RESIDUE REVIEWS

Residues of Pesticides and Other
Contaminants in the Total Environment

Editor
FRANCIS A. GUNTHER

JANE DAVIES GUNTHER

VOLUME 78

RESIDUE REVIEWS

Residues of Pesticides and Other Contaminants in the Total Environment

Editor
FRANCIS A. GUNTHER

Assistant Editor
JANE DAVIES GUNTHER

Riverside, California

VOLUME 78

SPRINGER-VERLAG
NEW YORK HEIDELBERG BERLIN
1981

Library of Congress Catalog Card Number 62-18595.
Printed in the United States of America.

New York: 175 Fifth Avenue, New York, N.Y. 10010
Heidelberg: 6900 Heidelberg 1, Postfach 105 280, West Germany

ISBN 0-387-90566-9 Springer-Verlag New York Heidelberg Berlin
ISBN 3-540-90566-9 Springer-Verlag Berlin Heidelberg New York

Foreword

Worldwide concern in scientific, industrial, and governmental communities over traces of toxic chemicals in foodstuffs and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published progress reports, and archival documentations. These three publications are integrated and scheduled to provide in international communication the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. Until now there has been no journal or other publication series reserved exclusively for the diversified literature on "toxic" chemicals in our foods, our feeds, our geographical surroundings, our domestic animals, our wildlife, and ourselves. Around the world immense efforts and many talents have been mobilized to technical and other evaluations of natures, locales, magnitudes, fates, and toxicology of the persisting residues of these chemicals loosed upon the world. Among the sequelae of this broad new emphasis has been an inescapable need for an articulated set of authoritative publications where one could expect to find the latest important world literature produced by this emerging area of science together with documentation of pertinent ancillary legislation.

The research director and the legislative or administrative advisor do not have the time even to scan the large number of technical publications that might contain articles important to current responsibility; these individuals need the background provided by detailed reviews plus an assured awareness of newly developing information, all with minimum time for literature searching. Similarly, the scientist assigned or attracted to a new problem has the requirements of gleaning all literature pertinent to his task, publishing quickly new developments or important new experimental details to inform others of findings that might alter their own efforts, and eventually publishing all his supporting data and conclusions for archival purposes.

The end result of this concern over these chores and responsibilities and with uniform, encompassing, and timely publication outlets in the field of environmental contamination and toxicology is the Springer-Verlag (Heidelberg and New York) triumvirate:

Residue Reviews (vol. 1 in 1962) for basically detailed review articles concerned with any aspects of residues of pesticides and other chemical contaminants in the total environment, including toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Residue Reviews* and the *Archives* are in identical formats and are subject to review, by workers in the field, for adequacy and value; manuscripts for the *Bulletin* are not reviewed and are published by photo-offset to provide the latest results without delay. The individual editors of these three publications comprise the Joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

That residues of pesticide and other contaminants in the total environment are of concern to everyone everywhere is attested by the reception accorded previous volumes of "Residue Reviews" and by the gratifying enthusiasm, sincerity, and efforts shown by all the individuals from whom manuscripts have been solicited. Despite much propaganda to the contrary, there can never be any serious question that pest-control chemicals and food-additive chemicals are essential to adequate food production, manufacture, marketing, and storage, yet without continuing surveillance and intelligent control some of those that persist in our foodstuffs could at times conceivably endanger the public health. Ensuring safety-in-use of these many chemicals is a dynamic challenge, for established ones are continually being displaced by newly developed ones more acceptable to food technologists, pharmacologists, toxicologists, and changing pest-control requirements in progressive food-producing economies.

These matters are of genuine concern to increasing numbers of governmental agencies and legislative bodies around the world, for some of these chemicals have resulted in a few mishaps from improper use. Adequate safety-in-use evaluations of any of these chemicals persisting into our foodstuffs are not simple matters, and they incorporate the considered judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

It is hoped that "Residue Reviews" will continue to serve as an integrating factor both in focusing attention upon those many residue matters requiring further attention and in collating for variously trained readers present knowledge in specific important areas of residue and related endeavors involved with other chemical contaminants in the total environment. The contents of this and previous volumes of "Residue Reviews" illustrate these objectives. Since manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of residue analytical chemistry, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology are being neglected; to the contrary, these apparent omissions are recognized, and some pertinent manuscripts are in preparation. However, the field is so large and the interests in it are so varied that the editors and the Advisory Board earnestly solicit suggestions of topics and authors to help make this international book-series even more useful and informative.

