RELATIONSHIP BETWEEN SOME INSECTICIDES AND LACTIC CULTURE ORGANISMS IN MILK

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ABSTRACT

RELATIONSHIP BETWEEN SOME INSECTICIDES
AND LACTIC CULTURE ORGANISMS IN MILK

By

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Sterile whole milks containing from <0.1 to 100 ppm of dieldrin and heptachlor were inoculated with Streptococcus lactis A254, S. lactis A62, Streptococcus cremoris E-8, Streptococcus diacetilactis 18-16 and Lactobacillus casei and incubated for 48 hr at appropriate temperatures. In addition, milks containing similar amounts of methoxychlor and malathion were inoculated with S. lactis A254 and L. casei and incubated for 48 hr at 32 C. The insecticides had no effect on the growth or production of lactic acid by the streptococci. Heptachlor, methoxychlor and malathion had neither adverse nor stimulative effect on the growth and lactic fermentation ability of L. casei in milk at 32 C. Dieldrin did not influence the lactic fermentation ability of L. casei but resulted in slight reduction in viable cell count in the milks containing 10 and 100 ppm.

In order to study the susceptibility of aldrin, DDT and lindane, to the biological action of lactic starter cultures, sterile whole milks containing 1 ppm of aldrin, DDT or lindane were inoculated with S. lactis A254,

S. lactis A62, S. cremoris E-8, S. diacetilactis 18-16, S. diacetilactis DRCI and L. casei. After two weeks of incubation at appropriate temperatures, the insecticides were extracted from the milks, cleaned, concentrated, and analyzed by gas chromatography using an electron capture detector. None of the organisms modified or degraded aldrin, DDT or lindane. This proves that commonly used lactic starter cultures neither help detoxify DDT or lindane, nor enhance the toxicity of aldrin in milk.

Since the growth of L. casei was slightly inhibited by 10 and 100 ppm of dieldrin in milk, possible uptake of dieldrin by L. casei was studied using ^{14}C-dieldrin. When L. casei was grown in Trypticase Soy Broth (TSB) containing 0.01-0.02 ppm of ^{14}C-dieldrin for 18 hr at 32 C the amount of ^{14}C-dieldrin retained with the harvested and washed cells was less than 0.2% of the total ^{14}C-dieldrin in the TSB. Since less than 0.2% of such a low concentration as 0.01-0.02 ppm of dieldrin was retained with the harvested cells, the count of the radioactivity obtained from the cells could be attributed to the residual radioactivity due to incomplete washing rather than to true uptake of ^{14}C-dieldrin into the cells. The population was about 10^8 cells/ml.

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INTRODUCTION

Pesticides are substances intended for preventing, destroying, repelling mitigating or regulating unwanted organisms or plants. Pesticides include insecticides, fungicides, herbicides, rodenticides, mematocides and plant regulators.

The President's Science Advisory Committee (1963) states that according to chemical composition, pesticides can be classified into (a) chlorinated hydrocarbons, (b) organic phosphorus compounds, (c) other organic compounds and (d) inorganic substances.

During the past twenty-five years pesticides have had an important role in improving the quality and quantity of man's food and fiber. The increased production and widespread use of pesticides have unintentionally caused new problems. Pesticides are designed to kill or interfere with the metabolism of unwanted target organisms. However, because of biochemical similarities among many species and inadequate selective specificity of the toxicant, pesticides may be destructive, not only to pests, but also to other plant and animal bodies. Consequently, extensive research has been performed to learn the effect of pesticides on man and his total living environment.

The acute toxicity of pesticides to human beings, animals and wildlife is well documented in the literature.

However, still more studies are necessary to determine the effects of pesticides on various other beneficial organisms which are utilized by man, and to determine the fate of pesticides in biological systems.

LITERATURE REVIEW

Pesticide Residues in the Human Diet

Pesticides can enter the body by ingestion, absorption through the intact skin, and by inhalation as indicated by the President's Science Advisory Committee (1963). Among these three routes of entrance, ingestion of food is the most important source of pesticide residues for the general public according to Richardson and Foter (1966), and Duggan and Dawson (1967). Crops may be contaminated with pesticide residues by direct application, by uptake from the treated soil, or by drift or runoff from applications to adjacent fields. Residues in milk and meat may result from direct application of insecticide to the animal, from the animal's ingestion of residue in feed and water, inhalation of toxic vapors, and absorption from applications to the animal's housing as stated by Lisk (1966), and Richardson and Foter (1966).

Dietary intake of pesticide residues in human food has been discussed by several investigators including Campbell et al. (1965), DuBois (1965), Cummings (1966), Richardson and Foter (1966), Duggan and Dawson (1967), Duggan and Weatherwax (1967), Duggan (1968) and Lindgren et al. (1968). The most frequently occurring pesticides

in the human diet are chlorinated hydrocarbon insecticides. Duggan (1968) pointed out that nearly 75 per cent of the total daily intake of chlorinated organic insecticides is from dairy and other animal products. Organic phosphate insecticides are not found as frequently in food as chlorinated hydrocarbon insecticides. Duggan and Weatherwax (1967) indicated that malathion is the most common organic phosphate insecticide in the diet and accounts for more than 80 per cent of the daily intake of organic phosphate pesticides. Although the tolerance for pesticides in milk is zero, the Food and Drug Administration (FDA) has established a series of actionable levels for the more commonly used insecticides such as aldrin, dichloro-diphenyl-trichloroethane (DDT), dieldrin, heptachlor, lindane and methoxychlor as pointed out by Campbell et al. (1965). Presence of various insecticides in milk has been discussed by Marth and Ellickson (1959a, b, c), Henderson (1965) and Heineman (1966).

The amount of residues in food products varies. According to the Food and Agriculture Organization (FAO) Working Party of the United Nations and the World Health Organization (WHO) Expert Committee on Pesticide Residues (1967) the "tolerance level" is the permitted concentration of a pesticide residue in or on a food. The FDA is responsible for establishing safe tolerances of pesticide residues on food products and for enforcing

such tolerances by preventing interstate and foreign transport of foods not meeting these tolerances. As an administrative principle, tolerances are set by the FDA at one hundredth of the lowest level which causes adverse effects in the most sensitive test animals whenever data on human toxicity are not available. After reviewing the data on which tolerances have been based, experimental evidence has proved inadequate in certain instances. In such cases the FDA has reassessed tolerance levels according to additional information available.

In deliberations on the toxicity of pesticide residues in the human diet, the FAO Working Party on Pesticide Residues (1967) pointed out the importance of sufficient information on pesticide losses, degradation or transformation during storage or processing of food before consumption. Certain changes in pesticides may result in either detoxification or increased toxicity. The FAO Working Party reported that in many instances little, if any, information was available on the post-harvest fate of pesticides on many crops.

Toxicity of Insecticides

Organic phosphorus compounds inhibit cholinesterase activity and thereby interfere with the transmission of impulses from nerve to ganglion and nerve to muscle. Most organic phosphorus insecticides have relatively high acute toxicities and have caused many fatal and nonfatal

poisonings in man. Many of them are degraded rapidly and thus seldom persist in the environment.

Acute toxicity of chlorinated hydrocarbon insecticides is associated with the central nervous sytem but toxic mechanisms are unknown. The toxic effects result in generalized convulsion, excitability, trembling, or paralysis, occasionally followed by death.

The LD_{50} values for individual insecticides are given in the Agriculture Handbook No. 313, "Suggested Guide for The Use of Insecticides to Control Insects Affecting Crops, Livestock, Households, Stored Products, and Forest Products" by Agricultural Research Service and Forest Service, U.S.D.A (1966), and in the "Guide to the Analysis of Pesticide Residues" published by the U.S. Public Health Service (1965). There are wide differences among species as well as sex of the same species in susceptibility to insecticides.

The President's Science Advisory Committee (1963) reported the chronic effect of insecticides based on the FDA feeding experiments. Mice fed as little as 0.5 ppm of dieldrin in the diet developed histological liver damage, and an increase to 10 ppm caused a fourfold increase in the frequency of liver tumors. One hundred per cent mortality of 14 nursing puppies occurred when several pregnant dogs were fed dieldrin at 0.6 mg/kg of body weight during pregnancy. No dieldrin was included

in the ration during lactation. Reproduction studies in rats showed that 2.5 ppm dieldrin and 50 ppm DDT in the diet reduced the number of young that survived the nursing period. With rats, 5 ppm of DDT in the diet produced histological changes in the liver, but there was no evidence of tumor induction. Similar results were obtained by many other researchers and these reports have been reviewed in "Evaluation of Some Pesticide Residue in Food" by the FAO Working Party on Pesticide Residues and the WHO Expert Committee on Pesticide Residues (1967).

Kupfer (1967) stated that the administration of organo-chlorine insecticides to various species of animals stimulated both the hepatic microsomal oxidations of drugs and the microsomal hydroxylation of steroids.

Boyd and Chen (1968) found that rats fed a low protein diet from the time of weaning were twice as susceptible to the toxic effect of lindane.

Metabolism of Chlorinated Hydrocarbon Insecticides

The literature on the metabolism of various chlorinated hydrocarbon insecticides in biological systems has been reviewed in "Scientific Aspects of Pest Control" by the National Academy of Sciences and National Research Council (1966), and in "Evaluation of Some Pesticide Residues in Food" by the FAO Working Party and the

WHO Expert Committee on Pesticide Residues (1967). The known metabolic products of DDT are dichloro-diphenyl-dichloroethane (DDD), dichloro-diphenyl-dichloro-ethene (DDE), and Dichloro-diphenyl-acetic acid (DDA). The liver and the intestinal flora are believed to be associated with the metabolism of DDT in animals. Conversion of DDT to DDD, DDE, and DDA is a detoxication process. Lindane is converted to pentachlorocyclohexane. Aldrin and heptachlor are epoxidized to dieldrin and heptachlor epoxide, respectively. Rat liver microsomes epoxidize aldrin and heptachlor in vitro. Epoxidation of aldrin or heptachlor is a toxicative process since the epoxides are more toxic than their precursors according to Westlake and San Antonio (1960), Krzeminski (1962) and Brooks (1967).

Storage of Chlorinated Hydrocarbon Insecticides in the Human Body

Due to the hydrophobic nature of chlorinated hydrocarbon insecticides, storage of these chemicals is mostly in adipose tissue. There are many reports on the storage of DDT and DDE in human fat including Hayes et al. (1956), Dale et al. (1963), Hunter et al. (1963), Hoffman et al. (1964), Egan et al. (1965) and Robinson et al. (1965). The amount of DDT reported varied from 2 to 30 ppm. The insecticide that has been studied most in regard to storage in the human body is DDT.

Durham (1965) studied the storage and excretion of DDT and its metabolites in volunteers who took known daily doses of DDT for 21 of the 48 months they were under observation. Men who received 3.5 mg/man/day stored increasing amounts of DDT and DDE and excreted increasing amounts of DDA in their urine for several months. They then reached a steady state of storage and excretion with no further change until the dosage was discontinued, after which the excretion diminished. The same phenomenon was observed in men who received 35 mg/man/day except they stored and excreted a great deal more DDT-derived material than the men given the lower level. After dosage was stopped, excretion decreased very slowly. It was also reported that no clinical effect associated with dosage was detected by the men themselves or by physical examination and laboratory testing.

However, the FAO Working Party and the WHO Expert Committee on Pesticide Residues (1967) stated that the possibility for DDT stored in the body to have deleterious consequences later in life could not be ruled out. In addition, there is no proof yet to refute that histological changes observed in the liver cells of test animals might also take place in man.

Several reports, including those of Dale et al. (1963), Hunter et al. (1963), Hoffman et al. (1964), Egan et al. (1965) and Robinson et al. (1965) are

available on the storage of dieldrin in human fat in the general population. The average concentration ranged between 0.03 and 0.3 ppm.

Hoffman et al. (1964) reported an average of 0.57 ppm of lindane in the analyses of 282 samples of human fat obtained from the general population of the United States. In the United Kingdom an average of 0.015 ppm of lindane was found in 20 human fat samples according to Robinson et al. (1965).

Even human milks contain some insecticides such as DDT, dieldrin and lindane as reported by West (1964), Egan et al. (1965), Quinby et al. (1965), and Curley and Kimbrough (1969).

Hayes (1966, 1968) stated that DDT, lindane, dieldrin and heptachlor epoxide were found not only in the adipose tissue of humans, but also in blood and in organs. Presence of these insecticides in blood was also reported by Dale et al. (1966, 1967).

Effect of Insecticide Residues on Microorganisms

The influence of insecticide residues on microorganisms has been studied by Lewis and Hammer (1946), Wilson and Choudhri (1946), Carlyle and Thorpe (1947), Smith and Wenzel (1947), Wilson (1948), Angelotti et al. (1954) and Bollen et al. (1954). With few exceptions, the studies have been limited to organisms important in

soil microbiology. The reports, in general, indicated that bacteria are not affected by the various insecticides. Further studies on the effect of chlorinated hydrocarbon pesticides on soil microorganisms were reviewed by Bollen (1961), Martin (1963) and Marth (1965), who also indicated that normal applications of the common insecticides have little effect on soil microbes.

Some fungi and protozoa were affected by some insecticides according to Kirkwood and Phillips (1946), Smith and Wenzel (1947), Wilson (1948), Bollen et al. (1954), Johnston (1957), Ukeles (1962) and Williams et al. (1963).

