THE USE OF DIOXY MP 14 (STABILIZED AQUEOUS CHLORINE DIOXIDE) TO CONTROL ENVIRONMENTAL AIRBORNE MICROORGANISMS

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A thesis submitted in fulfilment of the requirements for the degree of Magister Pharmaceuticiae in the School of Pharmacy, University of the Western Cape

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May 2009

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KEY WORDS

Microorganisms

Infectious diseases

Tuberculosis

Airborne pathogens

Biocide

Dioxy MP 14

Chlorine dioxide

Disinfection

Chicken pen

Misting system

ABSTRACT

THE USE OF DIOXY MP 14 (STABILIZED AQUEOUS CHLORINE DIOXIDE) TO CONTROL ENVIRONMENTAL AIRBORNE MICROORGANISMS

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M. Pharm Thesis, School of Pharmacy, University of the Western Cape.

Dioxy MP 14 is a locally developed form of stabilized chlorine dioxide in an aqueous medium. It has all the sanitizing properties of chlorine dioxide gas, a neutral compound of chlorine in the +IV oxidation state, which has been used extensively as a non-toxic sterilizing agent with various applications.

In this study, Dioxy MP14 was tested in a commercial chicken pen to determine its effectiveness as an environmental sanitizing agent. Control of environmental microbes in a chicken pen is important to ensure healthy birds and optimum egg production. The biocide was introduced via an overhead misting system with a variable dosing pump at various daily frequencies.

The effectiveness of environmental microorganism control was determined with air settle plates. The health and performance of the chickens were evaluated and compared to chickens in a control pen.

The results show a decrease in airborne microbial load in the treated pen. Better egg production and lower mortality of the chickens in the treated pen compared to the control pen, indicate effective environmental microbial control was

achieved with a residual 7.46 ppm Dioxy MP 14 at a daily dose given for 5 minutes every 2 hours.

This study was a pilot study, with encouraging results, for an extended study to investigate the feasibility of introducing Dioxy MP 14 through a misting system in a clinical environment (clinics and hospitals) to control airborne pathogens like *Mycobacterium tuberculosis* thereby reducing the infection risks for clinical workers and medical staff.

May 2009

DECLARATION

I declare that *The use of Dioxy MP 14 (stabilized aqueous chlorine dioxide) to control environmental airborne microorganisms* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Oluchi Nneka Mbamalu May 2009

Signed: . . . . . .

ACKNOWLEDGEMENTS

My sincere appreciation goes to the following:

Prof. Reinhard Uebel, my supervisor, for his insightful guidance and corrections throughout the duration of this work.

Med-Pride (Pty) Ltd for supply of the biocide used in the study and Magic Mist, South Africa, for installation and assistance in the use of the misting pump employed in the study.

Mr and Mrs Sakkie Botes, Chebani and Victoria of Bellevue Farms, Paarl, for the use of Bellevue Farms and for their generous assistance with the logistics involved throughout the data collection process of this study.

Prof. Pieter Gouws, for assistance with the microbiological aspects.

Mr Yunus Kippie, for technical assistance.

Kene Obikeze, for assistance with the statistical results.

School of Pharmacy, staff and colleagues, for the support and encouragement I received from everyone.

My family and friends, for the many sacrifices made towards the achievement of this goal and for their continuous love, patience and understanding.

Last but by no means the least, special thanks to the Almighty God for everything.

DEDICATION

To the Almighty God and my family

With many thanks

THE USE OF DIOXY MP 14 (STABILIZED AQUEOUS CHLORINE DIOXIDE) TO CONTROL ENVIRONMENTAL AIRBORNE MICROORGANISMS

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HOBr	Hypobromous acid
IU	International unit
KBr	Potassium bromide
KI	Potassium iodide
L	Litre
M	Molar
m	Metre
MCP	Manganese, Calcium and Phosphorus
µ	Micron
MDGs	Millennium Development Goals
MDR-TB	Multidrug resistant tuberculosis
mg	Milligram
mm	Millimetre
MORT	Mortality
N	Normal
$Na_2S_2O_3$	Sodium thiosulphate
NaOH	Sodium hydroxide
NPL	National Priorities List
o.stock	Old stock
PCE	Polychromatic erythrocytes
PROD %	Percentage production
ppm	Parts per million

