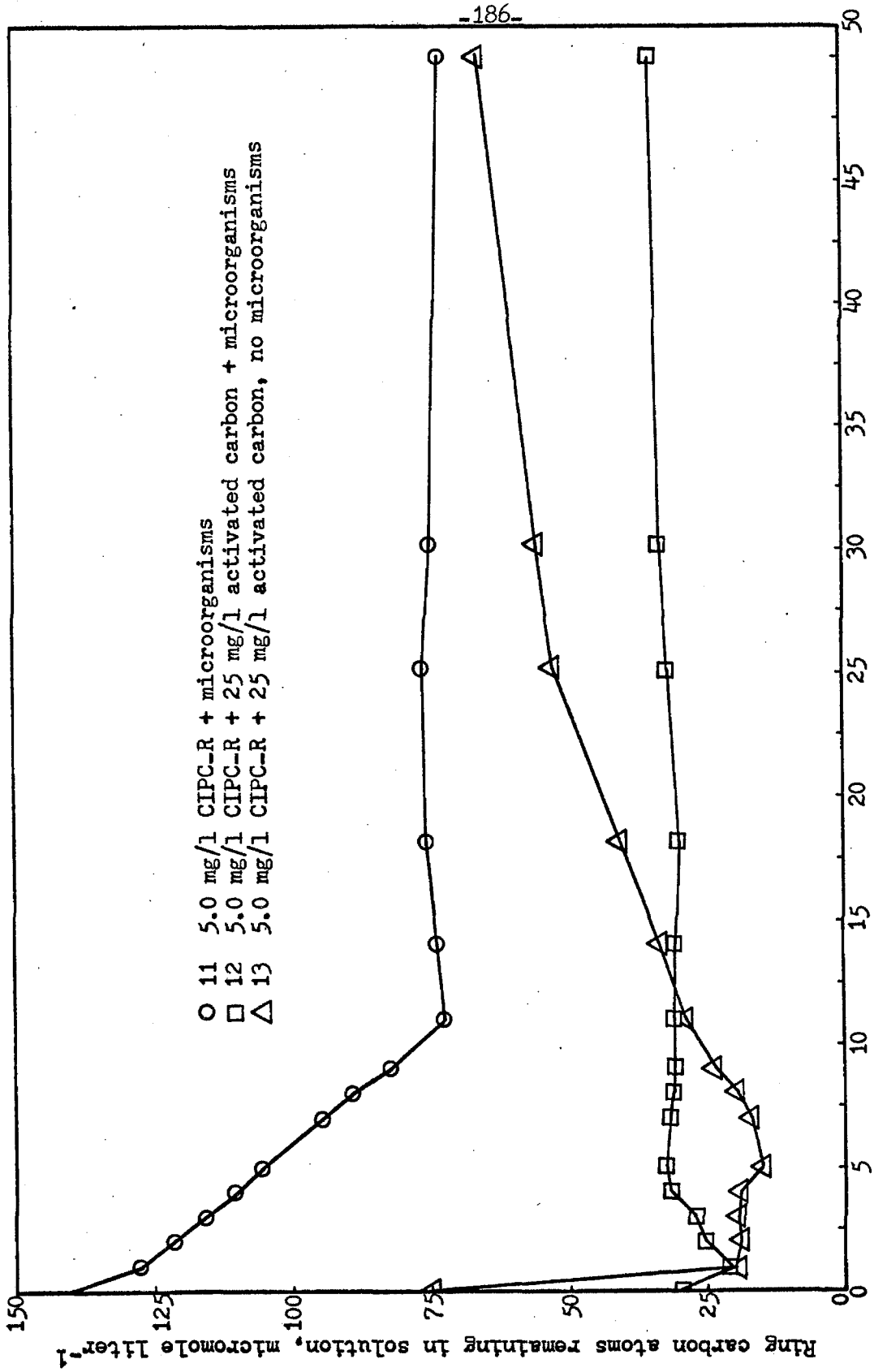


Figure 6-2. Degradation of adsorbed CIPC. Ring-carbon atoms remaining in solution.

solution remained relatively constant. Systems 10 and 13, initially sterile, became contaminated after five days. Following microbial contamination, the radioactivity in solution increased over the ensuing 25 days. For the CIPC-R culture (system 13) this increase continued for the duration of the experiment.

In addition to the analyses of the radioactivity in solution, measurements were made of the carbon-14 evolved as carbon dioxide in systems 1,2,6,7,10,11 and 12. In Table 6-3 and Figure 6-3 are presented the data obtained during the study. With the exception of system 12, less than 0.14 micromoles of radioactive carbon dioxide were collected during the first eight days. The total evolution of the isopropyl carbon atoms was greatest for the systems without activated carbon. In the case of the ring carbon atoms, the reverse was true. It was observed in Chapter 4, that little CO_2 arising from the degradation of CIPC remained in solution.

The initial stock solutions of radioactive CIPC were prepared with 95 percent ethanol. Subsequent dilutions were made with distilled water. Some ethanol, therefore, was present in each sample in the experimental series. For most of the systems, the ethanol concentration was approximately 0.18 moles per liter of sample. It is to be expected that ethanol would be readily metabolized by the microorganisms. The quantities of carbon dioxide that might be released could easily exhaust the barium hydroxide present in the absorbing solution. During the first five to seven days, large amounts of precipitate were evident in the carbon-dioxide traps. The barium hydroxide during this period may have been converted entirely to

Table 6-3

Carbon Atoms Evolved as Carbon Dioxide

Characteristic	System Number						
	1	2	6	7	10	11	12
Initial CIPC, mg/l	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Activated carbon, mg/l	0	0	25	25	25	0	25
Inoculated	Yes	Yes	Yes	Yes	No*	Yes	Yes
Type of carbon atoms measured	----------Isopropyl----------					Ring	Ring
Cumulative amount of carbon atoms evolved per liter of solution, micromole							
0.0 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0 days	0.00	0.00	0.00	0.00	0.00	0.02	0.78
2.0 days	0.00	0.00	0.00	0.00	0.00	0.04	0.98
3.0 days	0.00	0.00	0.00	0.00	0.00	0.06	1.03
4.0 days	0.01	0.01	0.00	0.01	0.00	0.08	1.06
4.5 days	0.01	0.01	0.01	0.10	0.01	0.09	1.07
5.0 days	0.02	0.01	0.01	0.01	0.01	0.09	1.08
5.5 days	0.02	0.02	0.01	0.01	0.01	0.01	1.09
5.9 days	0.02	0.02	0.01	0.01	0.01	0.11	1.10
6.5 days	0.03	0.03	0.02	0.02	0.01	0.12	1.11
7.0 days	0.04	0.04	0.02	0.02	0.01	0.13	1.12
7.5 days	0.04	0.04	0.02	0.02	0.01	0.13	1.13
8.0 days	0.05	0.05	0.02	0.03	0.01	0.14	1.15
8.5 days	0.06	0.06	0.03	0.03	0.02	0.15	1.22

Table 6-3(cont'd)

Characteristic	System Number						
	1	2	6	7	10	11	12
Cumulative amount of carbon atoms evolved per liter of solution, micromole (cont'd)							
9.0 days	0.08	0.07	0.03	0.04	0.02	0.15	1.27
9.5 days	0.23	0.07	0.04	0.04	0.03	0.16	1.29
10.0 days	0.26	0.08	0.04	0.05	0.03	0.17	1.33
11.0 days	0.30	0.09	0.07	0.05	0.06	0.19	1.39
12.1 days	0.34	0.12	0.13	0.07	0.09	0.20	1.44
13.1 days	0.39	0.30	0.29	0.11	0.13	0.22	1.51
14.0 days	0.47	0.34	0.38	0.17	0.17	0.23	1.56
15.0 days	0.58	0.36	0.43	0.25	0.22	0.27	1.61
16.1 days	0.76	0.41	0.52	0.37	0.28	0.32	1.66
17.0 days	0.96	0.47	0.61	0.43	0.33	0.36	1.71
18.1 days	1.16	0.58	0.71	0.46	0.39	0.42	1.83
21.1 days	1.66	0.95	1.03	0.72	0.44	0.62	2.05
23.0 days	1.93	1.50	1.32	0.93	0.50	0.78	2.28
25.1 days	2.21	2.05	1.64	1.24	0.57	0.95	2.47
28.0 days	2.64	2.82	2.02	1.72	0.64	1.17	2.74
30.1 days	2.89	3.24	2.23	2.00	0.72	1.33	2.92
35.2 days	3.42	4.31	2.76	2.79	0.86	1.79	3.48
37.2 days	3.63	4.63	2.91	3.01	0.98	1.96	3.71
50.0 days	4.79	6.35	3.60	4.21	1.25	3.16	5.26

*Visible evidence of contamination prior to 7-day sample.