Painter and Kilgore (1963) reported that none of 13 insecticides added to grape musts in wine fermentation had any measurable effect on the fermentation normally accomplished by the inoculated yeast Saccharomyces cerevisiae var. ellipsoideus.

Shaw and Robinson (1960) and Bartha et al. (1967) found that nitrification was not influenced adversely by chlorinated hydrocarbon insecticides.

Collins and Langlois (1968) observed inhibition of Pseudomonas fluorescens by 50 and 100 ppm of DDT in Trypticase Soy Broth (TSB), but no inhibition of P. fluorescens by corresponding amounts of DDT in skim milk. Dieldrin and heptachlor did not inhibit P. fluorescens in TSB or in skim milk. Heptachlor had a bactericidal

effect on Staphylococcus aureus in TSB but no adverse effect on S. aureus in skim milk. DDT reduced the growth of S. aureus in TSB but not in skim milk. Dieldrin showed no effect on S. aureus in TSB or in skim milk.

Transformation of Chlorinated Hydrocarbon Insecticides by Microorganisms

Kallman and Andrews (1963), reported the formation of DDD from DDT in the presence of S. cerevisiae from commercial yeast cakes in buffered sucrose medium. When DDE was also incubated with S. cerevisiae in order to validate a hypothesis that formation of DDD is preceded by the formation of DDE from DDT, no DDD was formed. Therefore, the hypothesis was invalidated and it was concluded that DDD is produced directly from DDT by reductive dechlorination.

Barker et al. (1965) observed the conversion of DDT to DDD by Proteus vulgaris, a bacterium isolated from the intestinal flora of a mouse. It was also suggested that DDE did not appear to be an intermediate in the production of DDD from DDT.

Stenersen (1965) isolated Aerobacter aerogenes, Alcaligenes faecalis, Escherichia coli, Bacillus brevis and Serratia marcescens from the excreta of flies and studied transformation of DDT by these organisms in meat extract bouillon. When growing anaerobically or under oxygen deficiency, the facultative anaerobes

S. marcescens, E. coli and one unidentified strain, converted to DDT to DDD and DDE. In aerated cultures neither the facultative anaerobes nor the obligate aerobes converted any DDT to DDD or other products.

From the study on dechlorination of DDT to DDD by Aerobacter aerogenes, Wedemeyer (1966) and Plimmer et al. (1968) indicated that reduced cytochrome oxidase was responsible for DDT dechlorination and that the presence of oxygen hindered dechlorination.

Mendel and Walton (1966) demonstrated that the normal flora of the gastrointestinal tract, rather than the liver as others have suggested, was the major source of the DDD that is found in animals fed DDT. When rats were given DDT by stomach tube, DDD occurred in the feces and livers of rats, but not in the feces and livers of rats injected intraperitoneally with DDT. Coliform bacteria isolated from feces of control animals could affect reductive dechlorination of DDT to DDD.

Johnson et al. (1967) found many pathogenic and saprophytic bacteria associated with plants which could convert DDT to DDD. Chacko et al. (1966) reported that several streptomyces species degraded DDT. Anaerobic dechlorination of DDT to DDD by soil microorganisms was studied by Guenzi and Beard (1967).

Gamma-pentachlorocyclohexane was identified as a decomposition product of lindane in soil. Yule et al.

(1967) stated that this product was 1000 times less toxic to insects than lindane and that soil microorganisms and alkaline soils are involved in the degradation process.

Bartha et al. (1967) studied the stability of aldrin, dieldrin, endrin, telodrin, DDT, DDD, and methoxychlor in soil and found no indication of microbial degradation during 30 days. However, Matsumura and Boush (1967) isolated Pseudomonas and Bacillus species, after examining more than 500 soil isolates from different locations which could degrade dieldrin. Partial hydrolysis of dieldrin by A. aerogenes in TSB was observed by Wedemeyer (1968).

Effect of Processing on Insecticides in Milk

Any process or manufacturing operation which detoxifies or reduces insecticide residues in milk would be of significant value. Some researchers investigated the possibility of removing insecticides form milk by ordinary manufacturing process.

In 1950 Mann found that pasteurization had little effect on the amount of DDT in milk. Langlois et al. (1964, 1965) noted that DDT and lindane in milk were essentially stable during pasteurization, homogenization, condensation and storage for four months. The only significant loss of DDT and lindane was caused by spray drying in the manufacture of spray dried whole milk

powder. During the manufacture of Swiss-type cheese detectable amounts of endrin, dieldrin, heptachlor, and lindane were removed in whey, resulting in less insecticide in the cheese product. Condensing of milk caused significant loss of heptachlor epoxide and dieldrin but no loss of heptachlor and endrin. Loss of heptachlor, heptachlor epoxide, dieldrin and endrin was observed during spray drying and drum drying of milk.

According to the study by Stemp and Liska (1966) 40-50 per cent of the telodrin residues were destroyed in evaporated milk and 10-20 per cent of the residues were destroyed during processing of milk for dry whole milk. Methoxychlor was stable throughout the processing treatments.

Kroger (1968) observed that freeze-drying and milk deodorization were not effective on the reduction or elimination of heptachlor epoxide, dieldrin and DDT from butteroil.

Ledford et al. (1968) also indicated that commercial steam distillation-vacuum processing removed a noticeable amount of lindane, but little heptachlor, dieldrin and DDT.

Comments and Objectives

With the persistent appearance of insecticides in milk it seemed desirable to understand the effect and fate of insecticides in milk in conjunction with the processing of dairy products. Marth and Ellickson (1959c) pointed out that little research had been done concerning the effects of insecticide residues on the processes employed in the manufacture of various dairy products and vice versa.

In the manufacture of fermented milk products such as sour cream, yoghourt, cultured buttermilk and cheese, inoculation of milk with lactic culture organisms and subsequent fermentation are essential processes. The main functions of lactic cultures are: to produce lactic acid and some flavor compounds. If milk happens to contain any antimetabolite or antimicrobial agent the lactic cultures may not grow or function properly. For example, antibiotics or sanitizing compounds are known to occur in milk sometimes and cause inhibition or retardation of growth and fermentation ability of lactic cultures as pointed out by Foster et al. (1957).

Being aware of (a) the presence of insecticides in milk, (b) inadequate selective toxicity of the insectides, and (c) the important role of lactic cultures in the manufacture of cultured dairy products, some people working

with lactic culture organisms have expressed concern about the effect of insecticides on these cultures.

As reviewed in the preceding section several reports are available regarding the effect of processing and storage of dairy products on insecticides from the physical point of view. From the microbiological standpoint, there is very little information concerning the effect of the fermentation process on insecticide residues in the manufacture of cultured dairy products.

In general, microorganisms are well known for their ability to decompose or modify various organic compounds. Furthermore, several organisms were reported to modify some insecticides. Consequently, it appeared conceivable that lactic culture organisms might also have the ability to modify some insecticides. Since transformation of insecticides does not necessarily reduce the toxicity, it would be meaningful to understand how some insecticides are affected by lactic culture organisms during fermentation in milk.

Knowledge of any chemical alteration in insecticide residues in food is essential to evaluate the chronic effect of the residues, and to assess realistic tolerance levels of the residues in foodstuffs.

The objectives of this research were to:

a) determine the effect of some insecticides on the growth and fermentation ability of commercially important lactic culture organisms.

(b) determine the ability of the lactic culture organisms to transform aldrin, DDT and lindane in milk, and

(c) determine the uptake of dieldrin by Lactobacillus casei.

MATERIALS AND METHODS

Effect of Insecticide Residues on Growth and Fermentation Ability of Lactic Culture Organisms

Dieldrin,[1] heptachlor,[2] and methoxychlor[3] were chosen as the chlorinated hydrocarbon insecticides for this study. Also included was one organophosphate insecticide, malathion.[4]

Stock solutions containing 10% of these insecticides were prepared by dissolving dieldrin, heptachlor, or methoxychlor in acetone, and by dissolving malathion in 95% ethyl alcohol. Subsequent 10-fold dilutions of the stock solutions were prepared using the solvents mentioned above so that 1.0 ml added to 100 ml of sterile whole milk would give milk containing 100, 10, 1, or 0.1 parts per million (ppm) of the insecticide. Preliminary experiments indicated that these solvents had no effect on the growth of the test organisms when used at

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[1]Dieldrin, 99+% pure, Shell Chemical Co., New York, New York.

[2]Heptachlor, 99+% pure, Applied Science Labs, Inc., State College, Pennsylvania.

[3]Methoxychlor, 100% pure, Shell Chemical Co., New York, New York.

[4]Malathion, 96% pure, American Cyanamid Co., Princeton, New Jersey.

concentrations up to 1% in the growth medium. The original milk usually contained less than 0.01 ppm, and always less than 0.05 ppm of insecticide.

The milk samples containing various amounts of insecticides were inoculated with 0.1% of an active litmus culture of Streptococcus lactis A62, S. lactis A254, Streptococcus cremoris E-8, Streptococcus diacetilactis 18-16, or Lactobacillus casei and were incubated at 32 C for 48 hr, except for samples inoculated with S. cremoris which were incubated at 25 C. Two control samples of milk with no added insecticides were included with each trial, one inoculated and one non-inoculated. The non-inoculated controls were included to check for contamination of samples throughout the experiment.

At 0, 24, and 48 hr of incubation, aliquots were withdrawn aseptically to determine the total plate count, pH and titratable acidity. Duplicate plates for a proper dilution were prepared using Plate Count Agar (Difco).[1] A Beckman pH Meter was used for pH measurement. For the determination of titratable acidity, a 9 ml aliquot from each sample was transferred to a "Mann Acidity Cup" using a 9 ml milk pipette, and diluted with 18 ml CO_2-free deionized water. After addition of 1 ml of phenolphthalein solution (1% in ethyl alcohol), the diluted milk sample was

[1]Plate Count Agar, Difco Laboratories, Detroit, Michigan.

titrated with 0.1N NaOH to first persistent pink. The acidity was calculated as per cent lactic acid.

Effect of Lactic Culture Organisms on Aldrin, DDT and Lindane in Milk

Preparation of samples.--To each of seven Erlenmeyer flasks, containing 25 ml sterile milk, was added 25 µg of aldrin[1] dissolved in acetone. Six individual samples were inoculated with 0.25 ml litmus milk culture of one of the following organisms: S. lactis A254, S. lactis A62, S. cremoris E-8, S. diacetilactis 18-16, S. diacetilactis DRCI or L. casei. The above cultures had been activated in litmus milk by four consecutive daily transfers prior to use. The 18 hr old cultures of the last transfer were used for inoculation. One remaining sample was not inoculated and was used as a control. The milk inoculated with S. cremoris E-8 was incubated at 25 C for two weeks. The remaining six milk samples were incubated at 32 C for two weeks. Samples containing DDT [2] and lindane[3] were prepared in the same manner.

After two weeks of incubation the insecticides were extracted from milk samples and cleaned. The techniques for the extraction and cleaning were adopted from the

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[1]Aldrin, 99+% pure, Applied Science Labs, Inc., State College, Pennsylvania.

[2,3]DDT, and lindane, 99+% pure, Applied Science Labs, Inc., State College, Pennsylvania.

methods recommended by Shell Chemical Company (1964) and by the FDA Pesticide Manual (1965).

Procedure for extraction, separation, partitioning and cleanup were performed separately but identically for each sample. Reference will be made to only one sample for the procedures mentioned above.

Extraction of insecticides from milk.--To the Erlenmeyer flask containing a milk sample, 30 ml of 95% ethyl alcohol and 15 ml of 5% potassium oxalate solution were added with thorough mixing after each addition. The milk fat containing the insecticide was then extracted from the milk with 100 ml anhydrous ethyl ether by shaking the mixture for 20 min on a Burrell Wrist-Action Shaker[1] with an automatic timer. The ethyl ether layer was decanted into a 600 ml beaker and the milk was re-extracted twice with 100 ml of hexane-ethyl ether (1:1, v/v). The extractions were combined and the solvent was evaporated from the extracted sample on a steam bath leaving fatty materials and the insecticide in the beaker.

Separation of insecticide and milk-fat.--The fatty materials along with the insecticide, were transferred to a 125 ml separatory funnel by washing the beaker five times, each time with 5 ml of hexane. Thirty milliliters

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[1]Burrell Wrist-Action Shaker with a timer, Burrell Corp., Pittsburgh, Pennsylvania.

of acetonitrile[1] were added to the separatory funnel, and the mixture was shaken vigorously for one minute. After the layers were separated, the lower layer (acetonitrile) was transferred into a one liter separatory funnel containing 700 ml of 2% NaCl solution and 100 ml of petroleum ether.[2] Partitioning with 30 ml of acetonitrile was repeated three more times, combining the acetonitrile extracts each time in the one liter separatory funnel. At this point fatty materials remained in the hexane layer and the insecticide was extracted into acetonitrile.