PTFE	Polytetrafluoroethylene
RNA	Ribonucleic acid
SABS	South African Bureau of Standards
SAMRC	South African Medical Research Council
SQASIG	Scottish Quality Assurance Specialist Interest Group
T° MIN	Minimum temperature
T° MAX	Maximum temperature
TB	Tuberculosis
THM	Trihalomethanes
TMA	Total microbial activity
ttl. mort.	Total mortality
US	United States
USDL	United States Department of Labour
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WRAI	Walter Reed Army Institute
XDR-TB	Extremely drug resistant tuberculosis

CHAPTER 1

INTRODUCTION

1.1 Background to and motivation for study

Microorganisms (microbes) refer to a group of organisms that are too small to be visible to the naked eye (Martin, 2000), hence the name 'micro' as opposed to 'macro'. Microorganisms have been studied for ages, not only because they have some contribution to bioremediation (i.e. the use of microorganisms to reduce concentration and hence toxicity caused by various chemical pollutants) (Dua *et al.*, 2002) but also because of their proclivity to cause infections (Barnes, 2006).

Microorganisms include bacteria, viruses, some fungi, mycoplasma, protozoa, microbial spores and rickettsiae (Martin, 2000). Disease causing organisms are called pathogens. They are a source of concern because of their relationship to infections / ill health and sanitation.

Human death from infectious diseases is immense and still rising (Mulder *et al.*, 2009). At least 17 million people die each year from infectious diseases (WHO, 1996). Infectious diseases continue to be a leading cause of childhood and adult morbidity and mortality in many parts of the world, and are devastating to the African population and economy, including South Africa, where they affect predominantly disadvantaged communities (SAMRC, 2008). A World Health

Organisation (WHO) survey ranked infections as one of the leading causes of death in the world and the leading cause of death in Africa. It was also rated as a major cause of death in children and one of the greatest disablers (WHO, 1999).

Tuberculosis is one of the infectious diseases that, despite efforts at eradication, has continued to plague man especially in developing regions of the world like Africa (Gomez & McKinney, 2004). Tuberculosis is an infectious disease caused by the bacillus, *Mycobacterium tuberculosis* (first identified in 1882 by Koch) and characterized by the formation of nodular lesions (tubercles) in tissues (Martin, 2000). It is second only to HIV/AIDS as an infectious killer of adults worldwide, causing nearly nine million cases of active TB disease and two million deaths every year (WHO, 2006a).

Worldwide, Africa accounts for 29% of TB cases and 34% of reported deaths due to TB (Lemos, 2008). Considering that the continent contributes only 11% of the world population (Lemos, 2008), the burden is quite heavy and cause for serious concern.

In the list outlining the major groups of causes of death in South Africa, infectious and parasitic diseases (as a group) were the highest cause of death in 2003 and 2004. Of the infectious diseases that contributed to mortality in these years, TB was the most mentioned. TB led the list in the leading underlying natural causes of death from 1997 till 2005 (Statistics South Africa, 2006; 2007).

Against this background, the need for an immediate control of TB and its spread cannot be over-emphasized. All possible efforts have to be employed and encouraged to bring TB under control.

As much as it needs to be controlled, even more worrisome is the menace of drug resistant tuberculosis of which two types exist – multidrug resistant (MDR) and extremely drug resistant (XDR) tuberculosis (Grare *et al.*, 2008).

Multidrug resistant tuberculosis refers to any strain / variant of TB that is unaffected by the two major first-line drugs used to fight TB - Isoniazid and Rifampicin, while extremely drug-resistant tuberculosis refers to a type of multi-drug resistant TB that develops when there is resistance to at least three of the six classes of second-line drugs (WHO, 2006b). XDR-TB is extremely difficult to treat as the patient has developed resistance to all but a few drugs. These forms of TB (MDR-TB and XDR-TB) are of great concern due to the possibility of transmission (Martinez *et al.*, 2008). Close contacts of diagnosed TB patients (such as family and friends) may have to be traced for screening and examination so as to reduce the incidence of transmission to others (Ndjeka *et al.*, 2008).

