FATE OF FOODBORNE BACTERIA IN PESTICIDE FORMULATIONS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Tat Yee Guan

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Food Science Department

May 2000

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Fate of Foodborne Bacteria in Pesticide Formulations

BY

Tat Yee Guan

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Tat Yee Guan

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Tat Yee Guan

DEDICATION

To the memory of my grandmother, who has played an influential role in shaping my life, whose beautiful soul and gentle kindness will live in my memory and heart forever.

ACKNOWLEDGEMENTS

First I would like to thank both my advisors, Dr. G. Blank, for his guidance during the entire research and assistance in the completion of this thesis, and Dr. A. H. Ismond, for her patience and on-going concern for my progress in the graduate studies. I would also like to extend a thank you to Dr. R. Van Acker for his participation in my examining committee.

Appreciation is extended to the University of Manitoba Bursary Programs which provided me with financial support.

There are many people in the Department of Food Science whom I would also like to thank. Jim Rogers for his technical support in computers, Randy Roller for his assistance in statistical analysis, Dr. Holley for his expertise and advise, Dr. Sapirstein for his expertise in statistical analysis, Arnie Hydamaka for hiring me as a part-time analyst which provided me financial support, Dr. Arntfield for her genuine encouragement and concern, Georgina Mejia, Aniko Bernatsky, and Amanda Penzick for their friendship and laughter. Thank you all for being there for me.

A heartfelt thanks to my family, Kim Pong Guan, Lee Fong Liew, Tat Jiin Guan, Dennis Guan, and Jonathan Guan. Thank you for your unending love, support, and encouragement from the very beginning of my masters studies. Thank you to my brothers and sister, who have been taking good care of the family while I was pursuing this path. Thank you to my best friend, Kevin Koo, who has been really supportive and caring at times of difficulty and discouragement. Last but certainly not the least, I would like to thank God for so many blessings. Thank you for Your grace, which is always sufficient for me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

The survival of *Escherichia coli* O157:H7, *Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Shigella flexneri,* and *Listeria monocytogenes* in the following pesticide solutions (reconstituted with tap water to mimic field application concentrations): Roundup, Poast & Merge, Gramoxone, Afolan, 2,4-D, Dithane M45, Benlate, Bravo 500, Ridomil 240EC, Thiram 75WP, Sevin XLR+, Lorsban 4E, Diazinon 500, Ambush 500EC, and Lagon 480E, was investigated following exposure for 1 and 24 h at 22°C using hydrophobic grid membrane filtration (HGMF). In general, *E. coli O157:H7, Salmonella spp.*, and *L. monocytogenes* (human isolate) survived and grew in Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC. *L. monocytogenes* (sheep isolate) also survived in Afolan and Bravo 500 but was not recovered in Lorsban 4E and Ambush 500EC. For *S. sonnei*, survival and growth were observed in Afolan, Bravo 500, and Ambush 500EC. In contrast, *S. flexneri* only survived in Afolan and Bravo 500. The growth of *E. coli* O157:H7, *S. typhimurium, S. sonnei*, and *L. monocytogenes* (human isolate) was investigated in Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC, over a 5-day period at 22°C. Highest growth (>10^6 colony forming units per ml; CFU/ml) was observed in Bravo 500 for all bacteria, regardless of inoculum levels (c. 10^2-10^4 CFU/ml). Increasing the incubation temperature from 20-22° to 31°C did not appear to enhance the growth of *E. coli* O157:H7 in Bravo 500. In Afolan, Lorsban 4E and Ambush 500EC, however, growth levels appeared higher at 31° compared to 20-22°C. Reducing the recommended field application concentration by half resulted in enhanced growth of *E. coli* O157:H7 in Afolan, however, no apparent increase was observed in

Bravo 500. In contrast, increasing the recommended field application concentration by 1.5 times resulted in reduced growth of *E. coli* O157:H7 with Afolan. Maximum growth levels in Bravo 500, however, appeared unchanged. In all cases, *E. coli* O157:H7 was shown to survive in Lorsban 4E and Ambush 500EC for 96 h. The pH of the pesticides (7.4-7.9) did not appear to influence the growth potential of the bacteria investigated. Pesticide minimum inhibition concentrations (MIC) relative to *E. coli* O157:H7 were also determined; the highest MIC occurred with Gramoxone. Pesticides in which MIC could not be established included Afolan, Bravo 500, Sevin XLR+, Lorsban 4E, Diazinon 500, and Ambush 500EC. The findings of this study clearly demonstrate that foodborne bacteria can not only survive in pesticide solutions, but also can increase in numbers. Also strain-to-strain differences exist.

INTRODUCTION

Pesticide use in agriculture and horticulture is widely accepted as necessary in the production and conservation of food sources. Unlike fertilizers, pesticides are not used for direct, yield-increasing purposes but only to secure attained production and quality. Indeed, pesticides are widely used to control infection of pests and diseases, and to kill unwanted weeds during the growing seasons and often even after harvest time. This usage can result in the presence of pesticide residues in food at the moment of consumption. For some time, this issue has raised health concerns from a consumer's point of view. Pesticide use has also raised another potential concern. That is, will the practice serve as a vector for the transfer of pathogenic bacteria to crops especially fruits and vegetables if post-emergent pesticides are used?

At the farm, water used for dilution of pesticides originates from rivers, lakes, streams, wells, or even dugouts. The presence of pathogenic microorganisms in such water bodies has been well documented and includes *Escherichia coli* O157:H7 (Isaacson et al., 1993; Kluger, 1998), *Listeria monocytogenes* (Al-Ghazali and Al-Azawi, 1988), *Salmonella typhimurium* (Levine et al., 1991), *Shigella sonnei* (Lindell and Quinn, 1973), *Campylobacter jejuni* (Levine et al., 1991), *Giardia lamblia* (Levine et al., 1991), and *Cryptosporidium parvum* (Smith, 1993; Casamore, 1991). Overall, the presence of microbial pathogens in farm usage waters has increased the awareness of potential health concerns (Dev et al., 1991; Isaacson et al., 1993).

Outbreaks of foodborne illness due to the consumption of fruits and vegetables have increased in recent years. In part this is due to the consumption of raw or minimally processed vegetables. Pathogens that are associated with these outbreaks include *E. coli* O157:H7 in apple cider (Besser et al., 1993), *L. monocytogenes* in cabbage and lettuce (Farber & Peterkin, 1991; Bendig & Strangeways, 1989), *Salmonella spp.* in bean sprouts, cantaloupes and tomatoes (Zhuang et al., 1995; Wei et al., 1995), and *Shigella spp.* in iceberg lettuce and green onions (Kapperud et al., 1995; Beuchat, 1996). There is a high potential for fruits and vegetables to become contaminated with pathogenic microorganisms because of their exposure to a wide variety of conditions during growth, harvest, processing, and distribution. Such conditions and sources of microbial contamination include contaminated irrigation water, human handling, contaminated containers, animal waste fertilizers, wild and domestic animal movement, postharvest washing of produce, improper cooking and/or holding temperature after cooking, improper storage, improper packaging, and contamination from other foods in food preparation areas (Madden, 1992). Earlier, pesticide application was suggested as a possible source of foodborne pathogen contamination of fruits and vegetables. This speculation is based on the fact that various soil and aquifer microorganisms are capable of degrading and growing on pesticides as the sole carbon and energy sources. For example, hydrolysis of the herbicide mecoprop and 2,4-dichlorophenoxyacetic acid by a microbial community comprised of *Pseudomonas spp., Alcaligenes sp., Flavobacterium sp.*, and *Acinetobacter calcoaceticus* has been demonstrated (Lappin et al., 1985).

Since bacterial pathogens are known to contaminate water, it is possible that the application of post emergent pesticides reconstituted with contaminated water could render fresh produce unsafe. Pathogens could either survive and/or reproduce on standing crops (Al-Ghazali & Al-Azawi, 1990) and if proper processing procedures are not followed, pathogens could be recovered on the finished products (Semanchek and Golden, 1996), many of which are consumed with minimal heat treatment.

The overall objectives of the research were: (1) to assess the ability of several foodborne pathogenic bacteria (Gram negative *Enterobacteriaceae: Escherichia coli* O157:H7, *Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Shigella flexneri*, and Gram positive *Listeria monocytogenes*) to survive and/or grow in aqueous pesticide solutions (common application concentration) intended for post emergent spray applications on fruits and vegetables; (2) to examine the effect of temperature and concentration on the growth/survival of *E. coli* O157:H7 in pesticide solutions; (3) to evaluate the relative effectiveness of various pesticides on *E. coli* O157:H7 inhibition via the establishment of minimum inhibitory concentrations (MIC).

LITERATURE REVIEW

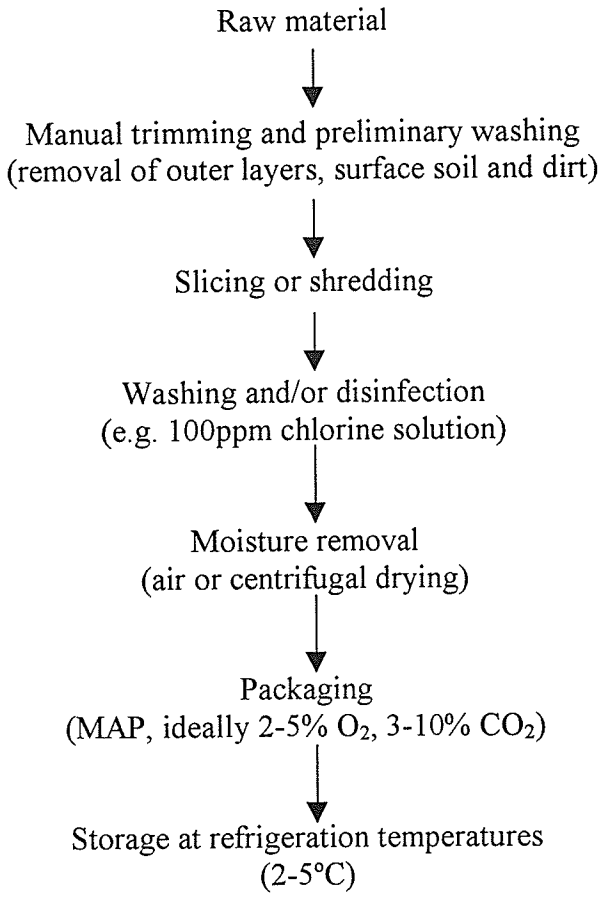
1. Minimally Processed Refrigerated Fruits and Vegetables

Minimally processed refrigerated (MPR) fruits and vegetables are gaining popularity with consumers and represent an expanding segment of the food industry. These ready-to-use (RTU) products are convenient and fresh for consumers. In particular, demand for fresh-cut produce has led to an increase in the quantity and variety of products available in the market. This has resulted in a rapid sales growth in North America over the past seven years, and growth up to 100% was reported in the U.S.A. (Neff, 1994). The sales of these RTU products are expected to comprise 26% of all produce sales in the U.S. in 1999 (Williams, Jr., 1999). Impressive sales in Canada, Europe, Asia, and Australia are transforming RTU products into a global phenomenon.

MPR fruits and vegetables may be simply trimmed produce (e.g. whole lettuce stripped of the outer layers) or may consist of trimmed, peeled, sliced, shredded, washed, or disinfected produce (e.g. dry coleslaw mix) (Francis et al., 1999). This fresh-cut produce may be combined with cooked vegetables, meats, pasta, or salad dressings. The final products are packaged and stored at refrigeration temperatures. A flow diagram for the production of MPR fruits and vegetables is shown in Figure 1.

The first step of production is removal of either outer layers or surface dirt, in order to reduce contamination of the raw products. Next, the fruit or vegetables are

Figure 1. A flow diagram for the production of minimally processed refrigerated fruits and vegetables.



Source: Francis et al. (1999)

sliced or shredded. Sliced or shredded products are thoroughly washed and are often dipped in antimicrobial/disinfectant solutions. This step reduces initial microbial load, thus reducing the rate of subsequent microbial spoilage and minimizing populations of potential pathogens. The washing agent can be water alone, but efficacy of washing is improved by adding antimicrobials, typically chlorine (100ppm) or citric and ascorbic acid (1%) in the wash water (Francis et al., 1999). The final step in minimal processing is removal of excess moisture added during washing, usually by centrifugation (Reyes, 1996).

MPR fruits and vegetables are usually sealed within semi-permeable films. When a sliced fruit or vegetable is packaged, it respires, thus modifying the gas composition inside the package. Ideally, O_2 levels will drop from 21% to 2-5%, and CO_2 levels will rise from 0.03% to 3-10% (O'Beirne, 1996). The gas composition, combined with refrigeration, slow down product respiration, retard microbial growth, delay physiological aging, and thus extend the shelf life of MPR products.

After packaging, MPR fruits and vegetables are stored at refrigeration temperatures (2-5°C). Ideally, modified atmosphere packaging, strict control of storage refrigeration, and a conservatively short shelf life of these fresh products should limit the opportunity for growth by pathogenic and spoilage microorganisms.

2. Pathogenic Bacteria Associated with Fruits and Vegetables

i. *Escherichia coli* O157:H7

a. Characteristics

E. coli is a Gram negative, motile, non-sporeforming rod-shaped bacterium. *E. coli* is a common inhabitant of the lower gastrointestinal tract of mammals (Francis et al., 1999). Despite the non-pathogenic status of the majority of strains, pathogenic serovars such as enterohemorrhagic *E. coli* O157:H7, have emerged as highly significant foodborne pathogens.

E. coli O157:H7 was first isolated from a patient in 1975 but was not identified as a human pathogen until 1982 (Riley et al., 1983). Gastroenteritis and hemorrhagic colitis (HC) malaises caused by this bacterium have classical symptoms of bloody diarrhea, while complications including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) have also been documented (Martin et al., 1986). TTP mainly affects adults while HUS is the leading cause of renal failure and death in young children (Karmali et al., 1983; Griffin & Tauxe, 1991; Erickson et al., 1995). In the United States, *E. coli* O157:H7 is a major cause of HC and HUS (Riley et al., 1983). The microorganism is highly virulent and numbers as low as < 15 organisms, have been reported to cause human infection (Sumner & Peters, 1997). Virulence factors include the production of high levels of cellular cytotoxins and capability of fimbrial adhesion (Varnum & Evans, 1991). Because the cellular cytotoxins are closely related to shiga toxin immunologically and functionally, they are called shiga-like toxins (SLTs). They also have been termed verotoxins because their cytotoxic activity could be demonstrated

in a line of African green monkey kidney cells known as Vero cells (Neill, 1997). Two distinct SLTs are produced, the first SLT-I (VT-1), may be neutralized by anti-shiga toxin, while the second SLT-II (VT-2) is not (Scotland et al., 1985). Most strains of *E. coli* O157:H7 produce either SLT-I and SLT-II, or only SLT-II, but a strain producing only SLT-I has also been described (Smith et al., 1987). SLT-II producing strains predominate in North American, the toxin produced being related, but not identical, to the SLT-II produced by UK isolates (Dickie et al., 1989).

Cattle, particularly calves, seem to be the principal reservoir of *E. coli* O157:H7 (Wells et al., 1991), since most outbreaks of illness have been associated with the consumption of undercooked beef and dairy products (Griffin & Tauxe, 1991). Transmission of the pathogen is principally via the fecal-oral route. The pathogen can be transmitted among humans by personal contacts typical of day-care settings (Sumner & Peters, 1997). Foods may become contaminated by fecal material containing the pathogen or cross-contaminated by soil, food, preparation surfaces, etc (Cieslak et al., 1993). Transmission of the organism by contaminated drinking and swimming water has also been reported (McGowan et al., 1989; Dev et al., 1991).

b. Outbreaks associated with fruits and vegetables

Since it was first recognized as a human pathogen in 1982, enterohemorrhagic *E. coli* O157:H7 has emerged rapidly as a recurrent causative agent in foodborne illnesses, with undercooked ground beef being the principal vehicle (Griffin & Tauxe, 1991; Padhye & Doyle, 1992). Other foods associated with *E. coli* O157:H7 outbreaks include

unpasteurized apple cider (Besser et al., 1993), apple juice (Steele et al., 1982), cantaloupe (Beuchat, 1996), mayonnaise (Erickson et al., 1995), and salad vegetables (Barnett et al., 1995; Wang et al., 1996).

The U.S. Food and Drug Administration (1993) does not regard foods with a pH below 4.6 to be potentially hazardous. Therefore foodborne outbreaks associated with apple cider is of particular concern as cider typically has a pH of 3.5-4.0, due to the presence of malic and lactic acid. In 1980, 13 Canadian children were diagnosed with HUS, which was linked to consumption of apple cider. *E. coli* O157:H7 was believed to be the causative agent because all patients developed bloody diarrhea and abdominal cramps prior to HUS onset (Steele et al., 1982). In the fall of 1991, fresh apple cider was again involved as a vehicle for *E. coli* O157:H7 infection in 18 people in Massachusetts (Besser et al., 1993). In 1996, unpasteurized apple cider and juice were implicated in two outbreaks of *E. coli* O157:H7 infection (CDC, 1996; CDC, 1997).

In 1995, an outbreak of *E. coli* O157:H7 infection among more than 70 people in Montana was associated with the consumption of leafy red, green, and romaine lettuce (Wang et al., 1996). An outbreak in 1994 involving *E. coli* O157:H7 was associated with broccoli as a salad bar item, possibly cross-contaminated with raw ground beef. At the University of Texas, 11 of 26 patients with HC were confirmed to have developed *E. coli* O157:H7 infections after consumption of broccoli (Barnett et al., 1995). In 1992, an outbreak of *E. coli* O157:H7 in Maine was also linked to the consumption of contaminated vegetables (Cieslak et al., 1993). The vegetables were contaminated in a

garden which was fertilized with cow and calf manure. This outbreak involved one woman and 4 children and eventually resulted in one death.

Perhaps one of the largest outbreaks occurred in July, 1996, where approximately 9,451 cases of *E. coli* O157:H7 infection were reported throughout Japan (Hara-Kudo et al., 1997; Bari et al., 1999). Among them, a major cluster occurred in Sakai City and was mainly linked with school lunches. Epidemiological investigation suggested that hydroponically grown radish (*Raphanus sativus L.*) sprouts were the suspect food item. This raised special concern about radish sprout-borne transmission of *E. coli* O157:H7.

c. Growth and survival in fruits and vegetables

Survival of *E. coli* O157:H7 in apple cider has been widely studied (Fisher & Golden, 1998a; Fisher & Golden, 1998b; Ingham & Uljas, 1998; Sage & Ingham, 1998; Semanchek & Golden, 1996; Miller & Kaspar, 1994; Besser et al., 1993; Zhao et al., 1993). The survival of *E. coli* O157:H7 in apple cider is dependent upon factors such as temperature, pH, and preservatives. Refrigeration has been reported to enhance rather than reduce the survival of *E. coli* O157:H7 in acidic foods (Ingham & Uljas, 1998; Zhao et al., 1993). Besser et al. (1993) determined that the survival time of *E. coli* O157:H7 in unpreserved, refrigerated (8°C) apple cider (pH below 4.0) was 20 days. These researchers also found that the addition of preservatives such as sodium benzoate (0.1%) reduced survival to less than 7 days. This finding was supported by Zhao et al. (1993) who reported that *E. coli* O157:H7 survived for 10 to 31 and 2 to 3 days in apple cider stored at 8 and 25°C, respectively. Their studies revealed that potassium sorbate had

minimal effect on the organisms, while survivors in cider containing sodium benzoate were detected for only 2 to 10 days or less than 1 to 2 days at 8 or 25°C, respectively. The authors suggested that the highest rates of inactivation occurred in the presence of a combination of 0.1% sodium benzoate and 0.1% potassium sorbate. However, later studies by Semanchek & Golden (1996) showed that *E. coli* O157:H7 is capable of survival in fresh apple cider for as long as 10 days at 20°C, while alcohol fermentation of fresh cider is an effective means of destroying this pathogen. They believed the inactivation of *E. coli* O157:H7 was attributed to the combined effects of pH and ethanol. Miller & Kaspar (1994) found that the pathogens were detectable in apple cider after 14 to 21 days at 4°C, and that survival was unaffected by the presence of potassium sorbate or sodium benzoate. This finding was supported by Fisher & Golden (1998b) who reported that *E. coli* O157:H7 survived for up to 18 days at 4, 10, and 25°C in unpreserved apple cider. The authors also studied the effects of various preservatives on the survival of the pathogen in apple cider and reported that at 4 and 10°C, 0.025% dimethyl bicarbonate was the most efficient. They also showed that *E. coli* O157:H7 was more resistant to preservatives at 4°C than at 25°C.

Ingham & Uljas (1998) studied the influence of previous storage conditions on the destruction of *E. coli* O157:H7 during heating of apple cider and juice. For example, short-term storage (≤ 6h) at room temperature of pH 3.4 apple cider and apple juice appeared to enhance the lethality of a subsequent pasteurization (61°C) step. Fisher & Golden (1998a) reported that no particular apple cultivar supported survival of *E. coli*

O157:H7. Results of this investigation suggested that *E. coli* O157:H7 was capable of slow growth at 25°C in apple cider when the pH was increased due to mold growth. Although acid adaptation is not a prerequisite for acid tolerance, acid adaptation of *E. coli* O157:H7 affects its survival in foods. Leyer et al. (1995) found that acid-adapted strains of *E. coli* O157:H7 demonstrated enhanced survival in shredded dry salami and apple cider when compared to unadapted cells.

Buchanan et al. (1999) studied *E. coli* O157:H7 contamination of intact apples and showed that internalization of the pathogen (infiltration of bacteria into the interior of apples especially the core region) greatly reduced the effectiveness of surface treatments. Treatment of the apples with 2,000mg/L sodium hypochlorite for 1 min reduced pathogen levels by 1 to 3 Log_{10} CFU/ml but did not eliminate the organisms, particularly from the outer core region. Janisiewicz et al. (1999) demonstrated that *E. coli* O157:H7 can grow at an exponential rate on freshly cut apple tissue. The pathogen has also been shown to grow rapidly in cantaloupe and watermelon cubes stored at 25°C (del Rosario & Beuchat, 1995). Packaging under modified atmosphere had no effect on survival or growth of *E. coli* O157:H7 in shredded lettuce or sliced cucumber (Abdul-Raouf, 1993). Growth occurred at both 12°C and 21°C; however, no growth was observed on shredded carrots held at the same temperatures.

Kusunoki et al. (1997) reported that germinating radish seeds stimulated the growth of *E. coli* O157:H7. Hara-Kudo et al. (1997) studied the contamination of radish sprouts after exposure to *E. coli* O157:H7-inoculated water. Their findings suggested

that *E. coli* O157:H7 in the edible parts of radish sprouts could pose a serious hazard if the seeds or hydroponic water was initially contaminated.

ii. *Salmonella spp.*

a. Characteristics

Salmonella, a genus of the family *Enterobacteriaceae*, is characterized as a Gram-negative, rod-shaped bacterium (Francis et al., 1999). Approximately 2,400 *Salmonella* serotypes are recognized; all are considered pathogenic to animals and humans (Harris et al., 1997). Symptoms of *Salmonella* infection include diarrhea, nausea, abdominal pain, vomiting, mild fever, and chills (ICMSF, 1996). *Salmonella* are mesophiles, with optimum growth temperatures of 35-43°C. Growth rate is substantially reduced at <15°C, while the growth of most *Salmonella* species is prevented at <7°C (Francis et al., 1999). *Salmonella* are facultatively anaerobic.

Animals, especially birds are the natural reservoirs for *Salmonella* (Sumner & Peters., 1997). In fact, they have been isolated from many mammals, poultry, reptiles, fish, amphibians, and insects (Francis et al., 1999). Hence, they are abundant in fecal material, sewage, and sewage-polluted ground water. Consequently, they may contaminate soil and various crops.

Salmonella is an important foodborne pathogen. There are between 40,000 and 60,000 reported cases of human salmonellosis per year world wide (Sumner & Peters, 1997). *Salmonella* spp. are the most frequently reported cause of foodborne outbreaks of

gastroenteritis in the U. S. (Zhuang et al., 1995). It has been difficult to establish an infectious dose for *Salmonella*. It was believed to be as high as 10^8-10^9 cells (LeMinor, 1984), but recent studies have demonstrated that much lower doses, ≤ 50 cells, can cause human infection, depending on the type of food, the virulence of the strain of *Salmonella* in question, and stomach contents of the victims (Doyle & Cliver, 1990).

b. Outbreaks associated with fruits and vegetables

Surveys of fresh produce have revealed the presence of several pathogenic *Salmonella* serotypes (Tamminga et al., 1978; Ercolani, 1976; Garcia-Villanova Ruiz et al., 1987). Tamminga et al. (1978) isolated *Salmonella* from various types of vegetables including eggplant, cauliflower, peppers, endive, and lettuce in the Netherlands. Ercolani (1976) reported that lettuce (68% of 120 samples) and fennel (72% of 89 samples) obtained in Italy yielded *Salmonella* serotypes such as *S. schotkmuelli, S. typhimurium, S. thompson, S. dublia, and S. anatum*. Between 1981 and 1983, Garcia-Villanova Ruiz et al. (1987) detected *Salmonella* in artichoke, beet leaves, celery, cardoon, cabbage, cauliflower, lettuce, parsley, and spinach in Spain. In a survey in Bangkok, Thailand, bean sprouts were also found to contain these pathogens (8.7% of 344 samples) (Jerngklinchan & Saitanu, 1993).

Poultry and other meat products, eggs, and dairy products, are the most commonly implicated food sources in salmonellosis outbreaks (Beuchat, 1996). Although fresh fruits and vegetables are implicated less frequently, outbreaks from these have been documented. Several outbreaks of salmonellosis in the U. S. have involved

fresh fruits, mainly melons. For example, *S. miami* and *S. bareilly* infections were linked to the consumption of precut watermelon in 1955 (Gayler et al., 1955). In more recent outbreaks, *S. oranienburg* and S. *javiana* were implicated with outbreaks associated with the consumption of watermelon (Blostein, 1991). In the U. S., four multistate outbreaks of salmonellosis, each involving 100 to 400 confirmed cases, have been reported with the consumption of fresh fruit and vegetables since 1990 (Wei et al., 1995). The consumption of contaminated cantaloupes was responsible for outbreaks of *S. chester* in 1990 and *S. poona* in 1991, and contaminated raw tomatoes were involved in outbreaks of *S. javiana* in 1990 and *S. montevideo* in 1993. The outbreak of *S. chester* associated with fresh cantaloupe from salad bars involved 30 states and resulted in two deaths (Beuchat, 1996). More than 25,000 individuals were eventually infected. In a survey conducted by the U. S. FDA, low numbers of pathogens were present on the skin of the melons (Ries et al., 1990). The second outbreak attributed to cantaloupe occurred in 15 states in the U.S. and in two Canadian provinces due to *S. poona*, with 241 confirmed cases in both countries. The cantaloupe was again associated with salad bars, thus suggesting that the pathogen can multiply while the cut cantaloupe is displayed in the salad bar.

An outbreak of 143 cases of infection caused by *S. saint-paul* in the UK in 1988 was attributed to raw bean sprouts (O'Mahony et al., 1990). In the same year, a large outbreak of salmonellosis occurred in Sweden and was again associated with the consumption of bean sprouts. The *Salmonellae* isolated from infected patients included *S. saint-paul, S. havana,* and *S. muenchen.* In 1994, an epidemic due to *S.*

bovismorbificans, which originated from Australian alfalfa seeds, was observed concomitantly in Sweden and Finland (Ponka et al., 1995). The total number of infections due to this outbreak in Sweden was 282. This epidemic was the third one due to sprouts contaminated with *Salmonella* in Finland in the 1990's.

c. Growth and survival in fruits and vegetables

An early study by Chung & Goepfert (1970) reported that a pH of 4.05 was the minimum at which *Salmonella* would grow under optimum laboratory conditions. This value was a function of the acid type, temperature, and oxygen supply. Many *Salmonella spp.* have been reported to grow well in fresh fruits and vegetables. Laboratory studies revealed that *S. montevideo* grew rapidly on the surface of whole tomatoes within 7 days and 1 day when stored at 20° and 30°C respectively, and survived at 10°C for 18 days (Zhuang et at., 1995). In chopped tomatoes (pH 4.1 ± 0.1), the pathogen increased significantly after storage for 96 or 22 h at 20° or 30°C respectively, and survived for 9 days at 5°C. Asplund & Nurmi (1991) also showed that *S. enteritidis, S. infantis*, and *S. typhimurium* were able to grow in fresh cut tomatoes (pH 3.99 to 4.37) at 22° and 30°C. Wei et al. (1995) found that stem scars and growth cracks of tomatoes provide protection to *S. montevideo*, i.e. the survival of the pathogen on these areas were greater than that on broken skin. The authors also indicated that treatment with 100 ppm of aqueous chlorine for up to 2 min failed to kill high levels of bacteria (10^8 CFU). Zhuang et al. (1995) recommended that in order to prevent problems, tomato dip tanks should be maintained at a temperature higher than the temperature of the tomatoes and at a free chlorine concentration of 200 ppm.

Salmonella has been associated with several serious outbreaks involving melon products served in salad bars (Ries et al., 1990; CDC, 1979, 1991; Gayler et al., 1955; Blostein et al., 1991). It was speculated that bacteria on the unwashed rind of the melon came in contact with the inferior of the fruit pieces during and after cutting. When the temperature became suitable, the pathogen reproduced (Madden, 1992). Research by Golden et al. (1993) indicated that a mixture of five *Salmonella* serotypes (*S. anatum, S. chester, S. havana, S. poona*, and *S. senftenberg*) grew on pieces of rind-free cantaloupe, watermelon, and honeydew melon at 23°C (respective pH of 6.67, 5.90, and 5.95) resulting in a 5 to 7 log increase in population within 24 h. No growth was recorded at 5°C.

Freshly pressed, unpasteurized apple cider can also harbor *Salmonella spp. S. typhimurium* has been isolated from cider samples that were associated with an outbreak of gastroenteritis (CDC, 1975). Subsequent studies showed that the pathogen grew in some apple juice depending on the variety of apple and the pH (Zhao et al., 1993). An increase of growth occurred at 22°C at a pH of 3.7 to 4.0 but not at a pH of 2.9 to 3.6. However, *Salmonella* could survive in pH 3.6 apple juice at 4°C for more than 30 days.