"Residue Reviews" attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these and other foreign chemicals in any segment of the environment. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of any "foreign" chemicals in our surroundings. Thus, manuscripts may encompass those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material. In addition, contaminant chemicals added in any manner to air, water, soil or plant or animal life are within this purview and these objectives.

Manuscripts are normally contributed by invitation but suggested topics are welcome. Preliminary communication with the editors is necessary before volunteered reviews are submitted in manuscript form.

Department of Entomology
University of California
Riverside, California
March 2, 1981

F.A.G.
J.D.G.

Table of Contents

Genetic engineering and biological detoxification of environmental pollutants

By

J. M. Pemberton*

Contents

I. Introduction

In modern agriculture and industry there is a heavy dependence on a wide range of synthetic chemical agents many of which appear to have no counterparts in nature. Many of these compounds, by their very nature and complexity, are resistant to degradation when released into soil, water, and air. Unfortunately the repeated use and release of such synthetics has become an everyday occurrance resulting in the phenomenon of environmental pollution. In a number of instances, as with the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and its potent contaminant TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), these pollutants can be mutagenic, teratogenic, and carcinogenic and any environmental accu-

* Department of Microbiology, University of Queensland, St. Lucia, Queensland 4067 Australia.

Residue Reviews, Volume 78

mulation poses a health hazard to both human and animal populations (SEILER 1978). A recognition of this problem has led to restrictions on the use and release of recalcitrant molecules, greater use of existing biodegradable compounds, and the development of nonpersistent, less toxic alternatives.

Whether or not a particular compound becomes an environmental pollutant depends to a large extent on its degradability. Where degradation does occur then this is usually carried out by microorganisms present in soil and water (ALEXANDER 1969). It is apparent from a variety of studies with soil microorganisms that naturally-occurring strains of bacteria and fungi have a limited capacity to degrade many of the novel compounds produced by chemical industries (KAUFMAN and KEARNEY 1976). However, current techniques of *in vivo* and *in vitro* genetic engineering can be used to construct laboratory strains of microorganisms, particularly bacteria, with the capacity to degrade recalcitrant molecules, such as the wide range of chlorinated aromatic and aliphatic compounds in use and as by-products of industry and agriculture.

II. Pollution problems unresolved

A number of major pollution problems have arisen directly and indirectly through the activities of large and expanding chemical industries throughout the world. In one particular area, production of pesticides, MUNNECKE (1979) has suggested three major categories of problems related to disposal and detoxification. First, there are the decontamination problems associated with industrial accidents involving high levels of toxic products or by-products of a particular industrial complex. A recent example was the explosion at a 2,4,5-trichlorophenol manufacturing plant in Seveso, Italy, in 1976. Substantial amounts of TCDD were released into the environment around the plant. Major efforts to decontaminate the area have proven unsuccessful (HAY 1978).

A second problem area is the disposal of cancelled, contaminated, or surplus pesticides. A prime example is the disposal of the United States Air Force stockpiles of Agent Orange which contains unacceptable levels of TCDD. Third is the reclamation of pesticide containers. Rinsing of these containers, prior to their disposal, has the potential for introducing many different chemicals into the environment.

Although pesticides are recognized as a major group of environmental pollutants there are a variety of other synthetic organic molecules that cause severe contamination of the terrestrial and aquatic environments. These molecules include the group of fire-retardants known as polybrominated biphenyls (PBBs) which are closely related to the ubiquitous and recalcitrant polychlorinated biphenyls (PCBs) (RISEBROUGH *et al* 1968). Another general contaminant is PVC (polyvinyl chloride) which was in very widespread use for many years until it was suspected of being a carcinogen (GOLDSMITH 1979). It is likely that many other synthetics in common use will be shown to be potent environmental

pollutants. In view of the extensive nature of environmental pollution by complex, recalcitrant synthetic molecules, new techniques are required in pollution control.

One approach, which I will concentrate on in this review, is to use *in vivo* and *in vitro* genetic engineering techniques to construct strains of bacteria with the capacity to degrade a range of recalcitrant molecules, in particular the wide range of novel halogenated aromatic and aliphatic compounds in use and as by-products of industry and agriculture.