A new WHO report (2008a) on the extent of drug resistance in TB intimates that in recent times, multidrug-resistant tuberculosis (MDR-TB) has been recorded at the highest rates ever. It is estimated that about 450,000 cases of MDR-TB occur worldwide annually (Amor *et al.*, 2008).

According to a South African government presentation at the 38th World Conference on Lung Health in Cape Town, 481 patients had been diagnosed with XDR TB by the end of October 2007. By the commencement of the conference (9th November 2007), 216 of these patients had died ("Global: Conference throws spotlight on growing TB threat", 2007). This underscores the severity of the situation.

At risk of infection too are the health care workers who have the responsibility of taking care of TB patients and the families and friends / associates of such patients. It is estimated that about one-third of the world's population is infected with TB (Larson & Narain, 2001). About 10% of these people develop active TB, which can be fatal, usually in the lungs. This can easily spread to other people (Joshi *et al.*, 2006).

As an occupational health hazard, TB is significant among health care workers in low and middle-income countries (Joshi *et al.*, 2006). The insufficiency of well-trained health workers around the globe has been an issue for a long time now with the crux felt especially among the developing countries (WHO, 2006c). In certain communities, this scenario where the people who look after the sick fall sick too, can be the cause of a total collapse of the health care system. For this reason, it is urgent and very important to find a way of protecting and preventing cross infection in health care workers (Joshi *et al.*, 2006).

Tuberculosis is spread mainly through infected air-borne droplets released into the air when an infected person coughs, sneezes, talks, spits, sings or does anything that

can release infected droplets into the surrounding air (Dye *et al.*, 2006). Anyone who inhales such infected droplet(s) can become infected, making health care workers a high-risk group. In fact, many cases could be said to be due to occupational exposure (Joshi *et al.*, 2006).

Hospitals are believed to be important focal points for the transmission of MDR-TB, often resulting in high mortality (Joshi *et al.*, 2006). It is believed that hospitals in developing countries play host to at least 90% of TB cases worldwide (van Gorkom, 1999; Jones-Lopez & Ellner, 2005). This is of great concern due to their deficient resources in prevention of TB transmission (Harries *et al.*, 1997; Pai *et al.*, 2006).

With about 8 million new cases of active tuberculosis diagnosed, and 2 million deaths occurring each year (Kim *et al.*, 2005), there is a need for intensified action against the TB infection. Among the recommendations put forward by the United States Department of Labour (2009) to prevent TB transmission is the implementation of an effective control program which minimizes exposures to TB (USDL, 2009).

It can therefore, not be overemphasized that research and investigation into means of controlling the spread of infection in healthcare facilities, is of vital importance. An alternative approach in controlling cross infection with airborne droplets is the use of effective disinfectants and biocides. These agents can also effectively be employed for decontamination of environments subjected to infectious pathogens (Fraise, 2007).

In this study, a proposed biocide was employed and tested for its efficacy to control environmental microorganisms in a poultry setting. It serves as a pilot study and model, under extreme environmental conditions, which in future can be extended to a hospital setting for environmental control of the TB bacillus.

1.2 Diseases and infections in the poultry

Diseases and infections have been a source of concern not only to the human population; they have also always been of major concern to the poultry industry. Risk of infections is increased with the high microbial load often associated with animal husbandry (Ruano *et al.*, 2001).

Poultry comprises all the birds that people keep for their use. Generally, this includes chicken, turkey, duck, goose, quail, pheasant, pigeon, guinea fowl, pea fowl, ostrich, emu and rhea (Butcher *et al.*, 2009). The poultry industry is a very big one. In South Africa for instance, the poultry industry contributes about 16% to the total gross value of agriculture with a growth in the range of 7% per annum for domestic demand (Esterhuizen, 2007). In several parts of the world, the poultry industry is acknowledged as a big contributor to the agricultural industry. Remarkable growth has been noticed over the years, with poultry food products surpassing that of cattle, and even with potential for further growth (Daghir, 2008). Poultry also serves as a source of income in developing communities of various developing countries in Africa (Alders *et al.*, 2005). Any factor that will affect the production output of poultry is therefore, a matter worthy of interest.