Partitioning with acetonitrile, water and petroleum ether.--By swirling the one liter separatory funnel gently in an inverted position, the insecticide was recovered in petroleum ether and the acetonitrile was removed in the aqueous layer (2% NaCl solution). The aqueous layer was transferred into another one liter separatory funnel and extracted with 100 ml of petroleum ether for 15 sec. The aqueous layer was discarded. The petroleum ether layer was added to the first one liter separatory funnel. The combined petroleum ether extracts were washed twice with 100 ml of 2% NaCl solution and the washings (salt solution of lower layer) were discarded. The petroleum ether

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[1]Acetonitrile, Grade for pesticide analysis, Fisher Scientific Company, Detroit, Michigan. Saturated with redistilled hexane before use.

[2]Petroleum ether, Nanograde for pesticide analysis, Mallinckrodt Chemical Works, St. Louis, Missouri.

extract was transferred to a 600 ml beaker. The separatory funnel was rinsed three times with 10 ml of petroleum ether and washings were combined in the beaker. The petroleum ether was evaporated on a steam bath, leaving the insecticide in the beaker.

Cleanup of extracted insecticide.--In addition to the removal of fatty materials by partitioning the milkfat containing the insecticide between acetonitrile and hexane, further cleanup of the extracted insecticide was required to remove other impurities prior to testing the sample by gas chromatography. Adsorption column chromatography was used for cleanup. The adsorbent was a Florisil[1]-Celite 545[2] mixture. Florisil was activated by heating at 130 C for 5 hr or longer before use. The Florisil-Celite 545 mixture consisted of 100 parts of activated Florisil, 12 parts of distilled water and 20 parts of Celite 545.

The chromatographic column[3] of 20 mm x 400 mm was packed, in order, with 1 in. of granular anhydrous sodium

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[1]Florisil, 60-100 mesh, Floridin Company, Tallahassee, Florida. A synthetic selective adsorbent, magnesia-silica gel.

[2]Celite 545, Johns-Manville Corporation, New York, New York. Celite 545 is a processed diatomite product.

[3]Chromatographic column, with Lab-Crest teflon stopcock, sintered glass disc, detachable joint with teflon seal. Made by the Lab-Crest Scientific Division of Fisher and Porter. Distributed by Brinkmann Instruments, Inc., Des Plaines, Illinois.

sulfate (Na_2SO_4), 10 g of Florisil-Celite 545 mixture, and 1 in of granular anhydrous Na_2SO_4. The packed column was washed with 50 ml of hexane. When the hexane reached the top layer of Na_2SO_4, the insecticide extract was transferred to the column using 5 ml of hexane and a pasteur pipette. A 500 ml Kuderna-Danish concentrator[1] was placed under the column to collect the effluent and the insecticide extract was allowed to enter the column. The beaker was rinsed three times with 5 ml, and one time with 30 ml of hexane. Each rinse was added to the column and allowed to enter the column. The insecticide extract was eluted from the column with an additional 350 ml of hexane at a rate of 4-5 ml per min. The hexane effluent in the Kuderna-Danish concentrator was evaporated to a final volume of 4-5 ml. The concentrate was transferred to a 25 ml volumetric flask and the final volume was adjusted to 25 ml with hexane which had been used to rinse the Kuderna-Danish concentrator.

The cleaned insecticide was saved for later analysis by gas liquid chromatography.

Analysis of insecticide.--Final analysis was made by gas liquid chromatography (GLC) using a F & M Hewlett-Packard Model 5750B Research Gas Chromatograph[2] equipped

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[1]Kuderna-Danish concentrator, Kontes Glass Company, Vineland, New Jersey.

[2]Gas Chromatograph, Hewlett-Packard Aanlytical Instruments, Palo Alto, California.

with an electron capture (EC) detector. The column for GLC was a 4' x 1/4" coiled glass column which was prepacked with 3.8% UC-W98 (a silicone rubber) on 80/100 mesh Diatoport S, and pretested by Hewlett-Packard. The carrier gas was a 90% argon-10% methane mixture [1] with a flow rate of 60-70 ml/min. Temperatures at the injection port, column, and EC detector were 255 C, 195-200 C and 193-196 C, respectively. The pulse interval on the EC detector was 50 μ sec and the chart speed of the recorder was 0.25"/min or 0.5"/min. The injection volume was 2-5 μl, depending on the kind of insecticide under investigation, and the volume of the samples injected was always the same as that of the controls. The effect of lactic starter culture organisms on insecticides was checked by comparing the chromatograms of insecticides from fermented milks with the chromatogram of insecticides from a non-fermented milk control.

The Uptake of Dieldrin by *Lactobacillus casei*

L. casei was activated by three daily transfers in Trypticase Soy Broth (TSB)[2] at 32 C. At the end of 18 hr incubation after the last transfer, 1 ml of culture was

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[1] 90 argon-10% methane, The Matheson Co., East Rutherford, New Jersey.

[2] Trypticase Soy Broth, BBL, Division of BioQuest, Division of Becton, Dickinson and Company, Cockeysville, Maryland.

added to 100 ml of TSB containing ^{14}C-dieldrin[1] with a specific activity of 72.4 milli curie/milli As a control, the same amount of L. casei culture was added to 100 ml of TSB containing no dieldrin. The samples were incubated at 32 C for 18 hr.

In order to measure the activity of ^{14}C-dieldrin in TSB, 0.5 ml of TSB containing ^{14}C-dieldrin, 14.5 ml of scintillator solution, and Cab-O-Sil[2] were added to a glass liquid scintillation vial. The amount of Cab-O-Sil was just enough to obtain a stable homogeneous gel. Then the contents were shaken vigorously and counted for radioactivity using a Packard-Tri-Carb Liquid Scintillation Spectrometer Model 3310.[3] The composition of the scintillation solution was 6 g/l of 2,5-diphenyloxazole (PPO), and 0.2 g/l of 1,4-bis-2-(4-methyl-5-phenyloxazole)(POPOP) in toluene. The number of viable cells was determined on Plate Count Agar.

Twenty-five milliliters of the 18 hr culture in TSB containing ^{14}C-dieldrin were washed with 25 ml of hexane in a stainless steel centrifuge bottle by shaking

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1 ^{14}C-dieldrin, Amersham/Searle Corporation, Des Plaines, Illinois.

[2]Cab-O-Sil, New England Nuclear Corp., Pilot Chemicals Division, Boston, Massachusetts.

[3]Packard Tri-Carb Liquid Scintillation Spectrometer Model 3310, Packard Instrument Company, Inc., Downers Grove, Illinois.

the mixture for 30 sec. Then the cells were harvested by centrifuging at 10,400 x g for 20 min using a Sorvall automatic refrigerated centrifuge.[1] The harvested cells were washed twice with 50 ml of physiological saline (0.89% NaCl), transferred to a liquid scintillation vial using 0.5 ml TSB, and prepared for radioactivity counting as described previously. The culture in the control TSB was treated in the same manner. Results are reported on a count per minute (cpm) per milliliter basis.

[1]Sorvall Superspeed, Model RC-2, Ivan Sorvall, Inc., Norwalk, Connecticut.

RESULTS

Effect of Insecticide Residues on Growth and Fermentation Ability of Lactic Culture Organisms

After individual cultures were inoculated into the milks containing 0, 0.1, 1, 10, and 100 ppm of the insecticide under study, the milks were incubated for 48 hr at appropriate temperatures as indicated in the Materials and Method section.

The tables 2 through 15 in the Appendix contain all the data on viable cell count, pH of the milks and per cent lactic acid in the milks at 0, 24 and 48 hr, for all of the combinations of cultures and insecticides tested.

Using the data obtained after the cultures were exposed to the insecticides in milk for 24 hr, the log of the viable cell count/ml, pH, and per cent lactic acid of the inoculated milks were plotted against the concentration of each insecticide tested. Because of the overlapping of the graphs, no more than two different lactic cultures could be represented in one figure, even when four different cultures were tested against the same kind of insecticide.

The graphs in Figure 1 describe the effect of different concentrations of dieldrin on viable cell count, pH and per cent lactic acid in whole milk when the milk was inoculated with S. lactis A254 or L. casei and incubated at 32 C for 24 hr.

The data in Figure 1-A show that 0.1 to 100 ppm of dieldrin in milk had no effect on the viable cell count of S. lactis A254. However, a slight reduction in the viable cell count of L. casei was observed in the milks containing 10 and 100 ppm of dieldrin. Figures 1-B and 1-C indicate that the presence of dieldrin in amounts up up 100 ppm in milk did not interfere with the reduction of pH or the production of lactic acid in milk by S. lactis A254 or L. casei.

As noted in Figure 2, 100 ppm of heptachlor in milk had neither an adverse nor a stimulative effect on viable cell counts or fermenting ability of S. lactis A254 or L. casei when inoculated into milk and incubated at 32 C for 24 hr.

In the milks containing 0.1, 1, 10, and 100 ppm of methoxychlor, the viable cell count, pH and per cent lactic acid were about the same as in the control milk containing no methoxychlor, when the milks were inoculated with S. lactis A254 or L. casei and incubated at 32 C for 24 hr. The results are depicted in Figure 3.

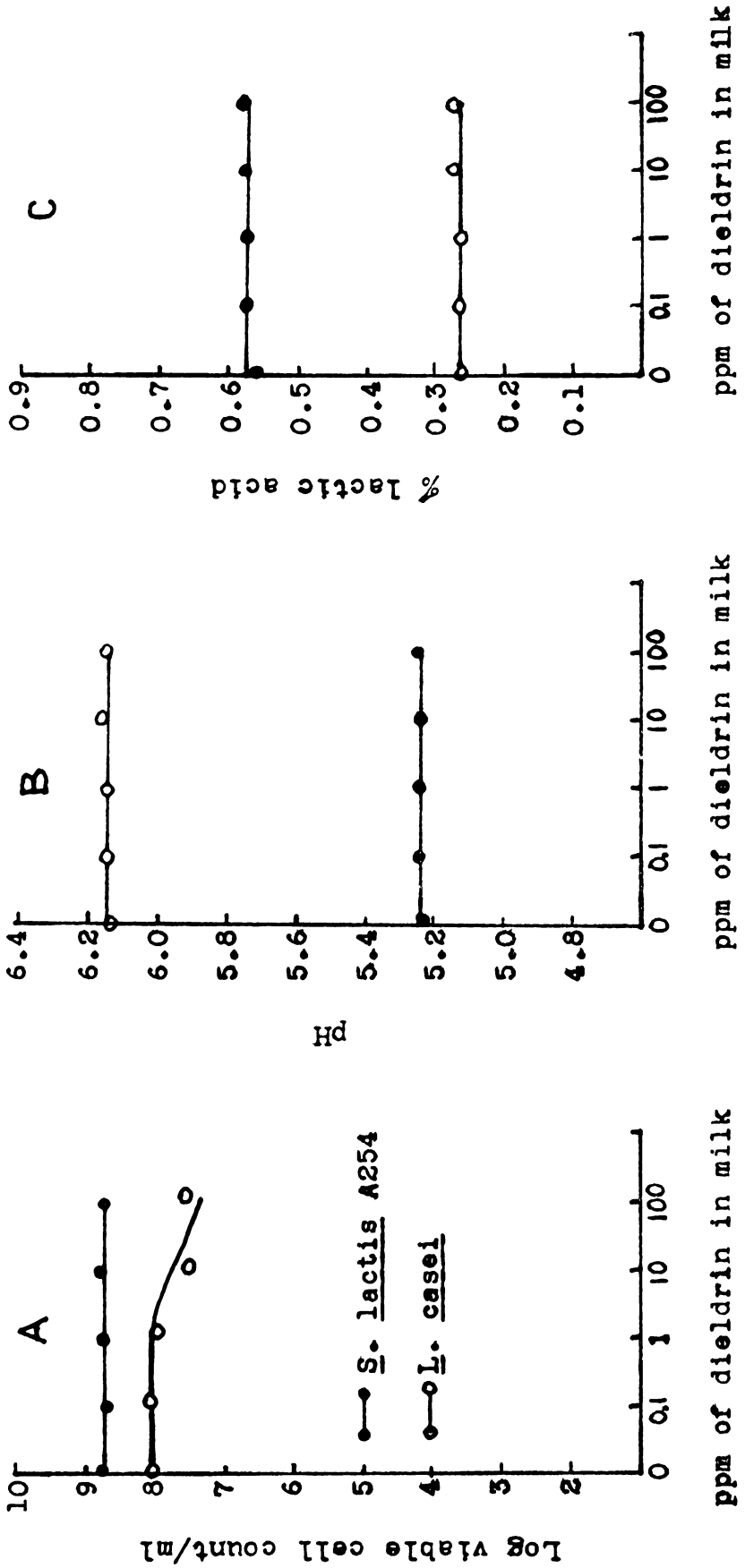

Figure 1. Effect of different concentrations of dieldrin on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A254 or Lactobacillus casei and incubated at 32 C for 24 hr.