Bacteria, which form the majority of biodegrading microorganisms found in soil and water, can exchange genetic determinants with other bacteria by at least three mechanisms. These naturally-occurring forms of genetic exchange, namely conjugation, transduction, and transformation, have been used successfully over the last 30 years to construct a variety of new strains of bacteria. Use of one or a combination of these mechanisms to construct genetically novel strains of bacteria is considered as *in vivo* genetic engineering.

III. *In vivo* genetic engineering

a) Plasmids and conjugation

Usually bacteria carry the bulk of their genetic determinants in a single chromosome. However, many bacterial strains have one or more minor chromosomes 1 to 2% the size of the main chromosome. Such small chromosomes (plasmids) are generally regarded as nonessential to the normal survival of the host cell. Most plasmids are transmissible from one bacterial cell to another by the process of conjugation (Fig. 1). During the conjugal process, the cell possessing the transmissible plasmid (the donor) produces a conjugal tube which attaches to a second cell which lacks the plasmid (the recipient). The donor cell duplicates its plasmid, transferring one copy to the recipient cell and retaining the other copy. When transfer via the conjugal tube is complete, the conjugating cells separate. In this way the plasmid and the genetic information it carries can be transferred rapidly to a population of cells which originally did not possess the plasmid.

Plasmids can be recognised by the functions they confer on their host cell. In the years since plasmids were first discovered a variety of bacterial characters have been shown to be carried on plasmids. The best known examples are the multiple drug resistance (R) plasmids which confer on their host simultaneous resistance to a variety of antibiotics. Antibiotic therapy directed against pathogenic species of bacteria harboring such R plasmids is particularly difficult and for this reason the evolution and spread of such plasmids is an undesirable occurrance.

b) Degradative plasmids

Plasmid involvement in the degradation of camphor was first reported by A. M. Chakrabarty and I. C. Gunsalus in 1971. Subsequent investiga-

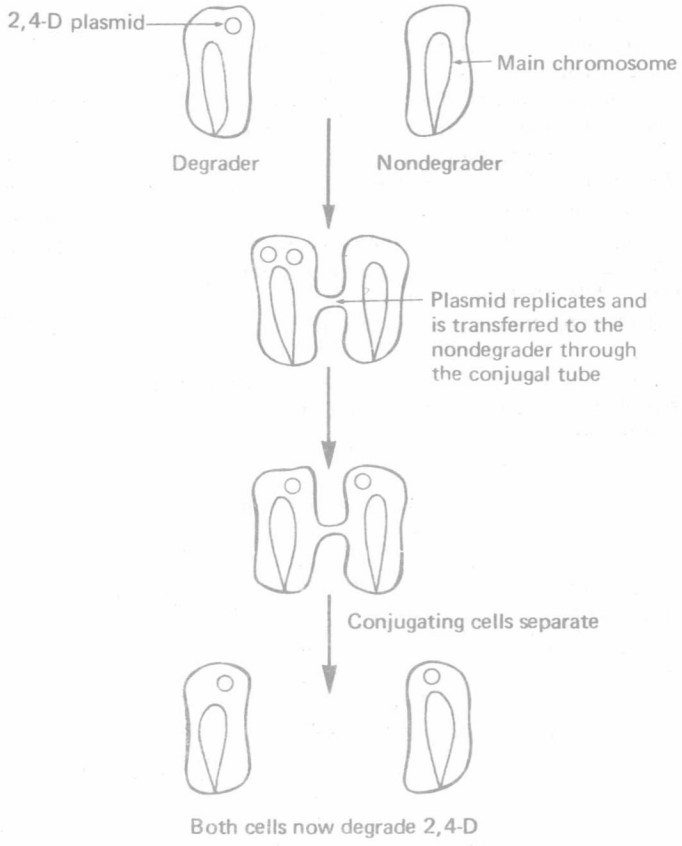

Fig. 1. Transfer of 2,4-D plasmids by conjugation.

tions have revealed plasmids which confer the ability to degrade other naturally-occurring aromatic and aliphatic compounds such as octane, naphthalene, salycylate, and toluate (CHAKRABARTY 1976). Such plasmids play a major role in the breakdown and recycling of naturally-occurring organic molecules; they do this by extending the range of complex organic compounds bacteria can use as sole sources of carbon and energy.