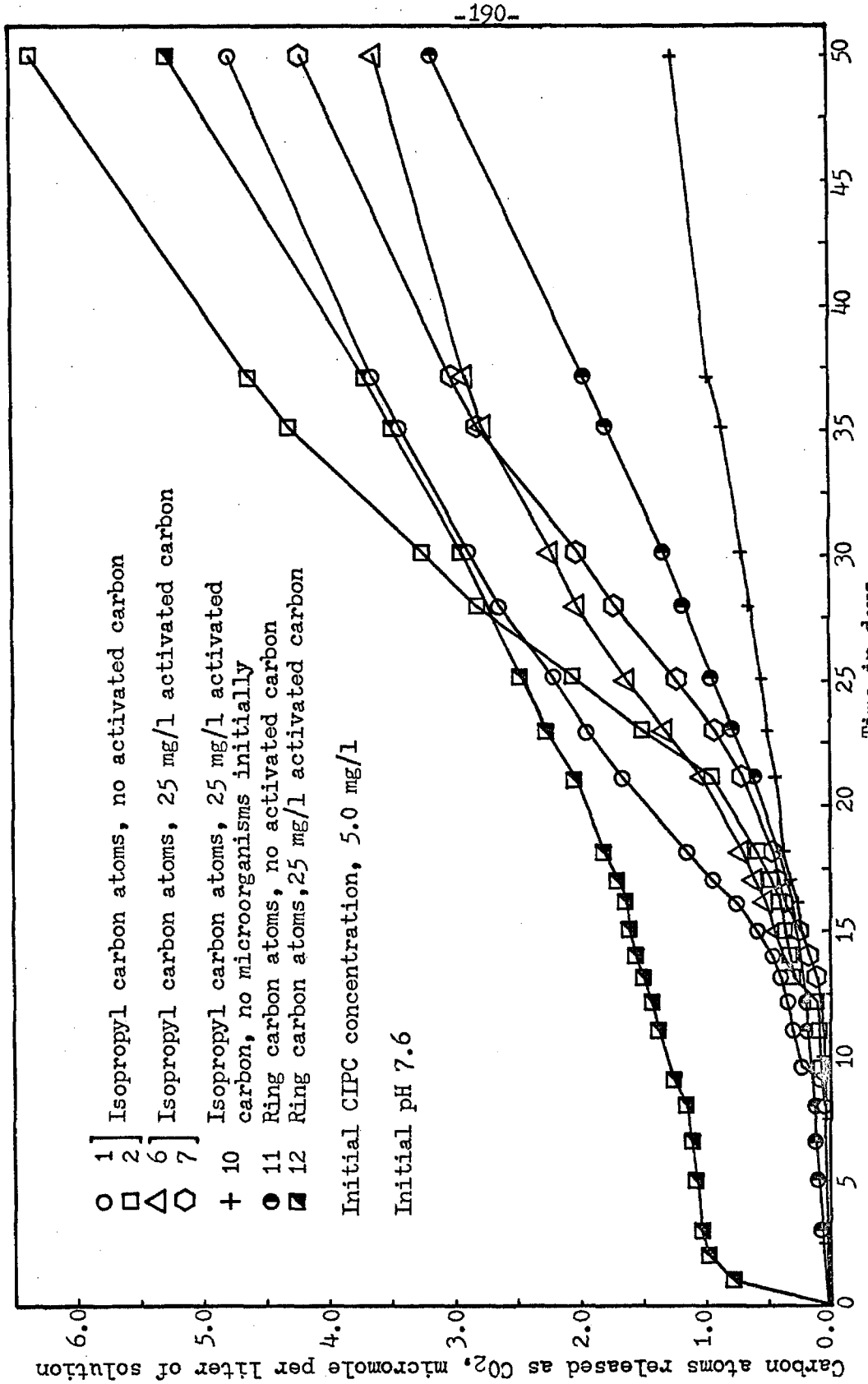


Figure 6-3. Cumulative amounts of carbon atoms released as carbon dioxide during the degradation of CIPC.

barium carbonate. Under these circumstances, some of the radioactive carbon dioxide may not have been collected. After seven days, the barium carbonate precipitate was noticeably less and it can be assumed that the carbon dioxide collection was complete.

A series of solutions containing known quantities of sodium carbonate labeled with carbon-14 was prepared to measure the lag time between the formation of carbon dioxide and its collection in the barium hydroxide traps. The solutions were acidified with concentrated hydrochloric acid and the vials containing barium hydroxide were removed periodically. Most of the carbon dioxide formed was collected within two hours. Essentially complete recovery was obtained within seven hours.

One additional kind of measurement was made during this study. On the eighteenth day, ten-milliliter aliquots of the culture suspensions were removed. The samples were centrifuged and one-milliliter portions of the supernatant were analyzed for the amount of radioactivity remaining in solution. The remainder of the samples were treated in the manner described in Section 3.2.1 to obtain samples of the cellular material and adsorbent suitable for radioactivity analysis. Results from these analyses are given in Table 6-4.

Since no satisfactory counting standard for cellular material was on hand, the counting efficiency could not be determined. The data, therefore, can be examined only qualitatively. It should be noted that the deposits of solid material on the planchets were generally quite thick, several millimeters in depth. For such material,

Table 6-4

Radioactivity Associated With Solid Matter After 18 Days

Characteristic	System Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Initial CIPC-I, mg/l	5.00	5.00	3.00	1.06	0.53	5.00	5.00	5.00	5.00	5.00	-	-	-
Initial CIPC-R, mg/l	-	-	-	-	-	-	-	-	-	-	5.00	5.00	5.00
Activated carbon, mg/l	0	0	0	0	0	25.0	25.0	12.4	12.4	25.0	0	25.0	25.0
Inoculated	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Volume of culture prior to sampling, ml	108	108	108	108	108	108	108	108	108	108	108	108	108
Sample volume, ml	10	10	10	10	10	10	10	10	10	10	10	10	10
Net counting rate, cpm*	537	462	165	499	121	4957	4211	6369	4973	9089	633	5627	11,110

*Background counting rate = 1.6 cpm

the self-absorption of beta radiation from carbon-14 would be high and the counting efficiency very low.

The counting rates for the systems without activated carbon were on the order of 500 counts per minute. Radioactivity levels for the other systems containing carbon ranged from 4000 to 11,000 counts per minute. Obviously, the activated carbon retained a significant quantity of the labeled carbon atoms. The highest levels of radioactivity were observed for systems 10 and 13 which were initially sterile. For these two systems, the counting rate was approximately twice as great as that for the corresponding systems inoculated with adapted organisms.

6.2 Discussion

Before considering the influence of activated carbon on the degradation of CIPC, the systems containing no activated carbon will be examined. Systems 1 and 2 contained 5.0 milligrams of CIPC-I per liter. As seen in in Figure 6-1, the rate of disappearance of the isopropyl-carbon atoms from solution was quite rapid for the first nine to ten days. Approximately 50 percent of the radioactivity vanished in nine days. Thereafter, the disappearance rate decreased sharply. Only an additional 25 percent of the labeled atoms was removed in the next 40 days. The systems in the fourth, fifth, and sixth stages of adaptation discussed in Section 4.1.2 initially had CIPC concentrations of 5.4 milligrams per liter. In those adaptation systems without the supplemental source of organic carbon, the relative initial rate of disappearance of the isopropyl-carbon atoms was

about the same as that in systems 1 and 2 of the activated carbon series. The rate of reduction of radioactivity in the fourth and fifth adaptation stages leveled off after four to five days. The isopropyl-carbon atoms from systems in the sixth adaptation stage continued to vanish from solution at a rapid rate for 20 days. At that time, only five percent of the isopropyl groups remained in solution. With the exception of the sixth adaptation stage, the disappearance from solution of the isopropyl-carbon atoms in systems 1 and 2 of the activated carbon series proceeded in a manner similar to that observed for the adaptation systems.

System 11 contained 5.0 milligrams of CIPC-R per liter. After 11 days, 51 percent of the radioactivity remained in solution in system 11. The radioactivity level then remained essentially constant for the rest of the experiment. Generally, the systems from the fourth, fifth, and sixth stages of adaptation to ring-labeled CIPC demonstrated slightly smaller decreases during the first 11 days. Eventually, the total percentage loss of radioactivity from solution was the same for the adaptation systems as for system 11 of the activated carbon series.

Systems 3,4, and 5 contained decreasing initial concentrations of CIPC-I. The relative losses of isopropyl-carbon atoms in these systems were significantly less than those observed for systems 1 and 2. For an initial CIPC-I concentration of 3.0 milligrams per liter, the observed radioactivity loss was only 22 percent after 50 days. Losses of 51 and 48 percent occured in the same period for

systems containing 1.06 and 0.53 milligrams of CIPC-I per liter respectively. The total amount of isopropyl-carbon atoms removed decreased with decreasing initial concentrations. This fact indicates that the overall rate of decomposition was dependent on the substrate concentration, as might be expected for organisms growing on a particular compound.