Escartin et al. (1989) found that *S. derby* and *S. typhi* increased approximately 1 log on sliced jicama, papaya, and watermelon stored at 25-27°C for 6 h. Piagentini et al. (1997) found that *S. hadar* increased by 4 to 6 log cycles on minimally processed

cabbage stored at 12 and 20°C respectively, after 10 days storage. In contrast, the population remained constant at 4°C during the storage time.

iii. *Listeria monocytogenes*

a. Characteristics

Five species of *Listeria* are currently recognized, but only *L. monocytogenes* is pathogenic to humans (Sumner & Peters, 1997). *Listeria monocytogenes* is a small, Gram-positive, rod-shaped bacterium, which is aerobic, motile at room temperature, and hemolytic (Bahk & Marth, 1990). The pathogen causes several diseases and problems in humans including meningitis, septicemia, stillbirths and abortions (ICMSF, 1996). The first reported listeric infections in humans appeared in 1929.

Of particular concern with *Listeria monocytogenes* is its ability to grow at refrigeration temperatures; the minimum temperature for growth is reported to be –0.4°C (Walker & Stringer, 1987). It grows best at 30-37°C (Sumner & Peters, 1997) but it can withstand freezing (Bahk & Marth, 1990). It is facultatively anaerobic; capable of survival and growth under the low O_2 concentrations found within modified atmosphere packages of ready-to-eat vegetables (Francis et al., 1999).

Listeria monocytogenes is considered ubiquitous in nature. It is frequently isolated from a large variety of environmental sources including soil, feces, sewage, silage, manure, water, mud, hay, animal feeds, dust, birds, animals, and humans (Gray & Killinger, 1966; Welshimer, 1968; Al-Ghazali & Al-Azawi, 1990; Nguyen-the & Carlin,

1994; Gunasena et al., 1995). It is also associated with plant materials such as shrubs, wild grasses, corn, cereals, and decaying vegetation (Welshimer & Donker-Voet, 1971). As with *E. coli O157:H7, L. monocytogenes* can be spread by person-to-person contact.

Since *Listeria monocytogenes* occurs widely in soil and in the agricultural environments, it is present naturally on many vegetables. Hence, contamination of vegetables by *L. monocytogenes* may occur through agricultural practices, such as irrigation with polluted water or fertilization using raw manure (Geldreich & Bordner, 1971; Nguyen-the & Carlin, 1994).

b. Outbreaks associated with fruits and vegetables

In 1967, Blenden & Szatalowicz (1967) reported that 731 cases of human listeriosis had been documented between 1933 and 1966 in the U. S. They stated that fresh produce such as lettuce and other fresh vegetables contaminated with *Listeria monocytogenes* may have been responsible for some of these cases. Ho et al. (1986) reported an outbreak of *L. monocytogenes* infection in Boston in 1979 involving 23 patients from 8 hospitals. Case patients preferred three foods (tuna fish, chicken salad, and cheese) more frequently than control patients. The only common foods served with these three foods were raw celery, tomatoes, and lettuce. It was concluded that consumption of these vegetables may have caused the listeriosis outbreak, although the vegetables were not analyzed for the presence of *L. monocytogenes*.

One major reported outbreak in North America occurred in 1981 in the Maritime Provinces (Prince Edward Island, Nova Scotia and New Brunswick) in Canada (Schlech et al., 1983). Thirty-four cases of perinatal listeriosis and seven cases of adult disease were diagnosed. Investigation revealed that the patients were more likely than the controls to have consumed coleslaw. Coleslaw taken from the refrigerator of a patient was positive for *Listeria monocytogenes* serotype 4b, which was the epidemic strain and the same strain isolated from the patient's blood. The coleslaw was commercially prepared with cabbage and carrots that came from local wholesalers and farmers. Two unopened packages of coleslaw obtained from two different local supermarkets yielded *L. monocytogenes* serotype 4b. Both packages came from the same processor. Investigation as to the source of cabbage revealed that one farmer who grew the cabbage also raised sheep. Two of his sheep had died of listeriosis in 1979 and 1981. The farmer used composted and fresh sheep manure to fertilize the fields in which cabbage was grown. After harvest, cabbage was kept in a cold-storage shed from October to early spring before shipment to the coleslaw processor. Since *L. monocytogenes* survives and grows at refrigeration temperatures, this information strongly indicates that the coleslaw was the vehicle of the outbreak.

The strong association of *L. monocytogenes* with vegetables led to several surveys in many countries. The U. S. Food and Drug Administration (Heisick et al., 1989) conducted a survey of 1,000 samples of 10 types of fresh produce at the retail level in the U. S. They found that 21 % of the potatoes, 14% of radishes, and 2% or less of cucumbers and cabbage were contaminated with *L. monocytogenes*. In the UK, Sizmur

& Walker (1988) detected *L. monocytogenes* in 4 of 60 prepackaged, ready-to-eat salads. The contaminated salad contained vegetables such as cabbage, celery, carrots, lettuce, cucumber, onion, leeks, watercress, and fennel. In the Netherlands, Beckers et al. (1989) detected *L. monocytogenes* in 11 of 25 samples of fresh cut vegetables, Harvey & Gilmour (1993) reported that 7 of 66 samples of salad vegetables and prepared salads produced in Northern Ireland contained the organism. Tomatoes and cucumbers surveyed in Pakistan also contained the bacterium (Vahidy, 1992). In Malaysia, Arumugaswamy et al. (1994) found a high percentage of bean sprouts (85%), sliced cucumbers (80%), and leafy vegetables (22.7%) were positive for *L. monocytogenes*. On a more positive side, Farber et al. (1989) did not detect *Listeria spp.* in 110 samples of lettuce, celery, tomatoes, and radishes analyzed in Canada. Likewise, Petran et al. (1988) did not detect *L. monocytogenes* in market samples of fresh and frozen vegetables.

c. Growth and survival in fruits and vegetables

Listeria monocytogenes has been shown to grow well on fresh produce stored at refrigeration temperatures. Connor et al. (1986) reported that *L. monocytogenes* grew in cabbage juice, reaching populations as high as 10^9 colony-forming units per millilitre (CFU/ml). Growth was also reported on asparagus, broccoli, and cauliflower stored at 4°C (Berrang et al., 1989), butternut squash at 4°C (Farber et al., 1998), cabbage at 5°C (Beuchat et al., 1986), chopped and packaged lettuce at 5°C (Steinbruegge et al., 1988; Beuchat & Brackett, 1990b), chicory endive at 6.5°C (Aytac & Gorris, 1994), whole rutabagas, butternut squash, onions, packaged Caesar salad, coleslaw mix, stir fry

vegetables at 10°C (Farber et al., 1998). Controlled-atmosphere storage does not seem to influence growth rates of the pathogen.

Growth of *L. monocytogenes* can occur on the surface of whole tomatoes held at 21°C, and at 10°C, the original population of the inocula remained constant for up to 2 weeks of storage, which is the normal shelf life expectancy (Beuchat & Brackett, 1991). *L. monocytogenes* population levels remained constant on whole rutabagas, onions, packaged Caesar salad, coleslaw mix, and stir fry vegetables at 4°C for 9 days (Farber et al., 1998). On the surface of fresh peeled potatoes packaged under vacuum, *L. monocytogenes* grew at 15°C but not at 4°C (Juneja et al., 1998). Beuchat & Brackett (1990a) reported that *L. monocytogenes* did not grow on raw carrots. In fact, carrot juice appeared to have a lethal effect on *L. monocytogenes* (Beuchat & Brackett, 1990a; Beuchat et al., 1994; Nguyen-the & Lund, 1990) and may serve as a potential sanitizer in processing of fresh produce (Beuchat, 1996). Babic et al. (1997) reported that freeze-dried spinach powder had an inhibitory effect on the growth of *L. monocytogenes*, but the inhibitory effect was greatly reduced when native or intrinsic microorganisms were almost eliminated by heating or irradiation. Their results indicated that if *L. monocytogenes* was present as a contaminant on fresh-cut spinach, its growth would probably be restricted by native microorganisms. Modified-atmosphere has been shown to extend the shelf life of vegetables, however this would allow more time for *L. monocytogenes* to grow (Berrang et al., 1989).

iv. *Shigella spp.*

a. Characteristics

The genus *Shigella* is a Gram negative, non-motile, non-sporeforming rod-shaped bacterium. *Shigella* is composed of four species: *S. sonnei, S. flexneri, S. dysenteriae*, and *S. boydii*; all are pathogenic to humans (Beuchat, 1996). *Shigella* spp. are the causative agents of shigellosis or bacillary dysentery, an illness characterized by frequent passage of stools containing blood and mucus and accompanied by painful abdominal cramps (Sumner & Peters, 1997). *Shigella* was first successfully isolated in 1898, and was shown to be the cause of bacterial dysentery which is distinct from amoebic dysentery. Bacillary dysentery is probably the most communicable bacterial diarrhea due to its low infectious dose (<100 CFU/g) (Sumner & Peters, 1997). This may explain why shigellosis is easily transmitted by personal contact. Secondary attacks or cross contamination can be very serious when introduced into a family or group of institutionalized people (DuPont et al., 1989). Shigellosis is also frequently transmitted by consumption of contaminated water and food.

Although shigellosis infection may be severe, the disease is normally self-limiting in healthy individuals (Bennish et al., 1990). The illness usually persists for up to 2 weeks and the patient recovers. Hence, in healthy individuals, the disease is not life threatening. However in malnourished young children, the weak elderly, and immunocompromised individuals, the illness can be fatal. In developing countries, shigellosis is a major cause of childhood mortality (Bennish et al., 1990). Untreated water supplies, poor sanitation, and overcrowded conditions all give rise to the spread of

shigellosis. The incidence of shigellosis in developed countries is low, mainly due to the widespread availability of sanitary and wastewater treatment facilities (Blaser et al., 1983). Outbreaks of shigellosis still occur in industrialized countries and are caused primarily by *S. sonnei* followed *by S. flexneri*. Most outbreaks of shigellosis occur during summer months (particularly via seafood), although outbreaks still occur throughout the year (Sumner & Peters, 1997).

The primary reasons for the spread of shigellosis in foods are poor personal hygiene of food handlers and abused holding temperatures of contaminated foods. Food and water usually become contaminated by fecal matter from an infected individual. People most frequently infected are those with poor personal hygiene, such as young children in day care centers, persons in nursing homes and mental institutions, and those in lower socio-economic groups who live under overcrowded conditions.

b. Outbreaks associated with fruits and vegetables

Foods implicated in outbreaks of shigellosis are often products that require hand processing or minimal heat treatment before consumption and are delivered fresh to consumers, such as fresh fruits and vegetables. Several large outbreaks of shigellosis have been attributed to the consumption of contaminated produce. Shredded lettuce was responsible for two gastroenteritis outbreaks caused by *S. sonnei* that took place simultaneously at two universities in Texas in 1986 (Martin et al., 1986). Infected students had eaten at self-serve salad bars and shredded lettuce was the only produce used in salads that were consumed by these students. Unlike most outbreaks of shigellosis,

these incidents were due to a contaminated food source and not by a food handler. It was believed the lettuce was contaminated in the field, although the exact point of contamination was not identified at the time of the outbreaks.

In another outbreak, 347 cases of *S. sonnei* gastroenteritis were again associated with eating shredded lettuce (Davis et al., 1988). All implicated restaurants received shredded lettuce from the same produce facility. An investigation revealed that a food handler in the plant was the source of contamination and temperature abuse of the shredded lettuce stored in plastic bags added to the problem.

In the spring of 1994, an increase in the number of domestic cases of *S. sonnei* infection occurred in several European countries, including Norway, Sweden, and the U. K. (Kapperud et al., 1995). In all these countries, epidemiological evidence incriminated imported iceberg lettuce of Spanish origin as the vehicle of transmission. An investigation indicated the contaminated lettuce was consumed from a salad bar, although no *Shigella* could be isolated from the suspected food source. Kapperud et al. (1995) suggested that the iceberg lettuce might have become contaminated in one or more of the following ways: (1) irrigation with inadequately treated sewage effluent or polluted water, (2) fertilization with contaminated sewage sludge or manure, (3) accidental flooding of cropland with polluted water following a period of heavy precipitation.

Shigella flexneri has resulted in two Midwestern U. S. outbreaks that were linked to the consumption of fresh green onions (Cook et al., 1995). The onions were traced to

shippers in California who obtained the green onions from a farm in Mexico. It was concluded that contamination may have taken place at harvest or during packing in Mexico.

In November of 1972, a large waterborne outbreak of shigellosis occurred among 289 students and 25 staff members of a junior high school in Stockport, Iowa (Lindell & Quinn, 1973). The school water supplies were implicated as the source of infection. The water supplies came from three bored wells located nearby the school building. At the time of outbreak, all three wells were found to be improperly covered. It was suspected that sewage from the school and surrounding area might have contaminated the wells.

c. Growth and survival in fruits and vegetables

Shigella spp. can survive in food and water under various environmental conditions. Early reports showed that *Shigella spp.* can survive for 50 days at room temperature (Taylor & Nakamura, 1964), 5-10 days in acidic foods, and 7-14 days in refrigerated, fermented milk (Wilson & Tanner, 1945). Other reports also found that *Shigella spp.* can survive from 3 weeks to 3 months in seafood, milk, cheese, cooking oil, eggs and soda (Sumner & Peters, 1997). Some studies also reported growth and survival of *Shigella spp.* on fruits and vegetables. Escartin et al. (1989) reported that *S. sonnei, S. flexneri*, and *S. dysenteriae* grew rapidly within 4-6 h at 22-27°C when inoculated onto the surface of freshly cut cubes of papaya, jicama, and watermelon. The pH of the produce was 5.69, 5.97, and 6.81, respectively. It was surprising that some strains of *Shigella* are able to tolerate acidic conditions less than pH 6.

Davis et al. (1988) revealed that *Shigella sonnei* can survive on lettuce at 5°C for 3 days without decreasing numbers, at 22°C it increased by more than 1,000-fold. Satchell et al. (1990) reported that *S. sonnei* can survive at refrigeration temperatures in shredded cabbage packaged and stored under a vacuum, modified-atmosphere, and aerobic conditions but decreased after 3 days at room temperature (24 ± 2°C), due to a decrease in pH (≈ 5.0 to 4.1).

A study by Rafii et al. (1995) examined the survival of *Shigella flexneri* on commercially packaged vegetables. Their results showed that the pathogen survived for several days at both ambient and refrigeration temperatures, and that the normal flora of the vegetables did not appear to eliminate the pathogen. Islam et al. (1993) reported that cucumbers can support the growth of *S. flexneri*. The authors also reported a 2-log increase over 6 h at 37°C, however, no reduction in population was observed when the vegetables were stored at 5°C for 72 h.

3. Vectors for Transmission of Pathogens to Fruits and Vegetables

Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields or orchards, or during harvesting, postharvest handling, processing, and distribution (Table 1). Pathogens capable of causing human infection

Table 1. Sources of pathogenic microorganisms on fruits and vegetables and conditions that influence their survival and growth

Preharvest:
Feces
Soil
Irrigation water
Green or inadequately composted manure
Air (dust)
Wild and domestic animals
Human handling
Postharvest:
Feces
Human handling (workers, consumers)
Harvesting equipment
Transport containers (field to packing shed)
Wild and domestic animals
Air (dust)
Wash and rinse water
Sorting, packing, cutting, and further processing equipment
Ice
Transport vehicles
Improper storage (temperature, physical environment)
Improper packaging (includes new packaging technologies)
Cross-contamination (other foods in storage, preparation, and display areas)
Improper display temperature
Improper handling after wholesale or retail purchase

Source: Beuchat (1996)

include bacteria, viruses, and parasites. They may be present in water used for irrigation or in manure used for fertilization or in soil where the produce is grown. Figure 2 illustrates various mechanisms by which fresh produce can become contaminated with pathogenic microorganisms.

i. Use of contaminated manure or sewage sludge as fertilizers

Benefits of using animal waste and sewage sludge for agricultural purposes include the higher nutrient content compared to synthetic fertilizers, and a solution to a waste disposal problem. However, these practices may also become a source of contamination of foodborne pathogens particularly to fresh produce that usually undergoes minimal heat treatment prior to consumption.

Dairy cattle, especially calves, are the principal reservoir of *E. coli* O157:H7. The pathogen is carried in both healthy and sick cattle, and isolation of sick cattle does not reduce the risk of transmission which is principally via the fecal-oral route (Garber et al., 1995). One important characteristic of *E. coli* O157:H7 is that, it may be shed in the feces on an intermittent basis (Wells et al., 1991). Therefore, controlling its spread among cattle is difficult. A survey of feces of dairy cattle in 14 states of the U. S. revealed that 22% of control herds and 50 % of case herds (herds in which *E. coli* O157:H7 was isolated previously) were positive for *E. coli* O157:H7 (Zhao et al., 1995). Populations ranging from $<10^2$ to 10^5 CFU/g of feces were detected in positive calves. Fecal carriage of other pathogenic bacteria by cattle, sheep, poultry and pigs has also been reported. In a Dutch study, *Listeria spp.* was isolated from the feces of 24% of 219 cows aborting due

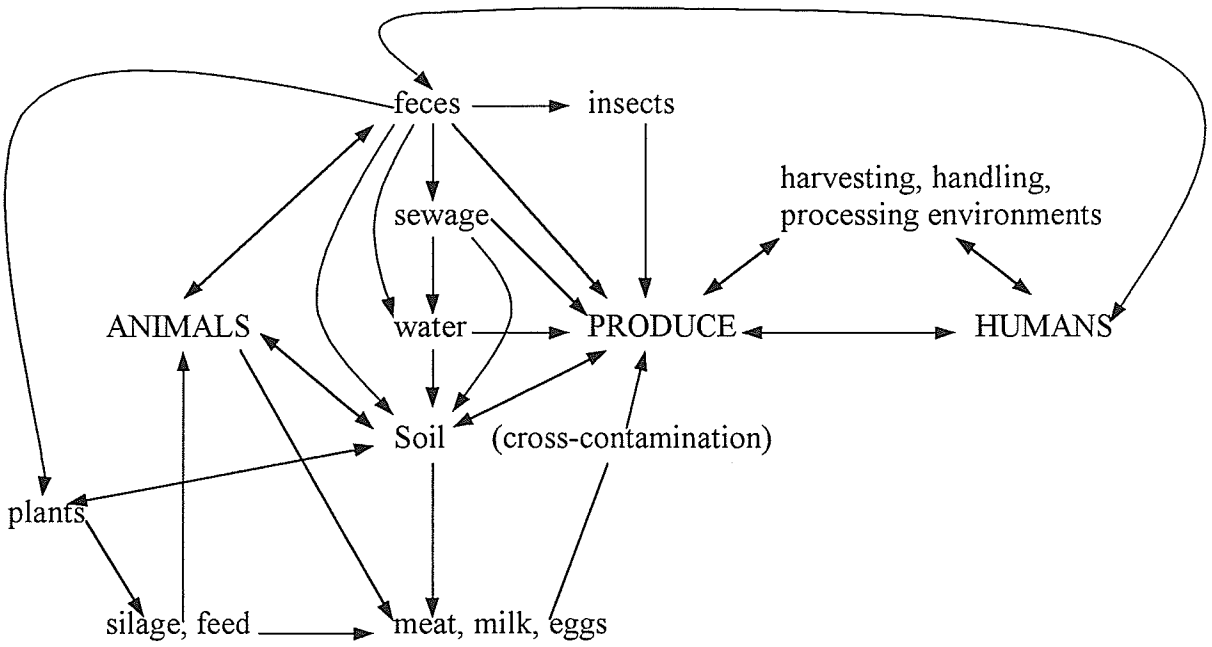


Figure 2. Mechanisms by which fresh fruits and vegetables can become contaminated with pathogenic microorganisms and serve as vehicles of human disease.

Source: Beuchat (1996)

to the pathogens, 14% of 85 cattle with *L. encephalitis*, 7% of 622 healthy cattle from herds with listeriosis (Bojsen-Moller, 1972). With sheep, *Salmonella spp.* were recovered from 4.7% of 192 fecal samples (Nabbut & Al-Nakhli, 1982).

Although the intestinal tracts of cattle have been identified as an important reservoir of *E. coli* O157:H7, there is only one report which pertains to the survival and growth characteristics of this pathogen in bovine feces. Wang et al. (1996) reported that *E. coli* O157:H7 could survive for 42 days and 49 days in bovine feces at 37°C having inocula of 10^2 (low) and 10^5 (high) CFU/g, respectively. The pathogen also survived for 49 and 56 days at 22°C with low and high inocula, respectively, plus 63 and 70 days at 5°C with low and high inocula, respectively. Fecal samples at 22 and 37°C had low moisture contents (about 10%) and a water activity, a_w, less than 0.5 near the end of the sampling periods. It was surprising that *E. coli* O157:H7 could survive and persist at this low a_w. Accelerated death of the pathogen at higher temperatures may in part be due to the reduction of a_w in the feces. Interestingly, survivors at each temperature retained their ability to produce both SLT-I and SLT-II. This study concluded that *E. coli* O157:H7 could survive well in bovine feces for a long period of time, depending on the temperature and perhaps a_w. Therefore, *E. coli* O157:H7-contaminated manure could increase the opportunity for pathogen transmission among cattle as well as produce if present on manure fertilized soil.

Depending on the survival rate of pathogens, animal feces containing foodborne pathogens could be an important source of reinfection in cattle herds and a source of

contamination to the environment and foods. Changes in farm management practices introduced in 1980s such as irrigating pastures and crops with manure slurry may contribute to an environment in which pathogenic microorganisms can persist (Hancock et al., 1994; Garber et al., 1995; Wang et al., 1996).

Alternately, sewage sludge is widely used as an agricultural fertilizer in many countries such as Iraq. Early studies in England and Iraq reported *L. monocytogenes* in sewage and sewage products (Kampelmacher & Van Noorle Jansen, 1974; Fenlon, 1985; Al-Ghazali & A-Azawi, 1986). The organism is also known to survive in sludge for long periods of time. Al-Azawi (1982) showed that *L. monocytogenes* could survive in stored sewage sludge cakes for at least 13 months with minimal death during storage. Later studies (Al-Ghazali & Al-Azawi, 1988) revealed that treatment of sewage did not kill *L. monocytogenes*. The organism was able to survive treatment and was present in low numbers (3-15 cells/g) in the sewage sludge cake. Another study showed that soils treated with sewage sludge cake became contaminated with *L. monocytogenes* (Al-Ghazali & Al-Azawi, 1990). Field experiments found that crops grown on treated soils became contaminated with the pathogen. *L. monocytogenes* was found on 10% of 50 alfalfa plants at harvest, although it was detected only in low numbers ($\leq$ 5 cells/g). The fact that these organisms did not lose virulence during long storage in soil could add to the risk of human health hazards via this route of transmission (Picard-Bonnand et al., 1988).

ii. **Use of reclaimed or polluted water for irrigation**

Water use and conservation in agricultural and food industries are of increasing concern because of increased cost of water and sewage fees that are based on the volume and biological oxygen demand (BOD) of the wastewater produced. To reduce usage and costs, industries are reconditioning wastewater for additional uses (Palumbo et al., 1996). Reconditioned water is currently being used for agricultural and urban landscape irrigation in the U. S. (Miller, 1991; Ramos-Cormenzana et al., 1994). Many food-processing plants plan to increase the use of reconditioned water for initial cleaning of vegetables and scalding water used in meat and poultry processing (Palumbo et al., 1999). In particular, wastewater reclamation was reported by Miller (1991) to be an effective means of decreasing pollution and supplementing available resources.

The occurrence of waterborne pathogens and their potential to survive and grow in reconditioned wastewater used for agricultural purposes is a public health concern. The protozoal parasite *Giardia lamblia* is the most commonly implicated agent in waterborne outbreaks; many of these outbreaks were associated with consumption of chlorinated but unfiltered surface water (Levine et al., 1991). *Shigella sonnei* is the most commonly implicated bacterial pathogen; the outbreaks were associated with water supplies that were contaminated by human waste (Levine et al., 1991). The parasite *Cryptosporidium* caused a large outbreak in Georgia in 1987, affecting 13,000 persons who drank from a chlorinated, filtered public water supply (Hayes et al., 1987). In the U.S., one large multistate outbreak linked to commercially produced ice made from contaminated well water caused illness with Norwalk-like virus among 5,000 persons in 1987 (CDC, 1987).

E. coli O157:H7 was first isolated from a water reservoir in Philadelphia, U. S., in 1986; wild life especially deer was believed to have contaminated the reservoir (McGowan et al., 1989). The pathogen caused a waterborne outbreak in UK in 1990 and the vehicle of transmission was the drinking water supply which was contaminated by cattle slurry (Dev et al., 1991). It also caused a large outbreak of HC in Africa in 1992; in this instance, patients consumed untreated water from rivers and dams that were contaminated by cattle carcasses and dung (Isaacson et al., 1993). In Canada, a study reported a link between gastrointestinal illness and the drinking of private well water contaminated by *E. coli* bacteria (University of Guelph press release, 1997). The study indicated that contamination of wells may result from farm practices and private sewage systems, and the problems could be exacerbated by poorly maintained wells. *Listeria monocytogenes* is also present in aqueous environments such as river waters and sewage sludge (Watkins & Sleath, 1981; Colburn et al., 1990). *Salmonella spp.* survived and were recovered from surface waters (Martinez-Manzanares et al., 1992; Black & Finch, 1993), wastewater (Martinez-Manzanares et al., 1992; Oragui et al., 1993), sea water (Morinigo et al., 1989), and bottled water (Warburton et al., 1994). *Vibrio spp.* was found in drinking water (Black & Finch, 1993; Lee et al., 1982), surface water (DePaola et al., 1984; Lee et al., 1982; Martinez-Manzanares et al., 1992; Motes et al., 1994) and ship ballast, bilge, and sewage (McCathy & Khambaty, 1994). The movement of animal wastes into surface waters is often recognized as a major factor contributing to the pollution of natural waters in many agricultural areas (Fernandez-Alvarez et al., 1991; Niemi & Niemi, 1991).

A preliminary survey showed that reconditioned water contained sufficient nutrients to support bacterial growth (Rajkowski et al., 1996). Laboratory studies indicated that reconditioned water from a meat plant (either nonchlorinated or with the residual chlorine neutralized by the addition of thiosulfate) would support the survival and growth of various Gram-negative bacteria including *Aeromonas hydrophila*, *Salmonella spp.*, and *Vibrio cholerae* (Palumbo et al., 1996; Rajkowski et al., 1996). Survival and growth were temperature dependent. Rajkowski & Rice (1998) indicated that *Pseudomonas aeruginosa* and *E. coli* O157:H7 also exhibit good survival and growth response. Palumbo et al. (1999) investigated the responses of three Gram-positive bacteria, *Enterococcus faecium*, *Staphylococcus aureus*, and *Listeria monocytogenes* in reconditioned water from a meat plant. They found that in contrast to Gram-negative bacteria that grew, the three Gram-positive bacteria survived with some slight increase in number in only nonchlorinated-reconditioned water. These results emphasized the importance of residual chlorine (0.2mg/L) in preventing the growth of these Gram-positive bacteria in potable and reconditioned water.

4. Pesticides

i. Types

Pesticides can be defined simply as "the killer of pests". The word pesticide is a very broad term, which covers a large number of more accurate names (Table 2). In most state and federal laws in the U. S., it is defined as any substance used for controlling, preventing, destroying, repelling, or mitigating any pest (Ware, 1986). Therefore, some groups of chemicals that do not actually kill pests are still included in pesticides, because

Table 2. Pesticide classes and uses.

Pesticide Class	Function
Acaricide	kills mites
Algicide	kills algae
Avicide	kills or repels birds
Bactericide	kills bacteria
Fungicide	kills fungi
Herbicide	kills weeds
Insecticide	kills insects
Larvicide	kills larvae (usually mosquito)
Miticide	kills mites
Molluscicide	kills snails and slugs (may include oysters, clams, mussels)
Nematicide	kills nematodes
Ovicide	destroys eggs
Pediculicide	kills lice (head, body, crab)
Piscicide	kills fish
Predicide	kills predators (usually coyotes)
Rodenticide	kills rodents
Silvicide	kills trees and brush
Slimicide	kills slimes
Termiticide	kills termites
Attractants	attract insects
Chemosterilants	sterilize insects or pest vertebrates (birds, rodents)
Defoliants	remove leaves
Desiccants	speed drying of plants
Disinfectants	destroy or inactivate harmful microorganisms
Feeding stimulants	cause insects to feed more vigorously
Growth regulators	stimulate or retard growth of plants or insects
Pheromones	attract insects or vertebrates
Repellents	repel insects, mites and ticks, or pest vertebrates (dogs, rabbits, deer, birds)

Source: Ware (1986)

they fit the definition. Among these are growth regulators (chemical compounds that stimulate or retard growth of plants and sometimes insects), desiccants (chemical compounds that speed the drying of plants), and defoliants (chemical compounds that remove leaves), etc (Table 2). Of all the groups listed in Table 2, the most widely used in the greatest volume in producing agricultural crops are the herbicides, insecticides, and fungicides. The world agrochemical market sales figures for pesticides in 1995 indicated that herbicides contributed to 48% of total sales, insecticides 28%, and fungicides 19%, and the others 4% (Copping & Hewitt, 1998). Pesticides can also be divided according to their trade name, common name, and family name (based on chemical groups).