A cursory glance at the list of compounds degraded by cells possessing these catabolic plasmids will show that many are constituents of crude oil. Since oil spills have become such a prevalent occurrance over recent years, FRIELLO *et al* (1976) constructed a strain possessing sufficient of these plasmids to enable it to rapidly degrade crude oil. The use of such strains in dissipating oil spills is debatable, nevertheless it does point to

an important principle when dealing with potential environmental pollutants. It is possible to construct bacterial strains which may be rare to nonexistent in nature and which are capable of the degradation of many poorly biodegradable, environmental pollutants.

c) Pesticide-degrading plasmids

The first indication that plasmids were involved in the environmental degradation of man-made synthetics came from the report of PEMBERTON and FISHER (1977) that a strain of *Alcaligenes paradoxus*, a ubiquitous saprophytic soil bacterium, possessed a transmissible plasmid conferring the ability to degrade the widely used herbicide 2,4-D (2,4-dichlorophenoxyacetic acid). The existence of such plasmids can explain why 2,4-D-degrading bacteria are so common in nature. The property can be transferred rapidly from one bacterial population to another endowing many populations with the ability to degrade what could potentially be quite a dangerous environmental pollutant.

Normally, plasmids that occur in one species of bacterium cannot be transferred to a different species of bacterium. These are known as narrow host range plasmids; the majority of naturally occurring plasmids fall into this category. However, there are a small number of closely related plasmids which transfer freely within and between different species and genera of bacteria, the so called broad host range plasmids. One of the remarkable features of the pesticide degrading plasmids that have been isolated from strains of *Alcaligenes paradoxus* and *A. eutrophus* is that the majority of them belong to this class of broad host range plasmids (PEMBERTON *et al* 1979). Pesticide-degrading plasmids can transfer freely among soil microorganisms without the constraints usually attached to the majority of other naturally-occurring plasmids.

When strains of *Alcaligenes eutrophus* are isolated as 2,4-D degraders the plasmid they contain confers only the ability to degrade the synthetic molecule 2,4-D. However, mutant strains can be isolated which degrade both 2,4-D and the naturally-occurring parental compound PAA (phenoxyacetic acid). The newly acquired or evolved character is also carried by the pesticide-degrading plasmid. Since the isolation of plasmid mutants that confer only the ability to degrade phenoxyacetic acid, it has been demonstrated that this evolution occurs in both directions. In the presence of the naturally occurring PAA the majority of plasmids in the soil microflora carry only the ability to degrade PAA, although one in a million plasmids also confers the ability to degrade 2,4-D. In the presence of 2,4-D, these few cells containing the 2,4-D plasmid have a selective advantage and overgrow the PAA degraders. The balance is now a million to one in favour of the 2,4-D degraders. Once evolved the plasmids can spread through the soil microbial population allowing rapid degradation of the available carbon source, be it the naturally-occurring PAA or its synthetic counterpart 2,4-D.

The evolution and spread of pesticide degrading plasmids in response to the introduction of a synthetic pollutant is an example of naturally-occurring genetic engineering which counters environmental pollution. It should be possible to duplicate the natural process in the laboratory with the production of bacterial strains capable of the degradation of other synthetic molecules, especially those relatively nonbiodegradable by-products of the chemical industry.

d) Strain construction

Isolation of naturally occurring plasmids involved in the degradation of synthetic halogenated aromatics, e.g., 2,4-D, has been followed by the isolation of laboratory-constructed strains which degrade a variety of unusual halogenated aromatics normally considered to be poorly biodegradable. REINEKE and KNACKMUSS (1979) demonstrated that by transferring the TOL (toluate utilising) plasmid to a strain of *Pseudomonas* which could degrade the haloaromatics 3-chlorobenzoate and 4-chlorophenol, they could produce strains capable of the complete degradation of such novel substrates as 4-chloro- and 3,5-dichloro-benzoate. The initial steps in the degradative pathway were coded for by the TOL plasmid genes and the remaining steps by the recipient *Pseudomonad* strain. This type of approach mimics the situation in nature, in the case of the 2,4-D plasmids the initial steps in the degradative pathway being plasmid-borne while the remaining steps appear to be located on the main chromosome. Already the use of *in vivo* genetic engineering techniques involving the conjugal transfer of broad host range plasmids has contributed significantly to an understanding and a resolution of a number of problems associated with environmental pollution by complex synthetic molecules. However, it should be possible to construct bacterial strains with an even wider range of metabolic activities against other recalcitrant molecules using not only conjugation but also transduction and transformation, the two other forms of genetic exchange between bacteria.