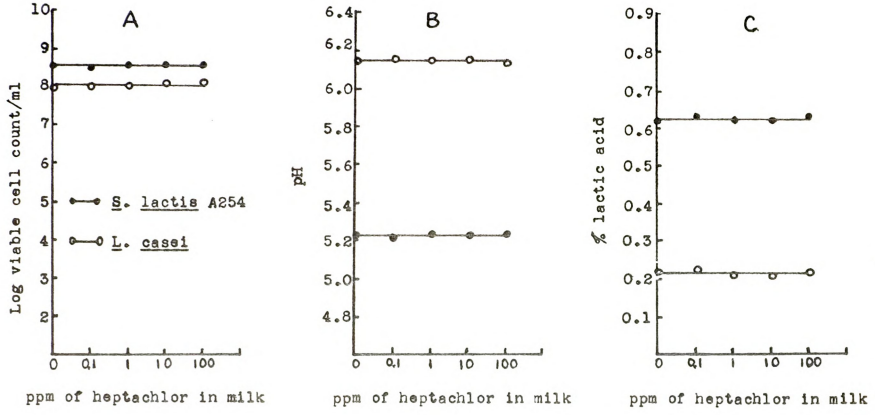

Figure 2. Effect of different concentrations of heptachlor on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A254 or Lactobacillus casei and incubated at 32 C for 24 hr.

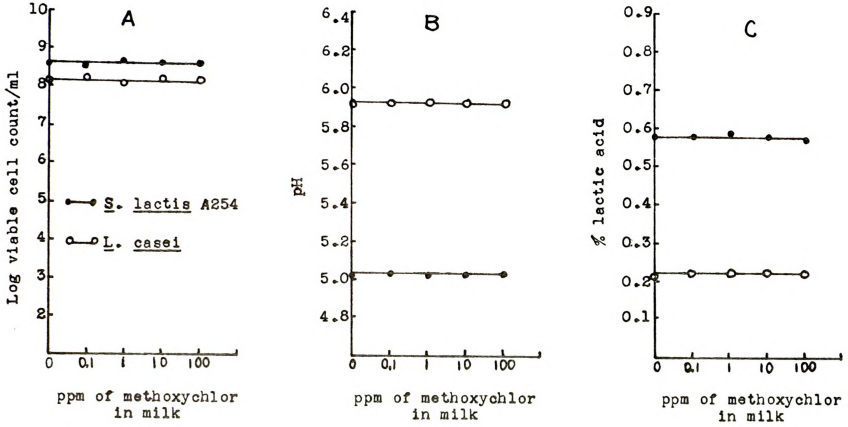

Figure 3. Effect of different concentrations of methoxychlor on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A254 or Lactobacillus casei and incubated at 32 C for 24 hr.

When S. lactis A254 or L. casei was grown for 24 hr at 32 C in milks containing 0.1 to 100 ppm of malathion, the malathion had no influence on the viable cell count or fermentation ability of the organisms. The results are illustrated in Figure 4.

Three additional strains of Streptococcus were exposed to dieldrin and to heptachlor to determine the uniformity of the resistant characteristic within the Streptococcus genus. As represented in Figures 5 and 6, both dieldrin and heptachlor at concentrations of 100 ppm in milk had no effect on the viable cell count or fermentation ability when the milks were inoculated with S. lactis A62 or S. diacetilactis 18-16 and incubated at 32 C for 24 hr.

Figures 7 and 8 show that 0.1 to 100 ppm of dieldrin or heptachlor in milk did not hinder the growth and lactic acid producing ability of S. cremoris E-8 when the organism was grown in the milk for 24 hr at 25 C.

At 0 hr of incubation the viable cell count, pH and titratable acidity (per cent lactic acid) were measured in order to note any unexpected initial variations which occasionally existed among the milk samples prior to inoculation with the lactic cultures. For example, as shown in Table 5 in the Appendix, the pH of the milk containing 0, 0.1, 1, 10 and 100 ppm of heptachlor was 6.46, 6.47, 6.45, 6.46 and 6.42, respectively

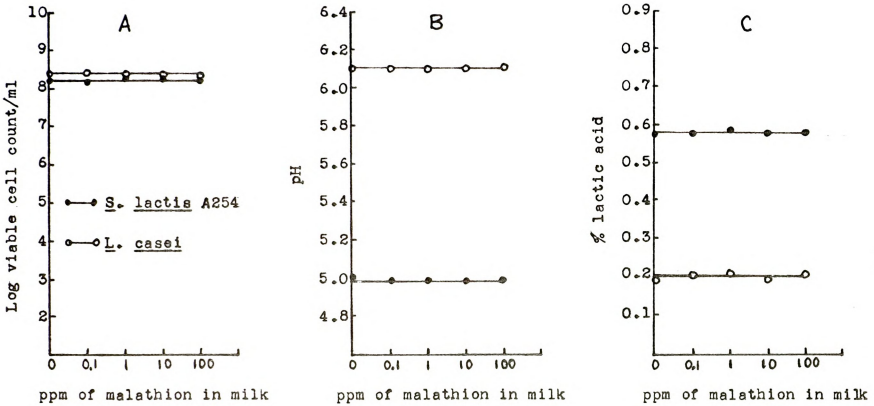

Figure 4. Effect of different concentrations of malathion on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A254 or Lactobacillus casei and incubated at 32 C for 24 hr.

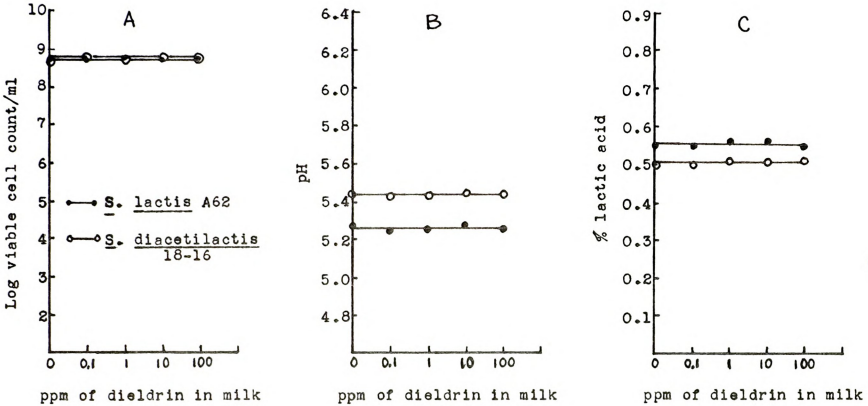

Figure 5. Effect of different concentrations of dieldrin on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A62 or Streptococcus diacetilactis 18-16 and incubated at 32 C for 24 hr.

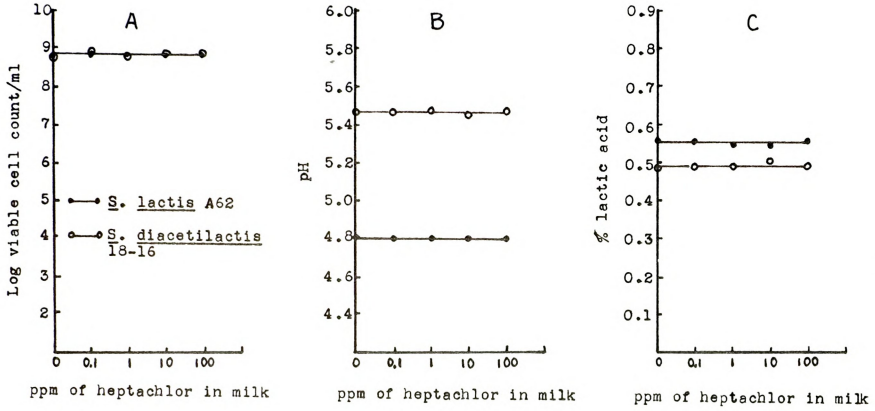

Figure 6. Effect of different concentrations of heptachlor on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus lactis A62 or Streptococcus diacetilactis 18-16 and incubated at 32 C for 24 hr.

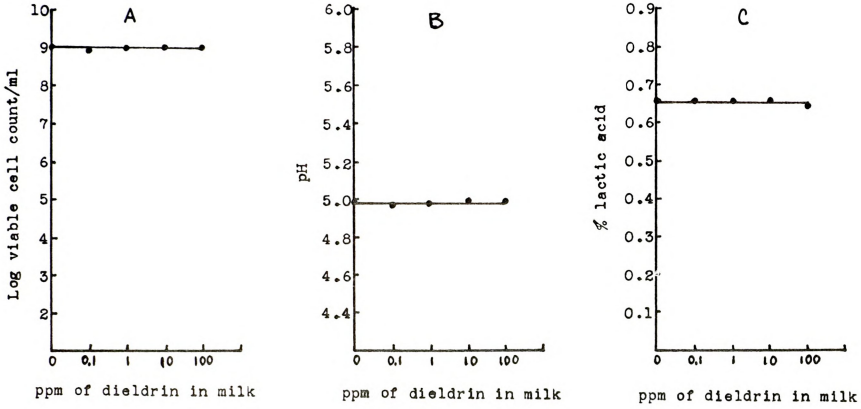

Figure 7. Effect of different concentrations of dieldrin on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus cremoris E-8 and incubated at 25 C for 24 hr.

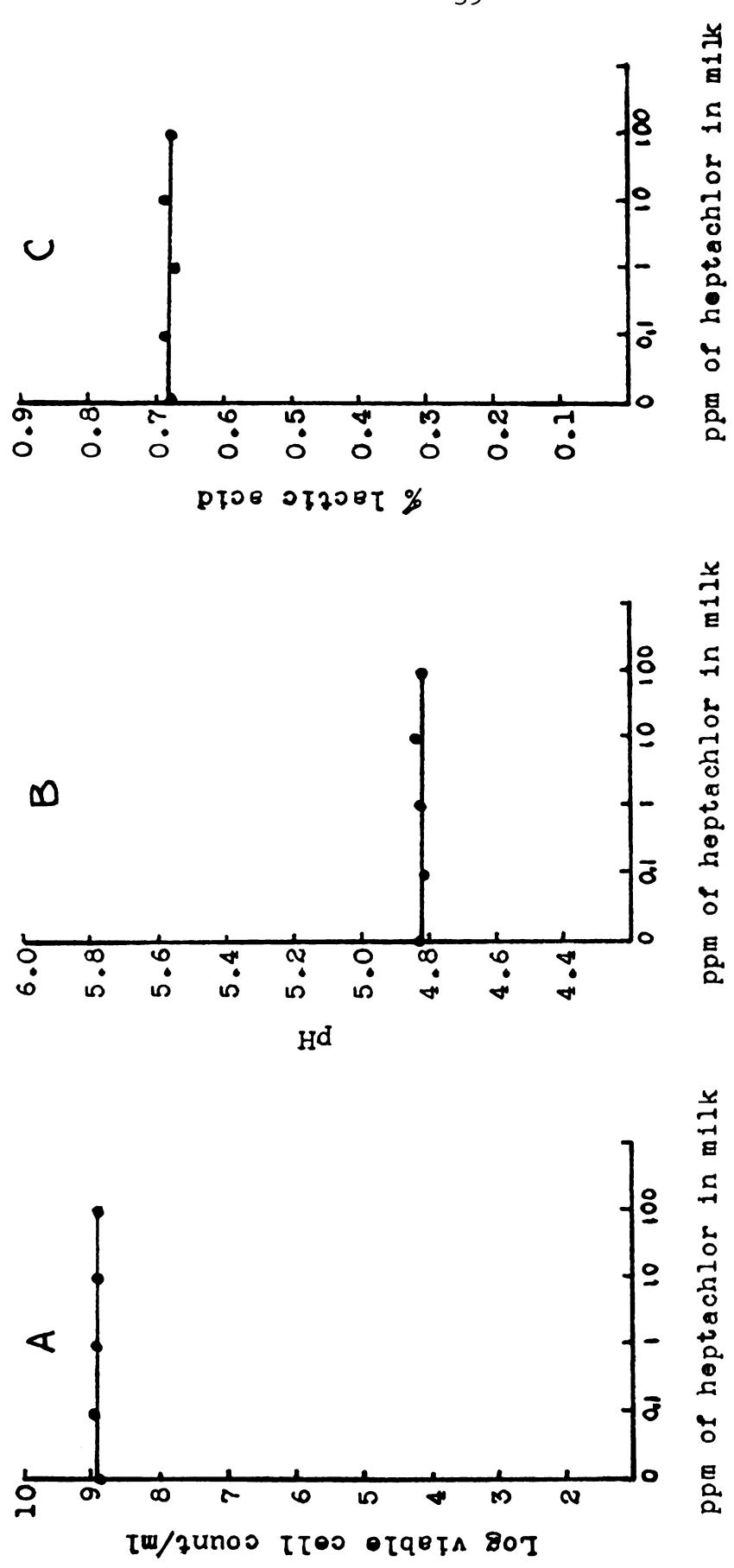

Figure 8. Effect of different concentrations of heptachlor on (A) viable cell count, (B) pH and (C) percent lactic acid in whole milk when the milk was inoculated with Streptococcus cremoris E-8 and incubated at 25 C for 24 hr.

at 0 hr of incubation. The lower pH in the milk containing 100 ppm of heptachlor was not due to the presence of the heptachlor since the preliminary study showed that addition of 100 ppm of an insecticide to milk did not change the pH of the milk. After L. casei was allowed to grow and ferment milk for 24 hr, the pH of the inoculated milk containing 100 ppm of heptachlor was 6.10 and the pH of the inoculated control was 6.14. However, the difference in pH in this case could not be attributed to the effect of the 100 ppm of heptachlor in the milk since there was initially the same degree of difference in pH between the two milks.

Although viable cell count, pH and per cent lactic acid were also determined at 48 hr of incubation, the data after 48 hr of incubation did not provide any more or different information that the data at 24 hr of incubation in respect to the effect of the insecticides on the growth and lactic acid fermentation ability of the cultures. Therefore, only the data at 24 hr of incubation are included in the graphs in Figures 1 through 8 in order to show the effect of different concentrations of insecticides on the growth and lactic acid producing ability of the cultures.