Seven experimental systems contained activated carbon. All of these systems had CIPC concentrations of 5.0 milligrams per liter prior to the addition of the adsorbent. The equilibrium conditions that existed prior to the inoculation of the CIPC-I systems were in close agreement with the data obtained in the adsorption studies (Section 5.1.2). Apparent equilibrium values for the CIPC-R systems did not agree with the previous results. A sharp decrease in the amount of radioactivity in solution was observed during the first day following inoculation. The sterile system 13 showed a continued decrease for the first five days. If the data at this time are assumed to represent equilibrium, good agreement is obtained with the adsorption study in Chapter 5. It appears, therefore, that equilibrium conditions were not obtained for the CIPC-R cultures prior to inoculation.

Following inoculation, the systems containing activated carbon followed a typical pattern. The number of labeled carbon atoms in solution increased markedly during the first five days. The levels of radioactivity stayed relatively constant or decreased slightly for the next 20 days. For those systems containing 12.4 milligrams of the

adsorbent per liter, a small increase in the radioactivity in solution was observed in the final portion of the experiment. A similar increase was noticed for the CIPC-R system containing 25 milligrams of activated carbon per liter. The radioactivity in solution for the CIPC-I systems with activated carbon concentrations of 25 milligrams per liter continued to decrease during this period. In all instances, the number of radioactive carbon atoms in solution after 50 days exceeded the equilibrium values before the addition of microorganisms.

Systems 10 and 13 also contained activated carbon, but were initially sterile. These systems became contaminated after five days. The radioactivity of the solutions increased greatly during the next 25 days. In the final 20 days, the number of isopropyl-carbon atoms in solution decreased slightly while a further increase in ring-carbon atoms in solution was observed. After 50 days, the total amount of radioactivity remaining in solution was roughly twice as great as the level of radioactivity in the corresponding inoculated systems.

A tentative hypothesis can now be advanced regarding the influence of activated carbon on the metabolism of CIPC. The addition of microorganisms to activated carbon-CIPC systems results in an increase in the amount of radioactivity in solution. The microorganisms or substances intimately associated with the organisms compete with the CIPC for sites on the adsorbent surface. A portion of the previously adsorbed CIPC is driven off the carbon surface and the amount in solution increases. In this study, the percentages of radioactivity in solution after five days was about the same for the CIPC-I

and CIPC-R systems containing 25 milligrams of activated carbon per liter. The ring- and isopropyl-carbon atoms were desorbed at the same rate initially. This fact would indicate that intact CIPC molecules were returning to solution during this time interval.

Following the period of increasing radioactivity, it is hypothesized that metabolism of the CIPC takes place only in the aqueous phase or at the exterior of the activated carbon. No degradation of CIPC occurs in the interior of the carbon particles. The adsorbent has effectively reduced the initial amount of substrate available to the microbial population. Degradation proceeds at the same rate as the rate in a system containing only the pesticide at a concentration equal to that in solution in the activated-carbon systems. As the CIPC in solution is degraded, additional quantities of the herbicide are desorbed. Since degradation is slow, it can be assumed that equilibrium conditions are essentially maintained at all times. A system exists where the CIPC in solution is being removed and, in order to attain equilibrium, a portion of the adsorbed CIPC returns to solution. The overall rate of disappearance of the isopropyl- or ring-carbon atoms from solution would be less in the presence of an adsorbent than for systems containing CIPC without an adsorbent.

The data shown in Figures 6-1 and 6-2, Section 6.1 are in general agreement with the hypothesis expounded in the preceding paragraphs. The disappearance from solution of the isopropyl groups in systems 1 and 2 containing 5.0 milligrams of CIPC per liter with no activated carbon was much faster than the removal observed in the

corresponding systems with activated carbon. After seven to nine days, the quantities of isopropyl segments remaining in systems 8 and 9 containing 12.4 milligrams of activated carbon per liter were greater than in systems 1 and 2. The rates of disappearance of the isopropyl moieties in systems 6 and 7 containing 25 milligrams of carbon per liter were also less than those observed for systems 1 and 2. It is conjectured that the amount of radioactivity in solution in systems 1 and 2 would have eventually decreased below the level in systems 6 and 7.

Following the initial period of desorption, approximately 5.2 micromole liter^{-1} of isopropyl-carbon atoms were in solution in systems 6 and 7. The subsequent removal of these carbon atoms paralleled the disappearance from solution of the isopropyl-carbon atoms in system 4. The latter initially held 5.0 micromole liter^{-1} of isopropyl-carbon atoms with no activated carbon. Systems 8 and 9 produced slightly different results. The increase in the radioactivity of the liquid phase indicated that more isopropyl segments were being desorbed than were being degraded. In such a complex system, the adsorption equilibrium is very likely influenced by the presence of metabolic intermediates and end products as well as the substances associated with the microorganisms. The findings in Chapter 4 indicated that some breakdown of the CIPC molecules may precede the metabolism of the isopropyl moiety. An intermediate formed in this manner might permit further desorption of CIPC while holding the isopropyl groups in solution. A net increase in the number of isopropyl-carbon atoms in solution might thereby result. The fact

that the systems containing larger amounts of adsorbent did not demonstrate a similar increase may be related to the biological growths. During the study, a more vigorous culture appeared to exist in systems 6 and 7 than in systems 8 and 9. More rapid degradation of the isopropyl groups may have occurred in systems 6 and 7 resulting in a net decrease in the radioactivity with time. For activated carbon concentrations of 12.4 and 24.8 milligrams per liter, the changes in the radioactivity of the solutions were small and can be considered in agreement with the earlier hypothesis.

Interpretation of the data obtained from the CIPC-R cultures is complicated by the fact that not all of the ring-carbon atoms are metabolized. In other words, some of the radioactive ring-carbon atoms remain in solution for extended periods of time. The slight increase in the radioactivity of the solution during the last 30 days of the experiment reflects the increase arising from desorption of CIPC-R and the decrease resulting from partial metabolism of the ring-carbon atoms.

Systems 10 and 13 were initially sterile but became contaminated after five days. The subsequent rise in the radioactivity content of the solutions greatly exceeded that in the inoculated cultures containing activated carbon. This increase continued for 30 days in the CIPC-I system and for 50 days in the CIPC-R system. All of the glassware employed in the sampling of the solutions was sterilized prior to its use. Since each system was sampled separately, contamination of the sterile systems with organisms from the inoculated cultures appears unlikely. The primary source of contamination

would appear to have been the atmosphere. During each sampling period, the systems were directly exposed to the laboratory atmosphere for a brief period. One other source of contamination was apparent in those systems with the carbon dioxide traps. The liquid scintillation vials suspended above the suspension were not sterilized and the periodic replacement of the vials could have introduced microorganisms to the systems. Whatever the source, it would not appear that the contaminant microorganisms readily utilized CIPC.

The ethanol present in the solutions might be expected to be preferred by the contaminant microorganisms over the CIPC as a substrate. Under these circumstances, substantial growth of the microorganisms might occur independently of any metabolism of CIPC. The resulting biological population or compounds associated with it might compete with the CIPC for adsorbent sites. A continuing desorption of CIPC could then result. The small decrease of 1.0 micromole $liter^{-1}$ in the quantity of isopropyl groups in solution during the last 20 days indicates that some degradation of CIPC has occurred. Partial metabolism of the ring-carbon atoms also may have been taking place since it has been shown that ring cleavage may precede degradation of the isopropyl group. The net effect of desorption and the persistence of some of the phenyl groups could certainly be an increase in the number of ring-carbon atoms in solution.

The data obtained from measurements of the carbon-14 dioxide evolved were presented in Figure 6-3, Section 6.1. As previously mentioned, the results from the analyses over the first five to seven days may not truly represent the total amount of radioactive carbon

dioxide evolved. Degradation of the ethanol present in the samples may have released sufficient CO_2 to exhaust the barium hydroxide during this period. During the remainder of the experiment excess barium hydroxide was present throughout each sampling period. Results from the seventh- through fifteenth-day samples showed that the evolution of isopropyl- and ring-carbon atoms as carbon dioxide was relatively slow. Extrapolation of these data suggests that low amounts of radioactive carbon dioxide were evolved during the first seven days. The results for this initial period, therefore, appear to be at least qualitatively correct.