Pesticides are biologically active in extremely small quantities, so the chemical has to be prepared in a form that is convenient to use and to distribute evenly over large areas. The pesticide is first manufactured in its relatively pure form, and is referred to as technical grade material. The technical grade is then formulated. Formulation is the processing of a pesticide by any method that will improve its properties of storage, handling, application, effectiveness, and safety (Ware, 1986). The formulated material will be used for direct application, or for dilution prior to application. Table 3 presents the major formulations used in agriculture, structural pest control, and households.

ii. Application methods

Pesticides can be applied in a variety of ways, depending on the type, their mode of action, or even their form. In order for pesticides to be used more efficiently, the actual target needs to be defined in terms of both time (timing of application such as seasons or

Table 3. Common formulations of pesticides[a]

1. Sprays (insecticides, herbicides, fungicides)
   a. Emulsifiable concentrates (also emulsible concentrates)
   b. Water-miscible liquids, sometimes referred to as liquids
   c. Wettable powders
   d. Water-soluble powders, e.g., prepackaged, tank drop-ins, for agricultural and pest control operator use
   e. Oil solutions, e.g., barn and corral ready-to-use sprays, and mosquito larvicides
   f. Soluble pellets for water-hose attachments
   g. Flowable or sprayable suspensions
   h. Flowable microencapsulated suspensions, e.g., Penncap M[R]
   i. Ultralow-colume concentrates (agricultural and forestry use only)
   j. Fogging concentrates, e.g., public health mosquito and fly abatement foggers

2. Dusts (insecticides, fungicides)
   a. Undiluted toxic agent
   b. Toxic agent with active diluent, e.g., sulfur, diatomaceous earth
   c. Toxic agents with inert diluent, e.g., home garden insecticide-fungicide combination in pyrophyllite carrier
   d. Aerosol dust, e.g., silica aerogel in aerosol form

3. Aerosols (insecticides, repellents, disinfectants)
   a. Pushbutton
   b. Total release

4. Granulars (insecticides, herbicides, algicides)
   a. Inert carrier inpregnated with pesticide
   b. Soluble granules, e.g., dry flowable herbicides

5. Fumigants (insecticides, nematicides, herbicides)
   a. Stored products and space treatment, e.g., liquids, gases, moth crystals
   b. Soil treatment liquids that vaporize

6. Impregnates (insecticides, fungicides, herbicides)
   a. Polymeric materials containing a volatile insecticide, e.g., No-Pest Strips[R], pet collars
   b. Polymeric materials containing non-volatile insecticides, e.g., pet collars, adhesive tapes, pet tags, livestock eartags
   c. Shelf papers containing a contact insecticide
   d. Mothproofing agents for woolens
   e. Wood preservatives
   f. Wax bars (herbicides)
   g. Insecticide soaps for pets

7. Fertilizer combinations with herbicides, insecticides, or fungicides

8. Baits (insecticides, molluscicides, rodenticides, and avicides)

9. Slow-release insecticides
   a. Microencapsulated materials for agriculture, mosquito abatement, and household
   b. Paint-on lacquers for pest control operators and home-owners
   c. Interior latex house paints for home use
   d. Adhesive tapes for pest control operators and home-owners
   e. Resin strips containing volatile organophosphate fumigant, e.g., No-Pest Strips^{R}

10. Insect repellents
   a. Aerosols
   b. B. Rub-ons (liquids, lotions, paper wipes, and sticks)

11. Insect attractants
   a. Food, e.g., Japanese beetle traps, ant and grasshopper baits
   b. Sex lures, e.g., pheromones for agricultural and forest pests

12. Animal systemics (insecticides, parasiticides)
   a. Oral (premeasured capsules or liquids)
   b. Dermal (pour-on or sprays)
   c. Feed-additive, e.g., impregnated salt block and feed concentrates

---

[a] This list is incomplete, but it contains most of the common formulations

Source: Ware (1986)

crop stages) and space (location of application in field). This proportion of emitted pesticide that reaches the target, and is in a form available to the pest, must be greatly increased (Matthews, 1992).

The aim of pesticide application is to distribute a small amount of active ingredient to the appropriate biological target with the minimum contamination of non-target organisms. Diversity of the target (insect, plant, soil, walls of dwelling, etc.) necessitates a variety of application techniques which can be summarized into five groups (Matthews, 1985): (1) release or propulsion through the air to the target either in the solid state as dusts or granules, or in the liquid state as sprays, (2) application directly to or injection into the plant, (3) injection into the soil, (4) release into irrigation water, (5) release into the air with diffusion to the target (fumigation). The majority of pesticides are applied as sprays; some are used for seed treatment and soil incorporation (Matthews, 1985). Selection of the application technique may be affected by the mode of action of the pesticide. In the case of insecticides, effectiveness may depend on direct contact, as for a stomach poison, or by having fumigant effects. One example is spraying the insecticide dieldrin to control locusts (Matthews, 1992).

The target for a herbicide can be (1) the weed seed to prevent germination or kill the seedling immediately after the seed germinates, (2) the roots, rhizomes or other underground tissues, (3) the stem, especially when applied to woody plants, (4) the foliage, and (5) the apical shoots (Matthews, 1992). Choice of application techniques will depend not only on the target but also on how easily the herbicide penetrates and it is

translocated in plants (systemic herbicide). Soil and foliar treatments are commonly used for herbicide application (Matthews, 1992). Herbicides can be categorized into pre-plant, pre-emergence, and post-emergence types (Copping & Hewitt, 1998). Pre-plant herbicide is a soil-acting pesticide applied before the crop is sown, sprayed or applied as granules to the soil surface. Pre-emergence herbicides are applied to the soil surface at the time the crop is sown or immediately after sowing, before the crop and weed emerge. Post-emergence herbicides are applied after the emergence of the weed and usually, but not necessarily, the crop as well. Post-emergence herbicides can be applied to the entire crop/weed canopy, otherwise known as an over-the-top application (Copping & Hewitt, 1998). Alternately, herbicides can be directed away from the crop at the weeds, a post-directed application. Localized patches of weeds can be controlled by spot treatment (Copping & Hewitt, 1998).

Fungicides can be applied as foliar sprays, or soil incorporation as a systemic fungicide, or seed treatment (Matthews, 1992). Foliar sprays are widely used to control mildew and scab on deciduous fruit. Seed treatment with fungicide ethirimol is also used to control mildew in barley (Matthews, 1992).

iii. Microbial degradation

After a pesticide is applied, it is subject to volatilization, water movement, sorption in soil, photodegradation, chemical degradation, man-mediated activities, and biodegradation. Biodegradation of chemicals by living organisms is one of the most important mechanisms for the breakdown of organic compounds. In fact,

microorganisms are the most important agents for such degradation (Bayarri et al., 1998). Microbial degradation of pesticides is a very specific and complex process. Rates of pesticide degradation in soil are a function of multiple factors including population densities and activity of pesticide-degrading microorganisms, pesticide bioavailability, and soil properties such as soil-binding, soil temperature, soil moisture content, soil organic matter, soil pH, soil depth, redox condition in soil, and prior application of pesticides (Parkin, 1994). Soil microorganisms have been reported capable of metabolizing pesticides; many utilize the organic compounds as a source of carbon or nitrogen or both. These soil microorganisms include *Pseudomonas spp., Rhodococcus spp., Alcaligenes eutrophus, Arthrobacter spp., Streptomyces spp., Flavobacterium spp., Nocardia spp., Moraxella spp., Desulfobacterium spp., Achromobacter spp., Acinetobacter spp.*, etc (Loos et al., 1967; Sethunathan & Yoshida, 1973; Rajagopal et al., 1984; Lappin et al., 1985; Karns et al., 1986; Chaudhy & Cortez, 1988; Chaudhy & Ali, 1988; Shelton, 1988; Aislabie et al., 1990; Turco & Konopka, 1990; Chapalamadugu & Chaudhry, 1991; Daugherty & Karel, 1994).

Reports pertaining to the microbial degradation of pesticides are common. However, the majority of such investigations tend to be focused on soil or aquatic microorganisms while the activity of microorganisms associated with food has been less well investigated. Peric et al. (1980) observed that diverse *Micrococcus spp.*, isolated from fermented sausages, could degrade the pesticides DDT and methoxychlor after 48 h at 37°C. Spiric et al. (1981; 1983) also isolated some diverse micrococci strains that could significantly degrade DDT and lindane in a nutrient medium. Mirna & Coretti

(1979) found a reduction of between 20 and 30% of lindane, and a degradation of DDT in trypticase soy broth to be the action of a micrococcus strain from a commercial meat starter culture. Sethunathan et al. (1969) also found that a *Clostridium sp.* could carry out anaerobic degradation of both DDT (dichlorodiphenyltrichloroethane) and lindane. Rajagopal et al. (1984) reported that a *Bacillus sp.* was able to degrade carbaryl (1-naphthyl, N-methylcarbamate), a carbamate insecticide widely used in rice fields. When degradation occurs, it is likely to result from enzymatic activity and may either occur immediately or only after a period of adaptation of the microorganism to the chemical. Under ideal conditions, the chemicals may be completely metabolized by one or more members of the microbial ecosystem, but in certain cases they may only be partially degraded to one or more toxic products (Chapalamadugu & Chaudhry, 1991).

Carbaryl is the active ingredient in the insecticide Sevin. It was first introduced in 1957, and is now one of the most widely applied carbamate insecticides (Larkin & Day, 1986). Chapalamadugu & Chaudhry (1991) used two *Pseudomonas* isolates to construct a bacterial consortium that completely catabolized carbaryl to CO_2. The microorganisms utilized these compounds as a sole source of carbon. Larkin & Day (1986) also reported that two *Pseudomonas spp.* and a *Rhodococcus sp.*, isolated from garden soil, could grow on carbaryl as sole carbon and nitrogen source at pH 6.8 but failed to metabolize carbaryl rapidly. At an alkaline pH and in aqueous solution, carbaryl hydrolyses to form 1-naphthol, methylamine, and carbon dioxide. It is more stable at an acid pH. The rate of carbaryl degradation was limited by the rate of chemical hydrolysis between pH 6.0 and

7.0. For many pesticides, hydrolysis is a primary route of degradation (Pappas et al., 1999).

A commonly used herbicide, 2,4-dichlorophenoxyacetate acid (2,4-D), has been found to be actively degraded by several strains of bacteria such as *Alcaligenes spp.*, *Flavobacterium spp.*, and *Arthrobacter spp.* (Loos et al., 1967; Daugherty & Karel, 1994). The herbicide can also be used as a growth regulator of citrus fruits (Orhon et al., 1989). Previous investigations indicated no practical chemical degradation method for 2,4-D and recommended a biological process for its removal from the environment; in fact, 2,4-D is reasonably biodegradable, provided that the medium contains biomass equipped with the necessary complement of enzymatic activity for its breakdown (Talinli, 1986). Orhon et al. (1989) found that 2,4-D remained non-biodegradable following short exposure to a microbial culture. However, biodegradation by the same culture may occur following acclimation of the chemical after 20-40 days. Upon acclimation, 2,4-D is biodegradable as a single substrate or when combined with other nutrient substrates (Orhon et al., 1989).

Degradation of diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate] by microorganisms has been reported in the presence of additional carbon sources and by the synergistic action of an *Arthrobacter sp.* and *Streptomyces sp.* (Sethunathan & Yoshida, 1973). A *Flavobacterium sp.* isolated from rice paddy water also metabolized diazinon as a sole carbon source and completely broke down the compound. This bacterium is also able to degrade the pesticide parathion.

Repeated use of pesticides could lead to enhanced rates of biodegradation and a subsequent loss of pesticide efficacy. Nevertheless, enhanced biological degradation provides an effective alternative to existing remedial technologies for the destruction of organic pollutants in the environment (Alexander, 1999). Increased rates of degradation have been reported for 2,4-D, carbofuran (Turco & Konopka, 1990; Parkin, 1994), some thiocarbamate herbicides, N-methylcarbamate and organophosphate insecticides (Shelton & Parkin, 1991).

I have to this point only discussed microbial degradation of pesticides by soil or aquatic microorganisms. However, no foodborne pathogens have been studied with regard to their ability to degrade pesticides.

Pesticides for agricultural purposes are usually diluted with water before use. At the farm, water sources are often bacteriologically contaminated and include rivers, lakes, streams, wells, or even dugouts. This information has formed the basis for my research: does pesticide use serve as a vector for the transfer of foodborne pathogens to crops, especially fruits and vegetables which usually undergo minimal heat treatment such as the MPR produce? This research is to determine the survival and growth of some pathogenic bacteria in solutions of various commercial pesticide formulations.

MATERIALS AND METHODS

1. Cultures, Growth Conditions, and Inocula Standardization

Escherichia coli O157:H7 strains 7236 (human isolate), 7283 (ground beef isolate), 7174 (bovine isolate), and *Salmonella enteritidis* phage type (PT) 8, were kindly donated by the Laboratory Center for Disease Control, Ottawa, Canada. *Salmonella typhimurium* strain 266 was provided by the University of Manitoba Culture Collection. *Listeria monocytogenes* ATCC strain 19112 (human isolate) and *L. monocytogenes* ATCC strain 19117 (sheep isolate) were obtained from the Food Product Development Center, Portage la Prairie, MB. *Shigella sonnei* 94 and *Shigella flexneri* 93 were provided by the Department of Microbiology Culture Collection, University of Manitoba, Winnipeg, MB.

All bacterial strains were maintained on trypticase soy agar slants (TSA, BBL, Cockeysville, MD) at 4°C. Bacterial cultures were activated by transferring loop inocula into 50 ml of trypticase soy broth (TSB, BBL) at room temperature (c. 20-22°C). Following two consecutive 24-h culture transfers, the cultures were sedimented by centrifugation at 1900 g for 10 min. The supernatants were discarded and the pellets were resuspended in sufficient 0.1% sterile peptone water (Peptone, Sigma, St. Louis, MO) in order to achieve an optical density of 0.6 at 600 nm (Ultrospec 2000, Pharmacia Biotech Inc., Baie d'Urfe, QU). This protocol (standardization) resulted in a bacterial population of approximately 10^8 colony-forming units per ml (CFU/ml) (verified by plate counts using trypticase soy agar following incubation for 24 h at 37°C).

2. Pesticides

A total of 15 pesticide formulations (products) were assessed. These included 5 products from each of: herbicide, insecticide, and fungicide (Table 4). All pesticides were kindly donated by Dr. R. Van Acker, Department of Plant Science, University of Manitoba. The pesticide formulations were diluted using sterile tap water in order to achieve common spray dilution rates (Table 4). All pesticide formulations were stored in the dark at room temperature. Pesticide solutions were freshly prepared prior to each assessment. Pesticide sterility was evaluated by hydrophobic grid membrane filtration (HGMF) as described in the following section. In this respect the pesticide formulation was diluted with 100 ml sterile tap water; 1 ml aliquots were subsequently evaluated using HGMF. Evaluations were performed in duplicate using TSA (24h, 37°C).

3. Short Time Survival

In order to assess the short time survival of each target microorganism in each pesticide solution, the following protocol was followed. Each standardized microbial culture was further diluted using sterile 0.1% peptone and a final 1 ml aliquot (c. 10^3 CFU/ml) was inoculated into a sterile test tube (18x150 mm; Fisher Scientific Co., Nepean, ON) containing 9 ml of a specified pesticide solution (previously diluted; see pesticides section). Tubes were vigorously mixed and statically incubated at room temperature (c. 20-22°C). The final bacterial concentration in each tube was c. 10^2 CFU/ml. Survivors were determined after incubation times of 1 and 24 h using a hydrophobic grid membrane filter (HGMF) technique (AOAC, 1990). This protocol consisted of placing a sterilized HGMF filter (ISO-GRID®, 0.45 μ, QA Life Sciences,

Table 4. Fifteen pesticide products investigated in this research, their common spray dilutions, and the pH values of the pesticide solutions.

Trade name	Company	Common name	Family name	Common spray dilution[a] (v/v* or w/v**)	pH
Herbicides					
Roundup	Monsanto	Glyphosphate	Glyphosphate	0.67%*	5.55
Poast &	BASF	Sethoxydim	Aryloxyphenoxy-propionate	0.26%*	4.34
Merge	BASF	Adjuvant	-	1.00%*	
Gramoxone	Zeneca Agro	Paraquat	Bipyridiniums	1.00%*	7.20
Afolan	AgrEvo	Linuron	Ureas	1.13%*	7.68
2,4-D amine	Interprovincial CO-OP	2,4-D	Phenoxy	1.57%*	7.15
Fungicides					
Dithane M45	Rohm & Haas	Mancozeb	Ethylenebis-dithiocarbamate	6.00 g/L**	7.34
Benlate	DuPont	Benomyl	Benzimadazole	4.67 g/L**	7.88
Bravo 500	Zeneca Agro	Chlorothalonil	Phthalimide	1.00%*	7.97
Ridomil 240EC	NCP[b]	Metalaxyl	Phenylamide	2.30%*	7.71
Thiram 75EP	Gustafson	Thiram	Dialkyl-dithiocarbamate	6.70 g/L**	9.21
Insecticides					
Sevin XLR+	RhonePoulenc	Carbaryl	Carbamate	0.47%*	8.06
Lorsban 4E	Dow Agro	Chlorpyrifos	Organophosphate	0.33%*	7.81
Diazinon 500	Green Cross	Diazinon	Organophosphate	0.73%*	3.70
Ambush 500EC	Zeneca Agro	Permethrin	Synthetic pyrethroid	0.05%*	7.44
Lagon 480E	UAP[c]	Dimethoate	Organophosphate	0.74%*	6.46

[a] These common spray dilutions were suggested by Dr. R. Van Acker, Department of Plant Science, University of Manitoba, according to Anonymous, 1997.
[b] Novartis Crop Protection [c] United Agr Prod

San Diego, CA) on the base of a filtration tower. Sterile peptone water (0.1%) was used to wash the prefilter and filter. One ml sample (culture suspended in pesticide solutions) or sample dilution was pipetted into the peptone water in the upper chamber of the tower. A vacuum was applied to draw the liquid from the upper chamber into the lower chamber of the filtration tower. Another 10-15 ml of sterile peptone water was added to the upper chamber and a vacuum was again applied to draw it through the metal prefilter into the lower chamber. Finally, a vacuum was applied to draw all liquid into the lower chamber of the tower through the ISO-GRID® membrane filter. Following filtration, the membrane filter was placed on the surface of pre-dried Trypticase Soy Fast green Agar (TSAF; 0.025% Fast Green added into TSA; Fast Green FCF, Sigma®, St. Louis, MO) plates by use of sterile forceps. Plates were incubated at 37°C in a thermostatically controlled incubator for 24-48 h. The filters were examined using a Quebec colony counter and counted for squares containing green or blue colonies (AOAC, 1990). The total number of positive squares was scored and converted to most probable numbers (MPN) using the MPN Conversion Table (AOAC, 1990). The MPN was multiplied by an overall dilution factor, if necessary, to obtain the total bacterial count per ml. Short time survival studies were performed using two trials. Each trial was performed in duplicate. The Fast Green, a dye, was incorporated into the agar to improve contrast when counting the colonies in the squares.

4. Growth Response in Pesticide Solutions

Escherichia coli O157:H7 (7236, human isolate), *Salmonella typhimurium* (266), *Listeria monocytogenes* (19112, human isolate), and *Shigella sonnei* (94) were evaluated for growth potential in solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC. These pesticides were chosen for investigation because they appeared to support an increase in population after 24 h of exposure as indicated in the short time survival studies. Each target microorganism was assessed using low (c. 10^2) and high (c. 10^4 CFU/ml) inocula. In this respect a 1 ml culture aliquot (c. 10^4 or 10^6 CFU/ml) was inoculated into a series of 250-ml Erlenmeyer flasks each containing 99 ml of pesticide solution (Table 4). All flasks were vigorously mixed and incubated in the dark at room temperature (c. 20-22°C) without agitation. Growth was evaluated at 0, 24, 48, 72, and 96 h using trypticase soy agar (TSA, BBL) following incubation at 37°C for 24 h. An automated spiral plater (Autoplate® 4000, Spiral Biotech, Bethesda, MD; 50 µl) was used. Counting of plates was assisted using an automated scanner and a computer reader (CASBA™ 4 System combines a high resolution CCD line scanner, Hewlett-Packard Co., and specialized Windows®-based software, CIA-BEN version 2.2, Spiral Biotech, Bethesda, MD). Initial and final pH's of pesticide solutions were determined using a Accumet® pH Meter 910 (Fisher Scientific Co., Nepean, ON).

The growth potential of *Escherichia coli* O157:H7 strain 7236 (low inoculum level) was also evaluated in pesticide solutions reconstituted at 0.5 and 1.5 times the recommended dosage (Table 4); all other remaining conditions were unchanged.

The impact of temperature on the growth of *E. coli* O157:H7 strain 7236 in pesticide solutions was additionally investigated. In this respect a low inoculum level (10^2 CFU/ml) was used in association with a storage temperature (thermostatically controlled incubator) of 31°C; all other remaining conditions were unchanged. All studies were performed using two trials. Each trial was performed in duplicate (n=4). Sterile tap water was used as the control medium for all growth studies.

5. Pesticide Minimum Inhibition Concentration (MIC)

Pesticide MIC relative to *Escherichia coli* O157:H7 strain 7236 (human isolate) was determined using a disc assay diffusion method. Petri dishes (100 x 15 mm) (Fisher Scientific, Canada) containing soft agar (consisting of 0.75% agar in TSB; Agar Granulated, BBL, Cockeysville, MD) poured to yield a layer ≤ 4mm, were surface-inoculated (0.2 ml) with an overnight suspension of *E. coli* O157:H7 (c. 10^9 CFU/ml). Plates were dried for 15 min in a laminar flow hood. Pesticide solutions were loaded (20 µl) by micropipette onto ¼″ sterile blank discs (Difco, Bacto, Detroit, MI) which were previously transferred to inoculated agar surfaces, eight discs per plate. Plates were incubated at 37°C for 24 h. Positive results were recorded at the highest concentration (usually a range) of pesticide that did not show clear inhibition zones. MIC values were established using pesticide increments as shown in Table 15.

6. Statistical Analysis

All experiments with the exception of the MIC study were performed in two trials each carried out in duplicate. All data were analyzed using Statistical Analysis System

software (SAS Institute, Inc., Cary, NC). The General Linear Model Procedure (PROC GLM) was used for the analysis of variance in order to determine significant differences ($p \leq 0.05$). Differences between means were compared using Duncan's Multiple Range Test.

RESULTS

1. Short Time Survival of Pathogens in Pesticide Solutions

i. *Escherichia coli* O157:H7

The short time survival results for *E. coli* O157:H7 strains in various pesticide solutions at room temperature (c. 21-22°C) following 1 and 24 h exposure are presented in Tables 5-7.

In the case of the human isolate (strain 7236), survivor levels in Afolan, 2,4-D, Benlate, Bravo 500, Thiram 75WP, Sevin XLR+, Lorsban 4E, Diazinon 500, and Ambush 500EC were not significantly different from the control after 1 h exposure ($p \leq 0.05$). In contrast, survivor levels in Gramoxone, Poast & Merge, Roundup, Ridomil 240EC, Lagon 480E, and Dithane M45 were significantly lower when compared to the control. No cell recovery was observed in Dithane M45. Over 24 h, population levels increased in some pesticide solutions, however, the majority declined. Pesticide solutions that showed a significant increase in the number of survivors included Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC; the highest survival level was observed in Bravo 500 (increase by more than 4 Log_{10} MPN/ml). Levels of survivors in these four pesticide solutions at 24 h were significantly different from each other, except for Lorsban 4E and Ambush 500EC. Pesticide solutions that contained a significant decrease in survivor levels after 24 h incubation included 2,4-D, Sevin XLR+, and Benlate. No recovery of survivors was observed in Roundup, Poast & Merge, Gramoxone, Dithane M45, Ridomil 240EC, Thiram 75WP, Diazinon 500, and Lagon 480E.

Table 5. Survival of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	Log_{10} (MPN/ml)[1, 2, 3] at time	
Pesticide	1h	24h
Control[4]	$2.05 \pm 0.03^{a}{}_{a}$	$2.05 \pm 0.03^{a}{}_{a}$
Roundup	$0.27 \pm 0.31^{d}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Poast & Merge	$0.51 \pm 0.37^{c}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Gramoxone	$1.34 \pm 0.13^{b}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Afolan	$2.16 \pm 0.07^{a}{}_{b}$	$3.62 \pm 0.23^{b}{}_{a}$
2,4-D	$2.06 \pm 0.03^{a}{}_{a}$	$0.57 \pm 0.11^{d}{}_{b}$
Dithane M45	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Benlate	$2.06 \pm 0.01^{a}{}_{a}$	$0.08 \pm 0.15^{f}{}_{b}$
Bravo 500	$2.14 \pm 0.12^{a}{}_{b}$	$6.76 \pm 0.05^{a}{}_{a}$
Ridomil 240EC	$0.12 \pm 0.24^{d, e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Thiram 75WP	$2.01 \pm 0.01^{a}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Sevin XLR+	$2.04 \pm 0.06^{a}{}_{a}$	$0.32 \pm 0.47^{e}{}_{b}$
Lorsban 4E	$1.90 \pm 0.09^{a}{}_{b}$	$2.49 \pm 0.02^{c}{}_{a}$
Diazinon 500	$2.07 \pm 0.04^{a}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Ambush 500EC	$2.07 \pm 0.04^{a}{}_{b}$	$2.57 \pm 0.10^{c}{}_{a}$
Lagon 480E	$0.08 \pm 0.15^{d, e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 1

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control

Table 6. Survival of *Escherichia coli* O157:H7 strain 7283 (ground beef isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$2.06 \pm 0.02^{a}{}_{a}$	$2.06 \pm 0.02^{a}{}_{a}$
Roundup	$0.08 \pm 0.15^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Poast & Merge	$0.08 \pm 0.15^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Gramoxone	$0.56 \pm 0.49^{c, d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Afolan	$2.13 \pm 0.06^{a}{}_{b}$	$5.81 \pm 0.08^{b}{}_{a}$
2,4-D	$2.07 \pm 0.07^{a}{}_{a}$	$2.04 \pm 0.10^{e}{}_{a}$
Dithane M45	$0.00 \pm 0.00^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Benlate	$2.05 \pm 0.04^{a}{}_{b}$	$3.57 \pm 0.18^{d}{}_{a}$
Bravo 500	$2.13 \pm 0.12^{a}{}_{b}$	$6.09 \pm 0.12^{a}{}_{a}$
Ridomil 240EC	$0.30 \pm 0.35^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Thiram 75WP	$1.09 \pm 0.94^{b, c}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Sevin XLR+	$1.27 \pm 0.62^{b}{}_{a}$	$1.77 \pm 0.06^{f}{}_{a}$
Lorsban 4E	$1.72 \pm 0.08^{a, b}{}_{b}$	$5.67 \pm 0.01^{c}{}_{a}$
Diazinon 500	$0.00 \pm 0.00^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Ambush 500EC	$2.08 \pm 0.09^{a}{}_{b}$	$5.86 \pm 0.09^{b}{}_{a}$
Lagon 480E	$1.14 \pm 1.00^{b, c}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 2

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

Table 7. Survival of *Escherichia coli* O157:H7 strain 7174 (bovine isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$2.05 \pm 0.02^{a}_{a}$	$2.05 \pm 0.02^{a}_{a}$
Roundup	$1.28 \pm 0.36^{b}_{a}$	$0.00 \pm 0.00^{e}_{b}$
Poast & Merge	$1.57 \pm 0.40^{b}_{a}$	$0.38 \pm 0.29^{e}_{b}$
Gramoxone	$0.97 \pm 0.39^{c}_{a}$	$0.00 \pm 0.00^{e}_{b}$
Afolan	$2.01 \pm 0.05^{a}_{b}$	$5.50 \pm 0.09^{b, c}_{a}$
2,4-D	$2.10 \pm 0.08^{a}_{a}$	$1.58 \pm 0.18^{d}_{b}$
Dithane M45	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{e}_{a}$
Benlate	$1.97 \pm 0.02^{a}_{b}$	$5.18 \pm 0.92^{c}_{a}$
Bravo 500	$2.06 \pm 0.10^{a}_{b}$	$5.66 \pm 0.10^{a, b}_{a}$
Ridomil 240EC	$0.25 \pm 0.33^{d}_{a}$	$0.00 \pm 0.00^{e}_{a}$
Thiram 75WP	$1.49 \pm 0.25^{b}_{a}$	$0.00 \pm 0.00^{e}_{b}$
Sevin XLR+	$2.08 \pm 0.04^{a}_{a}$	$1.52 \pm 0.36^{d}_{b}$
Lorsban 4E	$2.02 \pm 0.03^{a}_{b}$	$5.94 \pm 0.03^{a}_{a}$
Diazinon 500	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{e}_{a}$
Ambush 500EC	$2.08 \pm 0.05^{a}_{b}$	$5.90 \pm 0.06^{a, b}_{a}$
Lagon 480E	$0.08 \pm 0.15^{d}_{a}$	$0.00 \pm 0.00^{e}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 3

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

Survivor levels for *E. coli* O157:H7 strain 7283 (ground beef isolate) in pesticide solutions (Table 6) appeared similar to the human isolate with a few exceptions. Unlike the human isolate, survivor levels of this isolate in Thiram 75WP and Sevin XLR+ were significantly lower than the control at 1 h, while no recovery was observed in Diazinon 500 and Dithane M45. After 24 hours of pesticide exposure, the overall survival patterns of this isolate appeared similar to the human isolate. One major difference, however, was observed. Survivor levels in Benlate increased significantly by 24 h, which is in contrast to a significant decrease which was observed with the human isolate. In addition, survival levels for *E. coli* O157:H7 strain 7283 appeared higher than for the human isolate in Afolan, Lorsban 4E, and Ambush 500EC. For all three pesticide solutions, the increase was at least 3.6 Log_{10} MPN/ml as opposed to a 0.5 Log_{10} MPN/ml increase with the human isolate. The highest survivor level was again observed with Bravo 500.

Survivor levels for the bovine strain (7174) of *E. coli* O157:H7 appeared similar to the two previous strains. At 1 h, the pesticide survival pattern of this isolate was similar to the 7283 ground beef isolate, except with Sevin XLR+; with this pesticide solution the survivor number was not significantly different from the control. After an incubation period of 24 h, the survival levels of the 7174 isolate appeared similar to those for 7283, in terms of magnitude of increase when they were present in Afolan, Lorsban 4E, and Ambush 500EC.

ii. Salmonella spp.

The survival levels of *S. typhimurium* and *S. enteritidis* in pesticide solutions maintained at room temperature at 1 and 24 h are presented in Tables 8 and 9.

Overall, *Salmonella typhimurium* appeared to exhibit similar survival patterns in the pesticide solutions when compared to the *E. coli* O157:H7 strains, with some discrepancies. Unlike *E. coli* O157:H7, *S. typhimurium* survivor levels in Benlate and Lorsban 4E at 1 h were significantly lower than the control. No cell recovery was observed with Dithane M45, Diazinon 500, and Lagon 480E. Pesticide solutions in which there was a significant increase (Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC) in *S. typhimurium* levels were similar to those observed with *E. coli* O157:H7; only the magnitude of the increase in bacterial population differed. Bravo 500 contained the highest population (an increase of 5.34 Log_{10} MPN/ml from 1 to 24 h). Unlike *E. coli* O157:H7, no recovery of *S. typhimurium* was observed in Benlate at 24 h.

With regards to *Salmonella enteritidis*, survival patterns appeared less robust. At 1 h, only 2,4-D, Bravo 500, and Sevin XLR+ contained survivor levels similar to the control. In all other pesticide solutions, significantly lower survivor levels were observed. No cell recovery was observed in Poast & Merge, Dithane M45, Benlate, Ridomil 240EC, Diazinon 500, and Lagon 480E. At 24 h, *S. enteritidis* growth was significantly increased in Afolan, Bravo 500, and Ambush 500EC. As for the previous microorganisms examined, highest growth was observed in Bravo 500.