Effect of Lactic Culture Organisms on Aldrin, DDT and Lindane in Milk

Figure 9 shows the chromatogram of aldrin extracted from the noninoculated control milk. The milk

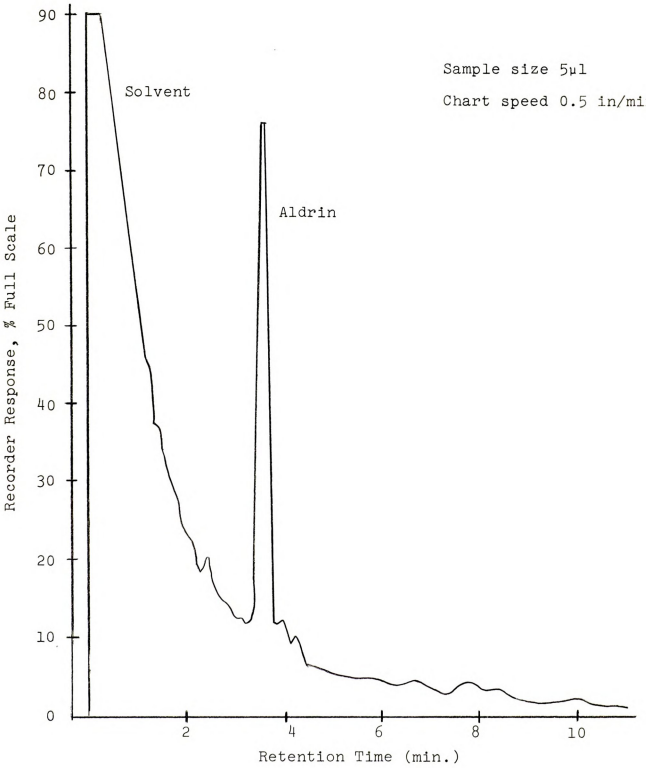

Figure 9. Chromatogram of noninoculated milk containing 1 ppm of aldrin.

contained 1 ppm of aldrin. Figure 10 shows the chromatogram of aldrin extracted from a milk sample which contained 1 ppm of aldrin and was inoculated with S. lactis A62. All the milks including the control were incubated at 32 C for two weeks.

When aldrin converts to dieldrin (a metabolic product of aldrin)or any other analog, the size of the peak representing aldrin becomes smaller and a new peak representing dieldrin or the analog appears on the chromatogram. Comparison of the chromatogram in Figure 10 with the chromatogram of the control in Figure 9 shows no difference between the two chromatograms. This indicates that aldrin was not degraded or converted to an analog when present in a milk substrate inoculated with S. lactis A62 and incubated at 32 C for two weeks.

The chromatograms of aldrin extracted from other milk samples containing aldrin and inoculated with S. lactis A254, S. diacetilactis 18-16, S. diacetilactis DRCI, S. cremoris E-8 or L. casei were all the same as those of the milk inoculated with S. lactis A62. This indicates that none of the above lactic culture organisms modified or degraded aldrin in milk. Since these chromatograms are all identical, only the chromatogram of the milk inoculated with S. lactis A62 is presented as a representative of the entire group.

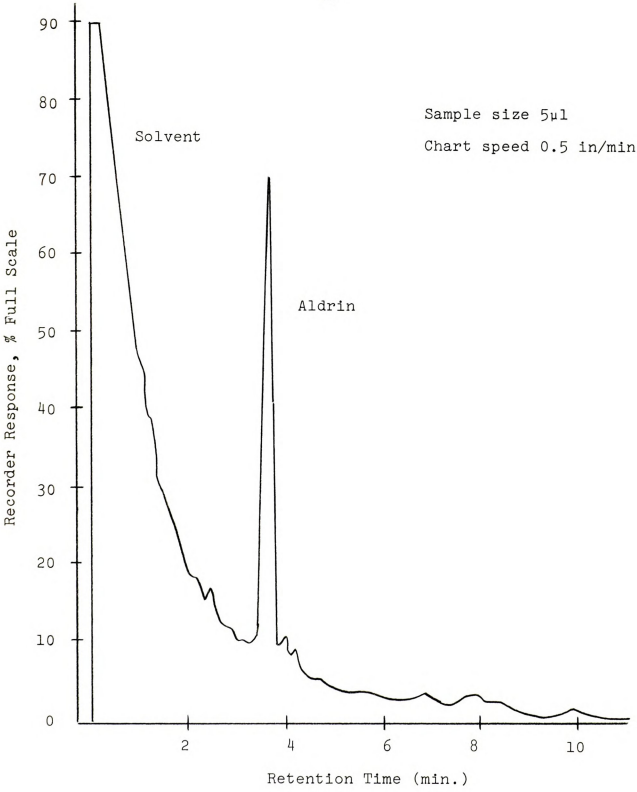

Figure 10. Chromatogram of milk containing 1 ppm of aldrin, inoculated with S. lactis A62 and incubated at 32 C for two weeks.

The effect of lactic culture organisms on DDT and lindane were also studied. The lactic culture organisms included S. lactis A62, S. lactis A254, S. diacetilactis 18-16, S. diacetilactis DRCI, S. cremoris E-8 and L. casei. Figure 11 shows the chromatogram of DDT from the noninoculated control milk. Figure 12 shows the chromatogram of DDT extracted from the milk sample inoculated with S. lactis A62. All the samples were incubated for two weeks at 32 C except the milk sample which was inoculated with S. cremoris and incubated at 25 C. In Figure 12 the height of the DDT peak is 37% full scale, which is the same as the height of the DDT peak in the chromatogram of the control in Figure 11. Thus it was apparent that DDT was stable even after two weeks of incubation with S. lactis A62. DDT was also stable in the presence of other lactic culture organisms, S. lactis A254, S. diacetilactis 18-16, S. diacetilactis DRCI, S. cremoris E-8 and L. casei.

The chromatogram of lindane extracted from a noninoculated control milk is shown in Figure 13. The chromatogram of lindane extracted from a milk sample inoculated with S. lactis A62 and incubated for two weeks is illustrated in Figure 14.

Chromatograms performed on the extracts from the milk samples inoculated with S. lactis A254, S. diacetilactis 18-16, S. diacetilactis DRCI, S. cremoris E-8

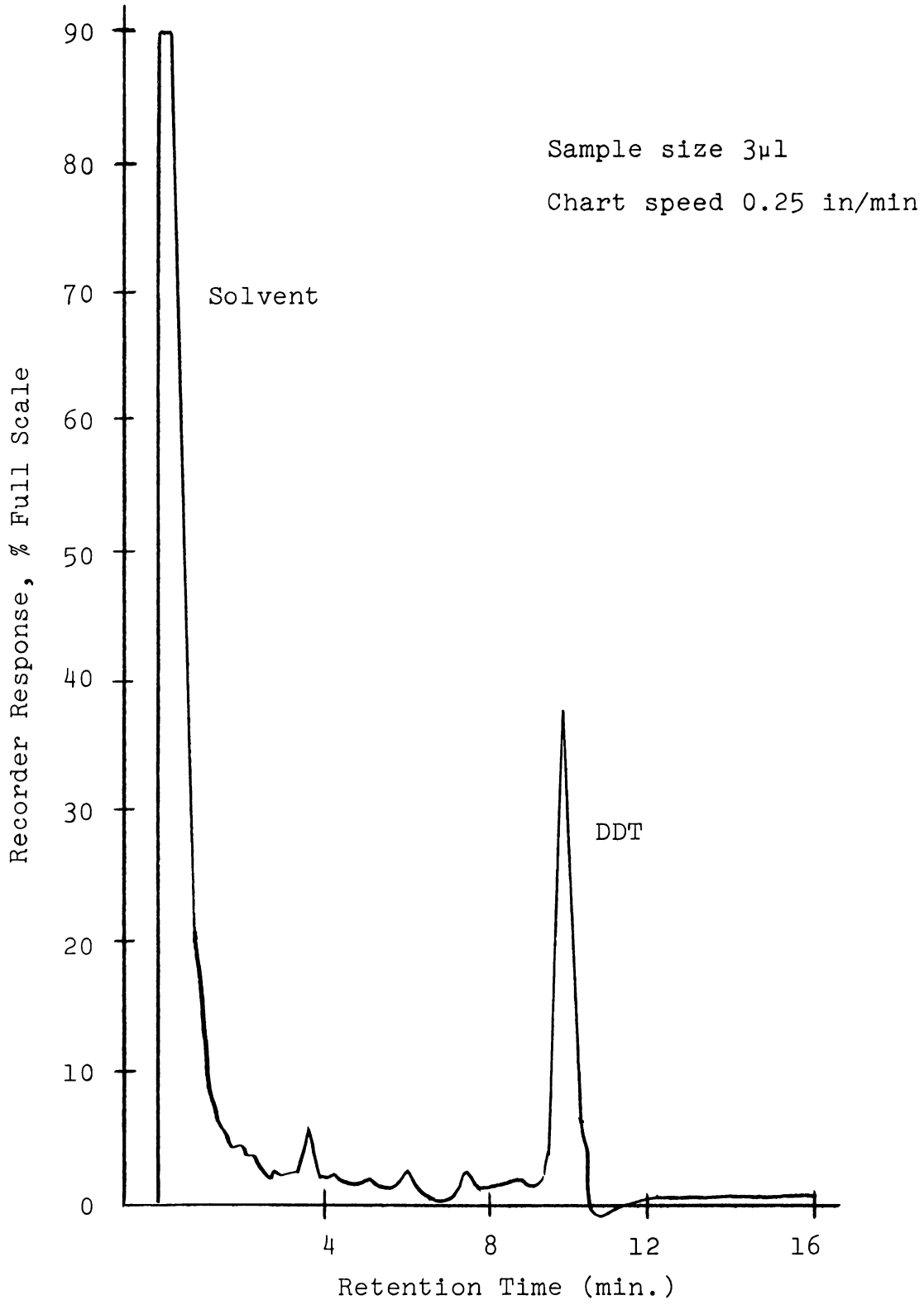

Figure 11. Chromatogram of noninoculated milk containing 1 ppm of DDT.

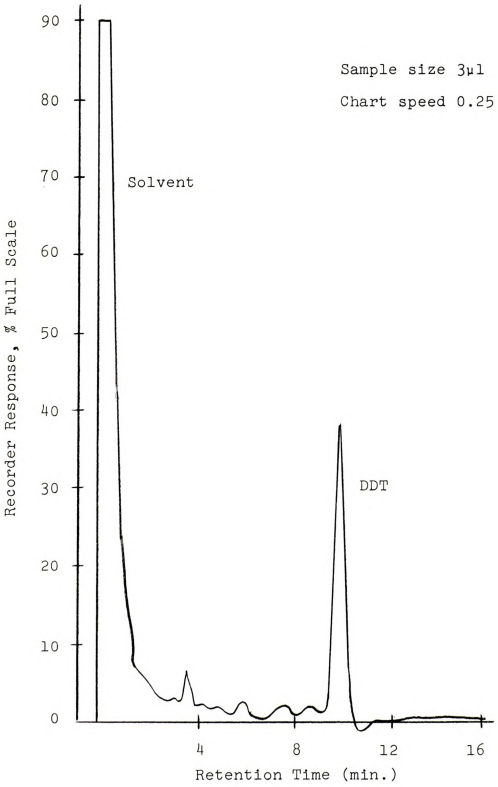

Figure 12. Chromatogram of milk containing 1 ppm of DDT, inoculated with S. lactis A62 and incubated for two weeks at 32 C.