A significant increase in the rate of carbon dioxide production was observed after about 15 days for most of the systems. The total amount of isopropyl-carbon atoms collected as carbon dioxide was greatest for systems 1 and 2 containing no activated carbon. Systems 6 and 7 containing 25 milligrams of carbon per liter produced somewhat smaller quantities of isopropyl-carbon dioxide. Evolution of isopropyl-carbon atoms was least in the initially sterile system 10. The degradation of the isopropyl group would appear to be greatest in the absence of activated carbon. The microorganisms in systems 6 and 7 seem to be able to degrade the isopropyl segment more readily than the contaminant organisms in system 10. These findings are consistent with the results from the measurements of the radioactivity remaining in solution and with the hypothesis expounded earlier in this section.

The collection of carbon dioxide in the CIPC-R cultures gave unusual results. Considerably more ring-carbon atoms were collected

in system 12 containing activated carbon than in system 11 from which it was absent. In the two days following inoculation, 0.98 micromoles of ring-carbon atoms were collected per liter of the activated carbon system. Only 0.04 micromoles could be accounted for in the system without carbon. For the remainder of the experiment, the release of ring-carbon dioxide in system 12 continued to exceed that in system 11. After 50 days, the total amounts of ring-carbon atoms collected were 3.16 and 5.26 micromoles per liter of solution for systems 11 and 12 respectively. The reason for the differences observed is not known. Some factor in the activated carbon system resulted in the rapid release of a large amount of radioactive carbon dioxide in spite of the apparently slow rate of degradation of the CIPC in this system.

Three of the systems without activated carbon were analyzed for both the radioactivity remaining in solution and that evolved as carbon dioxide. The disappearance of the labeled carbon atoms from solution in systems 1,2, and 11 is compared to the cumulative quantity of atoms collected from the gas phase in Table 6-5. The number of carbon atoms released as carbon dioxide relative to the number that disappeared from solution was significantly higher for the isopropyl moiety than for the ring structure. Conversely, a larger proportion of ring-carbon atoms would appear to be assimilated by the microorganisms.

Between the eighteenth and fiftieth day, more isopropyl-carbon atoms were captured as carbon dioxide than vanished from solution, as evidenced in Table 6-5. The number of ring-carbon atoms released as

Table 6-5

Comparison of Labeled Carbon Atoms Disappearing From Solution With Labeled Carbon Atoms Evolved as Carbon Dioxide

Characteristic	System Number		
	1	2	11
Initial CIPC-I, mg/l	5.00	5.00	-
Initial CIPC-R, mg/l	-	-	5.00
Initial concentration of labeled carbon atoms, micromole liter^{-1}	23.4	23.4	140
Labeled carbon atoms removed from solution, micromole liter^{-1}			
0 to 50 days	16.9	17.8	67
18 to 50 days	3.5	4.5	3
Labeled carbon atoms evolved as CO_2 per liter of solution, micromole			
0 to 50 days	4.8	6.4	3.2
18 to 50 days	3.6	5.8	2.7
Percentage of total number of labeled carbon atoms disappearing from solution eventually evolved as CO_2	28.3	35.7	4.7

carbon dioxide was only slightly less than the number disappearing from solution in the same time interval. These figures indicate that the bacterial cultures were in the endogenous phase of growth. The amount of substrate still available could not support continued growth. As a result, the bacteria utilized carbon-14 atoms stored in or on the bacteria cell itself.

Finally, the data from the radioactivity measurements on the solid material can be examined. Significant quantities of both isopropyl- and ring-carbon atoms were either incorporated into the cellular material or held on its surface. Those samples containing activated carbon gave much higher counting rates, reflecting the relatively large amounts of CIPC still adsorbed on the activated carbon. Microorganisms were originally absent from systems 10 and 13. The radioactivity of the particulate matter associated with these cultures was about twice as great as that for the corresponding inoculated cultures. Thus, more labeled-carbon atoms in the initially sterile samples remained on the adsorbent and, as shown in Figures 6-1 and 6-2, in solution than observed for the inoculated samples. These data further substantiate the supposition that the contaminant microorganisms do not metabolize CIPC as rapidly as the inoculated cultures.

The following statements summarize the findings of this chapter. Degradation of CIPC in those samples devoid of activated carbon generally followed the pattern established in the adaptation cultures discussed in Chapter 4. As might be expected, the degradation of CIPC was dependent on the initial substrate concentration. With decreasing concentrations of CIPC, the overall rate of

degradation tended to decrease. After ten days, the rate of metabolism of the isopropyl segment decreased markedly. This decrease was accompanied by an increase in the number of isopropyl-carbon atoms evolved as carbon dioxide. The quantities involved were such as to indicate that the microbial population was in the endogenous phase of growth. Approximately 35 percent of the isopropyl-carbon atoms that disappeared from solution were released to the gas phase as carbon dioxide. Only 5 percent of the ring-carbon atoms were captured as carbon dioxide. Although quantitative measurements could not be made on the biological cell material, some of the isopropyl- and ring-carbon atoms were apparently assimilated by the microorganisms.

The presence of activated carbon decreased the rate of disappearance of CIPC from the total system. It was hypothesized that metabolism of CIPC occurs primarily in the aqueous phase or at the activated carbon-water interface. The inoculation of an activated carbon-CIPC system at equilibrium results at first in some desorption of CIPC from the adsorbent. Competition between the surface of the microorganism and the CIPC for the adsorption sites may cause this desorption. The microorganisms subsequently utilize the CIPC in solution. As the CIPC molecules are broken down, the solution is replenished with CIPC from the activated carbon. The activated carbon acts as a reservoir for CIPC, releasing it to solution by desorption as equilibrium conditions dictate. These equilibrium conditions may be substantially different from those encountered in the simple activated carbon-CIPC system studied in Chapter 5. The presence

of biological material and metabolic intermediates complicates the process of adsorption.

The contaminant microorganisms may be considered to have influenced the system in a similar manner. These organisms may have employed the ethanol present as the primary source of nutrient. The increasing microbial population forced additional quantities of CIPC off the absorbent surfaces. Since the metabolism of CIPC was very slow in these cultures, the net effect was a continuing increase in the amount of radioactivity in solution.

The effect of solid surfaces on the growth of bacteria has been studied by others. Heukelekian and Heller (90) found that bacterial growth in dilute solutions of glucose and peptone was greatly enhanced by the presence of glass beads. Escherichia coli did not grow in a 2.5 milligram per liter solution of glucose and peptone. The addition of glass beads, four millimeters in diameter, enabled the bacteria to grow substantially in glucose-peptone concentrations as low as 0.5 milligrams per liter. Above substrate concentrations of 25 milligrams per liter, the glass beads had no appreciable effect on the growth. Sand grains had a similar effect on bacterial growth. It was concluded that the substrate tends to concentrate at the solid surfaces providing a region of enriched nutrition. The bacteria also tend to be adsorbed on the solid surfaces (78). The net effect is to bring the substrate and the organisms into more intimate contact.

Zobell (91) made similar observations with bacteria in sea water. The stimulation of growth with solid surfaces was more pronounced for colloidal and hydrophobic substrates. The beneficial effect of solid surfaces was observed for substrate concentrations up to ten milligrams per liter. While particles larger than bacteria enhance microbial growth, particles smaller than bacteria may hinder the growth (91). Zobell hypothesized that, in addition to the adsorption of nutrients and bacteria on the solid particles, the diffusion from the cell of exoenzymes and hydrolysates important in substrate metabolism may be retarded by the solid particles.

Lenhard and duPlooy (92) studied the adsorption and biological decomposition of the synthetic detergent manoxol in bottom muds. Muds demonstrating high adsorptive properties retarded the degradation of manoxol.

The studies just cited would not appear to be in conflict with the results contained in this chapter. The size of the activated carbon particles (1.65 microns) would not be expected to promote bacterial growth. Even if the exterior surfaces did promote growth, the substrate adsorbed in the interior to the carbon particles would not be directly affected. Two separate phenomena may be involved. In dilute solutions, the activated carbon adsorbs a substantial amount of the substrate (CIPC) effectively lowering the quantity available to the microorganisms. At the same time, the bacterial growth in such a system might be enhanced by the presence of the solid surfaces. That is, the growth of the bacteria in the presence of the adsorbent carbon would exceed that in systems

containing the same amount of substrate in solution, but devoid of solid surfaces.

The major findings of this chapter can be expressed in a few concluding remarks. The presence of activated carbon in the experimental systems reduced the over-all rate of microbial decomposition of the herbicide, CIPC. It is hypothesized that the degradation of CIPC takes place only in the aqueous phase or at the interface between the solution and the exterior of the activated carbon particles. Adsorption of CIPC on activated carbon, therefore, reduces the amount of this substrate accessible to the organisms. As CIPC is decomposed in solution, additional quantities of CIPC are desorbed from the activated carbon. The equilibrium conditions governing the adsorption of CIPC on activated carbon are influenced by the presence of the microorganisms and the metabolites.