Table 8. Survival of *Salmonella typhimurium* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$2.11 \pm 0.11^{a, b}{}_{a}$	$2.11 \pm 0.11^{a}{}_{a}$
Roundup	$0.63 \pm 0.57^{d}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Poast & Merge	$0.08 \pm 0.15^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Gramoxone	$2.09 \pm 0.04^{a, b}{}_{a}$	$1.24 \pm 0.11^{f}{}_{b}$
Afolan	$2.09 \pm 0.06^{a, b}{}_{b}$	$5.48 \pm 0.06^{b}{}_{a}$
2,4-D	$2.20 \pm 0.08^{a}{}_{a}$	$0.20 \pm 0.24^{g}{}_{b}$
Dithane M45	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Benlate	$0.12 \pm 0.24^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Bravo 500	$2.15 \pm 0.06^{a, b}{}_{b}$	$7.49 \pm 0.17^{a}{}_{a}$
Ridomil 240EC	$0.25 \pm 0.33^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Thiram 75WP	$1.26 \pm 0.06^{c}{}_{a}$	$0.00 \pm 0.00^{g}{}_{b}$
Sevin XLR+	$2.08 \pm 0.06^{a, b}{}_{a}$	$2.11 \pm 0.06^{e}{}_{a}$
Lorsban 4E	$1.88 \pm 0.12^{b}{}_{b}$	$3.09 \pm 0.15^{d}{}_{a}$
Diazinon 500	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$
Ambush 500EC	$2.09 \pm 0.02^{a, b}{}_{b}$	$4.42 \pm 0.08^{c}{}_{a}$
Lagon 480E	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{g}{}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 4

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

Table 9. Survival of *Salmonella enteritidis* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time	
	1h	24h
Control[4]	$2.05 \pm 0.03^{a, b}{}_{a}$	$2.05 \pm 0.03^{a}{}_{a}$
Roundup	$1.91 \pm 0.02^{c}{}_{a}$	$0.61 \pm 0.27^{g}{}_{b}$
Poast & Merge	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$
Gramoxone	$1.68 \pm 0.09^{e}{}_{a}$	$1.29 \pm 0.04^{f}{}_{b}$
Afolan	$2.02 \pm 0.03^{b}{}_{b}$	$4.57 \pm 0.15^{b}{}_{a}$
2,4-D	$2.11 \pm 0.01^{a}{}_{a}$	$0.12 \pm 0.24^{h}{}_{b}$
Dithane M45	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$
Benlate	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$
Bravo 500	$2.09 \pm 0.04^{a}{}_{b}$	$6.22 \pm 0.03^{a}{}_{a}$
Ridomil 240EC	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$
Thiram 75WP	$0.92 \pm 0.08^{g}{}_{a}$	$0.00 \pm 0.00^{h}{}_{b}$
Sevin XLR+	$2.10 \pm 0.03^{a}{}_{a}$	$2.22 \pm 0.02^{d}{}_{b}$
Lorsban 4E	$1.23 \pm 0.05^{f}{}_{b}$	$1.50 \pm 0.19^{e}{}_{a}$
Diazinon 500	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$
Ambush 500EC	$1.85 \pm 0.06^{d}{}_{b}$	$3.47 \pm 0.06^{c}{}_{a}$
Lagon 480E	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{h}{}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 5

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

iii. Shigella spp.

The survival of *S. sonnei* and *S. flexneri* in pesticide solutions at 1 and 24 h at room temperature are presented in Tables 10 and 11.

The survival patterns of *Shigella sonnei* resembled those of *E. coli O157:H7* and *Salmonella spp.* At 1 h of exposure, approximately one half of the pesticide solutions investigated exhibited survivor levels that were significantly lower than the control. No survivors were recovered in Dithane M45, Benlate, and Diazinon 500. After 24 h, significant increases in populations were observed in Afolan, Bravo 500, and Ambush 500EC. Bravo 500 contained the highest increase in population. Similar to *Salmonella spp.*, no survival of *Shigella sonnei* was observed in Benlate at either sampling time.

Overall, *Shigella flexneri* appeared to survive less well in the pesticide solutions compared to *S. sonnei*. At 1 h, only in Afolan and Bravo 500 was there no significant difference in bacterial survivors compared to the control. In all other pesticide solutions there was a significant decrease in bacterial survivors. At 24 h, bacterial survival was observed in Afolan, Bravo 500, Sevin XLR+, and Lorsban 4E. Again, Bravo 500 contained the highest increase in population.

Table 10. Survival of *Shigella sonnei* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$1.81 \pm 0.03^{a}{}_{a}$	$1.81 \pm 0.03^{a}{}_{a}$
Roundup	$1.56 \pm 0.30^{a, b}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Poast & Merge	$0.64 \pm 0.75^{d}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Gramoxone	$1.39 \pm 0.15^{b, c}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Afolan	$1.81 \pm 0.05^{a}{}_{b}$	$5.03 \pm 0.30^{b}{}_{a}$
2,4-D	$1.89 \pm 0.10^{a}{}_{a}$	$1.65 \pm 0.18^{e}{}_{a}$
Dithane M45	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Benlate	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Bravo 500	$1.89 \pm 0.02^{a}{}_{b}$	$5.66 \pm 0.04^{a}{}_{a}$
Ridomil 240EC	$0.15 \pm 0.17^{e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Thiram 75WP	$1.81 \pm 0.02^{a}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$
Sevin XLR+	$1.85 \pm 0.07^{a}{}_{a}$	$1.86 \pm 0.02^{d}{}_{a}$
Lorsban 4E	$1.37 \pm 0.05^{b, c}{}_{a}$	$0.08 \pm 0.15^{f}{}_{b}$
Diazinon 500	$0.00 \pm 0.00^{e}{}_{a}$	$0.00 \pm 0.00^{f}{}_{a}$
Ambush 500EC	$1.78 \pm 0.05^{a}{}_{b}$	$3.89 \pm 0.02^{c}{}_{a}$
Lagon 480E	$1.17 \pm 0.16^{c}{}_{a}$	$0.00 \pm 0.00^{f}{}_{b}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 6

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

Table 11. Survival of *Shigella flexneri* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time	
	1h	24h
Control[4]	$2.02 \pm 0.01^{a}{}_{a}$	$2.02 \pm 0.01^{a}{}_{a}$
Roundup	$1.03 \pm 0.22^{d}{}_{a}$	$0.00 \pm 0.00^{d}{}_{b}$
Poast & Merge	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$
Gramoxone	$0.76 \pm 0.19^{e}{}_{a}$	$0.00 \pm 0.00^{d}{}_{b}$
Afolan	$2.03 \pm 0.02^{a}{}_{a}$	$1.98 \pm 0.01^{b}{}_{a}$
2,4-D	$1.76 \pm 0.05^{b}{}_{a}$	$0.00 \pm 0.00^{d}{}_{b}$
Dithane M45	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$
Benlate	$0.00 \pm 0.00^{h}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$
Bravo 500	$2.03 \pm 0.03^{a}{}_{b}$	$2.44 \pm 0.08^{a}{}_{a}$
Ridomil 240EC	$0.22 \pm 0.29^{g}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$
Thiram 75WP	$0.57 \pm 0.11^{f}{}_{a}$	$0.00 \pm 0.00^{d}{}_{b}$
Sevin XLR+	$1.72 \pm 0.15^{b}{}_{a}$	$1.78 \pm 0.05^{c}{}_{a}$
Lorsban 4E	$1.45 \pm 0.08^{c}{}_{a}$	$0.08 \pm 0.15^{d}{}_{b}$
Diazinon 500	$0.08 \pm 0.15^{g, h}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$
Ambush 500EC	$1.20 \pm 0.17^{d}{}_{a}$	$0.00 \pm 0.00^{d}{}_{b}$
Lagon 480E	$0.08 \pm 0.15^{g, h}{}_{a}$	$0.00 \pm 0.00^{d}{}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 7

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

iv. Listeria monocytogenes

The survival of two strains of *Listeria monocytogenes* in pesticide solutions at room temperature at 1 and 24 h are presented in Tables 12 and 13.

In general, in terms of response in pesticide solutions, the human isolate of *Listeria monocytogenes* behaved similarly to *E. coli O157:H7* and *Salmonella spp*. The majority of pesticide solutions (11 out of 15) evaluated at 1 h contained a significant decrease in survivors compared to the control. Survivor levels in Afolan, 2,4-D, Bravo 500, and Sevin XLR+ were not significantly different compared to the control. Similarly, at 24 h most pesticide solutions contained significantly lower bacterial survivors than the control. Significant increase in growth of *L. monocytogenes* was observed in Afolan, Bravo 500 (the highest), Lorsban 4E, and Ambush 500EC. Similar to *Salmonella enteritidis*, this strain of *L. monocytogenes* was not recovered in Benlate at both sampling times.

In the case of the sheep isolate, the survival pattern differed. After 1 h of incubation, no survivors could be detected in Roundup, Thiram 75WP, Lorsban 4E and Ambush 500EC. Survivor levels in Sevin XLR+, Bravo 500, and Afolan were not significantly different from the control. After 24 h, survival/growth was only observed in Afolan, Bravo 500 (the highest), and Sevin XLR+. Significant increases in population (from 1 to 24 h) were only observed in Afolan and Bravo 500 (increase of c. 1 Log_{10} MPN/ml). No cell recovery was obtained in the remaining pesticide solutions.

Table 12. Survival of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$2.09 \pm 0.05^{a}_{a}$	$2.09 \pm 0.05^{a}_{a}$
Roundup	$0.20 \pm 0.24^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Poast & Merge	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Gramoxone	$1.88 \pm 0.07^{b}_{a}$	$1.38 \pm 0.09^{f}_{b}$
Afolan	$2.13 \pm 0.12^{a}_{b}$	$6.64 \pm 0.08^{b}_{a}$
2,4-D	$2.17 \pm 0.05^{a}_{a}$	$0.00 \pm 0.00^{g}_{b}$
Dithane M45	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Benlate	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Bravo 500	$2.16 \pm 0.04^{a}_{b}$	$7.49 \pm 0.05^{a}_{a}$
Ridomil 240EC	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Thiram 75WP	$0.79 \pm 0.37^{c}_{a}$	$0.00 \pm 0.00^{g}_{b}$
Sevin XLR+	$2.16 \pm 0.04^{a}_{a}$	$2.24 \pm 0.05^{e}_{a}$
Lorsban 4E	$1.88 \pm 0.16^{b}_{b}$	$3.68 \pm 0.15^{c}_{a}$
Diazinon 500	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$
Ambush 500EC	$1.71 \pm 0.14^{b}_{b}$	$3.47 \pm 0.16^{d}_{a}$
Lagon 480E	$0.00 \pm 0.00^{d}_{a}$	$0.00 \pm 0.00^{g}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 8

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

Table 13. Survival of *Listeria monocytogenes* strain 19117 (sheep isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	Log_{10} (MPN/ml)[1, 2, 3] at time 1h	24h
Control[4]	$2.56 \pm 0.02^{a}_{a}$	$2.56 \pm 0.02^{a}_{a}$
Roundup	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Poast & Merge	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Gramoxone	$0.76 \pm 0.56^{b}_{a}$	$0.00 \pm 0.00^{c}_{b}$
Afolan	$2.50 \pm 0.06^{a}_{b}$	$3.57 \pm 0.20^{a}_{a}$
2,4-D	$0.99 \pm 0.27^{b}_{a}$	$0.00 \pm 0.00^{c}_{b}$
Dithane M45	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Benlate	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Bravo 500	$2.59 \pm 0.04^{a}_{b}$	$3.52 \pm 0.21^{a}_{a}$
Ridomil 240EC	$0.22 \pm 0.29^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Thiram 75WP	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Sevin XLR+	$2.42 \pm 0.11^{a}_{a}$	$2.55 \pm 0.01^{b}_{a}$
Lorsban 4E	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Diazinon 500	$0.08 \pm 0.15^{c}_{a}$	$0.08 \pm 0.15^{c}_{a}$
Ambush 500EC	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$
Lagon 480E	$0.00 \pm 0.00^{c}_{a}$	$0.00 \pm 0.00^{c}_{a}$

[1] Results are means of two trials each performed in duplicate (n=4) ± standard deviations of means; refer to appendix 9

[2] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[3] Results followed by the same subscript for each treatment are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

[4] Sterile tap water as control.

2. Pesticide Minimum Inhibitory Concentrations relative to *E. coli* O157:H7

The pesticide minimum inhibitory concentrations (MIC) for *E. coli* O157:H7 strain 7236 (human isolate) are presented in Table 14.

Afolan, Bravo 500, Sevin XLR+, Lorsban 4E, Diazinon 500, and Ambush 500EC did not inhibit this particular strain of *E. coli* O157:H7, even when undiluted pesticide formulations were used. Among the pesticides examined, Gramoxone appeared to have the highest MIC for *E. coli* O157:H7 strain 7236 (human isolate).

Table 14. Minimum inhibitory concentration (MIC) range of pesticide solutions against *E. coli* O157:H7 strain 7236 (human isolate).

Pesticide	Formulation type[e]	MIC range	MIC determined using increments of:
Roundup	SL	1:3 to 1:4[a]	per 1 ml
Poast & Merge	EC	1:4 to 1:7[b]	per 1 ml
Gramoxone	SL	1:2200 to 1:2400[c]	per 100 ml
Afolan	SC	-[a, d]	
2,4-D amine	SL	1:13 to 1:17[b]	per 1 ml
Dithane M45	WP	1 g/L to 5 g/L[c]	per 1 g/L
Benlate	WP	3 g/L to 5 g/L[c]	per 2 g/L
Bravo 500	SC	-[a, d]	
Ridomil 240EC	EC	1:8 to 1:10[c]	per 1 ml
Thiram 75WP	WP	0.17 g/L to 0.22 g/L[c]	per 0.05 g/L
Sevin XLR+	SC	-[a, d]	
Lorsban 4E	EC	-[a, d]	
Diazinon 500	EC	-[a, d]	
Ambush 500EC	EC	-[a, d]	
Lagon 480E	EC	1:46 to 1:51[b]	per 1 ml

[a] Minimum of 3 trials with each trial performed in duplicate (minimum of n=6)
[b] Minimum of 5 trials with each trial performed in duplicate (minimum of n=10)
[c] Minimum of 10 trials with each trial performed in duplicate (minimum of n=20)
[d] No inhibition observed with undiluted pesticide formulation
[e] SL-water soluble liquid; EC-emulsifiable concentrate; SC-suspension concentrate; WP-wettable powder

3. Time-Course Growth Patterns in Pesticide Solutions

i. *Escherichia coli* O157:H7

Growth profiles for *E. coli* O157:H7 (human isolate) in pesticide solutions at 20-22°C using a high inoculum level are shown in Figure 3. After 96 h of incubation, growth levels in all pesticide solutions exceeded 10^6 CFU/ml. In the case of Bravo 500, growth was significantly higher than in any of the other pesticide solutions especially during the first 24 h of incubation. Growth profiles of *E. coli* O157:H7 in both Lorsban 4E and Ambush 500EC appeared similar to each other.

When the inoculum level was decreased to 10^2 CFU/ml, the population of *E. coli* O157:H7 in Bravo 500 was still the highest, followed by Afolan (Figure 4). Interestingly, the final population exceeded 10^7 CFU/ml at 96 h in Bravo 500 despite usage of a reduced initial inoculum, ostensibly due to excellent growth promoting characteristics of Bravo 500. Growth levels of *E. coli* O157:H7 in Lorsban 4E and Ambush 500EC up to 48 h appeared similar to the control. After 48 h, the growth rates in both pesticide solutions appeared to increase more rapidly.

Figure 3. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions at 22°C using a high inoculum. See appendix 10.

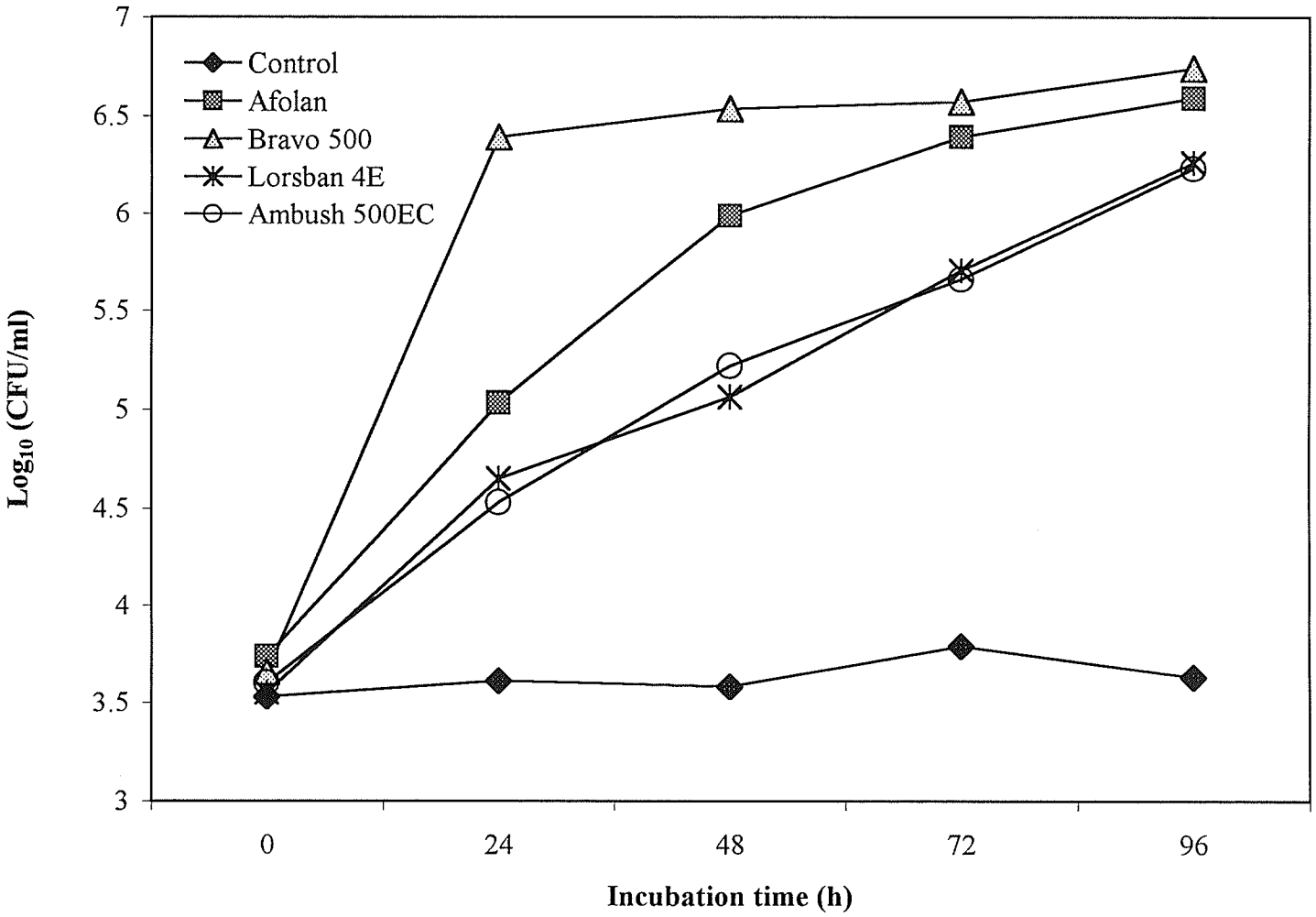
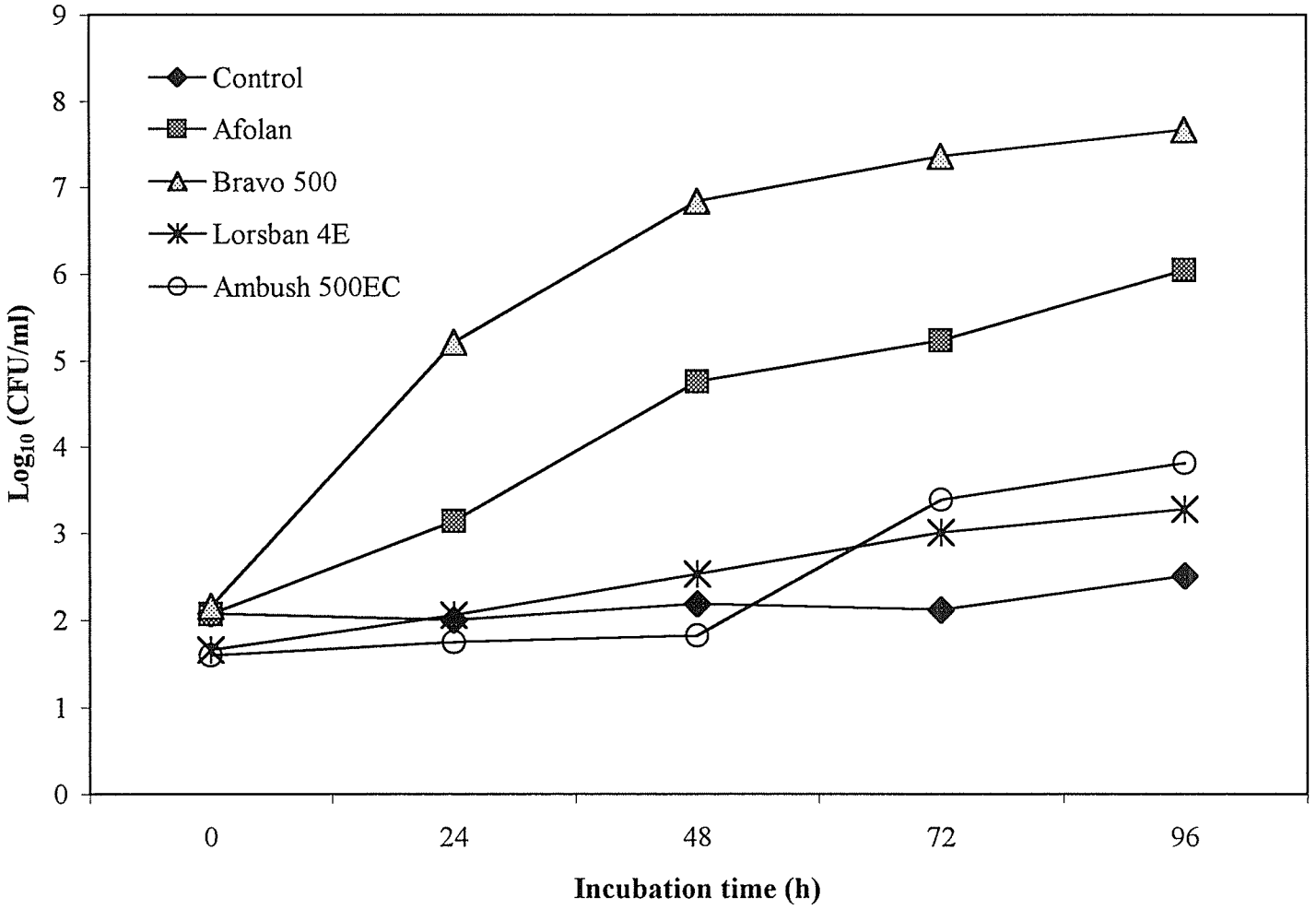


Figure 4. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions at 22°C using a low inoculum. See appendix 11.



ii. *Salmonella typhimurium*

The growth of *S. typhimurium* strain 266 in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC is presented in Figures 5 and 6.

At high initial inocula (10^3-10^4 CFU/ml), salmonellae growth appeared to increase rapidly in Afolan, Bravo 500, and Ambush 500EC, especially during the initial 48 h period. At 96 h, populations were highest (>10^6 CFU/ml) in Afolan and Ambush 500EC. In Bravo 500, salmonellae population increased by 2 Log_{10} CFU/ml within the first 24 h of growth and thereafter remained somewhat constant, a similar phenomenon was also observed for *E. coli* O157:H7 at high inocula.

When initial inoculum was decreased, the salmonellae population still increased relatively rapid especially in Bravo 500, Afolan, and Lorsban 4E. At 96 h of incubation, salmonellae levels were greater than 10^6 CFU/ml in Bravo 500, Afolan, and Lorsban 4E. Highest population levels were achieved in Bravo 500.

Figure 5. Growth of *Salmonella typhimurium* in pesticide solutions at 22°C using a high inoculum. See appendix 12.

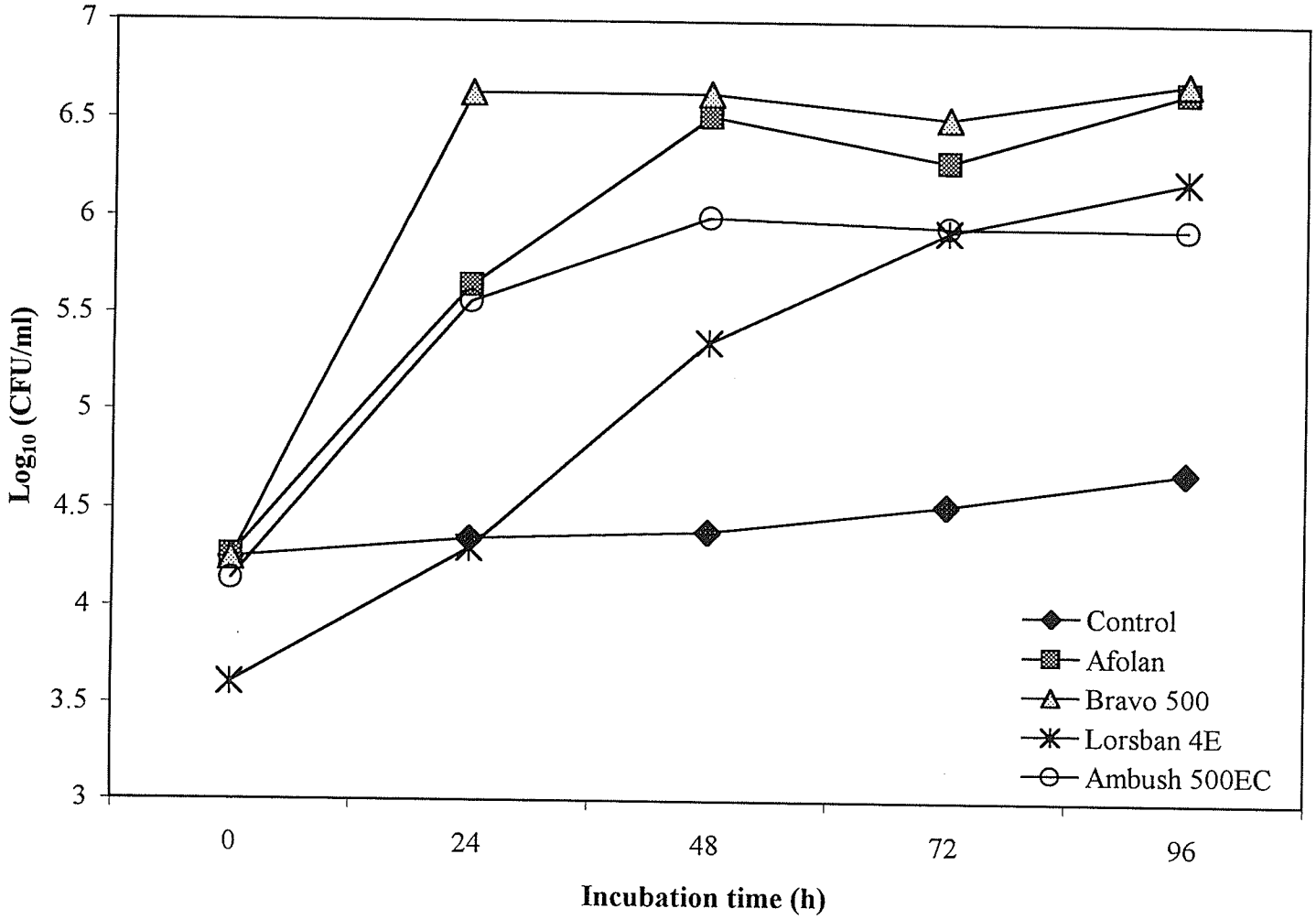
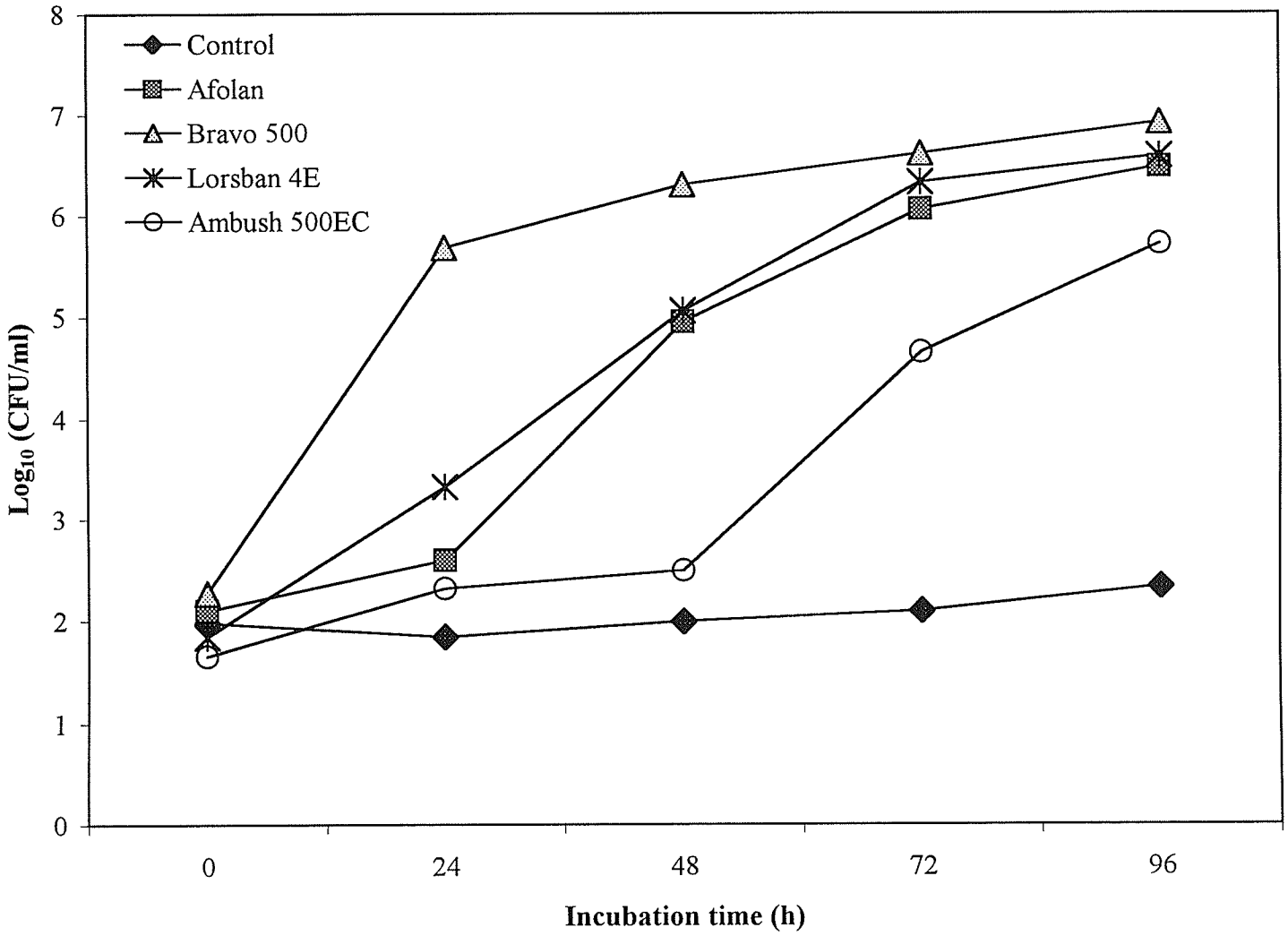


Figure 6. Growth of *Salmonella typhimurium* in pesticide solutions at 22°C using a low inoculum. See appendix 13.



iii. *Shigella sonnei*

The growth of *S. sonnei* in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC at two initial inoculum levels is presented in Figures 7 and 8.