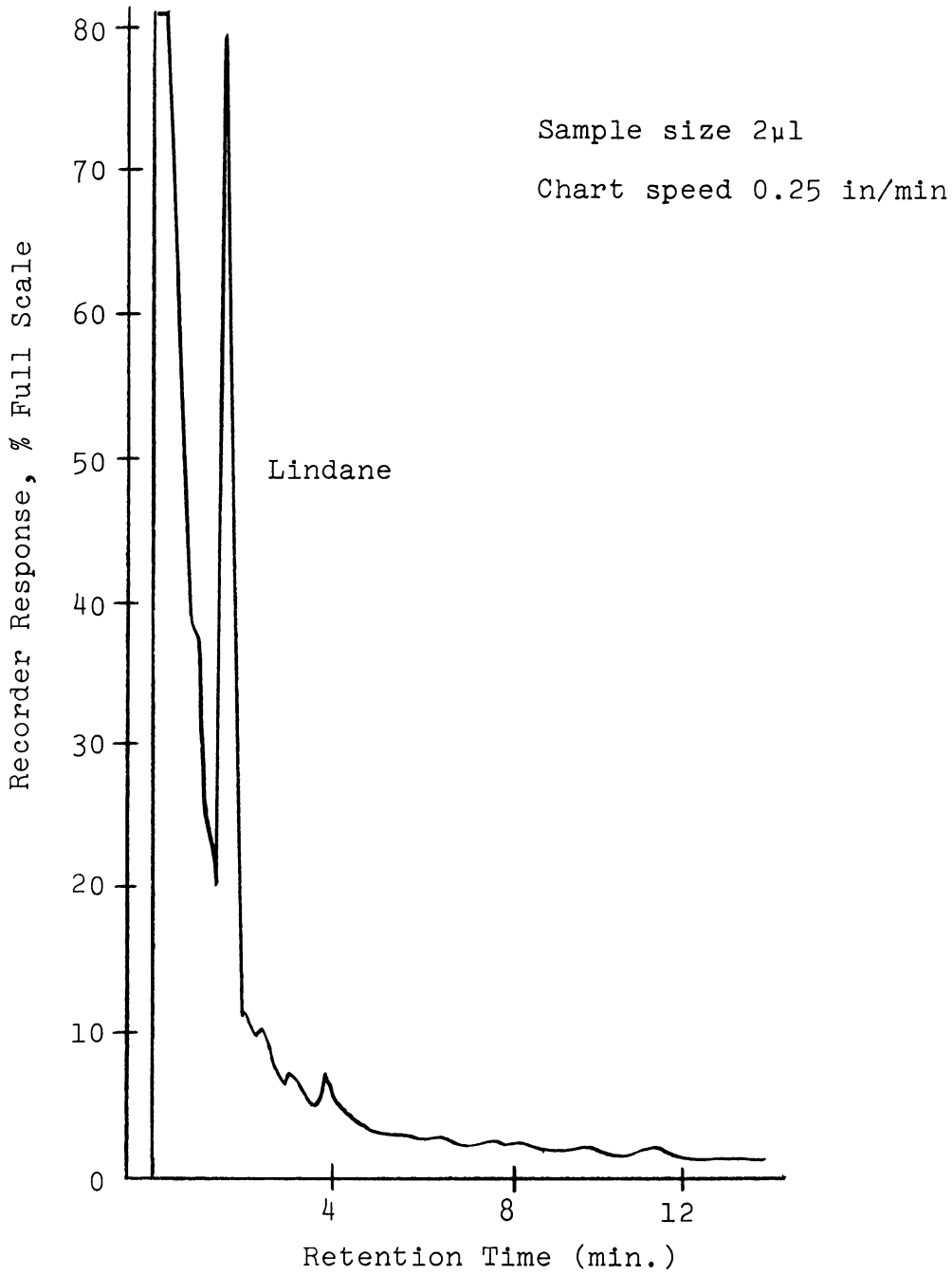

Figure 13. Chromatogram of a noninoculated milk containing 1 ppm of lindane.

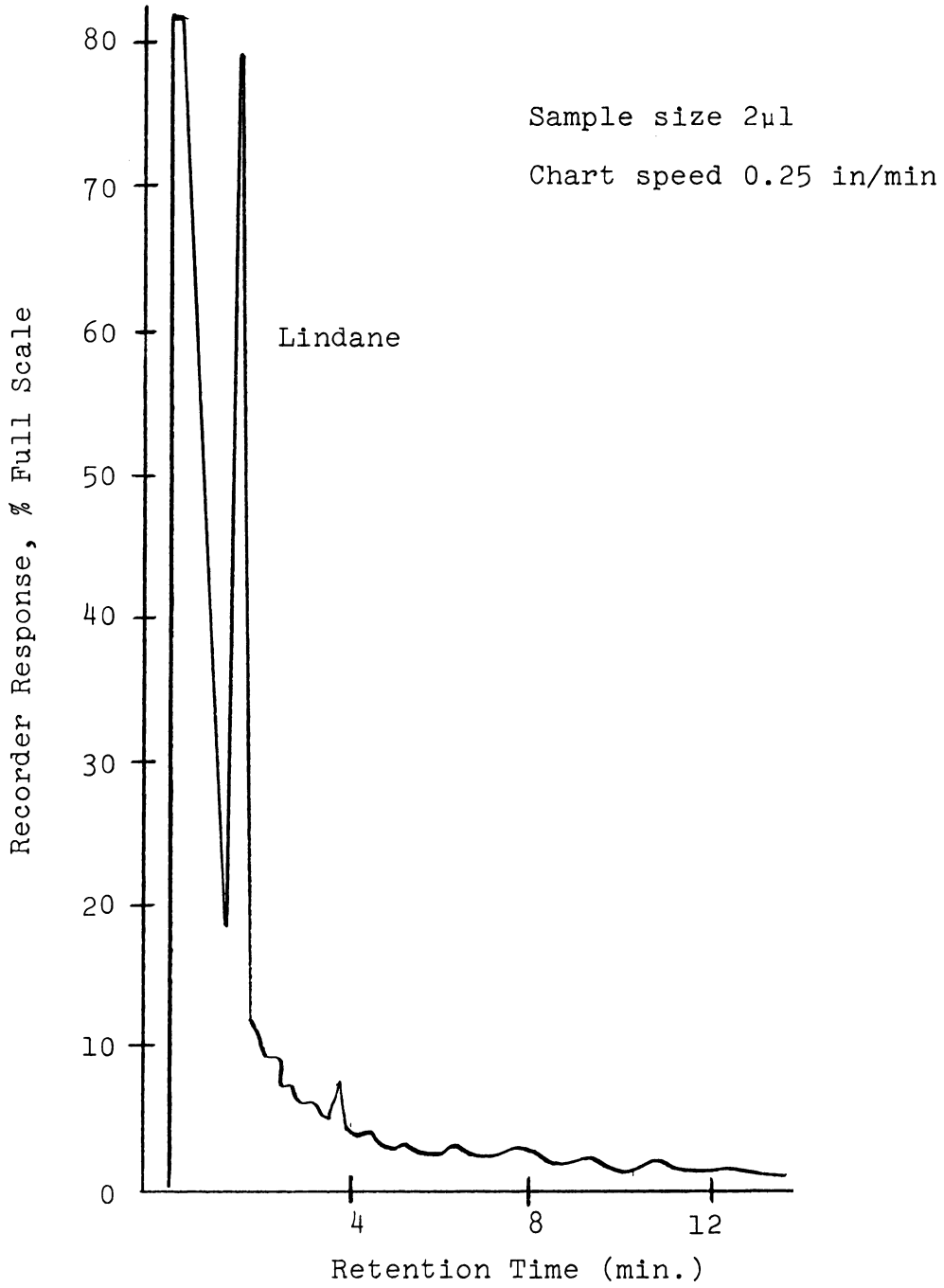

Figure 14. Chromatogram of milk containing 1 ppm of lindane, inoculated with S. lactis A62 and incubated for two weeks at 32 C.

or L. casei were the same as those obtained from the milk inoculated with S. lactis A62. A comparison of the chromatograms in Figures 13 and 14 indicates that lindane was also inert to the biological action of the lactic culture organisms.

When degradation or modification of DDT or lindane occurs the peak size representing DDT or lindane decreases and a new peak appears in the chromatogram to represent the newly formed analog. Such phenomena did not occur in the milk samples containing DDT or lindane when the milk samples were inoculated with the above lactic culture organisms and incubated for two weeks.

The Uptake of ^{14}C-dieldrin by Lactobacillus casei

When a study was performed on the effect of dieldrin, heptachlor, methoxychlor and malathion on the growth of L. casei, it was found that only dieldrin inhibited the growth of L. casei as illustrated in Figure 1-A. Therefore, possible uptake of dieldrin into the cells of L. casei was studied using ^{14}C-dieldrin. Table 1 contains data and calculations indicating the amount of uptake of ^{14}C-dieldrin in TSB by L. casei. Cells were grown for 18 hr at 32 C in TSB containing ^{14}C-dieldrin. After the cells were harvested and washed, the cells of L. casei retained less than 0.2% of the total ^{14}C-dieldrin in the TSB. For example, when there was about 68,000 cpm of

^{14}C-dieldrin in the TSB the harvested cells retained about 140 cpm of ^{14}C-dieldrin, which is 0.16% of 68,000 cpm. When the TSB growth medium contained 142,000 cpm of ^{14}C-dieldrin, the cells harvested from the TSB retained 280 cpm, which is 0.18% of the 142,000 cpm. The amount of ^{14}C-dieldrin remaining with the harvested cells was even less than 0.2% of the total ^{14}C-dieldrin in the growth medium. These results negate the idea that dieldrin may be absorbed into the cells of L. casei. The radioactivity counted from the harvested cells is probably due to the residual radioactivity resulting from incomplete washing.

TABLE 1.--Calculation of uptake of ^{14}C-dieldrin by Lactobacillus casei when grown in Trypticase Soy Broth (TSB) containing ^{14}C-dieldrin for 18 hr at 32 C.

	Trial I	Trial II
(a) *cpm of 1 ml TSB culture containing ^{14}C-dieldrin.	2,118	5,670
(b) cpm of 25 ml TSB culture containing ^{14}C-dieldrin = (a) x 25	67,966	141,750
(c) cpm of cells harvested from 25 ml of TSB containing ^{14}C-dieldrin	136	279
(d) cpm of cells harvested from 25 ml of TSB containing no ^{14}C-dieldrin	30	30
(e) per cent of ^{14}C-dieldrin retained by L. casei $= \frac{(c) - (d)}{(b)} \times 100$	0.16	0.18
(f) viable cell count per ml	24×10^7	22×10^7

*count per minute

DISCUSSION

Lactic starter cultures are essential in the manufacture of cheese and fermented milk products. When lactic cultures fail to grow or produce lactic acid, the failure is usually attributed to the presence of antibiotics, bacteriophages, free fatty acids, sanitizing chemicals in milk, or to some unknown factor. Failure of lactic starter cultures can be avoided by excluding milk containing inhibitory quantities of the above substances. Therefore, personnel in any culture company or dairy plant working with lactic starter cultures should know all the possible causes which can interfere with the activity of lactic starter cultures.

Some people even suspected that certain insecticides in milk, unintentionally present in excess of the actionable level set by the Food and Drug Administration (FDA), might inhibit the growth and fermentation ability of lactic starter cultures. However, there has been no experimental proof to refute or support such an opinion.

The purpose of the first part of the experiment was to study the effect of some insecticides on the growth and fermentation ability of several lactic starter cultures. Since lactic cultures produce lactic acid through the

fermentation of lactose in milk, the pH and per cent lactic acid developed in the inoculated milk are used as indices of the culture's fermentation ability. The growth was measured by viable cell count.

Data in Figures 1 through 4 show that none of the dieldrin, heptachlor, methoxychlor, and malathion in milk, ranging from 0.1 to 100 ppm had any effect on the viable cell count, pH and per cent lactic acid when the milk was inoculated with S. lactis A254 and incubated at 32 C.

When three additional Streptococci, S. lactis A62, S. diacetilactis 18-16 and S. cremoris E-8 were also exposed to 0.1 to 100 ppm of dieldrin and heptachlor, their growth and lactic acid fermentation ability were not affected.

Commercially important streptococci utilized as lactic starter cultures include S. lactis, S. diacetilactis, S. cremoris and S. thermophilus. In this study two strains of S. lactis, one strain of S. diacetilactis and one strain of S. cremoris were tested against two to four different insecticides. The chlorinated hydrocarbon insecticides can be classified into the following four groups: (1) aldrin, dieldrin and endrin; (2) DDT, DDD, DDE and methoxychlor; (3) heptachlor and heptachlor epoxide; and (4) lindane and BHC. The insecticides in each group are very closely related in their chemical

composition and structure. Three different groups are represented by dieldrin, heptachlor and methoxychlor, which were the insecticides selected for testing in this experiment.

Since all the lactic streptococci were not tested against all the insecticides which can possibly occur in milk, no absolute conclusion can be made concerning the effect of insecticide residues on lactic cultures. However, the results of this work prove that concentrations of insecticides far in excess of the amount normally occurring in milk will not interfere with normal growth and lactic fermentation ability of streptococci used in the manufacture of cultured dairy products.

In the current edition of Bergey's Manual of Determinative Bacteriology by Breed et al. (1957) S. diacetilactis is not listed as a separate species and some people designate it as S. lactis var. diacetilactis. However, research in recent years has established S. diacetilactis as a separate species. According to Foster et al. (1957) and Hammer and Babel (1956), S. diacetilactis is similar in most characteristics to S. lactis and differs from S. lactis in its ability to ferment citrate. Also S. diacetilactis uses more oxygen and produces much more CO_2 than S. lactis, and possesses a vigorous diacetyl reductase enzyme which is absent in S. lactis.

The results in Figures 2, 3, and 4 prove that up to 100 ppm of heptachlor, methoxychlor and malathion had neither an adverse nor a stimulative effect on the growth and lactic fermentation ability of L. casei in milk at 32 C. Dieldrin did not influence the lactic fermentation ability of L. casei but resulted in slight reduction in viable cell count (about one half log cycle) in the milks containing 10 and 100 ppm, as shown in Figure 1-2. Since the reduction of the viable cell count did not occur in the milks containing 0.1 and 1 ppm of dieldrin, and the concentrations between 1 and 10 ppm were not tested, it is not known exactly what is the lowest limiting concentration of dieldrin to cause reduction in viable cell count of L. casei in milk. However, the reduction in growth of L. casei at both 10 and 100 ppm was very slight and does not seem significant from a practical standpoint. L. casei is seldom used in lactic starter cultures but does have an important role in the ripening of Cheddar and some other varieties of cheese.