CHAPTER 7

SUMMARY AND CONCLUSIONS

The preceding chapters contain the results of studies on adsorption and microbial degradation of the pesticides isopropyl N-(3-chlorophenyl) carbamate (CIPC) and 2,4-dichlorophenoxyacetic acid (2,4-D) in aqueous solutions. The three studies comprising this research concerned the microbial degradation of 2,4-D and CIPC, the adsorption of these compounds on activated carbon and clay minerals, and the influence of activated carbon on the degradation of CIPC. In this chapter, the findings of the three major portions of the research are consolidated. These findings are then discussed with respect to their relevance to a better understanding of the behavior of pesticides in natural water systems. Finally, several areas for future research stemming from these studies are proposed.

7.1 Principal Findings of the Research

1. Only a small relative concentration of the pesticide 2,4-D was degraded by a mixed microbial population in a dilute medium of mineral salts. For aqueous solutions containing 0.1 and 1.0 milligrams of 2,4-D per liter, no more than 37 percent of the acetic acid moiety disappeared over a period of three to six months. The presence of large amounts of nutrient broth as a supplemental source of organic carbon had no appreciable effect on the rate of decomposition.

2. CIPC was metabolized much more rapidly than 2,4-D. Virtually complete degradation of the isopropyl-carbon portion of the CIPC molecule was observed in experimental systems that contained

nutrient broth. For a CIPC concentration of 1.0 milligrams per liter, 97 percent of the isopropyl groups disappeared from solution within eight days. At CIPC concentrations of 5.4 milligrams per liter, approximately 20 days were required to obtain the same percentage removals.

The rate of degradation of the isopropyl segments in the first through the fifth adaptation stages was appreciably slower in those systems in which nutrient broth was not present. In the fifth adaptation stage, for example, about 50 percent of the isopropyl-carbon atoms remained in solution after 21 days in the systems devoid of nutrient broth. The effect of nutrient broth on the rate of degradation may reflect the size of the microbial population, the presence in the nutrient broth of some substance contributing to the rapid growth of microorganisms metabolizing the isopropyl segment, or the presence of an abundant source of energy for the synthesis of induced enzymes. In the sixth adaption stage, systems without nutrient broth metabolized the isopropyl groups as rapidly as systems containing this additional carbon source. This change in the behavior of the systems was attributed to the presence of either a mutant bacterial strain or a contaminant organism capable of utilizing a portion of the CIPC molecule as a primary source of organic carbon. Subsequent microbial cultures, analyzed in conjunction with the studies on the influence of activated carbon on the degradation of CIPC, indicated that this organism did not survive the lengthy period between subcultures.

3. Total degradation of the ring segment of the CIPC molecule was not achieved. The major portion of the metabolism of the

ring-carbon atoms occured in the eight days following each inoculation. Thereafter, the rate of disappearance of the ring-carbon atoms from solution decreased considerably. The average total removals ranged from 38 to 62 percent for periods extending as long as 98 days. With the exception of the first adaptation stage, the rate and extent of metabolism of the ring structure were not affected by the presence of supplemental carbon in the form of nutrient broth.

Ultraviolet analyses of chloroform extracts indicated that a significant portion of the ring-carbon atoms remaining in solution in the inoculated cultures were associated with phenyl groups. However, in one contaminated system where 57.7 percent of the ring-carbon atoms remained in solution, the phenyl character of the system had been completely destroyed. The conclusion was reached that the phenyl groups in this system had been cleaved and a short chain, chlorinated hydrocarbon remained as an end product.

During the first seven to ten days of each adaptation stage, at least six ring-carbon atoms disappeared for every one of the isopropyl-carbon atoms that vanished from solution. This finding suggests the possibility that the CIPC molecules may undergo ring cleavage prior to the degradation of the isopropyl portion.

4. A partial metabolic pathway for the microbial degradation of CIPC can be proposed. Biological attack may be initiated at the amino nitrogen or the ester oxygen atom splitting the CIPC molecule into two portions. One portion contains the isopropyl group and may exist as a carboxylic acid. This fragment is completely metabolized. The second portion containing the 3-chlorophenyl group is converted to

4-chlorocatechol. Subsequent metabolism of this compound proceeds via the muconic acid pathway typical of simple aromatic compounds. A short chain intermediate, possibly consisting of two to four carbon atoms and a chlorine atom, may resist degradation by certain bacterial species.

It is possible that metabolism of the amino side chain may take place by a second route. In this event, the 3-chlorophenyl segment of the CIPC molecule may not be transformed into a degradable form. The ring portion would persist even though the isopropyl group was metabolized.

5. Clay minerals suspended in dilute pesticide solutions adsorbed very little 2,4-D and CIPC. At most, only one percent of an initial 2,4-D concentration of 0.13 milligrams per liter was adsorbed within 23 hours on 800 milligrams of montmorillonite per liter. Illite, kaolinite, and montmorillonite at concentrations as high as 800 milligrams per liter adsorbed no more than four percent of the CIPC present at a concentration of 0.40 milligrams per liter in 24 hours. Any effects of the hydrogen-ion concentration could not be discerned.

6. Powdered activated carbon readily adsorbed CIPC from aqueous solutions. In the well-stirred experimental systems, approximately 24 hours were required to reach equilibrium. Of the total equilibrium adsorption achieved in 24 hours, about 90 percent is attained in four hours. The adsorption equilibrium of the activated carbon-CIPC aqueous system can be represented closely by a Freundlich isotherm. For the Freundlich expression, $\frac{x}{m} = kC^{1/n}$, the constants n

and k were calculated to be 9.1 and 0.20 respectively. The ultimate capacity of the activated carbon for CIPC was estimated to be about 0.34 milligram of CIPC per milligram of carbon.

7. The adsorption of CIPC on activated carbon was independent of the hydrogen ion concentration over the pH range from 4.8 to 9.3. The acidity constant of the acid form of CIPC was estimated to be about 10^{-5}. Over most of the experimental pH range, therefore, CIPC would exist predominately in the molecular form.

8. The adsorption of CIPC on activated carbon appears to involve physical or weak chemical bonding. The heat of adsorption was estimated to be -7.0 kilocalories per mole. Adsorption processes involving electrostatic or weak chemical bonds typically have heats of adsorption of the same order of magnitude.

Furthermore, the adsorption of CIPC was reversible. Desorption of radioactively labeled CIPC was observed during the experiments conducted to determine the capacity of the activated carbon for the herbicide. This desorption of radioactive CIPC was evident in spite of the net adsorption of CIPC, both radioactive and non-radioactive molecules, observed during the same period of time. Such reversibility is an additional indication of physical or weak chemical bonding. The structure of CIPC would seem to be well suited for hydrogen bonding between the amino group and carbonyl oxygen atoms on the activated carbon surface.

9. Initial rates of adsorption for the CIPC-activated carbon system varied directly with the square root of time. This relationship is characteristic of many diffusion-controlled sorption processes.

The nominal activation energy was estimated to be about 1.7 kilocalories per mole, a value indicative of diffusion-controlled processes.

Attempts to fit the experimental data to several mathematical models were otherwise unsuccessful. Included among these models were expressions for diffusion of substances from well-stirred systems into spheres, cylinders and planes; diffusion to a surface with the reaction at the surface represented by Langmuir's equation; and a second-order surface reaction.

10. In a study on the effect of activated carbon on the degradation of CIPC, it was observed that the activated carbon reduced the rate of CIPC degradation. Following inoculation with microorganisms, the concentration of isopropyl- and ring-carbon atoms in solution rose sharply. After about 5 days, the concentration of isopropyl- and ring-carbon atoms in solution remained fairly constant. The same pattern was observed in initially sterile systems that became contaminated, probably with air-borne microorganisms. The increases in the concentrations of isopropyl- and ring-carbon atoms in these solutions were considerably greater than observed for the inoculated systems.

In systems containing 5.0 milligrams of CIPC per liter with no adsorbent, about 25 percent of the isopropyl-carbon atoms remained in solution after 50 days. After the same period of time, 55 to 60 percent of the isopropyl-carbon atoms were present in solution in systems containing 12.4 milligrams of activated carbon per liter. Degradation of the ring portion of the CIPC molecule likewise appeared to be adversely influenced by the activated carbon.

11. Significant quantities of the isopropyl- and ring-carbon atoms that vanished from solution in the activated carbon-CIPC studies were collected in the gas phase as carbon dioxide. In systems without activated carbon, about 28 to 36 percent of the isopropyl atoms and five percent of the ring atoms disappearing from solution were evolved as carbon dioxide. Between the eighteenth and fiftieth day, more of the isopropyl-carbon atoms were evolved as carbon dioxide than disappeared from solution. It was concluded that the microbial systems were in the endogenous phase of growth.