IV. Transduction

Transduction is the process of gene exchange between bacteria which is mediated by a virus (LOW and PORTER 1978). The virus attaches to the bacterial cell, injects its DNA, and the virus multiplies in the host cell producing anything up to 1,000 particles/cell. The host cell then lyses, releasing the virus particles which attach to other bacterial cells, and once again undergo their vegetative cycle. In rare instances, instead of packaging viral DNA in the virus particle during assembly of the virus particles, the virus packages bacterial DNA. Upon release from the host cell this rare viral particle then attaches to another bacterial cell and instead of injecting viral DNA, it injects bacterial DNA. In this way both plasmid and main chromosomal genes can be transferred from one bacterium to another

in the virus particle. There are at least two limitations on transduction. First, the amount of DNA transferred by any one viral particle is usually 1 to 2% the size of the main chromosome, or about the size of a plasmid. Second, transduction is usually restricted to very closely related strains of the same species of bacterium. Nevertheless transduction has proven to be particularly useful in strain construction for a number of species of bacteria.

V. Transformation

Transformation is the simplest form of genetic exchange and involves the release of DNA by the lysis of one bacterium and uptake of this DNA by a second bacterium. This type of gene exchange does not appear to be important in nature and is employed primarily as a research technique. Transformation suffers the same limitations as transduction, in that it tends to occur between related strains of the same species of bacterium and generally only small amounts of DNA are transferred. The major significance of this mechanism is that it forms an integral part of the *in vitro* genetic engineering technique (COHEN *et al* 1973).

VI. *In vitro* genetic engineering

a) Methodology

This technique provides almost unlimited scope for combining desirable degradative activities from many sources within a single bacterium. The technique involves cleavage of circular plasmid DNA at usually a single point using site specific restriction endonuclease enzymes (Fig. 2). The enzyme cuts each single DNA strand 5 to 6 base pairs apart and hence the ends of the resulting linear molecule are complementary and can reanneal. Using DNA (cloned) from any source bacterial, plant, or animal, cleaved with the same enzyme, mix it with the plasmid (vector) DNA, and allow the DNAs to reanneal. Since the restriction enzyme generates the same type of sticky, complementary ends in both the cloned and vector DNAs then they will reanneal to give a hybrid or chimeric molecule. The initial pairing between the plasmid and the cloned DNA is via hydrogen bonding, the final co-valent bonds between the plasmid and the cloned DNA are produced by treatment with polynucleotide ligase. The hybrid plasmid is then re-introduced into a bacterial host by the process of transformation, described previously. The bacterial cell now contains a self replicating entity (the plasmid vector) into which is inserted cloned DNA from almost any source. In this way degradative genes from one organism can be combined with the degradative genes of another organism, constructing a strain which is either rare or nonexistent in nature, as in the hypothetical DDT degrader cited in the example (Fig. 2). In this way co-metabolic activities of several bac-