At a high inoculum (10^4 CFU/ml), shigellae levels at 96 h were significantly higher in Bravo 500 and Afolan than in Ambush 500EC and Lorsban 4E. Bacterial populations reached 10^6 CFU/ml in all pesticide solutions by 72 h. At 96 h, the highest population was obtained in Bravo 500.

At low inoculum levels (10^1-10^2 CFU/ml), similar growth profiles were observed. However in Afolan, shigellae growth appeared stationary for the first 48 h of incubation.

Figure 7. Growth of *Shigella sonnei* in pesticide solutions at 22°C using a high inoculum. See appendix 14.

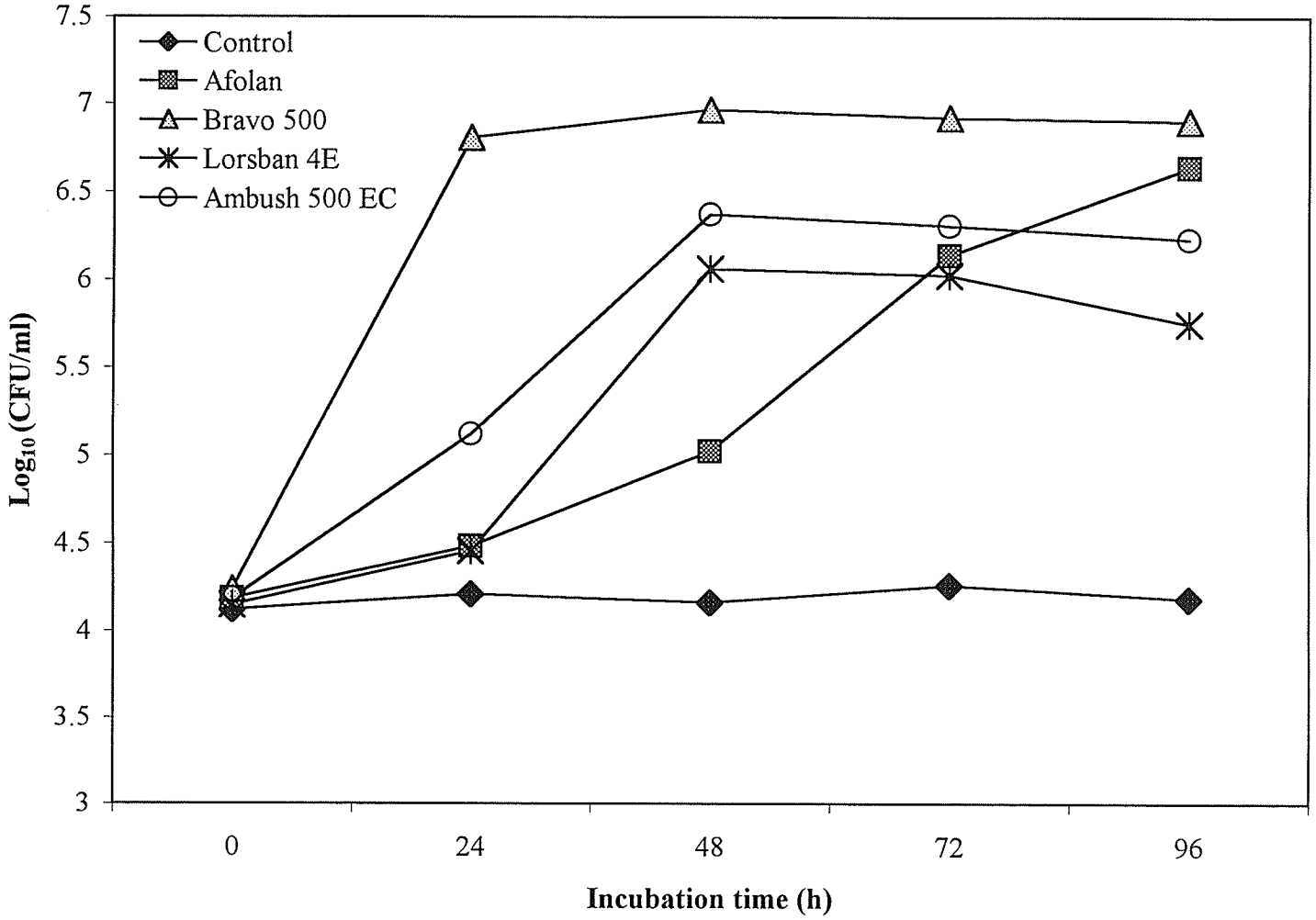
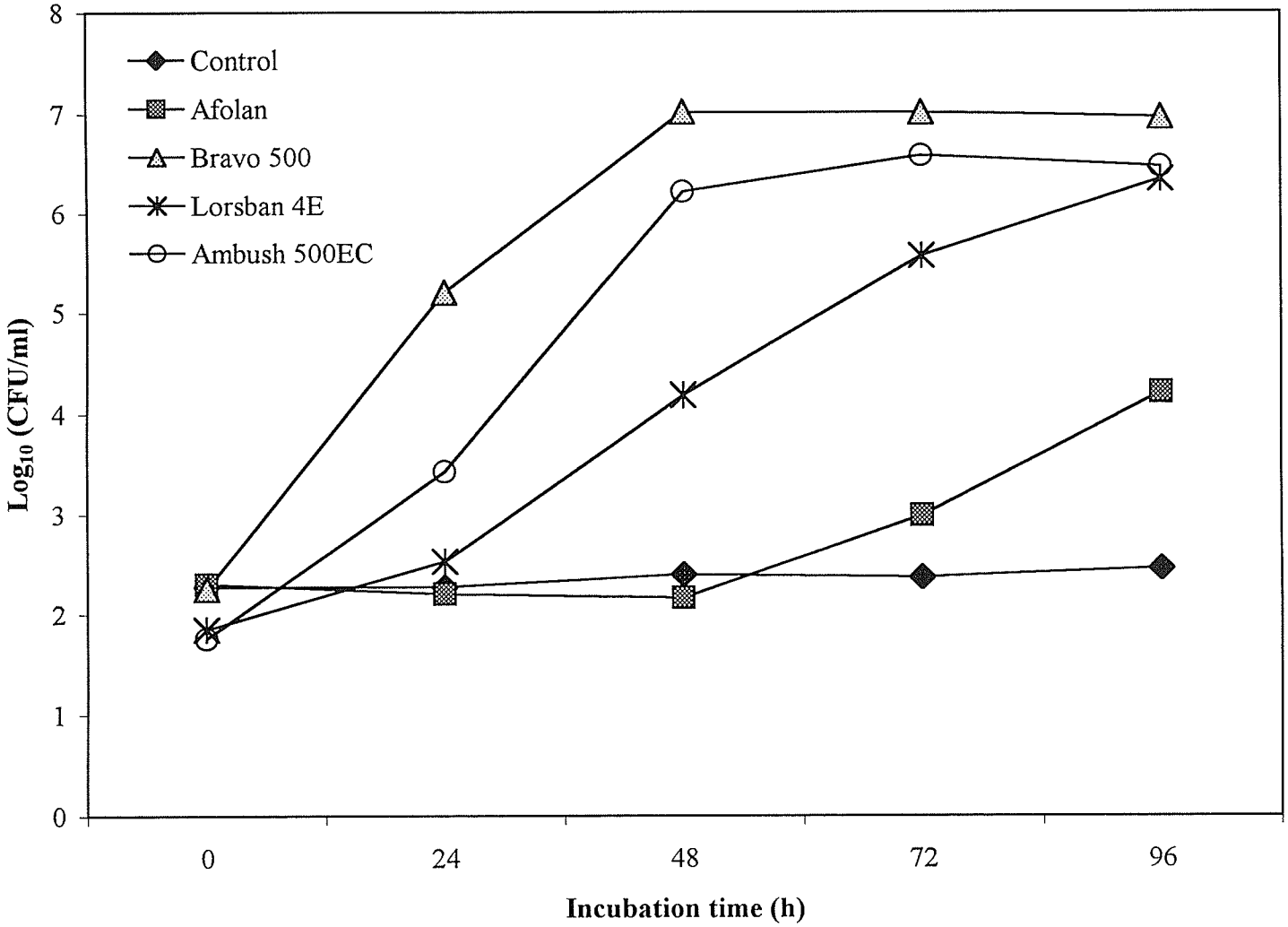


Figure 8. Growth of *Shigella sonnei* in pesticide solutions at 22°C using a low inoculum. See appendix 15.



iv. *Listeria monocytogenes*

The growth of *L. monocytogenes* strain 19112 (human isolate) in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC using high and low inocula is presented in Figures 9 and 10.

At high initial inocula (10^3-10^4 CFU/ml), the listeriae population increased in Bravo 500 by more than 2 logs within 48 h of incubation, thereafter maintaining a constant population level. In the case of Afolan, the listeriae level was similar to the control during the first 48 h, however, thereafter it increased by c. 1 log. In Lorsban 4E and Ambush 500EC, listeriae levels dropped within the first 24 h, and thereafter increased in Lorsban 4E. In Ambush 500EC, an increase in population was not obtained until after 48 h of growth. By 96 h, all growth levels were higher than in the control.

At low initial inocula (10^1-10^2 CFU/ml), listeriae growth in Bravo 500 still remained the highest at all sampling periods. Population levels in Afolan, Lorsban 4E, and Ambush 500EC increased but only slowly after 48 h of incubation. At the end of incubation, listeriae levels were significantly higher in Lorsban 4E and Afolan than in the control; in Ambush 500EC the population level was significantly lower. Population levels in Ambush 500EC appeared to increase only slightly (<0.5 Log10 CFU/ml) after 5 days of incubation.

Figure 9. Growth of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions at 22°C using a high inoculum. See appendix 16.

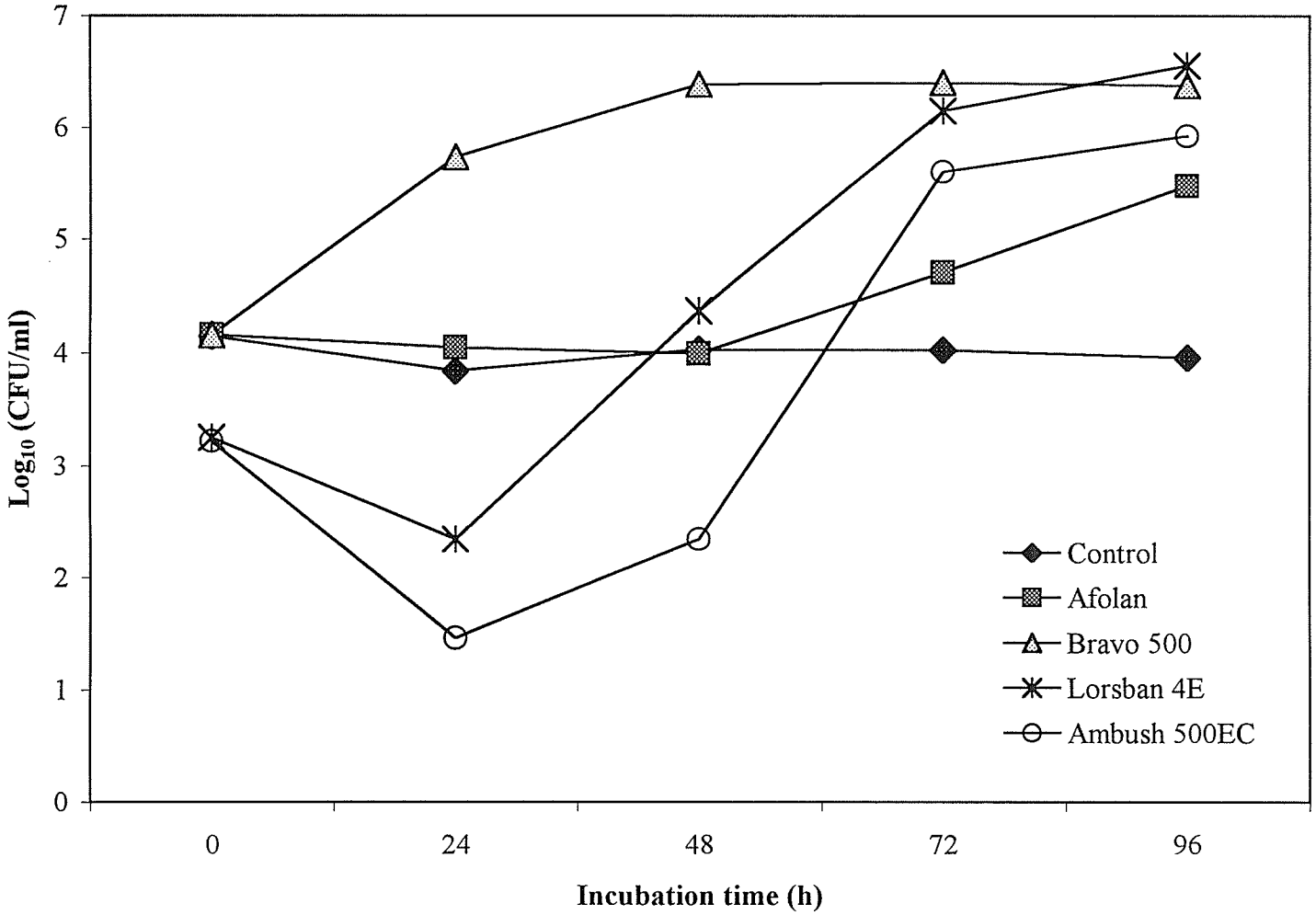
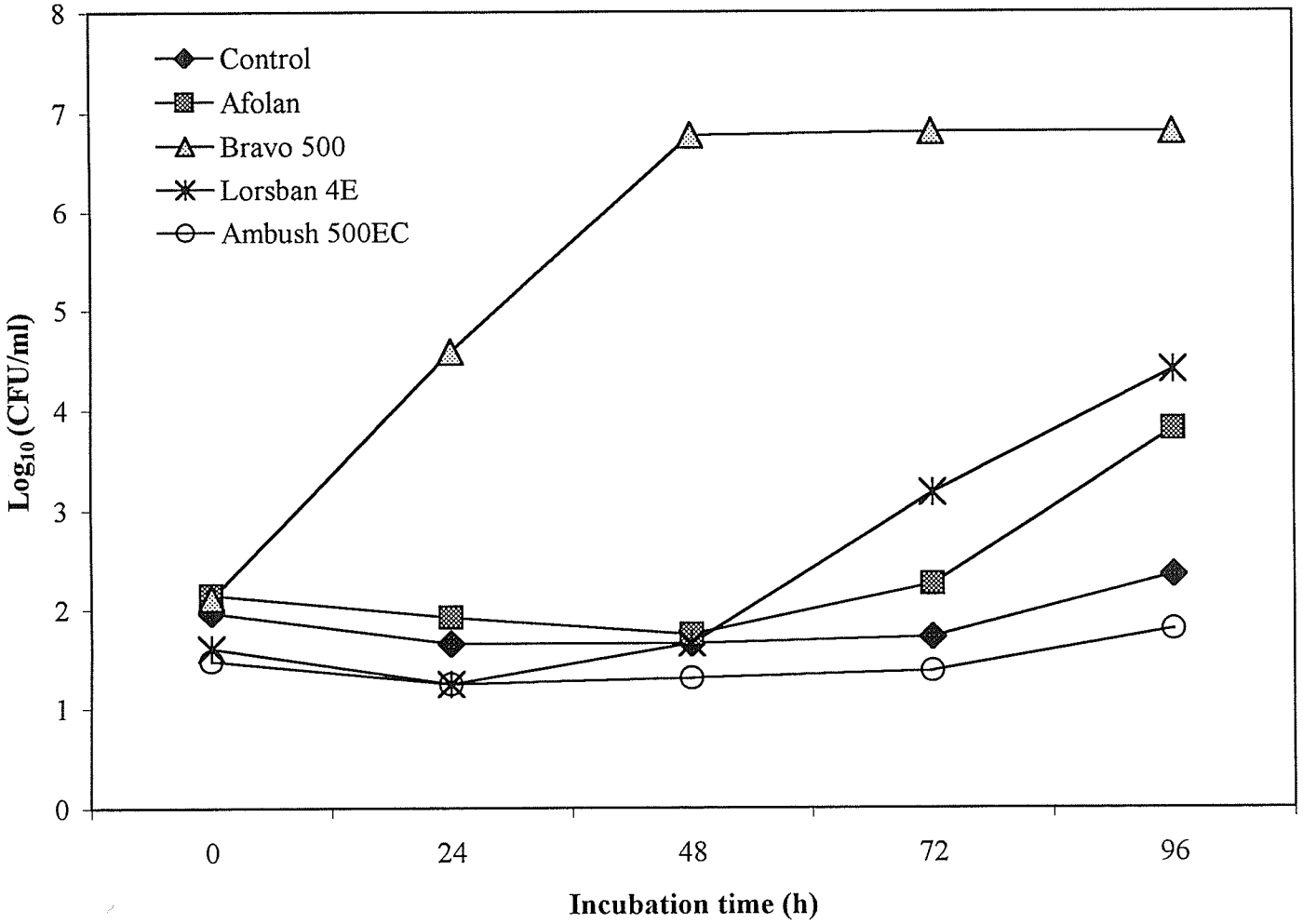


Figure 10. Growth of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions at 22°C using a low inoculum. See appendix 17.

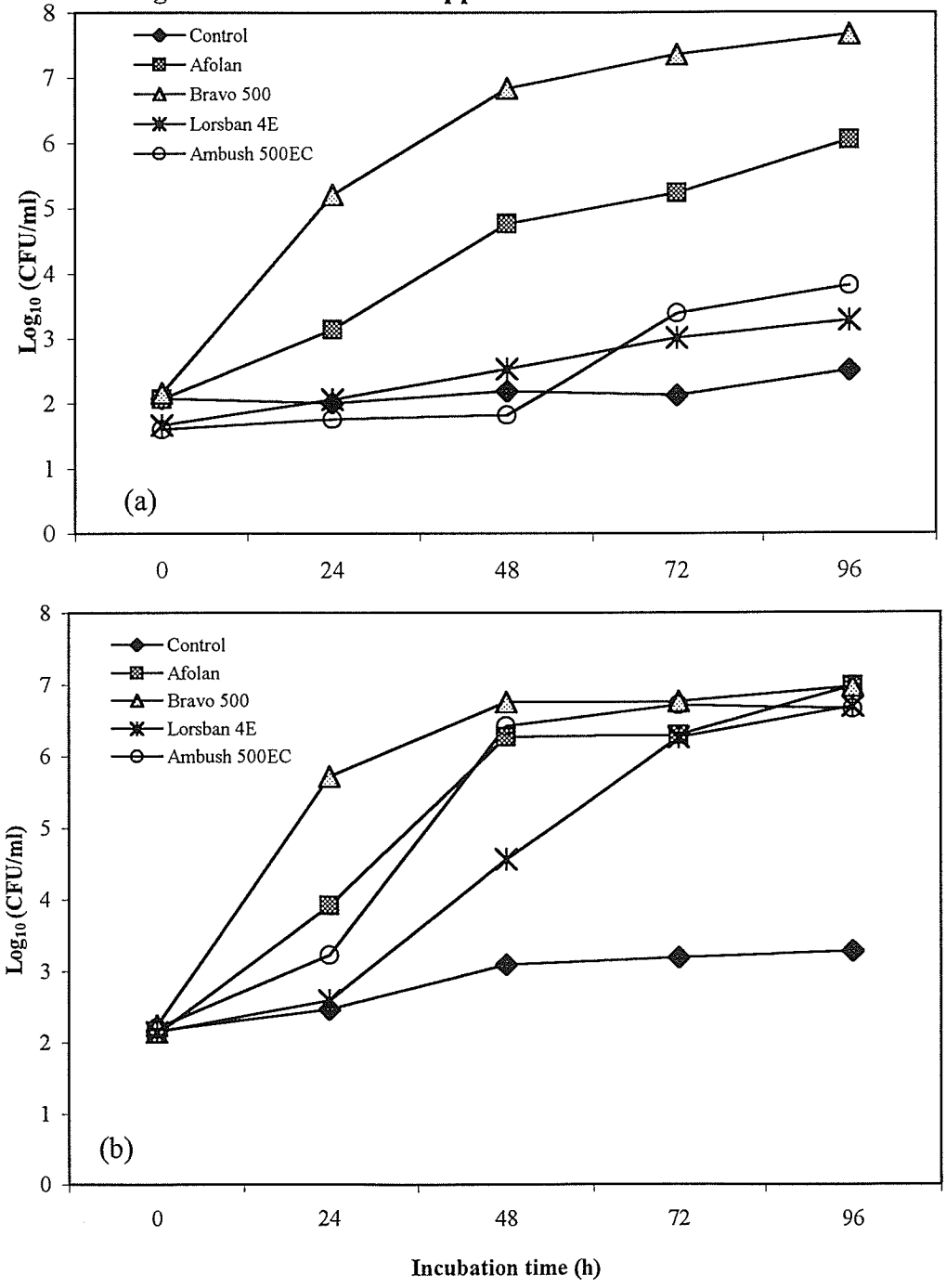


4. Effect of Incubation Temperature on Growth of *E. coli* O157:H7 in Pesticide Solutions

The growth pattern of *Escherichia coli* O157:H7 strain 7236 (human isolate) in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC at two temperatures using a low initial inoculum (c. 10^2 CFU/ml) is presented in Figure 11.

Overall growth at 31°C, especially in Afolan, Ambush 500EC and Lorsban 4E, proceeded more rapidly than at 22°C. This was particularly evident for *E. coli* O157:H7 when grown in Lorsban 4E. Regardless of the incubation temperature, *E. coli* O157:H7 was recovered from all pesticide solutions at all sampling periods. Highest population levels were obtained with Bravo 500 at 22°C. At 31°C, however, similar levels of *E. coli* O157:H7 were recovered from Afolan and Bravo 500, after 48 h of incubation.

Figure 11. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions at 22°C (a) and 31°C (b) using a low inoculum. See appendices 11 and 18.



5. Effect of Pesticide Concentration on Growth of *E. coli* O157:H7 in Pesticide Solutions

The growth pattern of *E. coli* O157:H7 strain 7236 (human isolate) in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC, at three application rates (0.5 recommended, commonly recommended, and 1.5 recommended), stored at 22°C using a low initial inoculum (c. 10^2 CFU/ml) is presented in Figure 12.

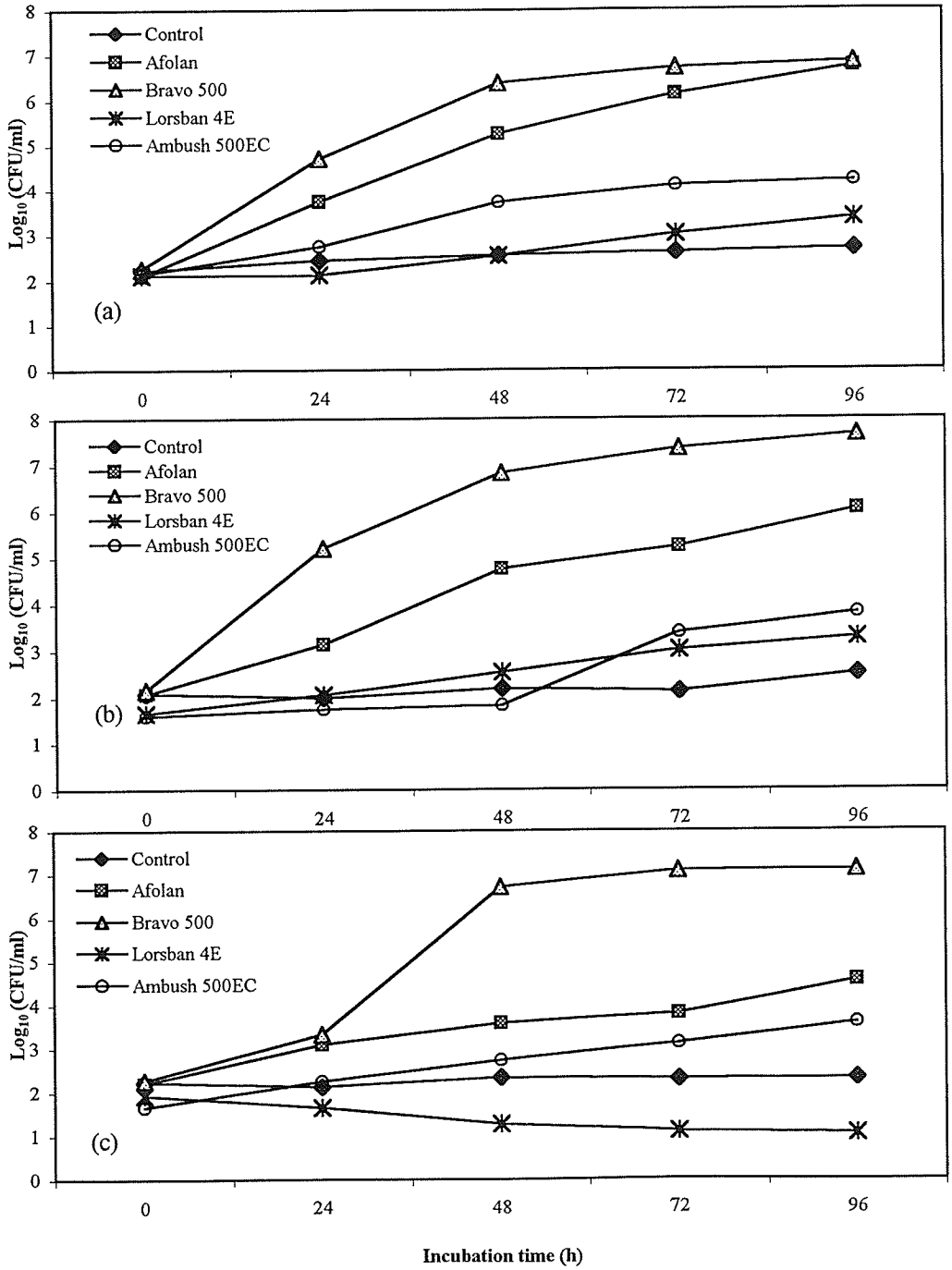
Overall, increasing the concentration of Bravo 500 had the least deleterious effect on the growth of *E. coli* O157:H7. At 1.5 times the recommended application rate, a lag phase of growth was observed during the first 24 h. Within the next 24 hour period, however, the population increased by at least 3 logs.

Increasing the concentration of the remaining pesticide solutions appeared to result in a decrease in the growth of *E. coli* O157:H7. This was particularly evident when *E. coli* O157:H7 was incubated in Lorsban 4E where recovery levels were below those of the control. Regardless of the concentration of pesticide solutions, *E. coli* O157:H7 was recovered throughout the incubation period in all pesticides examined.

6. pH of Pesticide Solutions

The initial pH values of the pesticide solutions are given in Appendix 21 to 27. Overall the pH of the pesticide solutions changed very little (less than 0.8) during the 5-day incubation, regardless of bacteria type, inocula level, pesticide concentration, or storage temperature.

Figure 12. Growth of *E. coli* O157:H7 strain 7236 at 0.5 times recommended pesticide concentrations (a), recommended pesticide concentrations (b), and 1.5 times recommended pesticide concentrations (c) at 22°C using low inocula. See appendix 19, 11, 20.



DISCUSSION

Microorganisms are well known for their ability to adapt to extreme or marginal environments, and there is now a large body of research to suggest that they can "learn" to cope with the multitude of toxic, man-made chemicals that have been released into their environment (Matsumura & Krishna Murti, 1982; Roberts et al., 1993; Motonaga et al., 1996, Racke, 1993; Maloney et al., 1988; Chapalamadugu & Chaudhry, 1991; Orhon et al., 1989; Sethunathan & Yoshida, 1973). Biodegradation of pesticides by soil microflora is perhaps the ultimate in adaptability.

In the present study, various pesticide formulations (commercial) were investigated relative to their ability to support microbial (pathogen) growth. This area of research was deemed important especially in the case where pesticides are applied (postemergence) to standing crops such as fruits and vegetables (many of which are eaten raw and/or are minimally processed). Since many pesticide formulations are invariably diluted with water prior to application according to the manufacturer's recommendations, it is possible that the use of non-potable or contaminated water obtained from on-farm sites (dugouts, wells, and aquifers) or near-by rivers, estuaries etc., could result in their contamination with potential pathogens. Obviously, the use of contaminated water alone could contribute to the spread of pathogens to crops, however, if pesticide formulations allow for microbial growth prior to their application, for example in the bulk or applicator tank, a more critical situation could arise.

The cultures used in this study are considered human pathogens and representatives have been isolated from foods including fruits and vegetables as etiologic agents responsible for food borne illness. It should also be pointed out that these bacteria were not subjected to enrichment (previous exposure to the pesticide solution or active ingredient) prior to their use as inocula and as such adaptive responses would not be expected.