12. It is hypothesized that the degradation of CIPC in the activated carbon system occurs principally in the aqueous phase or at the interface between the liquid and the exterior surface of the activated carbon. That is, little of the adsorbed CIPC is directly metabolized by the microbial population. The amount of substrate that is biologically available is reduced. For CIPC, the overall rate of degradation is greatly reduced by the presence of activated carbon.

13. Initial increases in the concentrations of CIPC in solution probably result from the displacement by bacterial cells of the CIPC adsorbed on the activated carbon surface. In effect, a new set of equilibrium conditions has been imposed on the CIPC-activated carbon system. As decomposition of the CIPC proceeds in the solution, additional quantities of CIPC desorb to maintain equilibrium. The net effect of the degradation and the desorption is an initial increase in the aqueous CIPC concentration followed by the gradual disappearance of the CIPC from the water. For organisms not grown on CIPC, the

initial increase may extend for several weeks as the rate of desorption exceeds the rate of degradation.

7.2 Interpretation of Pesticide Behavior in Natural Waters

The presence of organic micropollutants in drinking water supplies is of increasing concern to environmental health scientists and engineers. These micropollutants consist of a multitude of organic compounds. Neither the techniques nor the manpower are available to identify even a significant portion of these substances. Rather than expending great efforts to isolate and identify a few compounds, it would seem to be more advantageous to study the fundamental behavior of different classes of compounds in the water environment. Information on the effects on synthetic organic compounds of the physical, chemical, and biological processes active in natural waters will greatly assist in the characterization of the organic content of water supplies.

Because of their highly toxic nature, pesticides represent one of the more important classifications of micropollutants. Contained in this dissertation are the results of basic research on certain phenomena influencing the persistence of two herbicides, 2,4-D and CIPC, in water. A number of pesticides, including 2,4-D have been isolated from water supplies. While CIPC has not been found, it is certainly conceivable that this compound could enter drinking water supplies.

In laboratory systems, 2,4-D was very resistant to microbial degradation. The experimental systems contained a widely assorted,

microbial population. Microbial growth on supplemental sources of organic carbon was extensive. In spite of the excellent conditions for biological activity, 60 percent of the 2,4-D persisted for at least three to six months. A natural waterway would not be expected to provide as good a microbial environment as the experimental system. On this basis, it may be concluded that 2,4-D would not be materially degraded by the organisms present in a natural water system.

CIPC was considerably less resistant to biological degradation than 2,4-D. Nonetheless, the rates of degradation of CIPC are such that significant concentrations of the intact herbicide or its metabolic intermediates may persist for weeks or months in a natural water environment. One of the end products from the metabolism of CIPC persisted for at least three months in the experimental systems.

The persistence of pesticides in water supplies might be influenced by adsorption on suspended matter. Adsorbed pesticides might be deposited in bottom muds or transported downstream perhaps to be desorbed into the water. Removal of pesticides from drinking water might be facilitated if the compounds were adsorbed on the suspended matter. Conventional water treatment processes such as coagulation, sedimentation, and filtration would remove the offending substances.

Laboratory studies indicated that little, if any, 2,4-D or CIPC would be adsorbed on clay minerals. Illite, kaolinite, and montmorillonite were selected as being representative of the inorganic suspended solids in rivers. Clay concentrations as high as 800 milligrams per liter removed, at best, only minute quantities of the

herbicides from water solutions. These facts suggest that 2,4-D and CIPC would not be adsorbed on the suspended clays found in natural waterways.

The possibility that these two herbicides might be adsorbed on other types of particulate matter still exists. Indeed, both 2,4-D and CIPC can be adsorbed by activated carbon. Similar substances, undoubtedly with lesser adsorption capacities, might exist in water supplies. The influence of activated carbon on the degradation of CIPC was studied in this research. The results can be extended to the natural environment. If a biodegradable pesticide is adsorbed on suspended matter in a natural water system, the rate of microbial degradation of the pesticide probably will be reduced. The reduction in the degradation rate will be related to the amount of pesticide adsorbed. The more pesticide adsorbed, the slower the rate of degradation. In turn, the amount of pesticide adsorbed is affected by temperature, pH, competing compounds, and other factors.

In view of the studies on the biological degradation and physical adsorption of 2,4-D and CIPC, it would seem that appreciable quantities of these herbicides might reach water treatment plants. Activated carbon has been shown to be an effective means of removing organic pollutants, including certain pesticides, from water. Experiments with CIPC indicated that this compound also could be removed from contaminated water supplies with activated carbon. For a CIPC concentration of 1.0 milligrams per liter, eight milligrams of activated carbon per liter would reduce the CIPC concentration to 0.1 milligrams per liter or less within four hours at 20°C. The addition of

activated carbon prior to the flocculation process would thus provide sufficient contact time for most water treatment plants. Actual adsorbent dosages at 20°C can be estimated from the Freundlich isotherm developed previously.

The adsorption of organic matter is normally influenced by such factors as pH and temperature. The removal from solution of CIPC by activated carbon is independent of the pH over the range from 4.8 to 9.3. Temperature, on the other hand, does exert an appreciable effect on the adsorption process. With increasing temperature, the initial rate of adsorption increases, but the total amount adsorbed at equilibrium decreases. Temperature changes, therefore, would necessitate adjustments in the amount of activated carbon required to remove CIPC from contaminated water supplies.

This research concerned the behavior in aqueous solutions of only two pesticides, 2,4-D and CIPC. Hopefully, similar research will eventually enable environmental health scientists to establish the nature of the refractory substances known as organic micropollutants. Only then can their chronic and carcinogenic effects on the consumer be properly evaluated.

7.3 Proposals for Future Research

A number of questions were raised by this research, several of which will now be discussed. Consider first the metabolism of CIPC. While certain information was obtained about the metabolic pathway, the specific intermediates and end products were not identified. In itself, this information would be of scientific interest. More

important, however, the metabolites may also be harmful and should be identified. Particular attention should be focused on the phenyl compound possibly remaining after the isopropyl segment has been metabolized.

Studies on the influence of bacterial material and metabolic intermediates on the adsorption equilibria of the CIPC-activated carbon system should prove rewarding. Several kinds of bacteria could be employed separately and together. The effects observed with viable cells could be compared with the results using dead cells. Possible metabolites from the degradation of CIPC, such as 4-chlorocatechol and chloromuconic acid, might be studied.

The influence of adsorbents on the metabolism of organic matter warrants further study. One research proposal would be to examine the effect of particle size on the degradation of CIPC. The size of the activated carbon employed in this research was of the same order of magnitude as the bacteria. Larger adsorbent particles might greatly enhance bacterial growth, perhaps offsetting the effect of reduced substrate available to the microorganisms.

REFERENCES CITED

1. Anon.,"Synthetic Organic Chemicals, U.S. Production and Sales," U.S. Tariff Commission, Washington, D.C., 52 (1963).

2. Cottam, Clarence, "Pesticides and Water Pollution," Proceedings-The National Conference on Water Pollution, 222 (1960), Washington, D.C.

3. Woodward, Richard L., "Significance of Pesticides in Water Supplies," Journal of the Water Pollution Control Federation, 52:11, 1367 (1960).

4. Frear, Donald E.H., "Pesticide Index," College Science Publishers, State College, Pa., 193pp. (1961).

5. DeOng, E.R., "The Chemistry and the Uses of Pesticides," Second Edition, Reinhold Publishing Corporation, New York, 345pp. (1956).

6. Gunther, F.A. and Blinn, R.C., "Analysis of Insecticides and Acaricides," Interscience Publishers, Inc., New York, 696pp. (1955).

7. Metcalf, R.L., "Organic Insecticides," Interscience Publishers, Inc., New York, 392pp. (1955).

8. Shepard, H.H., "The Chemistry and Action of Insecticides," McGraw-Hill Book Company, Inc., New York, 504pp. (1951).

9. Henderson, C., Pickering, Q.H., and Tarzwell, C.M., "The Toxicity of Organic Phosphorus and Chlorinated Hydrocarbon Insecticides to Fish," Transactions of the Second Seminar on Biological Problems in Water Pollution, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio (1959).

10. Henderson, C., Pickering, Q.H., and Tarzwell, C.M., "Relative Toxicity of Ten Chlorinated Hydrocarbon Insecticides to Four Species of Fish," Transactions of the American Fisheries Society, 88, 23(1959).

11. Tarzwell, C.M., "The Toxicity of Some Organic Insecticides to Fishes," Proceedings of the Twelfth Annual Conference Southeastern Association of Game and Fish Commissioners, 233 (1958).

12. Pickering, Q.H., Henderson, C., and Lemke, A.E., "The Toxicity of Organic Phosphorus Insecticides to Different Species of Warmwater Fishes," Transactions of the American Fisheries Society, 91:2, 175 (1962).