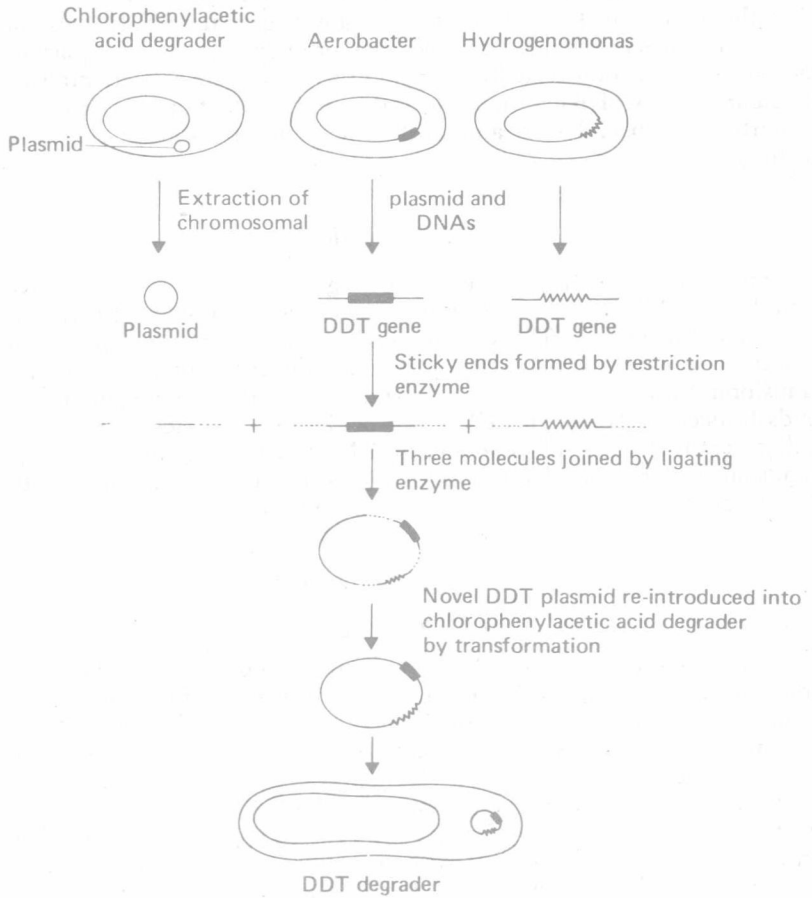

Fig. 2. Production of a DDT-degrading bacterium by *in vitro* genetic engineering; note the co-metabolic activities of *Aerobacter* and *Hydrogenomonas* are added to an organism capable of chlorophenyl acetic acid degradation (SOMERVILLE 1978, PFAENDER and ALEXANDER 1973).

teria can be combined within a single strain to produce a bacterium which has a complete degradative pathway for a novel substrate.

b) Co-metabolic activities combined

The term co-metabolism is used to describe the partial degradation of a chemical by a microorganism. Since the microorganism fails to degrade the molecule completely it gains little energy and carbon for

growth. In these circumstances growth is accomplished by simultaneous and complete degradation of another compound. Co-metabolic or incomplete degradation appears to result from a similarity between the synthetic molecule and a naturally-occurring, structurally related molecule which the organism can use as a sole source of carbon and energy. Two important examples serve to illustrate that the co-metabolic attack by a community of microorganisms can lead to complete degradation of novel synthetic molecules. In the first example DDT is co-metabolised first to dichlorodiphenyl-methane then to chlorophenylacetic acid by the concerted action of two microorganisms, *Aerobacter* and *Hydrogenomonas* (Pfaender and Alexander 1973). The much simpler end product chlorophenylacetic acid is readily broken down in the environment (Fig. 3).

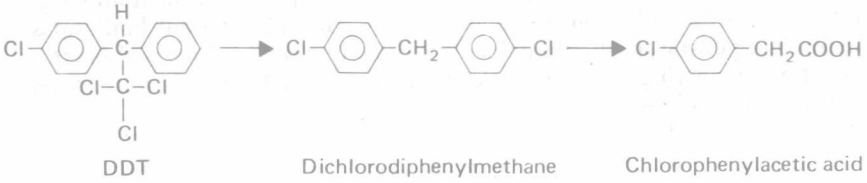

Fig. 3. Co-metabolism of DDT by the action of two bacteria, *Aerobacter* and *Hydrogenomonas* (Somerville 1978).

The degradative capacities of these two strains could be combined with an organism which degrades chlorophenylacetic acid to produce a single strain that degrades DDT, using *in vitro* genetic engineering (Fig. 2). The second example is the co-metabolic attack on 2,4,5-T by a *Pseudomonas* species which converts it to 3,5-dichlorocatechol which can now be readily broken down by microorganisms capable of degrading 2,4-D (Horvath 1970). In both of these examples it should be possible, by *in vitro* genetic engineering, to construct a bacterial strain capable of degrading completely both DDT and 2,4,5-T.

c) Other sources of DNA

By using *in vitro* genetic engineering techniques the source of the cloned DNA is not restricted to bacteria but DNA from any source bacterial, plant, insect, or animal can be cloned. Despite the extensive degradation of many organic compounds by soil microbial populations, a number of organochlorine insecticides do not appear to be degraded to any significant extent by microorganisms (Alexander 1969). However, one of the major forms of resistance to these chemicals in insects is an increased level of detoxification (Brown 1971, Brooks 1974). For those environmental pollutants or potential pollutants which are so complex as to preclude degradation by bacteria, as appears to be the case with