Initial investigations examined the short-time survival (≤ 24 h) of pathogens in pesticide formulations which were reconstituted with tap water to mimic recommended application rates. Short-term survival evaluations were performed since many manufacturers prefer that the spray solution, once made-up, be used within 24 h. A 7 day maximum in spray water is also recommended (Huang, 2000). The hydrophobic grid membrane filter (HGMF) was used since the technique allows for the recovery of low population levels and by using a washing technique decreases the 'carry-over' of pesticide onto recovery media. Decreasing 'carry-over' of pesticide would invariably lead to a more accurate estimation of survivors. A relatively low level (c. 10^2 CFU/ml) was formulated to supposedly mimic levels that are commonly found in non-potable water supplies (Grant, 1998; Falcao et al., 1993; Pal, 1992; Fernandez-Alvarez et al., 1991; Niemi & Niemi, 1991).

Overall results of the investigation indicated that, *E. coli O157:H7, Salmonellae, Shigellae* and *L. monocytogenes* not only exhibited short-term survival but also grew in solutions of the following pesticide formulations: Afolan (herbicide), Bravo 500

(fungicide), Lorsban 4E and Ambush 500EC (insecticides). Among the four formulations that were investigated for long-term growth potential (based on previous HGMF results), Bravo 500 solution consistently contained the highest bacterial population.

Following manufacture of the pure active ingredient (technical grade) the pesticide is processed into a usable form for direct application or for dilution followed by direct application. This process is referred to as formulation and involves any method which will improve its storage, handling, application, effectiveness or safety. The formulation is the form in which the pesticide is sold for use. The formulated pesticide is normally sold under a protected brand name.

The physical form of most commercial crop protection products which are sold, consist of emulsifiable concentrates which contain organic solvents (EC), water soluble liquids (SL), suspension concentrates for water based ingredients (SC) and wettable powders (WP). Afolan and Bravo 500 are sold as suspension concentrates while Ambush 500EC and Lorsban 4E are marketed as emulsifiable concentrates (PMRA, 1998).

Aside from the active ingredients (active substances), commercial formulations usually include additives and adjuvants. Pesticide additives or so-called inerts (formulant, coformulant) have various functions but overall increase pesticidal effectiveness by, for example, assisting the active ingredient into aqueous or solvent solutions. Many of the inerts function as surfactants, emulsifiers, dispersants,

solubilizers, antifoam/defoamers, and compatibilizers. Other important inerts include solvents, carrier/diluents, antifreeze, clay deactivators, preservatives, and thickeners/suspending agents (Hochberg, 1996). Adjuvants or tank mix additives consist of: surfactants, wetting agents, dispersants, emulsifiers, foaming agents, antifoams, pH buffers, polymeric adhesion promoters, film formers, antifreeze agents, alcohols, mineral oils, and modified vegetable oils. Overall, use of adjuvants provides more uniform application of the active ingredient upon a specific target, reduces water surface tension, improves ingredient stability, and allows better mixing of organics and aqueous systems. Needless to say the exact composition in terms of inerts and adjuvants in pesticide formulations is a closely guarded secret and falls under proprietary protection.

In this investigation, the breakdown or catabolism of the active ingredient by each microorganism for each pesticide formulation was not followed. Microbial utilization of inerts and adjuvants was also not determined. Therefore, the growth profiles noted in the solutions of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC may be due to utilization of all or part of the active ingredients and/or the inerts/adjuvants. Also, it can be assumed that, where survival and/or growth occurred, the active ingredients and/or the inerts/adjuvants were not inhibitory towards the target microorganisms, at least in the concentrations that were present.

Increasing the initial inoculum level from c. 10^2 to 10^4 CFU/ml for *E. coli* O157:H7 resulted in improved growth in the solutions of Ambush 500EC and Lorsban 4E. Similarly, an increase in inoculum resulted in improved growth in Ambush 500EC

and Afolan for *Salmonella typhimurium* and *Shigella sonnei*, respectively. For *Listeria monocytogenes*, the only Gram positive bacterium investigated in this research, increasing the inoculum level greatly improved its growth profile in Afolan. An improvement in growth as a result of increasing the initial inoculum indicated that perhaps some of the compounds present in the pesticide formulation were somewhat inhibitory. Therefore, the potential for pesticide solutions to support microbial growth is not only dependent upon the type or species of microorganisms present but also on the population level. This phenomenon is not unique to pesticides and has been observed with various compounds including antibiotics and food preservatives (Prescott et al., 1990). For example, increasing the spray concentration by 1.5 times the recommended rate resulted in a decrease in growth for *E. coli* O157:H7 in three of the four pesticide solutions tested, Bravo 500 being the exception. In contrast, decreasing the concentration to 0.5 times the recommended rate resulted in an increase in the level of growth for *E. coli* O157:H7 in three of the four pesticide solutions with the exception of Lorsban 4E. Therefore, concentration effects, as expected, are also important with regards to the ability of microorganisms to grow in pesticide solutions. Growth at different pesticide concentrations was investigated because dilution of pesticides at the farms may be approximate.

Pure cultures capable of utilizing various compounds including chlorinated aromatics have been isolated in many areas, and degradation pathways have been studied (Bumpus et al., 1993; Havens & Rase, 1991; Khan et al., 1988; Maloney et al., 1988; Engelhardt et al., 1972). References pertaining to the ability of the microorganisms used

in this study to degrade active ingredients, however, were not found. Overall, however, mixed cultures of soil borne microorganisms are more frequently involved in the biodegradation of soil-applied pesticides.

For example, Afolan, a urea herbicide commonly referred to as linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] has received considerable attention relative to its microbiological degradation. Studies demonstrated that a soil bacterium, *Bacillus sphaericus*, was able to degrade linuron and other related urea herbicides, and that the enzyme responsible for the degradation was an acylamidase (Wallnofer, 1969; Wallnofer & Bader, 1970; Engelhardt et al., 1971 & 1972). This enzyme was also shown to be linuron-inducible. In a later study by Roberts et al. (1993), they isolated a mixed bacterial culture that degraded linuron completely utilizing it as a source of both nitrogen and carbon. The authors suggested that a consortium of microorganisms was involved since no degradation was observed in pure culture; *Pseudomonas spp* were believed important components of the population.

Many soil isolates have been demonstrated to degrade Bravo 500, a wide-spectrum organochlorine fungicide whose active ingredient is chlorothalonil or TPN (2,4,5,6-tetrachloroisophthalonitrile)(Sato & Tanaka, 1987; Katayama et al., 1991; Motonaga et al., 1996). Most of these microorganisms degrade chlorothalonil in the presence of other carbon sources (Sato & Tanaka, 1987; Katayama et al., 1991). Since they do not utilize the chemical as an energy source, the process is known as cometabolism. These chlorothalonil-degrading bacteria include non-spore forming Gram

positive and negative rods (Sato & Tanaka, 1987; Katayama et al., 1991). No particular bacterial strains, however, have been identified to be involved in this cometabolism process. Although potential pathways of chlorothalonil degradation have been proposed, no specific enzymes involved in its metabolism have been identified.

Organophosphate insecticides including parathion, malathion, diazinon and chlorpyrifos have also been reported to be biodegradable (Racke, 1993; Daughton & Hsieh, 1977; Bourquin, 1977; Gunner & Zuckerman, 1968). Lorsban 4E, whose active ingredient is chlorpyrifos [*O,O*-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate], is among the top 25 world leading crop protection products (Ramos Tombo & Blaser, 1999). Research evidence indicates that it is primarily cometabolism that characterizes the microbial metabolism of chlorpyrifos in soil (Racke, 1993). Microbial adaptation for its catabolism, however, has not been reported to occur in soil (Racke & Coats, 1988 & 1990; Racke et al., 1990). However, several direct investigations on the microbial metabolism of chlorpyrifos reported a lack of its metabolism in microbial culture (Hirakoso, 1969; Sethunathan & Pathak, 1972; Lal et al., 1987; Racke & Coats, 1988). On the contrary, a few researchers have reported some microbial metabolism of the pesticide. Jones & Hastings (1981) observed metabolism of chlorpyrifos with several soil fungi, including *Trichoderma harzianum, Penicillium multicolor, P. vermiculatum, Mucor sp.* Ivashina (1986) reported that the dissipation of the chemical was more rapid in a sucrose-supplemented media containing *Trichoderma sp.* and a glucose-supplemented media containing *Bacillus sp.* than in control media containing no microorganisms. Lal & Lal (1987) observed some degree of degradation by the yeast

Saccharomyces cervisiae. Shaker et al. (1988) reported metabolism by two strains of lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Bumpus et al. (1993) demonstrated the mineralization of chlorpyrifos by the fungus, *Phanerochaete chrysosporium*. Microbial enzymes have been shown to hydrolyze chlorpyrifos. Munnecke & Hsieh (1975) first reported the ability of parathion hydrolase, an organophosphorus ester-hydrolyzing enzyme isolated from a mixed microbial culture, to hydrolyze chlorpyrifos. This finding was in agreement with a report by Havens & Rase (1991) who also observed chlorpyrifos degradation by a parathion hydrolase obtained from *Pseudomonas diminuta*.

Ambush 500EC, whose active ingredient is a synthetic pyrethroid named permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclo-propanecarboxylate] is a commonly used insecticide. Few reports exist on microbially mediated pyrethroid degradation, in particular, by pure cultures (Khan et al., 1988; Lord et al., 1982; Sakata et al., 1992). Chiaki et al. (1987) described the isolation of a soil isolate *Bacillus sp.* and both the purification and characterization of its esterase which catalyzed the hydrolytic cleavage of a permethrin analog. Maloney et al. (1988) showed that pure cultures *of Bacillus cereus, Pseudomonas fluorescens,* and *Achromobacter sp.* were capable of transforming permethrin in the presence of Tween 80. The isolates were unable to grow or transform permethrin in the absence of Tween 80 and apparently used it as their primary carbon source during the cometabolism of the insecticide. A later study by Maloney et al. (1993) found that permethrin detoxification could be achieved

with a cell-free microbial enzyme system. This permethrin-hydrolyzing enzyme, obtained from *Bacillus cereus,* was characterized as a carboxylesterase.

Temperature was also demonstrated to play an important role in the growth of *E. coli* O157:H7 in various pesticide solutions. When temperature was increased from 20-22° to 31°C, *E. coli* O157:H7 growth was enhanced especially in Lorsban 4E and Ambush 500EC. This is to be expected since microbial activity increases with increasing temperature up to an optimum value. Growth at the higher incubation temperature (31°C) was investigated because this temperature (and even higher temperature) can be encountered during the summer months. Moorman (1994) stated that within the range of temperature conditions normally encountered in cultivated soils (0 to 35°C), the rate of pesticide degradation generally increases with temperature. Walker & coworkers (Smith & Walker, 1977; Walker, 1974, 1976, 1978; Walker & Smith, 1979) considered soil temperature to be the most important environmental factor influencing pesticide degradation rate in soils. Biodegradation studies of herbicide EPTC (S-ethyl dipropyl thiocarbamate) showed that the chemical was degraded more rapidly at 15 and 25°C than at 5°C in soil, with a more pronounced effect at 25°C (Obrigawitch et al., 1982).

Results from studies examining MIC values for *E. coli* O157:H7 strain 7236 (human isolate) indicated that pesticide formulations of Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC did not exhibit any inhibition on the pathogen, at least with the current inoculum level. Survival of this bacterium after 1 and 24 h exposure would then be expected. The non-toxic nature of these four pesticide formulations on the pathogen

was also shown during time-course studies. Interestingly, the HGMF survival levels of *E. coli* O157:H7 at 24 h in Lorsban 4E and Ambush 500EC were not significantly different from one another and were significantly lower compared to Bravo 500 and Afolan. This pattern was similarly displayed during the 96 h time-course study. Two pesticide formulations: Diazinon and Sevin XLR+ also showed no inhibitory effects (no MIC values established) towards *E. coli* O157:H7 yet survival levels of this bacterium at 24 h (HGMF) were minimum to zero. This discrepancy can be partially attributed to the difference in the inoculum levels used. Survival studies (HGMF) performed at 1 and 24 h used inoculum levels of approximately 10^2 CFU/ml while MIC values were established by spreading 0.2ml inoculum containing 10^9 CFU/ml over the surface of an agar plate (c. 10^6 CFU/cm^2). It would appear, therefore, that increasing the inoculum level increased the resistancy of *E. coli* O157:H7 towards certain pesticide formulations, a phenomenon which is well recognized (and which was also demonstrated in this study) in assessing microbial susceptibility to antimicrobials (Prescott et al., 1990). It is interesting to note that Gramoxone appeared to be a potent antimicrobial considering its relatively high MIC. The fact that no survivors were obtained after one hour exposure (HGMF) confirms this observation. Although the MIC range values established for the various formulations help to explain the survival and growth of *E. coli* O157:H7, it should be pointed out that these values may not reflect the inhibitory activity of the active ingredients (active substances). Since inerts and adjuvants are also incorporated into the formulations, it is possible that either or both of these compounds contributed to the established MIC values. Obviously, where no MIC value was established, absence of

inhibitory compounds, regardless of their nature and/or insufficient concentration, would be expected.

The initial pH values of solutions of the four pesticide formulations used during the 96 hour time-course study were similar to each other (pH difference of less than 0.5 unit). In all cases the pH increased slightly over the 96 hour course of the growth studies, regardless of inoculum level. It is therefore concluded that pH was not a mitigating factor in this study.

Based on the results of this study, low levels of pathogenic bacteria, including *E. coli* O157:H7, *Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Shigella flexneri*, and *Listeria monocytogenes*, which are frequently found in polluted waters (c. 10^2 CFU/ml), can increase substantiality in pesticide solutions and thereby constitute a health hazard if applied to a standing crop. This is especially of concern considering that numerous studies have confirmed survival and even growth of these bacteria on fruits and vegetables.

CONCLUSIONS

In this study, various foodborne bacteria were shown to survive and/or grow in the solutions of pesticide formulations. Survival and in particular growth of pathogens in the solutions of various commercial pesticide formulations including Afolan, Bravo 500, Lorsban 4E, and Ambush 500EC may contribute to a potential health hazard to standing crops such as fruits and vegetables. Based on the results of this study, several conclusions can be made:

1. The potential for pathogens (*E. coli* O157:H7, *Salmonella spp.*, *Shigella spp.*, and *L. monocytogenes*) to grow and/or survive in a pesticide solution is influenced by the product type and concentration, the initial concentration of the contaminating microbial population, the types of microorganisms (genus and species and perhaps strain), and temperature of pesticide (spray solution) storage.

2. Bravo 500 solution supported the greatest growth of *E. coli* O157:H7, *S. typhimurium, L. monocytogenes* 19112, and *S. sonnei*. Some pesticides (Afolan, Bravo 500, Sevin XLR+, Lorsban 4E, Diazinon 500, and Ambush 500EC) did not exhibit an MIC value against *E. coli* O157:H7 in the concentrated form. In this research, it appeared that the pH was not a factor affecting growth of pathogens.

3. In view of the fact that pathogenic bacteria can grow in the solutions of selected pesticide formulations, the storage time for the spray solution, once made-up, should

be no longer than 24 h unless a potent biocide is added to the formulation. This recommendation is also endorsed by some pesticide manufacturers.

4. Active ingredients and/or inerts/adjuvants should be assessed for their ability to sponsor microbial growth. If long term storage of spray solutions is to be maintained safely, compounds favoring growth should be removed or substituted, especially if a biocide is not added.

RECOMMENDATIONS FOR FUTURE STUDIES

1. Analysis for the degradation of active compounds in the solutions of pesticide formulations exhibiting microbial growth.

2. Examination of the survival and/or growth of mixed cultures in the solutions of pesticide formulations.

3. On farm surveys of applicator tanks for the presence of foodborne pathogens and a concomitant microbial evaluation of on farm water supplies.

4. Examination of the fate of pathogenic microorganisms after pesticide application on fruits and vegetables.

5. Examination of the survival and/or growth patterns of other pathogenic microorganisms, such as *Giardia lamblia, Campylobacter jejuni, Cryptosporidium parvum,* etc, in the solutions of pesticide formulations.

REFERENCES

Abdul-Raouf, U., L. R. Beuchat, and M. S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Applied and Environmental Microbiology 59:1999-2006.

Aislabie, J., A. J. Bej, H. Hurst, S. Rothenburger, and R. M. Atlas. 1990. Microbial degradation of quinoline and methylquinolines. Applied and Environmental Microbiology 56:345-351.

Al-Azawi, S. K. 1982. Microbiological aspects of the disposal of sewage waste to agricultural land. M. Sc. Thesis. University of Aberdeen, Scotland.

Alexender, M. 1999. Biodegradation and Bioremediation. 2nd edition. Academic Press, San Diego, CA. pp.xiii,1-7.

Al-Ghazali, M. R., and S. K. Al-Azawi. 1986. Detection and enumeration of *Listeria monocytogenes* in a sewage treatment plant in Iraq. Journal of Applied Bacteriology 60:251-254.

Al-Ghazali, M. R., and S. K. Al-Azawi. 1988. Effects of sewage treatment on the removal of *Listeria monocytogenes*. Journal of Applied Bacteriology 65:203-208.

Al-Ghazali, M. R., and S. K. Al-Azawi. 1990. *Listeria monocytogenes* contamination of crops grown on soil treated with sewage sludge cake. Journal of Applied Bacteriology 69:642-647.

Anonymous, 1997. In: Guide to Crop Protection 1997. Manitoba Agriculture and Food. Winnipeg, MB.

AOAC, 1990. Official methods of analysis: agricultural chemicals; contaminants; drugs. Ed. K. Helrich. 15th edition. Vol 1. AOAC, Inc. Arlington, Virginia.

Arumugaswamy, R. K., G. Rusul Rahamat Ali, and S. Nadzriah Bte Abd. Hamid. 1994. Prevalence of *Listeria monocytogenes* in foods in Malaysia. International Journal of Food Microbiology 23:117-121.

Asplund, K., and E. Nurmi. 1991. The growth of salmonellae in tomatoes. International Journal of Food Microbiology 13:177-182.

Aytac, S. A., and L. G. M. Gorris. 1994. Survival of *Aeromonas hydrophila* and *Listeria monocytogenes* on fresh vegetables stored under moderate vacuum. World Journal of Microbiology and Biotechnology 10:670-672.

Babic, I., A. E. Watada, and J. G. Buta. 1997. Growth of *Listeria monocytogenes* restricted by native microorganisms and other properties of fresh-cut spinach. Journal of Food Protection 60:912-917.

Bahk, J., and E. M. Marth. 1990. Listeriosis and *Listeria monocytogenes*. Academic Press, New York. pp. 247-257.

Bari, M. L., H. Kusunoki, H. Furukawa, H. Ikeda, K. Isshiki, and T. Uemura. 1999. Inhibition of growth of *Escherichia coli* O157:H7 in fresh radish (*Raphanus sativus* L.) sprout production by calcinated calcium. Journal of Food Protection 62:128-132.

Barnett, B. J., M. Schwartze, D. Sweat, S. Lea, J. Taylor, B. Bibb, G. Pierce, and K. Hendricks. 1995. Outbreak of *Escherichia coli* O157:H7, Waco, Texas. Epidemic Intelligence Service, 44th Ann. Con., CDC, Atlanta, GA. pp. 17-18.

Bayarri, S., A. Herrera, A. A. Arino, R. Lazaro, and P. Conchello. 1998. A study of the degradation in vitro of organochlorine compounds by the meat starter micro-organism *Micrococcus varians*. Extended Summaries: 1st European Pesticide Residues Workshop. pp.147-151.

Beckers, H. J., P. H. In't Veld, P. S. S. Soentoro, and E. H. M. Delfgou-van Asch. 1989. The occurrence of *Listeria* in food. In: Foodborne listeriosis: proceedings of a symposium, September 7. Behr, Hamburg. Wiesbaden, Germany. pp.85-97.

Bendig, J. W. A., and J. E. M. Strangeways. 1989. *Listeria* in hospital lettuce. Lancet 8648:616-617.

Bennish, M. L., J. R. Harris, B. J. Wojtyniak, and M. Struelens. 1990. Death in shigellosis: incidence and risk factors in hospitalized patients. Journal of Infectious Diseases 161:500-506.

Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1989. Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. Applied and Environmental Microbiology 55:2167-2171.

Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. Journal of American Veterinary Medicine Association 269:2217-2220.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection 59:204-216.

Beuchat, L. R., and R. E. Brackett. 1990a. Inhibitory effects of raw carrots on *Listeria monocytogenes*. Applied and Environmental Microbiology 56:1734-1742.

Beuchat, L. R., and R. E. Brackett. 1990b. Growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging, temperature and time. Journal of Food Science 55:755-758,870.

Beuchat, L. R., and R. E. Brackett. 1991. Behaviour of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. Applied and Environmental Microbiology 57:1367-1371.

Beuchat, L. R., R. E. Brackett, and M. P. Doyle. 1994. Lethality of carrot juice *to Listeria monocytogenes* as affected by pH, sodium chloride and temperature. Journal of Food Protection 57:470-474.

Beuchat, L. R., R. E. Brackett, D. Y. Y. Hao, and D. E. Conner. 1986. Growth and thermal inactivation of *Listeria monocytogenes* in cabbage and cabbage juice. Canadian Journal of Microbiology 32:791-795.

Black, E. K., and G. R. Finch. 1993. Detection and occurrence of waterborne bacterial and viral pathogens. Water Environmental Research 65:295-300.

Blaser, M. J., R. A. Pollard, and R. A. Feldman. 1983. *Shigella* infections in the United States, 1974-1980. Journal of Infectious Diseases 147:771-775.

Blenden, D. C., and F. T. Szatalowicz. 1967. Ecological aspects of listeriosis. Journal of American Veterinary Medicine Association 151:1761-1766.

Blostein, J. 1991. An outbreak of *Salmonella javiana* associated with consumption of watermelon. Journal of Environmental Health 56:29-31.

Bojsen-Moller, J. 1972. Human listeriosis: diagnostic and epidemiological and clinical studies. Acta Pathogenic Microbiology Scand Section B. Supplement. 229:72-155.

Bourquin, A. W. 1977. Degradation of malathion by saltmarsh microorganisms. Applied and Environmental Microbiology 33:356-362.

Buchanan, R. L., S. G. Edelson, R. L. Miller, and G. M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. Journal of Food Protection 62:444-450.

Bumpus, J. A., S. N. Kakar, and R. D. Coleman. 1993. Fungal degradation of organophosphorous insecticides. Applied Biochemistry and Biotechnology 39:715-726.

Casamore, D. 1991. The epidemiology of human cryptosporidiosis and the water routes of infection. Water Science and Technology 24:157-164.

Centers for Disease Control. 1975. *Salmonella typhimurium* outbreak traced to a commercial apple cider-New Jersey. Morbidity and Mortality Weekly Report 24:87-88.

Centers for Disease Control. 1979. *Salmonella oranienburg* gastroenteritis associated with consumption of precut watermelons. Morbidity and Mortality Weekly Report 28:522-523.

Centers for Disease Control and Prevention. 1987. Outbreak of gastroenteritis-Pennsylvania and Delaware. 36:709-711.

Centers for Disease Control and Prevention. 1991. Multi-state outbreak of *Salmonella poona* infections-United States and Canada. Morbidity and Mortality Weekly Report 40:549-552.

Centers for Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infection associated with drinking unpasteurized commercial apple cider-October, 1996. Morbidity and Mortality Weekly Report 45:975.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider-Connecticut and New York, October, 1996. Morbidity and Mortality Weekly Report 46:4-8.

Chapalamadugu, S., and G. R. Chaudhry. 1991. Hydrolysis of carbaryl by a *Pseudomonas sp.* and construction of a microbial consortium that completely metabolizes carbaryl. Applied and Environmental Microbiology 57:744-750.

Chaudhry, G. R., and A. N. Ali. 1988. Bacterial metabolism of carbofuran. Applied and Environmental Microbiology 54:1414-1419.

Chaudhry, G. R., and L. Cortez. 1988. Degradation of bromacil by a *Pseudomonas sp.* Applied and Environmental Microbiology 54:2203-2207.

Chiaki, S., K. Nishizawa, M. Sugimoto, K. Sonada, and F. Kishimoto. 1987. A novel microorganism, a novel esterase and method for preparing the same. European patent application 0243167, Sumitomo Chemical Company Ltd.

Chung, K. C., and J. M. Geopfert. 1970. Growth of *Salmonella* at low pH. Journal of Food Science 35:326-328.

Cieslak, P. R., T. J. Barrett, and P. M. Griffin. 1993. *Escherichia* coli O157:H7 infection from a manured garden. Lancet 342:367.

Colburn, K. G., C. A. Kaysner, C. Abeyta, Jr., and M. M. Wekell. 1990. *Listeria* species in a California coast estuarine environment. Applied and Environmental Microbiology 56:1007-1011.

Conner, D. E., R. E. Brackett, and L. R. Beuchat. 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Applied and Environmental Microbiology 52:59-63.

Cook, K. A., T. Boyce, C. Langkop, K. Kuo, M. Swartz, D. Ewert, E. Sowers, J. Wells, and R. Tauxe. 1995. Scallions and shigellosis: a multistate outbreak traced to imported green onions. Epidemic Interlligence Service 44th Annu. Conf., Mar. 27-31, CDC, Atlanta, GA. p. 36.

Copping, L. G., and H. G. Hewitt. 1998. In: Chemistry and Mode of Action of Crop – Protection Agents. The Royalty Society of Chemistry, Cambridge. pp.7-8,19-20.

Daughton, C. G., and D. P. H. Hsieh. 1977. Parathion utilization by bacterial symbionts in a chemostat. Applied and Environmental Microbiology 34:175-184.

Daugherty, D. D., and S. F. Karel. 1994. Degradation of 2,4-dichlorophenoxyacetic acid by *Pseudomonas cepacia* DBO1(pRO101) in a dual-substrate chemostat. Applied and Environmental Microbiology 60:3261-3267.

Davis, H., J. P. Taylor, J. N. Perdue, G. N. Stelma, Jr., J. M. Humphreys, Jr., R. Roundtree, III, and K. D. Greene. 1988. A shigellosis outbreak traced to commercially distributed shredded lettuce. American Journal of Epidemiology 128:1312-1321.

del Rosario, B. A., and L. R. Beuchat. 1995. Survival and growth of enterohemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. Journal of Food Protection 58:105-107.

DePaola, A., M. W. Presnell, R. E. Becker, M. L. Motes, Jr., S. R. Zywno, J. F. Musselman, J. Taylor, and L. Williams. 1984. Distribution of *Vibrio cholerae* in the Apalachicola (Florida) Bay estuary. Journal of Food Protection 47:549-553.

Dev, V. J., M. Main, and I. Gould. 1991. Waterborne outbreak of *Escherichia coli* O157:H7. Lancet 337:1412.

Dickie, N., J. I. Speirs, and M. Akhtor. 1989. Purification of a serogroup O157:H7 verotoxin and its detection in North America hemorrhagic colitis isolates. Journal of Clinical Microbiology 27:1973-1978.

Doyle, M. P., and D. O. Cliver. 1990. *Salmonella.* Academic Press, San Diego, CA. pp. 185-204.

DuPont, H. L., M. M. Levine, R. B. Hornick, and S. B. Formal. 1989. Inoculum size in shigellosis and implications for expected mode of transmission. Journal of Infectious Diseases 159:1126-1128.

Engelhardt, G., P. R. Wallnofer, and R. Plapp. 1971. Degradation of linuron and some other herbicides and fungicides by a linuron-inducible enzyme obtained *from Bacillus sphaericus.* Applied Microbiology 22:284-288.

Engelhardt, G., P. R. Wallnofer, and R. Plapp. 1972. Identification of *N, O*-dimethylhydroxylamine as a microbial degradation product of the herbicide, linuron. Applied Microbiology 23:664-666.

Ercolani, G. L. 1976. Bacteriological quality assessment of fresh marketed lettuce and fennel. Applied and Environmental Microbiology 31:847-852.

Erickson, J. P., J. W. Stamer, M. Hayes, D. N. Mckenna, and L. A. Van Alstine. 1995. An assessment of *Escherichia coli* O157:H7 contamination risks in commercial mayonnaise from pasteurized eggs and environmental sources, and behavior in low-pH dressings. Journal of Food Protection 58:1059-1064.

Escartin, E. F., A. C. Ayala, and J. S. Lozano. 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. Journal of Food Protection 52:471-472.

Falcao, D. P., S. R. Valentini, and C. Q. F. Leite. 1993. Pathogenic or potentially pathogenic bacteria as contaminants of fresh water from different sources in Araraquara, Brazil. Water Research 27:1737-1741.

Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiology Reviews 55:476-511.

Farber, J. M., G. W. Sanders, and M. A. Johnston. 1989. A survey of various foods for the presence of *Listeria* species. Journal of Food Protection 52:456-458.

Farber, J. M., S. L. Wang, Y. Cai, and S. Zhang. 1998. Changes in populations of *Listeria monocytogenes* inoculated on packaged fresh-cut vegetables. Journal of Food Protection 61:192-195.

Fenlon, D. R. 1985. Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. Journal of Applied Bacteriology 59:537-543.

Fernandez-Alvarez, R. M., S. Carballo-Cuervo, M. C. de la Rosa-Jorge, and J. Rodriguez-de Lecea. 1991. The influence of agricultural run-off on bacterial populations in a river. Journal of Applied Bacteriology 70:437-442.

Fisher, T. L., and D. A. Golden. 1998a. Fate of *Escherichia coli* O157:H7 in ground apples used in cider production. Journal of Food Protection 61:1372-1374.

Fisher, T. L., and D. A. Golden. 1998b. Survival of *Escherichia coli* O157:H7 in apple cider as affected by dimethyl dicarbonate, sodium bisulfite, and sodium benzoate. Journal of Food Science 63:904-906.

Food and Drug Administration. 1993. Food code, Pub. No. PB94-113941AS. U. S. Public Health Service, U. S. Dept. of Commerce, Technology Administration, National Technical Information Service, Springfield, VA.

Francis, G. A., C. Thomas, and D. O'Beirne. 1999. The microbiological safety of minimally processed vegetables. International Journal of Food Science and Technology 34:1-22.

Garber, L. P., S. J. Wells, D. D. Hancock, M. P. Doyle, J. Tuttle, J. A. Shere, T. Zhao. 1995. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. Journal of American Veterinary Medicine Association 207:46-49.

Garcia-Villanova Ruiz, B., R. Galvez Vargas, and R. Garcia-Villonova. 1987. Contamination on fresh vegetables during cultivation and marketing. International Journal of Food Microbiology 4:285-291.

Gayler, G. E., R. A. MacCready, J. P. Reardon and B. F. McKernan. 1955. An outbreak of salmonellosis traced to watermelon. Public Health Reports. 70:311-313.