13. Surber, E.W. and Pickering, Q.H., "Acute Toxicity of Endothal, Diquat, Hyamine, Dalapon, and Silvex to Fish," The Progressive Fish-Culturist, 164 (1962).

14. Durham, W.F., "The Toxicity of Chemicals Used in Mosquito Control," Proceedings of the 44th Annual Meeting of the New Jersey Mosquito Extermination Association, Atlantic City, New Jersey (1957).

15. Fitzhugh, O.G., "Physiological Effects of Organic Phosphate Pesticides," Proceedings of the Conference on Physiological Aspects of Water Quality, Washington, D.C., 135 (1960).

16. McKee, J.C. and Wolf, H.W., "Water Quality Criteria," Second Edition, The Resources Agency of California, State Water Quality Control Board, Sacramento, California (1963).

17. Anon.,"Chloro-IPC and Its Use for Weed and Grass Control," Columbia-Southern Chemical Corporation, Pittsburgh, Pennsylvannia.

18. Hayes, W.J. Jr.,"Clinical Handbook on Economic Poisons,"U.S. Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center, Atlanta, Georgia (1963).

19. Newman, A.S., DeRose, H.R., and DeRigo, H.T., "Persistence of Isopropyl N-Phenyl Carbamate in Soils," Soil Science, 66, 393 (1948).

20. Jones, L.W., "Stability of DDT and Its Effects on Microbial Activities of Soil," Soil Science, 73,237 (1952).

21. Alexander, Martin, "Microbiology of Pesticides and Related Hydrocarbons," Principles and Applications in Aquatic Microbiology, Rudolfs Research Conference, John Wiley and Sons, Inc., 15 (1964).

22. Bailey, G.W. and White, J.L., "Review of Adsorption and Desorption of Organic Pesticides by Soil Colloids, With Implications Concerning Pesticide Bioactivity," Journal of Agricultural and Food Chemistry, 12, 324 (1964).

23. Cohen J.M. and Bendixen, T.W., "Progress Report on Lysimeter Studies of the Possibility of Ground Water Contamination by the Nematocide, D-D Mixture," U.S. Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, Pre-publication copy (1962).

24. Audus, L.J., "The Biological Detoxication of 2,4-Dichlorophenoxyacetic Acid in Soil," Plant and Soil, 2, 31 (1949).

25. Audus, L.J., "Biological Detoxication of 2,4-Dichlorophenoxyacetic Acid in Soils: Isolation of an Effective Organism," Nature, 166, 356 (1950).

26. Audus, L.J.,and Symonds, K.V., "Further Studies on the Breakdown of 2,4-Dichlorophenoxyacetic Acid by a Soil Bacterium," Annuals of Applied Biology, 42 174 (1955).

27. Evans, W.C. and Smith, B.S., "The Photochemical Inactivation and Microbial Metabolism of the Chlorophenoxyacetic Acid Herbicides," Biochemical Journal, 57,XXX (1954).

28. Jensen, H.I., "Detoxication of Hormone Herbicides by Soil Bacteria," Nature 170, 39 (1952).

29. Steenson, T.J. and Walker, N., "Observations on the Bacterial Oxidation of Chlorophenoxyacetic Acids," Plant and Soil, 8, 17 (1956).

30. Fernly, H.N. and Evans, W.C., "Metabolism of 2,4-Dichlorophenoxyacetic Acid by Soil Pseudomonas; Isolation of α-Chloromuconic Acid as an Intermediate," Biochemical Journal, 73, 22p (1959).

31. Audus, L.J., "The Biological Detoxication of Hormone Herbicides in Soil," Plant and Soil, 3,170 (1951).

32. Gaunt, J.K. and Evans, W.C., "Metabolism of 4-chloro-2-methylphenoxyacetic Acid by a Soil Micro-organism," Biochemical Journal, 79, 25p (1961).

33. Evans, W.C. and Moss, P., "The Metabolism of the Herbicide, p-Chlorophenoxyacetic Acid by A Soil Microorganism. The Formation of a β-Chloromuconic Acid on Ring Fission," Biochemical Journal, 65, 8p (1957).

34. Audus, L.J., "Fate of Sodium 2,4-Dichlorophenoxyethyl-sulfate in the Soil," Nature, 170, 886 (1952).

35. Proctor, M.H., "Some Steps in the Degradation of Naphthalene Acetic Acid," Plant and Soil, 18, 338 (1963).

36. Korte, F., "Metabolic Studies with ^{14}C-Labelled Drin-Insecticides," Vth Annual Pesticide Congress, London (July, 1963).

37. Korte, F., Ludwig, G., and Vogel, J., "Umwandlung von Aldrin-^{14}C und Dieldrin-^{14}C Moskito-Larven," Annalen der Chemie, Justus Liebigs, 655, 135 (1962).

38. Ludwig, G., Weis, J., and Korte, F., "Excretion and Distribution of Aldrin-^{14}C and Its Metabolites After Oral Administration for a Long Period of Time," Life Sciences, 3, 123 (1964).

39. Martin, J.P., Harding, R.B., Cannell, G.H., and Anderson, L.D., "Influence of Five Annual Field Applications of Organic Insecticides on Soil Biological and Physical Properties," Soil Science, 87, 334 (1959).

40. Shaw, W.M. and Robinson, B., "Pesticide Effects in Soils on Nitrification and Plant Growth," Soil Science, 90, 320 (1960).

41. Briunsma, J., "The Action of 4,6-Dinitro-o-cresol (DNOC) in Soil. I. The Emergence from Cress Seed in DNOC-treated Soil Mixtures," Plant and Soil, 12, 249 (1960).

42. Webb, H.J., "Water Pollution Resulting from Agricultural Activities," Journal of the American Water Works Association, 54:1, 83 (1962).

43. Nicholson, H.P.,"Insecticide Pollution of Water Resources," Journal of the American Water Works Association, 51:8, 981 (1959).

44. Weaver, L., Gunnerson, C.G., Breidenbach, A.W., and Lichtenberg, J.J., "Chlorinated Hydrocarbon Pesticides in Major U.S. River Basins," Public Health Reports, 80:6, 481 (1965).

45. Breidenbach, Lichtenberg, J.J., Henke, C.F., Smith, D.J., Eichelberger, J.W., and Stierli, H., "The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters," Public Health Service Publication No. 1241, Washington, D.C. (1964).

46. Anon., "Pollution-Caused Fish Kills in 1962," Public Health Service Publication No. 847, Washington, D.C. (1962).

47. Hoffman, C.H., "Physiological Effects of Insecticides on Aquatic Life," Proceedings-Conference on Physiological Aspects of Water Quality (1960).

48. Anon. HEW-A46, U.S. Department of Health, Education, and Welfare, Public Health Service, Washington, D.C. (March 19, 1964).

49. Anon. HEW-A72, U.S. Department of Health, Education, and Welfare, Public Health Service, Washington, D.C. (April 3,1964).

50. Anon., HEW-B82, U.S. Department of Health, Education, and Welfare, Public Health Service, Washington, D.C. (July 10, 1964).

51. Toth, R.C., "Huge Fish Kills Cease on Lower Mississippi," Los Angeles Times, 18 (December 21, 1964).

52. Welch, E.B. and Spindler, J.C., "DDT Persistence and Its Effects on Aquatic Insects and Fish After an Aerial Application," Journal of the Water Pollution Control Federation, 36:10, 1285 (1964).

53. Carollo, J.A., "The Removal of DDT from Water Supplies," Journal of the American Water Works Association, 37:12, 1310 (1945).

54. Cohen, J.M., Kamphake, L.J., Lemke, A.E., Henderson, C., and Woodward, R.L., "Effect of Fish Poisons on Water Supplies. I. Removal of Toxic Materials," Journal of the American Water Works Association, 52, 1551 (1960).

55. Cohen, J.M., Rourke, G.A., Woodward, R.L., "Effect of Fish Poisons on Water Supplies. II. Odor Problems," Journal of the American Water Works Association, 53, 49 (1961).

56. Cohen, J.M., Pickering, Q.H., Woodward, R.L., and Van Heuvelen, W., "Effect of Fish Poisons on Water Supplies. III. Field Study at Dickinson," Journal of the American Water Works Association, 53, 233 (1961).

57. Schwartz, H.G., Jr., "Removal of a Selected Organic Pesticide by Specific Water Treatment Unit Processes," Master of Science Thesis, Washington University, St. Louis, Missouri (1962).

58. Robeck, G.A., Dostal, K.A., Cohen, J.M., and Kriessl, J.F., "Effectiveness of Water Treatment Processes in Pesticide Removal," U.S. Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, pre-publication copy (1964).

59. Buescher, C.A., Dougherty, J.H., and Skrinde, R.T., "Chemical Oxidation of Selected Organic Pesticides," Journal of the Water Pollution Control Federation, 36, 1005 (1964).