In general, lactic starter cultures are mixed strains of S. lactis and/or S. cremoris plus one strain of Leuconostoc to ferment citric acid. Some Lactobacilli are used as lactic starter cultures only for special occasions. Lactobacillus acidophilus is used to make acidophilus milk. Lactobacillus bulgaricus is used for

the manufacture of Bulgarian milk, and is also used in combination with S. thermophilus for the manufacture of yoghourt and Swiss cheese. Only the most commonly used lactic starter cultures were included in this study and it is not known how insecticides would affect the growth and lactic fermentation of L. acidophilus and L. bulgaricus, but presumably their response would be similar to that of L. casei.

In contrast to the results of this study, Bradley and Li (1968) reported that the presence of dieldrin in milk reduced acid production by lactic cultures in the manufacture of Cheddar cheese. According to their data, the reduction of titratable acidity due to the presence of dieldrin was 0.01-0.02 per cent lactic acid at the stage of draining. However, prior to inoculation, the titratable acidity of the milk containing dieldrin was lower than that of the control milk by 0.01-0.05 per cent lactic acid. In this case, the initially lower acidity in milk containing dieldrin seems to account for the lower acidity of the same milk after inoculation and incubation, compared with the acidity of the control milk.

This study was restricted to the effect of some insecticides on the growth and lactic fermentation ability of culture organisms commonly used in dairy products. Thus it cannot be predicted, from the results of this

work, how lactic cultures would react to the presence of insecticides in substrates other than milk.

Collins and Langlois (1968) found that heptachlor and DDT showed no effect on Staphylococcus aureus in skim milk, although heptachlor was bactericidal and DDT was inhibitory to S. aureus in Trypticase Soy Broth (TSB) at 100 ppm. Pseudomonas fluorescens was inhibited by 100 ppm of DDT in TSB but not in skim milk. Dieldrin had no effect on the growth of S. aureus and P. fluorescens either in TSB or in skim milk. Speculation based on the observation by Collins and Langlois (1968) suggests that lactic starter cultures would not be affected by insecticides in milk, although they might be adversely affected by some insecticides in certain broth media. Furthermore, 100 ppm of heptachlor or DDT in milk is an unrealistic concentration.

There are no other reports with which the results of this study can be compared. The soil bacteria were not affected by various chlorinated hydrocarbon insecticides as discussed in the Literature Review. The observation by Collins and Langlois (1968) and the results of this study indicate that insecticides certainly do not have a strong or a wide spectrum of antibacterial activity.

Because of the widespread use, various insecticides have persistently appeared in nearly all the milk, and it

is extremely difficult to obtain milk from any species which is completely free of all insecticides. The actionable amount of DDT, DDE, DDD, lindane, BHC and methoxychlor in milk is 0.05 ppm in milk or 1.25 ppm in milk fat; and for aldrin, dieldrin, heptachlor, heptachlor epoxide and endrin the actionable amount is 0.01 ppm in milk or 0.25 ppm in milk fat. When no test insecticide was added to milk used in this investigation the milk was designated as the control, containing less than 0.05 ppm of the test insecticide under study and confirmed by chromatographic analysis.

It is unlikely that any insecticide will occur in milk in concentrations much above the actionable level. One hundred parts per million of dieldrin or heptachlor in milk is 10,000 times higher than the actionable level recognized by the FDA and 100 ppm of methoxychlor in milk is 5,000 times higher than the actionable level. Therefore, the effect of concentrations above 100 ppm of insecticide was not routinely tested in this study.

Malathion was included in this study since it is the most widely used organophosphate insecticide. There is no actionable level for organophosphate insecticides in milk. This is because organophosphate insecticides seldom survive the biochemical reactions of the mammalian system to appear in milk. Consequently the organophosphate

insecticides are of little concern in milk, except when absorbed into milk from the environment.

When the susceptibility of bacteria to a chemical agent is tested, if the initial population is very high, the bacteria may overcome the effect of the chemical agent. In this experiment, 0.1% of culture was inoculated into milk samples although frequently 0.5% to 1% of lactic starter culture is inoculated into milk for the production of fermented milk products.

In this experiment, the samples inoculated with S. cremoris were incubated at 25 C and the samples inoculated with S. lactis, S. diacetilactis or L. casei were incubated at 32 C. The incubation temperature of 32 C and 25 C were chosen for convenience within the temperature range which allows active growth of the cultures. In commercial practice milks inoculated with lactic cultures are incubated at temperatures ranging from 21 to 45 C, depending on the organism used and product desired. The optimum growth temperature for S. lactis, S. diacetilactis and L. casei is about 30 C but temperatures of 20 to 25 C are more favorable to Leuconostoc dextranicum and Leuconostoc citrovorum which are used along with the lactic starter cultures.

In one group of experiments methoxychlor was dissolved in butteroil, which was then mixed with non-fat dry milk solids and water, reconstituted into milk and

used as a growth medium. When this milk was inoculated with S. lactis A254 and L. casei there was no effect by methoxychlor on the growth and lactic acid producing ability of the cultures. When the same experiments were performed in cereal milk (half and half), similar results were obtained even at concentrations up to 1000 ppm of methoxychlor in cereal milk.

Chlorinated hydrocarbon insecticides are chemically and physically most stable and persistent among the pesticides. Presently, only slight modification of a few chlorinated hydrocarbon insecticides is known to occur after they are sprayed in commercial application. In spite of the continuous effort, there is little progress in finding a way to remove or destroy the unwanted residual insecticides which pollute human food and environment.

As discussed in the Literature Review, aldrin, DDT, heptachlor, and lindane are the few chlorinated hydrocarbon insecticides which are known to be susceptible to the metabolism of some living organisms. Metabolism of aldrin to dieldrin, and heptachlor to heptachlor epoxide is a toxicative process. Modification of DDT and lindane to their analogs is a detoxication process.

The second part of this experiment was to determine if lactic starter cultures would be able to modify aldrin, DDT or lindane in milk and alter the toxicity of

the insecticides. When five lactic streptococci and L. casei were allowed to grow in the milks containing 1 ppm of aldrin, DDT or lindane for two weeks, none of the organisms modified or degraded aldrin, DDT or lindane This proves that commonly used lactic starter cultures neither help detoxify DDT or lindane in milk, nor enhance the toxicity of aldrin.

Stenerson (1965) observed the conversion of DDT to DDD by Escherichia coli in meat extract bouillon. Mendel and Walton (1966) and Langlois (1967) demonstrated the ability of E. coli to dechlorinate DDT to DDD in TSB but Langlois (1967) found that when E. coli was inoculated into skim milk containing DDT there was little conversion of DDT to DDD. According to the observation by Langlois (1967) milk seems to protect an insecticide from the action of bacteria, even when an organism has the potential to react on the insecticide. Studies on the modification of insecticides by microorganisms have been mainly devoted to DDT in synthetic broth media rather than in food substrate. The lactic starter cultures are Gram-positive, whereas most of the bacteria which have been reported to alter DDT are Gram-negative organisms such as E. coli, Aerobacter aerogenes, Proteus vulgaris, Serratia marcescens, and some species of Pseudomonas, Erwinia, Achromobacter and Xanthomonas. Wedemeyer (1966) and Johnson (1969) suggested that the

cytochrome system in microorganisms is directly responsible for the reductive dechlorination of DDT to DDD. Lactic streptococci and lactobacilli do not have cytochrome systems.

The results of this research, in combination with observations of other investigators, lead to the conclusion that modification or degradation of insecticides in milk by lactic starter cultures is not likely to occur. Subsequently, the effect of the fermentation process on insecticides in milk should be of little concern in assessing a reasonable "tolerance level" for insecticides in milk and in the evaluation of acceptable daily intake.

With the analytical techniques used in this study, 0.01 µg/ml of the insecticides and related analogs could be detected.

Since the growth of L. casei was slightly inhibited by 10 and 100 ppm of dieldrin in milk, uptake of dieldrin by L. casei was studied using ^{14}C-dieldrin. Studying the uptake of ^{14}C-dieldrin by L. casei in milk rather than in TSB might have given better correlation between the results of this part of the work and the results of the study on the effect of dieldrin on L. casei. However, no practical method was available to harvest L. casei cells from coagulated milk samples. When L. casei was grown in TSB containing 0.01 to 0.02 ppm of ^{14}C-dieldrin for 18 hr the amount of ^{14}C-dieldrin retained with the harvested

and washed cells was less than 0.2% of the total ^{14}C-dieldrin in the TSB. In handling radioactive material, several washings do not accomplish complete removal of residual radioactivity. Since less than 0.2% of such a low concentration as 0.01-0.02 ppm of dieldrin was retained with the harvested cells, the count of the radioactivity obtained from the cells could be attributed to the residual radioactivity due to incomplete washing rather than to true uptake of ^{14}C-dieldrin into the cells. The population of the cells was about 10^8 cells/ml.

It is not known why chlorinated hydrocarbon insecticides have little effect on lactic starter cultures and why they were stable against the activity of lactic cultures. From the known information, however, some speculation can be made. Although the mechanism of the toxicity by chlorinated hydrocarbon insecticides is not elucidated yet, these chemicals are known to be nerve poisons. Bacteria are not known to possess a nerve system and there is no experimental evidence to prove that the insecticides have antibacterial activity.

Kostenbauder (1968) explained that (a) the degree of antimicrobial activity of a chemical agent can often be correlated with the rate at which the chemical agent gains access to the site at which it acts and (b) the distribution of antimicrobial agents between aqueous and non-aqueous phases is an important factor.

The hydrophobic nature of chlorinated hydrocarbon insecticides and their negligible solubility in water limit their availability in the aqueous phase of milk. Wettability of bacteria indicates that bacterial cell walls are more hydrophilic than hydrophobic. Actually, when the TSB cultures of S. lactis A62 and L. casei were skaken with hexane and the layers allowed to separate, no organisms were observed in the hexane layer when subjected to microscopic examination. Salton (1964) mentioned in his discussion of "solubility properties" of the bacterial cell wall, that walls of many Gram-positive bacteria are insoluble in many organic solvents including alcohols, ethers and acetone. Salton (1964) also cited that the isolated walls of Streptococcus faecalis and Corynebacterium diphtheriae were insoluble in 90% (w/w) phenol, while the isolated cell wall of E. coli was soluble.

All the lactic starter cultures are Gram-positive and there seems to be little chance for such cultures to stay in the lipid phase in which the insecticides are present.

With high concentrations of insecticides in milk there could be some contact between lactic cultures and insecticides. However, several points should be considered: (a) the cell wall of Gram-positive bacteria contains about 2% lipid compared with about 20% lipid in the cell wall of Gram-negative bacteria (Luria, 1960),

(b) the nature of the cell wall of Gram-positive bacteria as discussed above and (c) the hydrophobic nature of chlorinated hydrocarbon insecticides. In view of these facts an assumption could be drawn that the insecticides would have little access into the cells of lactic starter cultures.

SUMMARY AND CONCLUSIONS

The purpose of the first part of this research was to study the effect of some insecticides on the growth and fermentation ability of the lactic starter cultures. Streptococcus lactis A254, S. lactis A62, Streptococcus cremoris E-8, Streptococcus diacetilactis 18-16 and Lactobacillus casei were grown for 48 hr in sterile whole milks containing 0, 0.1, 1, 10 and 100 ppm of dieldrin, and heptachlor. S. lactis A254 and L. casei were also grown for 48 hr in sterile whole milk containing 0, 0.1, 1, 10 and 100 ppm of methoxychlor and malathion. Observation of viable cell count, pH and per cent lactic acid in the milks at 24 and 48 hr incubation indicated that dieldrin, heptachlor, methoxychlor and malathion had no effect on the growth and lactic fermentation ability of the streptococci tested.

Since all the lactic streptococci were not tested against all the insecticides which can possibly occur in milk, no absolute conclusion can be made concerning the effect of insecticide residues on lactic cultures. However, the results of this work prove that concentrations of insecticides far in excess of the amount normally occurring in milk will not interfere with normal growth

and lactic fermentation ability of streptococci commonly used in the manufacture of cultured dairy products.

Heptachlor, methoxychlor and malathion had no influence on the growth and lactic acid producing ability of L. casei. Dieldrin did not affect the lactic fermenting ability of L. casei but caused slight reduction in viable cell count of L. casei in the milks containing 10 and 100 ppm. However, the reduction in growth of L. casei was very slight and does not seem significant from a practical standpoint. L. casei is seldom used in lactic starter cultures but does have an important role in the ripening of Cheddar and other varieties of cheese.

The second part of this research was to determine if lactic starter cultures would be able to modify aldrin, DDT or lindane in milk and alter the toxicity of the insecticides. When S. lactis A62, S. lactis A254, S. cremoris E-8, S. diacetilactis 18-16, S. diacetilactis DRCI, and L. casei were allowed to grow in the milks containing 1 ppm of aldrin, DDT or lindane for two weeks, none of the organisms modified or degraded aldrin, DDT or lindane. This proves that commonly used lactic starter cultures neither help detoxify DDT or lindane in milk, nor enhance the toxicity of aldrin. Degradation of DDT or lindane is a detoxication process and modification of aldrin and heptachlor to their epoxides is a toxicative process. Among chlorinated hydrocarbon

insecticides, aldrin, DDT, lindane and heptachlor are the few which are known to be susceptible to the metabolism of some living organisms. Thus the results of this research lead to the conclusion that modification or degradation of insecticides in milk by lactic starter cultures are not likely to occur. Consequently the effect of the fermentation process on insecticides in milk should be of little concern in assessing a reasonable tolerance level for insecticides in milk and in the evaluation of acceptable daily intake.