Geldreich, E. E., and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market: a review. Journal of Food Technology 34:184-195.

Golden, D. A., E. J. Rhodehamel, and D. A. Kautter. 1993. Growth of *Salmonella spp.* in cantaloupe, watermelon and honeydew melons. Journal of Food Protection 56:194-196.

Grant, M. A. 1998. Analysis of bottled water for *Escherichia coli* and total coliforms. Journal of Food Protection 61:334-338.

Gray, M. L., and Killinger, A. H. 1966. *Listeria monocytogenes* and listeric infections. Bacteriological Review 30:309-382.

Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *E. coli* O157:H7, other enterohaemorrhagic *E. coli*, and the associated haemolytic syndrome. Epidemiological Reviews 13:60-98.

Gunasena, D. K., C. P. Kodikara, K. Ganepola, and S. Widanapathirana. 1995. Occurrence of *Listeria monocytogenes* in food in Sri Lanka. Journal of the National Science Council of Sri Lanka 23:107-114.

Gunner, H. B., and B. M. Zuckerman. 1968. Degradation of "diazinon" by synergistic microbial action. Nature (London) 217:1183-1184.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiology Infection 113:199-207.

Hara-Kudo, Y., H. Konuma, M. Iwaki, F. Kasuga, Y. Sugita-Konishi, Y. Ito, and S. Kumagai. 1997. Potential hazard of radish sprouts as a vehicle of *Escherichia coli* O157:H7. Journal of Food Protection 60:1125-1127.

Harris, I. T., P. J. Fedorka-Cray, J. T. Gray, L. A. Thomas, and K. Ferris. 1997. Prevalence of *Salmonella* organisms in swine feed. Journal of American Veterinary Medicine Association 210:382-385.

Harvey, J., and A. Gilmour. 1993. Occurrence and characteristics of *Listeria* in foods produced in Northern Ireland. International Journal of Food Microbiology 19:193-205.

Havens, P. L., and H. F. Rase. 1991. Detoxification of organophosphorus pesticide solutions. In: Emerging Technologies in Hazardous Waste Management II. ACS, Washington, DC. pp.261-281.

Hayes, E. B., T. D. Matte, and T. R. O'Brien. 1987. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. New England Journal of Medicine 320:1372-1376.

Heisick, J. E., D. E. Wagner, M. L. Nierman, and J. T. Peeler. 1989. *Listeria spp.* found on fresh market produce. Applied and Environmental Microbiology 55:1925-1927.

Hirakoso, S. 1969. Inactivating effects of microorganisms on insecticidal activity of Dursban. Japanese Journal of Experts in Medicine 39:17-20.

Ho, J. L., K. N. Shands, G. Friedland, P. Ecklind, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Archive International Medicine 146:520-524.

Hochberg, E. G. 1996. The market for agricultural pesticide inert ingredients. In: Pesticide Formulation and Adjuvant Technology. Eds. C. L. Foy and D. W. Pritchard. CRC Press, Boca Raton, FL. pp.203-208.

Huang, C. 2000. Personal communication via email. Zeneca Agro, 3-75 Scurfield Blvd, Winnipeg, MB.

Ingham, S. C., and H. E. Uljas. 1998. Prior storage conditions influence the destruction of *Escherichia coli* O157:H7 during heating of apple cider and juice. Journal of Food Protection 61:390-394.

International Commission on Microbiological Specifications for Foods. 1996. Microbiological specifications of food pathogens. In: Microorganisms in Foods. Eds. T. A. Roberts, A. C. Baird-Parker and R. B. Tompkin. Blackie Academic & Professional, London.

Isaacson, M., P. H. Canter, P. Effler, L. Arntzen, P. Bomans, and R. Heenan. 1993. Haemorrhagic colitis epidemic in Africa. Lancet 341:961.

Islam, M. S., M. K. Hasan, and S. I. Khan. 1993. Growth and survival of *Shigella flexneri* in common Bangladeshi foods under various conditions of time and temperature. Applied and Environmental Microbiology 59:652-654.

Ivashina, S. A. 1986. Interaction of Dursban with soil microorganisms. Agrokhimia 8:75-76.

Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Applied and Environmental Microbiology 65:1-5.

Jerngklinchan, J., and K. Saitanu. 1993. The occurrence of salmonellae in bean sprouts in Thailand. Southeast Asian Journal of Tropical Medicine & Public Health 24:114-118.

Jones, A. S., and F. L. Hasting. 1981. Soil microbe studies. In: Field and Laboratory Evaluations of Insecticides for Southern Pine Beetle Control. Eds. F. L. Hastings and J. E. Coster. U. S. Dept Agriculture, Southern Forest Experiment Station, Forest Service, SE-21. pp.13-35.

Juneja, V. K., S. T. Martin, and G. M. Sapers. 1998. Control of *Listeria monocytogenes* in vacuum-packaged pre-peeled potatoes. Journal of Food Science 63:911-914.

Kampelmacher, E. H., and L. M. Van Noorle Jansen. 1974. Occurrence of *L. monocytogenes* in effluents. In: VI International Symposium of listeriosis. Sutton Bonington, England. 23:66-70.

Kapperud, G., L. M. Rorvik, V. Hasseltvedt, E. A. Hoiby, B. G. Iversen, K. Staveland, G. Johnsen, J. Leitao, H. Herikstad, Y. Andersson, G. Langeland, B. Gondrosen, and J. Lassen. 1995. Outbreak of *Shigella sonnei* infection traced to imported to iceberg lettuce. Journal of Clinical Microbiology 33:609-614.

Karmali, M. A., B. T. Steele, M. Petric, and C. Lim. 1983. Sporadic cases of haemolytic uremic syndrome associated with faecal cytotoxin and citotoxin-producing *E. coli.* Lancet i:619-620.

Karns, J. S., W. W. Mulbry, J. O. Nelson, and P. C. Kearney. 1986. Metabolism of carbofuron by a pure bacterial culture. Pesticide Biochemistry and Physiology. 25:211-217.

Katayama, A., H. Isemura, and S. Kwatsuka. 1991. Population change and characteristics of chlorothalonil-degrading bacteria in soil. Journal of Pesticide Science 16:239-245.

Khan, S. U., R. M. Behki, R. I. Tapping, and M. Humayoun Akhtar. 1988. Deltamethrin residues in an organic soil under laboratory conditions and its degradation by a bacterial strain. Journal of Agricultural and Food Chemistry 36:636-638.

Kluger, J. 1998. Killer bacteria *E. coli* O157:H7. Time Magazine. August 3. Canadian edition. pp.30-36.

Kusunoki, H., M. L. Bari, T. Kajikawa, and T. Uemura. 1997. Growth of *Escherichia coli* O157:H7 in the presence of germinating radish (*Raphanus sativus* L) seeds, p. 207. In 123rd Proceedings of the Japanese Society of Veterinary Science, 2-4 April 1997.

Lal, S., and R. Lal. 1987. Bioaccumulation, metabolism, and effects of DDT, fenitrothion, and chlorpyrifos on *Saccharomyces cerevisiae*. Archive of Environmental Contamination and Toxicology 16:753-757.

Lal, S., D. M. Saxena, and R. Lal.1987. Uptake, metabolism and effects of DDT, fenitrothion and chlorpyrifos on Tetrahymena pyriformis. Pesticide Science 21:181-191.

Lappin, H. M., M. P. Greaves, and J. H. Slater. 1985. Degradation of the herbicide mecoprop [2-(2-methyl-4-chlorophenoxy)propionic acid] by a synergistic microbial community. Applied and Environmental Microbiology 49:429-433.

Larkin, M. J., and M. J. Day. 1986. The metabolism of carbaryl by three bacterial isolates, *Pseudomonas spp.* (NCIB 12042 & 12043) and *Rhodococcus sp.* (NCIB 12038) from garden soil. Journal of Applied Bacteriology 60:233-242.

Lee, J. V., D. J. Bashford, T. J. Donovan, A. L. Furnis, and P. A. West. 1982. The incidence of *Vibrio cholerae* in water, animals and birds in Kent, England. Journal of Applied Bacteriology 52:281-291.

LeMinor, L. 1984. Genus III. *Salmonella*. Baltimore, MD: Williams and Wilkins. pp. 427-458.

Levine, W. C., W. T. Stephenson, and G. F. Craun. 1991. Waterborne disease outbreaks, 1986-1988. Journal of Food Protection 54:71-78.

Leyer, G. J., L-L. Wang, and E. A. Johnson. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Applied and Environmental Microbiology 61:3752-3755.

Lindell, S. S., and P. Quinn. 1973. *Shigella sonnei* isolated from well water. Applied Microbiology 26:424-425.

Loos, M. A., J. M. Bollag, and M. Alexander. 1967. Phenoxyacetate herbicide detoxication by bacterial enzymes. Journal of Agricultural & Food Chemistry 15:858-860.

Lord, K. A., M. McKinley, and N. Walker. 1982. Degradation of permethrin in soils. Environmental Pollution Series A Ecology and Biology 29:81-90.

Madden, J. M. 1992. Microbial pathogens in fresh produce-the regulatory perspective. Journal of Food Protection 55:821-823.

Maloney, S. E., A. Maule, and A. R. W. Smith. 1988. Microbial transformation of the pyrethroid insecticides: permethrin, deltamethrin, fastac, fenvalerate, and fluvalinate. Applied and Environmental Microbiology 54:2874-2876.

Maloney, S. E., A. Maule, and A. R. W. Smith. 1993. Purification and preliminary characterization of permethrinase from a pyrethroid-transforming strain of *Bacillus cereus*. Applied and Environmental Microbiology 59:2007-2013.

Martin, M. L., D. Shipman, M. E. Potter, J. G. Wachsmuth, K. Wells, K. Hedberg, and R. V. Tauxe. 1986. Isolation of *E. coli* O157:H7 from dairy cattle associated with two cases of haemolytic uremic syndrome. Lancet ii:1043.

Martinez-Manzanares, E., M. A. Morinigo, D. Castro, M. C. Balebona, M. S. Munoz, and J. J. Borrego. 1992. Relationship between indicators of fecal pollution in shellfish-growing water and the occurrence of human pathogenic microorganisms in shellfish. Journal of Food Protection 55:609-614.

Matsumura, F., and C. R. Krishna Murti. 1982. In: Biodegradation of Pesticides. Plenum Press, New York, NY. pp.67-87.

Matthews, G. A. 1985. Application from the ground. In: Pesticide Application: Principles and Practice. Ed. P. T. Haskell. Clarendon press, Oxford. p.95.

Matthews, G. A. 1992. In: Pesticide Application Methods. John Wiley & Sons Ltd, New York. pp. 15-36.

McCathy, S. A., and F. M. Khambaty. 1994. International dissemination of epidemic *Vibrio cholerae* by cargo ship ballast and other nonpotable waters. Applied and Environmental Microbiology 60:2597-2601.

McGowan, K. L., E. Wickersham, and N. A. Strockbine. 1989. *Escherichia coli* O157:H7 from water. Lancet 29:967-968.

Miller, K. J. 1991. U. S. water reuse: current status and future trends. Municipal wastewater reuse: selected readings on water reuse EPA 430/09-91-022:18-24. U. S. Environmental Protection Agency, Washington, D.C.

Miller, L. G., and C. W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. Journal of Food Protection 57:460-464.

Mirna, A., and K. Coretti. 1979. Influence of processing on the degradation of pesticides in meat products. Meat Science 3:97-108.

Moorman, T. B. 1994. Pesticide degradation by soil microorganisms: environmental, ecological, and management effects. In: Soil Biology: Effects on Soil Quality. Eds. J. L. Hatfield and B. A. Stewart. CRC Press, Boca Raton, FL. pp.121-165.

Morinigo, M. A., R. Cornax, M. A. Munoz, P. Romero, and J. J. Borrego. 1989. Viability of *Salmonella* species in natural waters. Current Microbiology 18:267-273.

Motes, M., A. DePaola, S. Zywno-van Ginkel, and M. McPhearson. 1994. Occurrence of toxigenic *Vibrio cholerae* O1 in oysters in Mobile Bay, Alabama: an ecological investigation. Journal of Food Protection 57:975-980.

Motonaga, K., K. Takagi, and S. Matumoto. 1996. Biodegradation of chlorothalonil in soil after suppression of degradation. Biology and Fertility in Soils 23:340-345.

Munnecke, D. M., and D. P. H. Hsieh. 1975. Development of microbial systems for the disposal of concentrated pesticide suspensions. Med Facul Landbouww Rijksuniv Gent 40:1237-1247.

Nabbut, N. H., and H. M. Al-Nakhli. 1982. Incidence of salmonellae in lymph nodes, spleens and feces of sheep and goats slaughtered in the Riyadh public abattoir. Journal of Food Protection 45:1314-1317.

Neff, J. 1994. Salad says for pre-cut produce. Food Business 7:18-22.

Neill, M. A. 1997. Overview of verotoxigenic *Escherichia coli*. Journal of Food Protection 60:1444-1446.

Nguyen-the, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition 34:371-401.

Nguyen-the, C., and B. M. Lund. 1990. The lethal effect of carrot on *Listeria* species. Journal of Applied Bacteriology 70:479-488.

Niemi, R. M., and J. S. Niemi. 1991. Bacterial pollution of waters in pristine and agricultural lands. Journal of Environmental Quality 20:620-627.

O'Beirne, D. 1996. Living foods crack the ageing process. Irish Scientist Yearbook 4:33.

Obrigawitch, T., R. G. Wilson, A. R. Martin, and F. W. Roeth. 1982. The influence of temperature, moisture, and prior EPTC application on the degradation of EPTC in soils. Weed Science 30:175-181.

O'Mahony, M., J. Cowden, B. Smyth, D. Lynch, M. Hall, B. Rowe, E. K. Teare, R. E. Tettmar, A. M. Coles, R. J. Gilbert, E. Kingcott, and C. L. R. Bartlett. 1990. An outbreak of *Salmonella saint-paul* infection associated with beansprouts. Epidemiology Infection 104:229-235.

Oragui, J. I., H. M. Arridge, D. D. Mara, H. W. Pearson, and S. A. Silva. 1993. Enumeration of salmonellae in wastewater by the MPN technique. Water Research 27:1697-1699.

Orhon, D., I. Talinli, and O. Tunay. 1989. The fate of 2,4-D in microbial cultures. Water Research 23:1423-1430.

Padhye, N. V., and M. P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. Journal of Food Protection 55:555-565.

Pal, D. 1992. Microbial pollution in water and its effect on fish. Journal of Aquatic Animal Health 4:32-39.

Palumbo, S. A., J. Call, B. Huynh, and J. Fanelli. 1996. Survival and growth potential of *Aeromonas hydrophila* in reconditioned pork-processing plant water. Journal of Food Protection 59:881-885.

Palumbo, S. A., A. Pickard, and J. E. Call. 1999. Fate of gram-positive bacteria in reconditioned, pork-processing plant water. Journal of Food Protection 62:194-197.

Pappas, C. J., N. B. Kyriakidis, N. B., and P. E. Athanasopoulos. 1999. Degradation of parathion methyl on field-sprayed apples and stored apples. Journal of AOAC International 82:359-363.

Parkin, T. B. 1994. Modeling environmental effects on enhanced carbofuran degradation. Pesticide Science 40:163-168.

Peric, M. J. Raseta, M. S. Visacki, and A. Spiric. 1980. Degradation of organochlorine pesticides as influenced by micrococci isolated from fermented sausages. Technologija Mesa 21:132-133.

Pest Management Regulatory Agency. August, 1998. Regulatory information on pesticide products. Canadian Centre for Occupational Health and Safety.

Petran, R. L., E. A. Zottola, and R. B. Gravini. 1988. Incidence of *Listeria monocytogenes* in market samples of fresh and frozen vegetables. Journal of Food Science 53:1238-1240.

Piagentini, A. M., M. E. Pirovani, D. R. Guemes, J. H. Di Pentima, and M. A. Tessi. 1997. Survival and growth of *Salmonella hadar* on minimally processed cabbage as influenced by storage abuse conditions. Journal of Food Science 62:616-618.

Picard-Bonnand, E., J. Collin, and B. Carbonnelle. 1988. Study on the preservation of *Listeria monocytogenes* in different sorts of soil. Abstracts of the 10th International Symposium. The University Medical School. P'ecs, Hungary. p. 97.

Ponka, A., Y. Andersson, A. Siitonen, B. de Jong, M. Jahkola, O. Haikala, A. Kuhmonen, and P. Pakkala. 1995. *Salmonella* in alfalfa sprouts. Lancet 345:462-463.

Prescott, L. M., J. P. Harley, and D. A. Klein. 1990. The control of microorganisms. In: Microbiology. Wm. C. Brown. Dupuque, IA. pp.234-249.

Racke, K. D. 1993. Environmental fate of chlorpyrifos. Reviews of Environmental Contamination and Toxicology 131:1-151.

Racke, K. D., and J. R. Coats. 1988. Degradation of organophophorus insecticides in soil: specificity of enhanced microbial degradation. Journal of Agricultural and Food Science 36:193-199.

Racke, K. D., and J. R. Coats. 1990. Enhanced biodegradation of pesticides in the environment. ACS Symposium Series 426. American Chemical Society. Washington, DC.

Racke, K. D., D. A. Laskowski, and M. R. Schultz. 1990. Resistance of chlorpyrifos to enhanced biodegradation in soil. Journal of Agricultural and Food Chemistry 38:1430-1436.

Rafii, F., M. A. Holland, W. E. Hill, and C. E. Cerniglia. 1995. Survival of *Shigella flexneri* on vegetables and detection by polymerase chain reaction. Journal of Food Protection 58:727-732.

Rajagopal, B., V. R. Rao, G. Nagendrappa, and N. Sethunathan. 1984. Metabolism of carbaryl and carbofuran by soil-enrichment and bacterial cultures. Canadian Journal of Microbiology 30:1458-1466.

Rajkowski, K. T., and E. W. Rice. 1998. Recovery and survival of *Escherichia coli* O157:H7 in reconditioned water. Submitted for publication.

Rajkowski, K. T., E. W. Rice, B. Huynh, and J. Patsy. 1996. Growth of *Salmonella spp.* and *Vibrio cholerae* in reconditioned wastewater. Journal of Food Protection 59:577-581.

Ramos-Cormenzana, A., A. Castillo, C. Incerti, and L. F. Gomez-Palma. 1994. Bacteriological indicators of fecal contamination: result of a loading experiment with untreated urban wastewater. Journal of Applied Bacteriology 76:95-99.

Ramos Tombo, G. M., and H. U. Blaser. 1999. Chirality in agrochemistry: an established technology and its impact on the development of industrial stereoselective catalysis. In: Pesticide Chemistry and Bioscience: The Food-Environment Challenge Eds. G. T. Brooks and T. R. Roberts. The Royal Society of Chemistry. Cambridge, UK. pp.33-54.

Reyes, V. G. 1996. Improved preservation systems for minimally processed vegetables. Food Australia 48:87-90.

Ries, A. A., S. Zaza, C. Langkop, R. V. Tauxe, and P. A. Balke. 1990. A multistate outbreak of *Salmonella chester* linked to imported cantaloupe. Abstract of Interscience Conference on Anitimicrobial Agents and Chemotherapy p. 238.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, E. S. Olscott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine 308:681-685.

Roberts, S. J., A. Walker, N. R. Parekh, S. J. Welch, and M. J. Waddington. 1993. Studies on a mixed bacterial culture from soil which degrades the herbicide linuron. Pesticide Science 39:71-78.

Sage, J. R., and S. C. Ingham. 1998. Evaluating survival of *Escherichia coli* O157:H7 in frozen and thawed apple cider: potential use of a hydrophobic grid membrane filter-SD-39 agar method. Journal of Food Protection 61:490-494.

Sakata, S., N. Mikami, and H. Yamada. 1992. Degradation of pyrethroid optical isomers by soil microorganisms. Journal of Pesticide Science 17:181-189.

Satchell, F. B., P. Stephenson, W. H. Andrews, L. Estela, and G. Allen. 1990. The survival of *Shigella sonnei* in shredded cabbage. Journal of Food Protection 53:558-562.

Sato, K., and H. Tanaka. 1987. Degradation and metabolism of a fungicide, 2,4,5,6-tetra-chloroisophthalonitrile (TPN) in soil. Biology and Fertility of Soil 3:205-209.

Schlech, W. F., P. M. Lavigne, R. A. Bostolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis-evidence for transmission by food. New England Journal of Medicine 308:203-206.

Scotland, S. M., H. R. Smith, and B. Rowe. 1985. Two distinct toxins active on vero cells from *Escherichia coli* O157. Lancet ii:885-886.

Semanchek, J. J., and D. A. Golden. 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. Journal of Food Protection 59:1256-1259.

Sethunathan, N., E. M. Bautista, and T. Yoshita. 1969. Degradation of benzene hexachloride by a soil bacterium. Canadian Journal of Microbiology 15:1349-1354.

Sethunathan, N., and M. D. Pathak. 1972. Increased biological hydrolysis of diazinon after repeated application in rice paddies. Journal of Agricultural and Food Chemistry 20:586-589.

Sethunathan, N., and T. Yoshida. 1973. A *Flavobacterium sp.* that degrades diazinon and parathion. Canadian Journal of Microbiology 19:873-875.

Shaker, N., S. Abo-Donia, Y. A. El-Shaheed, and A. Ismail. 1988. Effect of lactic acid bacteria and heat treatments on pesticides contaminated milk. Egypt Journal of Dairy Science 16:309-317.

Shelton, D. R. 1988. Mineralization of diethylthiophosphoric acid by an enriched consortium from cattle dip. Applied and Environmental Microbiology 54:2572-2573.

Shelton, D. R., and T. B. Parkin, 1991. Effect of moisture on sorption and biodegradation of carbofuran in soil. Journal of Agricultural and Food Science 39:2063-2068.

Sizmur, K., and C. W. Walker. 1988. *Listeria* in prepacked salads. Lancet i:1167.

Smith, J. L. 1993. *Cryptosporidium* and *Giardia* as agents of foodborne disease. Journal of Food Protection 56:451-461.

Smith, H. R., B. Rowe, and R. J. Gross. 1987. Haemorrhagic colitis and vero-cytotoxin producing *Escherichia coli* in England and Wales. Lancet i:1062-1065.

Smith, A. E., and A. Walker. 1977. A quantitative study of asulam persistence in soil. Pesticide Science 8:449-456.

Spiric, A., V. Visacki, J. Raseta, M. Peric, and N. Rajkovic. 1981. Kinetics of decomposition of DDT in meat products under the action of selected micrococci. Technologija Mesa 22:344.

Spiric, A., V. Visacki, J. Raseta, M. Peric, and N. Rajkovic. 1983. Sixth International Congress of Food Science and Technology 2:70.

Steele, B. T., N. Murphy, and C. P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. Journal of Pediatrics 101:963-965.

Steinbrugge, E. G., R. B. Maxcy, and M. B. Liewen. 1988. Fate of *Listeria monocytogenes* on ready to serve lettuce. Journal of Food Protection 51:596-599.

Sumner, S. S., and D. L. Peters. 1997. Microbiology of vegetables. In: Processing Vegetables: Science and Technology. Eds. D. S. Smith, J. N. Cash, W. –K. Nip, and Y. H. Hui. Technomic Publishing Co., Pennsylvania. pp. 87-107.

Talinli, I. 1986. Fate of phenol and 2,4-D acid in activated sludge systems. Ph. D. thesis, Istanbul Technical University, Department of Civil Engineering, Istanbul.

Tamminga, S. K., R. R. Beumer, and E. K. Kampelmacher. 1978. The hygienic quality of vegetables grown in or imported into the Netherlands: a tentative survey. Journal of Hygiene Camb 80:143-154.

Taylor, B. C., and M. Nakamura. 1964. Survival of shigellae in food. Journal of Hygiene Camb 62:303-311.

Turco, R. F., and A. Konopka. 1990. Biodegradation of carbofuran in enhanced and non-enhanced soils. Soil Biology and Biochemistry 22:195-201.

University of Guelph press release. May 22, 1997. *E. coli*-contaminated well water linked to gastrointestinal illness.

Vahidy, R. 1992. Isolation of *Listeria monocytogenes* from fresh fruits and vegetables. (Abstract.) HortScience 27:628.

Varnam, A. H., and M. G. Evans. 1991. Foodborne Pathogens-An Illustrated Text. Wolfe Publishing, England. pp.101-500.

Walker, A. 1974. A simulation model for prediction of herbicide persistence. Journal of Environmental Quality 3:396-401.

Walker, A. 1976. Simulation of herbicide persistence in soil. II. Simazine and linuron in long-term experiments. Pesticide Science 7:50-58.

Walker, A. 1978. Simulation of the persistence of eight soil-applied herbicides. Weed Research 18:305-313.

Walker, A., and A. E. Smith. 1979. Persistence of 2,4,5-T in a heavy clay soil. Pesticide Science 10:151-157.

Walker, S. J., and M. F. Stringer. 1987. Growth of *Listeria monocytogenes* and *Aeromonas hydrophila* at chill temperatures. Campden Food Preservation Research Association Technical Memorandum no. 462. CFPRA: Chipping Campden, UK.

Wallnofer, P. 1969. The decomposition of urea herbicides by *Bacillus sphaericus*, isolated from soil. Weed Research 9:333-339.

Wallnofer, P. R., and J. Bader. 1970. Degradation of urea herbicides by cell-free extracts of *Bacillus sphaericus*. Applied Microbiology 19:714-717.

Wang, G., T. Zhao, and M. P. Doyle. 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Applied and Environmental Microbiology 62:2567-2570.

Warburton, D. W., B. Bowen, and A. Konkle. 1994. The survival and recovery of *Pseudomonas aeruginosa* and its effect upon salmonellae in water: methodology to test bottled water in Canada. Canadian Journal of Microbiology 40:987-992.

Ware, G. W. 1986. Pesticide formulations. In: Fundamentals of Pesticides: A Self-Instruction Guide. Thomson Publications, Fresno. pp.8-11,23-39.

Watkins, J., and K. P. Sleath. 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. Journal of Applied Bacteriology 50:1-9.

Wei, C. I., T. S. Huang, J. M. Kim, W. F. Lin, M. L. Tamplin, and J. A. Bartz. 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. Journal of Food Protection 58:829-836.

Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like-toxin-producing *E. coli* from dairy cattle. Journal of Clinical Microbiology 29:985-989.

Welshimer, H. J. 1968. Isolation of *Listeria monocytogenes* from vegetation. Journal of Bacteriology 95:300-303.

Welshimer, H. J., and J. Donker-Voet. 1971. *Listeria monocytogenes* in nature. Applied Microbiology 21:516-519.

Williams, J. Jr. 1999. Ensuring the safety and quality of fresh-cut produce. Scope 14:1,3-5.

Wilson, F. L., and F. W. Tanner. 1945. Behavior of pathogenic bacteria in fermented milks. Food Research International 10:122-134.

Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Applied and Environmental Microbiology 59:2526-2530.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber.1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Applied and Environmental Microbiology 61:1290-1293.

Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Applied and Environmental Microbiology 61:2127-2131.