60. Faust, S.D. and Aly, O.M., "Water Pollution by Organic Pesticides," Journal of the American Water Works Association, 56:3, 267 (1964).

61. Aly, O.M. and Faust, S.D., "Studies on the Fate of 2,4-D and Ester Derivatives in Natural Surface Waters," Journal of Agricultural and Food Chemistry, 12:6, 541 (1964).

62. Chambers, C.W., Tabak, H.H., and Kabler, P.W., "Degradation of Aromatic Compounds by Phenol-Adapted Bacteria," Journal of the Water Pollution Control Federation, 35:12, 1517 (1963).

63. Tabak, H.H., Chambers, C.W., and Kabler, P.W., "Microbial Metabolism of Aromatic Compounds," Journal of Bacteriology, 87:4, 910 (1964).

64. Bogan, R.H., Okey, R.W., and Vargas, D.J., "Pesticides in Natural Waters," Research, 14, 269 (1961).

65. Okey, R.W. and Bogan, R.H., "Synthetic Organic Pesticides- An Evaluation of Their Persistence in Natural Waters," Proceeding of the Eleventh Industrial Waste Conference, Corvallis, Oregon (1963).

66. Okey, R.W. and Bogan, R.H., "Apparent Involvement of Electronic Mechanisms in Limiting the Microbial Metabolism of Pesticides," Water Pollution Control Federation meeting, Seattle, Washington (1963).

67. Weber, W.J. and Morris, J.C., "Kinetics of Adsorption on Carbon from Solution," Journal of the Sanitary Engineering Division, ASCE, 89 SA2, 31 (1963).

68. Weber, W.J. and Morris, J.C., "Kinetics of Adsorption on Carbon from Solution-Closure," Journal of the Sanitary Engineering Division, ASCE, 89SA6, 53 (1963).

69. Weber, W.J. and Morris, J.C., "Equilibria and Capacities for Adsorption on Carbon," Journal of the Sanitary Engineering Division, ASCE, 90:SA3, 79 (1964).

70. Weber, W.J. and Rumer, R.R., "Intraparticle Transport of Sulfonated Alkylbenzenes in a Porous Solid: Diffusion with Non-Linear Adsorption," pre-publication copy (1965).

71. Friedlander, G. and Kennedy, J.W., "Nuclear and Radiochemistry," John Wiley and Sons, Inc., 468 pp. (1962).

72. Gray, P.H.H. and Thornton, H.G., "Soil Bacteria That Decompose Certain Aromatic Compounds," Zentr. Bakteriol. Parasit., Abt.II, 73, 74 (1928).

73. Shelley, R.N. and Umberger, C.J., "Behavior of Acidic Organic Compounds in Nonaqueous Media of Ion Exchange Resins," Analytical Chemistry, 31, 593, (1959).

74. Smith, J.N., Spencer, B., and Williams, R.T., "Studies in Detoxication. 34. The Metabolism of Chlorobenzene in the Rabbit. Isolation of Dihydrodihydroxychlorobenzene, p-Chlorophenylglucuronide, 4-Chlorocatechol Glucuronide, and p-Chlorophenylmercaptic Acid," Biochemical Journal, 47, 284, (1950).

75. Parke, D.V. and Williams, R.T., "Studies in Detoxication. 63 "The Metabolism of Halogenobenzenes," Biochemical Journal, 59, 415 (1955).

76. Bilbo, A.J. and Wyman, G.M., "Steric Hindrance to Coplanarity in o-Fluorobenzidines," Journal of the American Chemical Society, 75, 5313, (1953).

77. Kerr, P.F. et. al., "American Petroleum Institute Project 49-Clay Mineral Standards," Columbia University, 1-8, (1949-1950).

78. Mitchell, Peter, "Physical Factors Affecting Growth and Death," Bacterial Physiology edited by C.H. Werkman and P.W. Wilson, Academic Press, Inc., 173 (1951).

79. McMichael, F.C. and McKee, J.E., "Report of Research on Wastewater Reclamation at Whittier Narrows," W.M. Keck Laboratory of Environmental Health Engineering, California Institute of Technology, Pasadena (1965).

80. Dixon, M. and Webb, E.C., "Enzymes," Academic Press Inc., 782 pp. (1958).

81. Umbreit, W.W., "Modern Microbiology," W.H. Freeman and Company, 507 pp. (1962).

82. Fruton, J.S. and Simonds, S., "General Biochemistry," second edition, John Wiley and Sons, Inc., 1077 pp. (1961).

83. Stanier, R.Y. and Ingraham, J.L., "Protocatechuic Acid Osidase," Journal of Biological Chemistry, 210, 799 (1954).

84. MacDonald, D.L., Stanier, R.Y., and Ingraham, J.L., "The Enzymatic Formation of β -Carboxymuconic Acid," Journal of Biological Chemistry, 210, 809 (1954).

85. Hayaishi, O., Katagiri, M., and Rothberg, S., "Studies on Oxygenases. Pyrocatechase," Journal of Biological Chemistry, 229, 905 (1957).

86. Daniels, F. and Alberty, R.A., "Physical Chemistry," John Wiley and Sons, Inc., 671 pp. (1959).

87. Steel, E.W., "Water Supply and Sewerage," fourth edition, McGraw-Hill Book Company, Inc., 311 (1960).

88. Pauling, Linus, "The Nature of the Chemical Bond," third edition, Cornell University Press, 498 (1960).

89. Crank, J., "The Mathematics of Diffusion," Oxford at the Clarendon Press, 347 pp. (1956).

90. Heukelekian, H. and Heller, A., "Relation Between Food Concentration and Surface for Bacterial Growth," Journal of Bacteriology, 40, 547 (1940).

91. Zobell, C.E., "The Effect of Solid Surfaces Upon Bacterial Activity," Journal of Bacteriology, 46, 39 (1943).

92. Lenhard, G. and duPlooy, A., "Studies on the Removal of Synthetic Detergents from the Water Phase of Bottom Deposits by Physical Adsorption and Biochemical Degradation," Hydrobiologia, 22, 317 (1963), Biological Abstracts, 45, 3298 (1964).

APPENDIX I

GLOSSARY OF CHEMICAL NOMENCLATURE FOR PESTICIDES CITED IN THIS THESIS.

Trade Name	Chemical Name
Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene
Aminotriazole	3-Amino-1,2,4-triazole
BHC	1,2,3,4,5,6-Hexachlorocyclohexane
Chlordane	1,2,4,5,6,7,8,8-Octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene.
Chlorothion	0,0-Dimethyl 0-(3-chloro-4-nitrophenyl) phosphorothioate.
CIPC	Isopropyl N-(3-chlorophenyl) carbamate.
2,4-D	2,4-Dichlorophenoxyacetic acid.
Dalapon	2,2-Dichloropropionic acid, sodium salt.
DDD	2,2-bis(p-chlorophenyl)-1,1-dichloroethane.
DDE	Dichlorodiphenyl dichloroethylene.
DDT	Dichloro-diphenyl-trichloroethane.
Diazinon	0,0-Diethyl 0-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate.
Dieldrin	1,2,3,4,10,10,-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endoexo-5,8-dimethanonaphthalene.

Trade Name	Chemical Name
Diquat	1,1'-Ethylene-2,2'-dipyridinium dibromide.
Endothal	Disodium 3,6-endoxohexahydrophthalate.
Endrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4-endo,endo-5,8-dimethanonaphthalene.
Ferbam	Ferric dimethyldithiocarbamate
Guthion	0,0-diethyl S-4-oxo-1,2,3-benzotriazin-3(4H)-yl-methyl phosphorodithioate.
Heptachlor	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene.
IPC	Isopropyl N-phenylcarbamate
Lindane	Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane.
Malathion	S-(1,2-bis(ethoxycarbonyl) ethyl) 0,0-dimethyl phosphorodithioate.
Maneb	Manganous ethylenebisdithiocarbamate
MCPA	4-Chloro-2-methylphenoxyacetic acid.
Methyl parathion	0,0-Dimethyl-0-p-nitrophenyl phosphorothioate.
Para-oxon	0,0-Diethyl-0-p-nitrophenyl phosphate
Parathion	0,0-Diethyl-0-p-nitrophenyl phosphorothioate.
Rotenone	Derrin
Sulfoxide	1,2-Methylenedioxy-4-(2-(octylsulfinyl)propyl) benzene.

Trade Name	Chemical Name
2,4-5-T	2,4,5-Trichlorophenoxyacetic acid.
TEPP	Tetraethyl pyrophosphate
Thanite	Isobornyl thiocyanoacetate
Toxaphene	Octachlorocamphene
Zineb	Zinc ethylene bisdithiocarbamate
Ziram	Cyclohexylamine complex