In order to study the possible uptake of dieldrin by L. casei the organism was grown for 18 hr in Trypticase Soy Broth containing 0.01-0.02 ppm of ^{14}C-dieldrin. The harvested and washed cells retained less than 0.2% of the total ^{14}C-dieldrin in the TSB. Such a low level of radioactivity counted from the harvested cells could be attributed to the residual radioactivity due to incomplete washing rather than to true uptake of ^{14}C-dieldrin into the cells.

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APPENDIX

CHEMICAL NOMENCLATURE OF THE INSECTICIDES

Aldrin	1, 2, 3, 4, 10-hexachloro-1,4, 4a, 5, 8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene.
BHC	1, 2, 3, 4, 5, 6-hexachlorocyclohexane consisting of several isomers and containing a specified percentage of gamma isomer.
DDA	2,2-bis(p-chlorophenyl) acetic acid.
DDA (TDE)	1, 1-dichloro-2, 2-bis(p-chlorophenyl)ethane
DDE	1, 1-dichloro-2, 2-bis(p-chlorophenyl)ethane
DDT	1, 1, 10-trichloro-2, 2-bis(p-chlorophenyl) ethane
Dieldrin	1, 2, 3, 4, 10, 1--hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene.
Endrin	1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-endo-5, 8-dimethanonaphthalene.
Heptachlor	1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a, tetrahydro-4, 7-methanoindene.
Heptachlor epoxide	2,3-eopxy-1, 4, 5, 6, 7, 8, 8-heptachloro-hexahydro-4, 7-endomethanoindene.
Lindane	gamma isomer of 1, 2, 3, 4, 5, 6-hexachloro-cyclohexane.

Malathion	S-[1, 2-bix(ethoxycarbonyl)ethyl] O, O-dimethyl phosphorodithioate.
Methoxychlor	1, 1, 1-trichloro-2, 2-bis(p-methoxyphenyl) ethane.

TABLE 2.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of dieldrin as indicated, when the milk was inoculated with Streptococcus lactis A254 and incubated at 32 C.

Incubation time (hr)	ppm of dieldrin added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	71×10^4	53×10^4	51×10^4	64×10^4	71×10^4
24	63×10^7	50×10^7	58×10^7	56×10^7	48×10^7
48	57×10^7	54×10^7	58×10^7	71×10^7	59×10^7
	pH of milk				
0	6.42	6.42	6.42	6.42	6.44
24	5.22	5.22	5.22	5.22	5.22
48	5.02	5.03	5.04	5.04	5.02
	% lactic acid in milk				
0	0.14	0.14	0.14	0.14	0.14
24	0.56	0.57	0.57	0.57	0.58
48	0.69	0.70	0.69	0.68	0.70

TABLE 3.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of dieldrin as indicated, when the milk was inoculated with Lactobacillus casei and incubated at 32 C.

Incubation time (hr)	ppm of dieldrin added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	18×10^4	18×10^4	82×10^3	13×10^4	14×10^4
24	12×10^7	13×10^7	10×10^7	31×10^6	37×10^6
48	27×10^7	24×10^7	20×10^7	20×10^7	13×10^7
	pH of milk				
0	6.48	6.49	6.49	6.49	6.48
24	6.12	6.13	6.12	6.14	6.13
48	5.51	5.50	5.50	5.53	5.53
	% lactic acid in milk				
0	0.14	0.15	0.15	0.15	0.15
24	0.26	0.26	0.26	0.27	0.27
48	0.44	0.45	0.44	0.43	0.43

TABLE 4.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of heptachlor as indicated, when the milk was inoculated with Streptococcus lactis A254 and incubated at 32 C.

Incubation time (hr)	ppm of heptachlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	73×10^4	69×10^4	62×10^4	65×10^4	70×10^4
24	42×10^7	34×10^7	41×10^7	39×10^7	40×10^7
48	48×10^7	47×10^7	63×10^7	61×10^7	65×10^7
	pH of milk				
0	6.45	6.45	6.45	6.54	6.45
24	5.21	5.21	5.22	5.22	5.22
48	5.05	5.05	5.06	5.07	5.06
	% lactic acid in milk				
0	0.15	0.15	0.15	0.15	0.15
24	0.61	0.62	0.61	0.61	0.62
48	0.67	0.67	0.67	0.66	0.68

TABLE 5.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of heptachlor as indicated, when the milk was inoculated with *Lactobacillus casei* and incubated at 32 C.

Incubation time (hr)	ppm of heptachlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	39×10^4	38×10^4	39×10^4	31×10^4	38×10^4
24	11×10^7	11×10^7	92×10^6	96×10^6	11×10^7
48	35×10^7	34×10^7	38×10^7	37×10^7	40×10^7
	pH of milk				
0	6.46	6.47	6.45	6.46	6.42
24	6.14	6.14	6.13	6.13	6.10
48	5.42	5.42	5.42	5.42	5.40
	% lactic acid in milk				
0	0.14	0.14	0.14	0.15	0.16
24	0.21	0.22	0.20	0.20	0.21
48	0.46	0.46	0.46	0.45	0.46

TABLE 6.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of methoxychlor as indicated, when the milk was inoculated with Lactobacillus lactis A254 and incubated at 32 C.

Incubation time (hr)	ppm of methoxychlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	28×10^4	37×10^4	29×10^4	39×10^4	44×10^4
24	40×10^7	40×10^7	43×10^7	39×10^7	35×10^7
48	62×10^7	55×10^7	49×10^7	63×10^7	65×10^7
	pH of milk				
0	6.48	6.48	6.48	6.48	6.48
24	5.02	5.02	5.02	5.02	5.02
48	4.78	4.78	4.78	4.78	4.78
	% lactic acid in milk				
0	0.14	0.14	0.14	0.14	0.14
24	0.58	0.58	0.59	0.58	0.57
48	0.64	0.65	0.65	0.64	0.64

TABLE 7.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of methoxychlor as indicated, when the milk was inoculated with Lactobacillus casei and incubated at 32 C.

Incubation time (hr)	ppm of methoxychlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	24×10^4	25×10^4	23×10^4	27×10^4	27×10^4
24	16×10^7	18×10^7	12×10^7	16×10^7	16×10^7
48	68×10^7	80×10^7	68×10^7	74×10^7	78×10^7
	pH of milk				
0	6.45	6.45	6.45	6.45	6.45
24	5.92	5.92	5.92	5.92	5.92
48	5.06	5.02	5.01	5.01	5.05
	% lactic acid in milk				
0	0.13	0.13	0.13	0.13	0.13
24	0.21	0.22	0.22	0.22	0.21
48	0.49	0.50	0.51	0.51	0.49

TABLE 8.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of malathion as indicated, when the milk was inoculated with Streptococcus lactis A254 and incubated at 32 C.

Incubation time (hr)	ppm of malathion added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	24×10^4	24×10^4	24×10^4	24×10^4	24×10^4
24	16×10^7	17×10^7	16×10^7	17×10^7	15×10^7
48	12×10^7	12×10^7	12×10^7	14×10^7	14×10^7
	pH of milk				
0	6.4	6.4	6.4	6.4	6.4
24	5.0	4.98	4.98	4.98	4.98
48	4.75	4.75	4.78	4.73	4.75
	% lactic acid in milk				
0	0.16	0.16	0.16	0.16	0.16
24	0.58	0.58	0.59	0.58	0.58
48	0.66	0.66	0.65	0.66	0.66

TABLE 9.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of malathion as indicated, when the milk was inoculated with Lactobacillus casei and incubated at 32 C.

Incubation time (hr)	ppm of malathion added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	24×10^4	24×10^4	24×10^4	24×10^4	24×10^4
24	22×10^7	24×10^7	21×10^7	21×10^7	19×10^7
48	26×10^7	34×10^7	38×10^7	28×10^7	24×10^7
	pH of milk				
0	6.5	6.5	6.5	6.5	6.5
24	6.1	6.1	6.1	6.1	6.1
48	5.3	5.3	5.3	5.3	5.3
	% lactic acid in milk				
0	0.14	0.14	0.14	0.14	0.14
24	0.19	0.20	0.20	0.18	0.20
48	0.40	0.42	0.40	0.41	0.41

TABLE 10.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of dieldrin as indicated, when the milk was inoculated with *Streptococcus lactis* A62 and incubated at 32 C.

Incubation time (hr)	ppm of dieldrin added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	86×10^4	98×10^4	96×10^4	87×10^4	88×10^4
24	57×10^7	50×10^7	55×10^7	54×10^7	54×10^7
48	55×10^7	61×10^7	61×10^7	64×10^7	47×10^7
	pH of milk				
0	6.43	6.43	6.43	6.43	6.43
24	5.27	5.25	5.25	5.27	5.26
48	5.01	5.01	5.00	5.00	5.00
	% lactic acid in milk				
0	0.14	0.14	0.14	0.14	0.15
24	0.55	0.55	0.56	0.56	0.55
48	0.63	0.64	0.65	0.64	0.64

TABLE 11.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of dieldrin as indicated, when the milk was inoculated with Streptococcus diacetilactis 18-16 and incubated at 32 C.

Incubation time (hr)	ppm of dieldrin added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	84×10^4	82×10^4	79×10^4	85×10^4	72×10^4
24	51×10^7	55×10^7	48×10^7	57×10^7	57×10^7
48	36×10^7	45×10^7	37×10^7	33×10^7	46×10^7
	pH of milk				
0	6.43	6.44	6.43	6.43	6.44
24	5.44	5.43	5.43	5.44	5.44
48	5.28	5.28	5.28	5.28	5.28
	% lactic acid in milk				
0	0.16	0.16	0.16	0.16	0.16
24	0.50	0.50	0.52	0.51	0.52
48	0.56	0.57	0.56	0.56	0.56

TABLE 12.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of heptachlor as indicated, when the milk was inoculated with Streptococcus lactis A62 and incubated at 32 C.

Incubation time (hr)	ppm of heptachlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	10×10^5	10×10^5	10×10^5	11×10^5	11×10^5
24	61×10^7	72×10^7	70×10^7	71×10^7	60×10^7
48	65×10^7	63×10^7	61×10^7	59×10^7	64×10^7
	pH of milk				
0	6.42	6.42	6.42	6.42	6.42
24	4.80	4.80	4.80	4.80	4.80
48	4.64	4.60	4.63	4.65	4.65
	% of lactic acid in milk				
0	0.14	0.14	0.15	0.15	0.15
24	0.56	0.56	0.55	0.55	0.56
48	0.58	0.59	0.59	0.58	0.59

TABLE 13.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of heptachlor as indicated, when the milk was inoculated with Streptococcus diacetilactis 18-16 and incubated at 32 C.

Incubation time (hr)	ppm of heptachlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	95×10^4	89×10^4	93×10^4	10×10^5	84×10^4
24	65×10^7	77×10^7	60×10^7	72×10^7	70×10^7
48	69×10^7	57×10^7	56×10^7	77×10^7	78×10^7
	pH of milk				
0	6.43	6.43	6.42	6.43	6.43
24	5.47	5.47	5.47	5.45	5.46
48	5.32	5.32	5.32	5.31	5.31
	% lactic acid in milk				
0	0.14	0.14	0.14	0.13	0.14
24	0.49	0.49	0.49	0.50	0.49
48	0.55	0.56	0.54	0.54	0.55

TABLE 14.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of dieldrin as indicated, when the milk was inoculated with Streptococcus cremoris E-8 and incubated at 25 C.

Incubation time (hr)	ppm of dieldrin added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	51×10^4	50×10^4	48×10^4	57×10^7	56×10^4
24	96×10^7	88×10^7	96×10^7	95×10^7	93×10^7
48	15×10^7	12×10^7	14×10^7	16×10^7	13×10^7
	pH of milk				
0	6.44	6.43	6.43	6.42	6.42
24	4.99	4.97	4.98	4.99	4.99
48	4.99	4.98	4.99	4.99	4.99
	% lactic acid in milk				
0	0.15	0.14	0.15	0.14	0.14
24	0.66	0.66	0.66	0.66	0.64
48	0.66	0.67	0.67	0.66	0.66

TABLE 15.--Viable cell count per ml, pH, and per cent lactic acid of whole milk containing various concentrations of heptachlor as indicated, when the milk was inoculated with *Streptococcus cremoris* E-8 and incubated at 25 C.

Incubation time (hr)	ppm of heptachlor added to milk				
	0	0.1	1	10	100
	viable cell count per ml of milk				
0	90×10^4	92×10^4	85×10^4	92×10^4	86×10^4
24	81×10^7	91×10^7	82×10^7	78×10^7	87×10^7
48	61×10^7	48×10^7	44×10^7	68×10^7	62×10^7
	pH of milk				
0	6.42	6.43	6.43	6.41	6.41
24	4.83	4.82	4.83	4.84	4.83
48	4.45	4.45	4.45	4.46	4.46
	% lactic acid in milk				
0	0.13	0.14	0.14	0.13	0.13
24	0.68	0.69	0.68	0.69	0.68
48	0.72	0.72	0.72	0.72	0.71