APPENDICES

Appendix 1. Survival of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	0,1	4,3	0,0	0,0
Poast & Merge	6,6	0,3	0,0	0,0
Gramaxone	14,26	28,23	0,0	0,0
Afolan	$1.28x10^2$, $1.73x10^2$	$1.27x10^2$, $1.52x10^2$	$3.40x10^3$, $2.20x10^3$	$6.00x10^3$, $7.00x10^3$
2,4-D amine	$1.18x10^2$, $1.09x10^2$	$1.05x10^2$, $1.24x10^2$	3,4	5,3
Dithane M45	0,0	0,0	0,0	0,0
Benlate	$1.21x10^2$, $1.12x10^2$	$1.12x10^2$, $1.18x10^2$	1,0	2,1
Bravo 500	$1.59x10^2$, $1.90x10^2$	$1.04x10^2$, $1.12x10^2$	$4.96x10^6$, $5.64x10^6$	$5.78x10^6$, $6.83x10^6$
Ridomil 240EC	0,0	1,3	0,0	0,0
Thiram 75WP	$9.70x10^1$, $1.00x10^2$	$1.03x10^2$, $1.05x10^2$	0,0	0,0
Sevin XLR+	$1.15x10^2$, $1.29x10^2$	$1.04x10^2$, $9.30x10^1$	10,2	0,0
Lorsban 4E	$7.60x10^1$, $6.20x10^1$	$8.70x10^1$, $9.80x10^1$	$3.05x10^2$, $3.32x10^2$	$3.01x10^2$, $3.09x10^2$
Diazinon 500	$1.14x10^2$, $1.03x10^2$	$1.21x10^2$, $1.30x10^2$	0,0	0,0
Ambush 500EC	$1.33x10^2$, $1.19x10^2$	$1.04x10^2$, $1.11x10^2$	$2.86x10^2$, $3.26x10^2$	$4.31x10^2$, $4.90x10^2$
Logan	0,0	1,2	0,0	0,0
Control[b]	$1.36x10^2$, $1.21x10^2$	$1.04x10^2$, $109x10^2$	$1.36x10^2$, $1.21x10^2$	$1.04x10^2$, $109x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 2. Survival of *Escherichia coli* O157:H7 strain 7283 (ground beef isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	0,0	2,0	0,0	0,0
Poast & Merge	0,0	2,1	0,0	0,0
Gramaxone	11,8	2,0	1,0	0,0
Afolan	$1.50x10^2$, $1.47x10^2$	$1.37x10^2$, $1.12x10^2$	$5.48x10^5$, $5.34x10^5$,	$7.27x10^5$, $7.70x10^5$
2,4-D amine	$9.40x10^1$, $1.14x10^2$	$1.32x10^2$, $1.29x10^2$	$1.10x10^2$, $8.30x10^1$	$1.40x10^2$, $1.18x10^2$
Dithane M45	0,0	0,0	0,0	0,0
Benlate	$1.14x10^2$, $1.29x10^2$	$1.01x10^2$, $1.11x10^2$	$4.96x10^3$, $5.54x10^3$	$2.53x10^3$, $2.71x10^3$
Bravo 500	$1.70x10^2$, $1.77x10^2$	$1.00x10^2$, $1.16x10^2$	$1.26x10^6$, $1.78x10^6$	$1.00x10^6$, $1.01x10^6$
Ridomil 240EC	0,0	3,5	0,0	0,0
Thiram 75WP	74,74	1,4	0,0	0,0
Sevin XLR+	5,6	54,75	60,66	49,60
Lorsban 4E	$6.70x10^1$, $4.60x10^1$	$5.40x10^1$, $4.80x10^1$	$4.66x10^5$, $4.62x10^5$	$4.70x10^5$, $4.59x10^5$
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$1.18x10^2$, $9.10x10^2$	$1.48x10^2$, $1.25x10^2$	$9.59x10^5$, $6.07x10^5$	$6.59x10^5$, $7.37x10^5$
Logan	103,92	0,4	0,0	0,0
Control[b]	$1.24x10^2$, $1.14x10^2$	$1.21x10^2$, $1.10x10^2$	$1.24x10^2$, $1.14x10^2$	$1.21x10^2$, $1.10x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 3. Survival of *Escherichia coli* O157:H7 strain 7174 (bovine isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	7,15	27,47	0,0	0,0
Poast & Merge	15,18	78,87	1,2	4,4
Gramaxone	3,7	16,23	0,0	0,0
Afolan	$9.20x10^1$, $1.06x10^2$	$9.50x10^1$, $1.17x10^2$	$2.97x10^5$, $3.30x10^5$,	$4.03x10^5$, $2.47x10^5$
2,4-D amine	138,145	95, 129	21,44	46,50
Dithane M45	0,0	0,0	0,0	0,0
Benlate	$8.80x10^1$, $9.20x10^1$	$9.60x10^1$, $9.60x10^1$	$2.40x10^4$, $2.46x10^4$	$9.59x10^5$, $9.23x10^5$
Bravo 500	$9.40x10^1$, $1.31x10^2$	$9.40x10^1$, $1.45x10^2$	$3.78x10^5$, $3.99x10^5$	$4.45x10^5$, $6.42x10^5$
Ridomil 240EC	0,5	1,2	0,0	0,0
Thiram 75WP	16,23	47,54	0,0	0,0
Sevin XLR+	109,129	114,132	14,19	66,68
Lorsban 4E	$1.02x10^2$, $1.00x10^2$	$1.06x10^2$, $1.18x10^2$	$9.59x10^5$, $8.94x10^5$	$8.29x10^5$, $8.12x10^5$
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$1.06x10^2$, $1.15x10^2$	$1.40x10^2$, $1.23x10^2$	$7.09x10^5$, $9.59x10^5$	$7.47x10^5$, $7.97x10^5$
Logan	0,0	1,2	0,0	0,0
Control[b]	$1.08x10^2$, $1.12x10^2$	$1.09x10^2$, $1.20x10^2$	$1.08x10^2$, $1.12x10^2$	$1.09x10^2$, $1.20x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 4. Survival of *Salmonella typhimurium* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	MPN/ml[a] at time			
	1h		24h	
	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	12,14	2,0	1,0	0,0
Poast & Merge	0,0	1,2	0,0	0,0
Gramaxone	115,140	116,121	13,15	20,23
Afolan	$1.13x10^2$, $1.38x10^2$	$1.37x10^2$, $1.10x10^2$	$2.64x10^5$, $2.84x10^5$	$3.66x10^5$, $3.00x10^5$
2,4-D amine	130,143	176,192	0,3	0,2
Dithane M45	1,0	0,0	0,0	0,0
Benlate	0,3	0,0	1,0	2,1
Bravo 500	$1.23x10^2$, $1.67x10^2$	$1.29x10^2$, $1.56x10^2$	$2.40x10^7$, $4.84x10^7$	$3.83x10^7$, $2.13x10^7$
Ridomil 240EC	1,5	0,2	0,0	0,1
Thiram 75WP	17,22	16,18	0,0	0,0
Sevin XLR+	113,128	101,139	131,151	111,132
Lorsban 4E	$9.50x10^1$, $9.80x10^1$	$6.00x10^1$, $6.00x10^1$	$1.30x10^3$, $1.88x10^3$	$8.00x10^2$, $1.20x10^3$
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$1.28x10^2$, $1.18x10^2$	$1.24x10^2$, $1.19x10^2$	$2.84x10^4$, $3.27x10^4$	$2.06x10^4$, $2.50x10^4$
Logan	0,0	0,0	0,0	0,0
Control[b]	$1.59x10^2$, $1.62x10^2$	$1.05x10^2$, $1.06x10^2$	$1.59x10^2$, $1.62x10^2$	$1.05x10^2$, $1.06x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 5. Survival of *Salmonella enteritidis* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	MPN/ml[a] at time			
	1h		24h	
	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	82,85	77,79	4,9	2,4
Poast & Merge	0,0	0,0	0,0	0,0
Gramaxone	51,61	38,43	21,21	17,20
Afolan	$1.05x10^2$, $1.17x10^2$	$9.80x10^1$, $1.02x10^2$	$2.99x10^4$, $5.40x10^4$	$2.57x10^4$, $4.70x10^4$
2,4-D amine	130,131	129,130	0,3	0,0
Dithane M45	0,0	0,0	0,0	0,0
Benlate	0,0	0,0	0,0	0,0
Bravo 500	$1.28x10^2$, $1.34x10^2$	$1.13x10^2$, $1.17x10^2$	$1.72x10^6$, $1.80x10^6$	$1.56x10^6$, $1.60x10^6$
Ridomil 240EC	0,1	0,0	0,0	0,0
Thiram 75WP	8,11	7,8	0,0	0,0
Sevin XLR+	127,141	117,121	173,176	155,163
Lorsban 4E	17,20	15,17	28,50	18,40
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$7.60x10^1$, $8.10x10^1$	$6.10x10^1$, $6.40x10^1$	$3.19x10^3$, $3.46x10^3$	$2.50x10^3$, $2.84x10^3$
Logan	0,0	0,0	0,0	0,0
Control[b]	$1.11x10^2$, $1.25x10^2$	$1.06x10^2$, $1.09x10^2$	$1.11x10^2$, $1.25x10^2$	$1.06x10^2$, $1.09x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 6. Survival of *Shigella sonnei* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	13,50	48,57	0,0	0,0
Poast & Merge	15,25	0,0	0,0	0,0
Gramaxone	32,33	16,21	0,0	0,0
Afolan	$6.80x10^1$, $7.50x10^1$	$5.80x10^1$, $6.20x10^1$	$5.19x10^4$, $7.05x10^4$	$2.03x10^5$, $1.77x10^5$
2,4-D amine	89,101	62,63	26,43	56,65
Dithane M45	0,0	0,0	0,0	0,0
Benlate	0,0	0,0	0,0	0,0
Bravo 500	$7.50x10^1$, $7.70x10^1$	$7.20x10^1$, $8.40x10^1$	$4.40x10^5$, $4.09x10^5$	$5.08x10^5$, $4.91x10^5$
Ridomil 240EC	2,2	0,0	0,0	0,0
Thiram 75WP	62,68	65,68	0,0	0,0
Sevin XLR+	78,82	56,72	69,69	73,76
Lorsban 4E	20,24	24,27	0,1	1,2
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$5.30x10^1$, $6.10x10^1$	$5.80x10^1$, $6.90x10^1$	$7.83x10^3$, $8.12x10^3$	$7.83x10^3$, $7.37x10^3$
Logan	16,22	9,15	0,0	0,0
Control[b]	61,73	65,66	61,73	65,66

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 7. Survival of *Shigella flexneri* in pesticide solutions after contact times of 1 and 24 hours at 22°C.

Pesticide	MPN/ml[a] at time			
	1h		24h	
	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	12,20	6,9	0,0	0,0
Poast & Merge	0,0	0,0	0,0	0,0
Gramaxone	8,9	4,4	0,0	0,0
Afolan	110,113	103,108	93,93	100,98
2,4-D amine	60,66	52,53	1,1	0,0
Dithane M45	0,0	0,0	0,0	0,0
Benlate	0,0	0,0	0,0	0,0
Bravo 500	109,117	103,103	310,215	283,308
Ridomil 240EC	2,4	0,0	0,1	0,0
Thiram 75WP	4,5	3,3	0,0	0,0
Sevin XLR+	66,71	34,48	64,67	53,58
Lorsban 4E	30,33	22,29	1,2	0,0
Diazinon 500	0,2	0,0	0,0	0,0
Ambush 500EC	18,25	11,12	1,1	0,0
Logan	1,2	0,1	0,0	0,0
Control[b]	106,109	103,105	106,109	103,105

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 8. Survival of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	1,2	1,3	1,1	0,0
Poast & Merge	0,0	0,0	0,0	0,0
Gramaxone	67,93	68,80	18,23	28,29
Afolan	$9.30x10^1$, $1.32x10^2$	$1.64x10^2$, $1.70x10^2$	$3.91x10^6$, $5.37x10^6$,	$3.60x10^6$, $4.70x10^6$
2,4-D amine	133,169	131,156	0,0	0,0
Dithane M45	0,0	0,0	0,0	0,0
Benlate	0,0	0,0	0,0	0,0
Bravo 500	$1.30x10^2$, $1.53x10^2$	$1.36x10^2$, $1.63x10^2$	$2.63x10^7$, $3.01x10^7$	$3.37x10^7$, $3.30x10^7$
Ridomil 240EC	0,0	0,0	0,0	0,0
Thiram 75WP	6,8	2,15	0,0	0,0
Sevin XLR+	132,137	151,162	166,205	156,171
Lorsban 4E	$9.80x10^1$, $1.01x10^2$	$4.70x10^1$, $6.90x10^1$	$4.00x10^3$, $3.45x10^3$	$7.71x10^3$, $4.79x10^3$
Diazinon 500	0,0	0,0	0,0	0,0
Ambush 500EC	$3.40x10^1$, $4.50x10^1$	$6.40x10^1$, $6.80x10^1$	$1.90x10^3$, $2.80x10^3$	$2.96x10^3$, $4.55x10^3$
Logan	0,0	0,0	0,0	0,0
Control[b]	$1.29x10^2$, $1.38x10^2$	$1.08x10^2$, $1.14x10^2$	$1.29x10^2$, $1.38x10^2$	$1.08x10^2$, $1.14x10^2$

[a] Each trial was performed in duplicate

[b] Sterile tap water as control

Appendix 9. Survival of *Listeria monocytogenes* strain 19117 (sheep isolate) in pesticide solutions after contact times of 1 and 24 hours at 22°C.

	MPN/ml[a] at time			
	1h		24h	
Pesticide	Trial 1	Trial 2	Trial 1	Trial 2
Roundup	0,0	0,0	0,0	0,0
Poast & Merge	0,0	0,0	0,0	0,0
Gramaxone	0,5	12,18	0,0	0,1
Afolan	$2.62x10^2$, $3.07x10^2$	$3.43x10^2$, $3.53x10^2$	$2.40x10^3$, $4.00x10^3$	$2.80x10^3$, $7.00x10^3$
2,4-D amine	6,8	8,24	0,1	0,1
Dithane M45	0,0	0,0	0,0	0,0
Benlate	0,0	0,0	0,0	0,0
Bravo 500	$3.52x10^2$, $3.56x10^2$	$4.10x10^2$, $4.25x10^2$	$2.10x10^3$, $4.00x10^3$	$2.50x10^3$, $6.00x10^3$
Ridomil 240EC	0,2	1,4	0,0	1,1
Thiram 75WP	0,0	0,0	0,0	0,0
Sevin XLR+	181,262	310,320	345,347	352,363
Lorsban 4E	0,0	0,0	0,0	1,1
Diazinon 500	0,0	0,2	0,0	0,2
Ambush 500EC	0,0	0,1	0,0	0,1
Logan	0,0	0,0	0,0	0,0
Control[b]	$3.46x10^2$, $3.47x10^2$	$3.75x10^2$, $3.82x10^2$	$3.46x10^2$, $3.47x10^2$	$3.75x10^2$, $3.82x10^2$

[a] Each trial was performed in duplicate
[b] Sterile tap water as control

Appendix 10. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions at 22°C using a high inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	3.53 ± 0.02 [d]
	Afolan	3.74 ± 0.01 [a]
	Bravo 500	3.66 ± 0.04 [b]
	Lorsban 4E	3.55 ± 0.07 [c, d]
	Ambush 500EC	3.60 ± 0.01 [c]
24	Control[3]	3.61 ± 0.03 [e]
	Afolan	5.03 ± 0.07 [b]
	Bravo 500	6.39 ± 0.03 [a]
	Lorsban 4E	4.65 ± 0.04 [c]
	Ambush 500EC	4.53 ± 0.03 [d]
48	Control[3]	3.58 ± 0.08 [d]
	Afolan	5.99 ± 0.02 [b]
	Bravo 500	6.54 ± 0.01 [a]
	Lorsban 4E	5.06 ± 0.29 [c]
	Ambush 500EC	5.22 ± 0.10 [c]
72	Control[3]	3.79 ± 0.06 [c]
	Afolan	6.39 ± 0.07 [a]
	Bravo 500	6.57 ± 0.05 [a]
	Lorsban 4E	5.70 ± 0.33 [b]
	Ambush 500EC	5.66 ± 0.03 [b]
96	Control[3]	3.63 ± 0.05 [d]
	Afolan	6.59 ± 0.12 [b]
	Bravo 500	6.74 ± 0.06 [a]
	Lorsban 4E	6.26 ± 0.14 [c]
	Ambush 500EC	6.23 ± 0.01 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time period are not significantly different
($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 11. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	2.08 ± 0.04 [a]
	Afolan	2.07 ± 0.05 [a]
	Bravo 500	2.16 ± 0.07 [a]
	Lorsban 4E	1.66 ± 0.04 [b]
	Ambush 500EC	1.60 ± 0.11 [b]
24	Control[3]	2.00 ± 0.04 [c]
	Afolan	3.14 ± 0.05 [b]
	Bravo 500	5.21 ± 0.01 [a]
	Lorsban 4E	2.05 ± 0.02 [c]
	Ambush 500EC	1.75 ± 0.07 [d]
48	Control[3]	2.19 ± 0.08 [d]
	Afolan	4.76 ± 0.04 [b]
	Bravo 500	6.83 ± 0.05 [a]
	Lorsban 4E	2.53 ± 0.01 [c]
	Ambush 500EC	1.82 ± 0.22 [e]
72	Control[3]	2.12 ± 0.10 [e]
	Afolan	5.23 ± 0.14 [b]
	Bravo 500	7.35 ± 0.09 [a]
	Lorsban 4E	3.01 ± 0.01 [d]
	Ambush 500EC	3.39 ± 0.06 [c]
96	Control[3]	2.51 ± 0.02 [e]
	Afolan	6.04 ± 0.13 [b]
	Bravo 500	7.67 ± 0.21 [a]
	Lorsban 4E	3.28 ± 0.07 [d]
	Ambush 500EC	3.81 ± 0.17 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 12. Growth of *Salmonella typhimurium* in pesticide solutions at 22°C using a high inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	4.25 ± 0.01 [a]
	Afolan	4.26 ± 0.03 [a]
	Bravo 500	4.25 ± 0.02 [a]
	Lorsban 4E	3.61 ± 0.04 [c]
	Ambush 500EC	4.14 ± 0.03 [b]
24	Control[3]	4.35 ± 0.08 [c]
	Afolan	5.65 ± 0.03 [b]
	Bravo 500	6.63 ± 0.15 [a]
	Lorsban 4E	4.30 ± 0.19 [c]
	Ambush 500EC	5.57 ± 0.07 [b]
48	Control[3]	4.39 ± 0.06 [d]
	Afolan	6.52 ± 0.03 [a]
	Bravo 500	6.63 ± 0.07 [a]
	Lorsban 4E	5.36 ± 0.17 [c]
	Ambush 500EC	6.00 ± 0.05 [b]
72	Control[3]	4.53 ± 0.03 [d]
	Afolan	6.29 ± 0.11 [b]
	Bravo 500	6.51 ± 0.07 [a]
	Lorsban 4E	5.93 ± 0.15 [c]
	Ambush 500EC	5.96 ± 0.04 [c]
96	Control[3]	4.70 ± 0.17 [c]
	Afolan	6.65 ± 0.01 [a]
	Bravo 500	6.70 ± 0.10 [a]
	Lorsban 4E	6.20 ± 0.12 [b]
	Ambush 500EC	5.95 ± 0.04 [b]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 13. Growth of *Salmonella typhimurium* in pesticide solutions at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	1.98 ± 0.05 [b, c]
	Afolan	2.10 ± 0.03 [a, b]
	Bravo 500	2.27 ± 0.06 [a]
	Lorsban 4E	1.84 ± 0.17 [c]
	Ambush 500EC	1.65 ± 0.31 [d]
24	Control[3]	1.84 ± 0.03 [e]
	Afolan	2.59 ± 0.07 [c]
	Bravo 500	5.68 ± 0.04 [a]
	Lorsban 4E	3.32 ± 0.13 [b]
	Ambush 500EC	2.31 ± 0.11 [d]
48	Control[3]	1.99 ± 0.02 [d]
	Afolan	4.94 ± 0.34 [b]
	Bravo 500	6.30 ± 0.02 [a]
	Lorsban 4E	5.05 ± 0.09 [b]
	Ambush 500EC	2.49 ± 0.10 [c]
72	Control[3]	2.09 ± 0.07 [e]
	Afolan	6.05 ± 0.20 [c]
	Bravo 500	6.61 ± 0.12 [a]
	Lorsban 4E	6.32 ± 0.13 [b]
	Ambush 500EC	4.65 ± 0.23 [d]
96	Control[3]	2.33 ± 0.05 [d]
	Afolan	6.48 ± 0.09 [b]
	Bravo 500	6.92 ± 0.04 [a]
	Lorsban 4E	6.58 ± 0.09 [b]
	Ambush 500EC	5.71 ± 0.17 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 14. Growth of *Shigella sonnei* in pesticide solutions at 22°C using a high inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	4.12 ± 0.04 [b]
	Afolan	4.18 ± 0.05 [a, b]
	Bravo 500	4.24 ± 0.04 [a]
	Lorsban 4E	4.15 ± 0.04 [a, b]
	Ambush 500EC	4.18 ± 0.10 [a, b]
24	Control[3]	4.21 ± 0.04 [d]
	Afolan	4.48 ± 0.26 [c]
	Bravo 500	6.81 ± 0.02 [a]
	Lorsban 4E	4.45 ± 0.01 [c]
	Ambush 500EC	5.12 ± 0.04 [b]
48	Control[3]	4.16 ± 0.06 [e]
	Afolan	5.02 ± 0.06 [d]
	Bravo 500	6.97 ± 0.03 [a]
	Lorsban 4E	6.06 ± 0.03 [c]
	Ambush 500EC	6.38 ± 0.03 [b]
72	Control[3]	4.26 ± 0.04 [d]
	Afolan	6.14 ± 0.11 [c]
	Bravo 500	6.92 ± 0.03 [a]
	Lorsban 4E	6.03 ± 0.03 [c]
	Ambush 500EC	6.31 ± 0.17 [b]
96	Control[3]	4.18 ± 0.03 [e]
	Afolan	6.64 ± 0.08 [b]
	Bravo 500	6.90 ± 0.04 [a]
	Lorsban 4E	5.75 ± 0.02 [d]
	Ambush 500EC	6.23 ± 0.12 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 15. Growth of *Shigella sonnei* in pesticide solutions at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	2.28 ± 0.09 [a]
	Afolan	2.30 ± 0.06 [a]
	Bravo 500	2.26 ± 0.14 [a]
	Lorsban 4E	1.84 ± 0.07 [b]
	Ambush 500EC	1.75 ± 0.03 [b]
24	Control[3]	2.27 ± 0.05 [c]
	Afolan	2.20 ± 0.04 [c]
	Bravo 500	5.20 ± 0.12 [a]
	Lorsban 4E	2.53 ± 0.29 [c]
	Ambush 500EC	3.43 ± 0.22 [b]
48	Control[3]	2.40 ± 0.06 [d]
	Afolan	2.16 ± 0.06 [e]
	Bravo 500	7.01 ± 0.04 [a]
	Lorsban 4E	4.18 ± 0.14 [c]
	Ambush 500EC	6.21 ± 0.08 [b]
72	Control[3]	2.37 ± 0.06 [e]
	Afolan	2.99 ± 0.08 [d]
	Bravo 500	7.01 ± 0.04 [a]
	Lorsban 4E	5.57 ± 0.02 [c]
	Ambush 500EC	6.58 ± 0.05 [b]
96	Control[3]	2.46 ± 0.10 [e]
	Afolan	4.21 ± 0.09 [d]
	Bravo 500	6.97 ± 0.09 [a]
	Lorsban 4E	6.34 ± 0.07 [c]
	Ambush 500EC	6.47 ± 0.02 [b]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 16. Growth of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions at 22°C using a high inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	4.15 ± 0.02 [a]
	Afolan	4.16 ± 0.02 [a]
	Bravo 500	4.16 ± 0.03 [a]
	Lorsban 4E	3.25 ± 0.08 [b]
	Ambush 500EC	3.22 ± 0.02 [b]
24	Control[3]	3.84 ± 0.04 [c]
	Afolan	4.05 ± 0.01 [b]
	Bravo 500	5.73 ± 0.04 [a]
	Lorsban 4E	2.34 ± 0.14 [d]
	Ambush 500EC	1.46 ± 0.17 [e]
48	Control[3]	4.03 ± 0.05 [c]
	Afolan	3.40 ± 0.05 [c]
	Bravo 500	6.39 ± 0.01 [a]
	Lorsban 4E	4.37 ± 0.04 [b]
	Ambush 500EC	2.34 ± 0.07 [d]
72	Control[3]	4.03 ± 0.05 [e]
	Afolan	4.71 ± 0.14 [d]
	Bravo 500	6.40 ± 0.03 [a]
	Lorsban 4E	6.15 ± 0.02 [b]
	Ambush 500EC	5.60 ± 0.07 [c]
96	Control[3]	3.96 ± 0.04 [e]
	Afolan	5.48 ± 0.03 [d]
	Bravo 500	6.38 ± 0.01 [b]
	Lorsban 4E	6.56 ± 0.01 [a]
	Ambush 500EC	5.92 ± 0.23 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 17. Growth of *Listeria monocytogenes* strain 19112 (human isolate) in pesticide solutions at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	1.97 ± 0.16 [a]
	Afolan	2.15 ± 0.05 [a]
	Bravo 500	2.11 ± 0.04 [a]
	Lorsban 4E	1.60 ± 0.28 [b]
	Ambush 500EC	1.48 ± 0.12 [b]
24	Control[3]	1.65 ± 0.22 [c]
	Afolan	1.91 ± 0.10 [b]
	Bravo 500	4.58 ± 0.17 [a]
	Lorsban 4E	1.24 ± 0.07 [d]
	Ambush 500EC	1.24 ± 0.07 [d]
48	Control[3]	1.65 ± 0.09 [b]
	Afolan	1.74 ± 0.27 [b]
	Bravo 500	6.77 ± 0.03 [a]
	Lorsban 4E	1.65 ± 0.24 [b]
	Ambush 500EC	1.30 ± 0.09 [c]
72	Control[3]	1.72 ± 0.27 [d]
	Afolan	2.26 ± 0.17 [c]
	Bravo 500	6.81 ± 0.03 [a]
	Lorsban 4E	3.17 ± 0.05 [b]
	Ambush 500EC	1.38 ± 0.15 [e]
96	Control[3]	2.35 ± 0.13 [d]
	Afolan	3.81 ± 0.30 [c]
	Bravo 500	6.81 ± 0.01 [a]
	Lorsban 4E	4.40 ± 0.28 [b]
	Ambush 500EC	1.80 ± 0.06 [e]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 18. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) in pesticide solutions incubated at 31°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	2.16 ± 0.09 [a]
	Afolan	2.11 ± 0.06 [a]
	Bravo 500	2.23 ± 0.20 [a]
	Lorsban 4E	2.15 ± 0.12 [a]
	Ambush 500EC	2.20 ± 0.04 [a]
24	Control[3]	2.46 ± 0.10 [d]
	Afolan	3.92 ± 0.25 [b]
	Bravo 500	5.71 ± 0.03 [a]
	Lorsban 4E	2.59 ± 0.23 [d]
	Ambush 500EC	3.22 ± 0.28 [c]
48	Control[3]	3.09 ± 0.07 [d]
	Afolan	6.27 ± 0.20 [b]
	Bravo 500	6.76 ± 0.04 [a]
	Lorsban 4E	4.56 ± 0.16 [c]
	Ambush 500EC	6.42 ± 0.15 [b]
72	Control[3]	3.19 ± 0.05 [c]
	Afolan	6.29 ± 0.16 [b]
	Bravo 500	6.76 ± 0.11 [a]
	Lorsban 4E	6.26 ± 0.18 [b]
	Ambush 500EC	6.71 ± 0.09 [a]
96	Control[3]	3.27 ± 0.03 [c]
	Afolan	6.97 ± 0.06 [a]
	Bravo 500	6.96 ± 0.03 [a]
	Lorsban 4E	6.69 ± 0.10 [b]
	Ambush 500EC	6.66 ± 0.06 [b]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 19. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) at 0.5 times the recommended pesticide concentrations at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	2.22 ± 0.06 [a, b]
	Afolan	2.10 ± 0.13 [b]
	Bravo 500	2.26 ± 0.11 [a]
	Lorsban 4E	2.11 ± 0.09 [a, b]
	Ambush 500EC	2.15 ± 0.07 [a, b]
24	Control[3]	2.45 ± 0.01 [d]
	Afolan	3.74 ± 0.06 [b]
	Bravo 500	4.71 ± 0.02 [a]
	Lorsban 4E	2.13 ± 0.15 [e]
	Ambush 500EC	2.75 ± 0.11 [c]
48	Control[3]	2.55 ± 0.16 [d]
	Afolan	5.25 ± 0.28 [b]
	Bravo 500	6.38 ± 0.14 [a]
	Lorsban 4E	2.53 ± 0.01 [d]
	Ambush 500EC	3.72 ± 0.14 [c]
72	Control[3]	2.62 ± 0.23 [e]
	Afolan	6.13 ± 0.14 [b]
	Bravo 500	6.71 ± 0.10 [a]
	Lorsban 4E	3.02 ± 0.02 [d]
	Ambush 500EC	4.10 ± 0.13 [c]
96	Control[3]	2.70 ± 0.17 [d]
	Afolan	6.74 ± 0.12 [a]
	Bravo 500	6.85 ± 0.02 [a]
	Lorsban 4E	3.38 ± 0.06 [c]
	Ambush 500EC	4.20 ± 0.02 [b]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 20. Growth of *Escherichia coli* O157:H7 strain 7236 (human isolate) at 1.5 times the recommended pesticide concentrations at 22°C using a low inoculum.

Time (h)	Pesticide	Viable count[1] ± standard deviation (Log_{10} CFU/ml)[2]
0	Control[3]	2.23 ± 0.10 [a]
	Afolan	2.20 ± 0.09 [a]
	Bravo 500	2.27 ± 0.17 [a]
	Lorsban 4E	1.93 ± 0.25 [b]
	Ambush 500EC	1.65 ± 0.09 [b]
24	Control[3]	2.13 ± 0.08 [b]
	Afolan	3.10 ± 0.05 [a]
	Bravo 500	3.34 ± 0.03 [a]
	Lorsban 4E	1.65 ± 0.33 [c]
	Ambush 500EC	2.26 ± 0.09 [b]
48	Control[3]	2.34 ± 0.03 [d]
	Afolan	3.57 ± 0.06 [b]
	Bravo 500	6.70 ± 0.04 [a]
	Lorsban 4E	1.27 ± 0.06 [e]
	Ambush 500EC	2.73 ± 0.15 [c]
72	Control[3]	2.29 ± 0.03 [d]
	Afolan	3.80 ± 0.26 [b]
	Bravo 500	7.09 ± 0.16 [a]
	Lorsban 4E	1.10 ± 0.10 [e]
	Ambush 500EC	3.11 ± 0.17 [c]
96	Control[3]	2.32 ± 0.24 [d]
	Afolan	4.56 ± 0.22 [b]
	Bravo 500	7.11 ± 0.07 [a]
	Lorsban 4E	1.05 ± 0.09 [e]
	Ambush 500EC	3.59 ± 0.22 [c]

[1] Values represent means of two trials each performed in duplicate (n=4)
[2] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)
[3] Sterile tap water as control

Appendix 21. Initial and final pH of *E. coli* O157:H7 strain 7236 grown in pesticide solutions at 22°C using low and high inocula.

Pesticide	Initial	Final	
		Low	High
Afolan	7.98	8.27	8.30
Bravo 500	7.95	8.32	8.32
Lorsban 4E	7.81	8.28	8.27
Ambush 500EC	8.09	8.29	8.32

Appendix 22. Initial and final pH of *S. typhimurium* grown in pesticide solutions at 22°C using low and high inocula.

Pesticide	Initial	Final	
		Low	High
Afolan	7.98	8.26	8.26
Bravo 500	7.95	8.29	8.30
Lorsban 4E	7.81	8.30	8.29
Ambush 500EC	8.09	8.29	8.30

Appendix 23. Initial and final pH of *S. sonnei* grown in pesticide solutions at 22°C using low and high inocula.

Pesticide	Initial	Final	
		Low	High
Afolan	7.98	8.26	8.33
Bravo 500	7.95	8.30	8.29
Lorsban 4E	7.81	8.30	8.29
Ambush 500EC	8.09	8.35	8.29

Appendix 24. Initial and final pH of *L. monocytogenes* strain 19112 grown in pesticide solutions at 22°C using low and high inocula.

Pesticide	Initial	Final	
		Low	High
Afolan	7.98	8.30	8.28
Bravo 500	7.95	8.28	8.28
Lorsban 4E	7.81	8.28	8.31
Ambush 500EC	8.09	8.29	8.31

Appendix 25. Initial and final pH of *E. coli* O157:H7 strain 7236 grown in pesticide solutions (ca. 1.5 times the recommended applied dose) at 22°C using low inocula.

Pesticide	Initial	Final
Afolan	7.44	8.20
Bravo 500	7.66	8.23
Lorsban 4E	7.62	8.22
Ambush 500EC	7.90	8.17

Appendix 26. Initial and final pH of *E. coli* O157:H7 strain 7236 grown in pesticide solutions (ca. 0.5 times the recommended applied dose) at 22°C using inocula.

Pesticide	Initial	Final
Afolan	7.78	8.24
Bravo 500	7.40	8.19
Lorsban 4E	7.69	8.24
Ambush 500EC	7.91	8.23

Appendix 27. Initial and final pH of *E. coli* O157:H7 strain 7236 grown in pesticide solutions at 31°C using low inocula.

Pesticide	Initial	Final
Afolan	7.98	8.37
Bravo 500	7.95	8.33
Lorsban 4E	7.81	8.29
Ambush 500EC	8.09	8.34