The chicken is a very common domestic animal. At a population of more than 24 billion in 2003 (Perrins, 2003), they are the most populous of the birds. Humans make use of chicken as a source of food, in the form of meat and eggs.

Chickens and other birds in the poultry class are affected by a number of infectious diseases. One of such diseases is Tuberculosis (WRAI, 1988). The primary route of transmission of tuberculosis from infected animals is through the aerosol route (Fleming & Hunt, 2000). This makes it easier for infection to be transmitted via infected bedding in addition to transmission through coughing (WRAI, 1988). Disinfection with the appropriate effective disinfectant can rid the bedding of the TB bacterium as well as other infective microorganisms (Rutala *et al.*, 1991).

Microbes in general, can affect chick quality and hence production (Temperley & Limper). Hygiene and sanitation play a crucial role in the prevention and control of disease outbreak and / or spread in poultry production premises (Meroz & Samberg, 1995). The ultimate disinfectant should be bactericidal, fungicidal, virucidal and sporicidal (Temperley & Limper). Chlorine dioxide has been reported to have such characteristics (Isomoto *et al.*, 2006; Vandekinderen *et al.*, 2009).

1.3 Disinfection in South Africa

In South Africa, disinfectants are controlled under Act 54 of 1972 and regulated by the South African Bureau of Standards (SABS). It is required that all disinfectants and detergent-disinfectants are registered with the regulatory body (SABS, 2008a).

Disinfectants in South Africa are regulated by the SABS and controlled, along with foodstuffs and cosmetics, under Act 54 of 1972 (Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972), described:

The Act, as amended by No. 39 of 2007: Foodstuffs, Cosmetics and Disinfectants Amendment Act, 2007, serves "to control the sale, manufacture, [and] importation and exportation of foodstuffs, cosmetics and disinfectants; and to provide for [incidental] matters connected therewith.".

Under the act, the term 'disinfectant' means any article or substance used or applied or intended to be used or applied as a germicide, preservative or antiseptic, or as a deodorant or cleansing material which is not a cosmetic (Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972).

The quality of disinfectants is tested and assured by the SABS. Standards are important because they ensure that only suitable products, processes and services are used in any setting. By so doing, they shield consumers from the reach and danger of unsafe products and services (SABS, 2008b).

Dioxy MP 14 is registered by the South African Bureau of Standards as bactericidal and sporicidal (Product Information Fact Sheet: Dioxy MP 14).

1.4 Work plan and objectives

In the study, an aqueous disinfectant (Dioxy MP 14) will be investigated for its effectiveness in airborne microbial control in a poultry farm. The biocide will be dispersed, using a misting pump, in the form of a mist in the poultry environment / pen. The outcome of the study will determine whether this method can be introduced into a contaminated hospital setting such as a tuberculosis ward.

Two pens housing between 2000 and 2500 chicken will be used for investigating the efficacy of Dioxy MP 14 against general / common environmental microbes.

In the first part of the investigation, the aqueous biocide solution (at four different concentrations), will be released into the pen on different days. The dispersal of the biocide will be done by an overhead high pressure misting system via pipes that run the length and breadth of the pen. The first pen (Pen A) will receive the biocide while the second (Pen B), which will serve as the control, will not receive the biocide – the overhead pipes for spraying the biocide are not present in pen B. Nutrient agar and potato dextrose agar plates will be exposed (settle plate method) in both pens for 5 minutes before and after initial spraying with the biocide and then every hour for about 6 hours without further spraying. The plates will be incubated and the number of colony forming units (CFUs) units determined.

Following standard microbiological procedure, the nutrient agar plates for the growth of bacteria will be incubated at 37°C for 24 hours; the potato dextrose agar plates (for the growth of yeasts and moulds) will be incubated at 25°C for 5 days (White &

Hood, 1931a). Microbiological activity will be assessed by checking the plates for Total Microbial Activity (TMA) and yeast and mould counts. The result from the test /experimental pen (pen A) will be compared to that from the control pen (pen B).

Another aspect of the first part of the investigation is the control experiment. Water (the solvent for the biocide solution) will be sprayed at regular intervals instead of the biocide. The nutrient agar and potato dextrose agar plates will be exposed twice daily again, incubated at 37°C for 24 hours and 25°C for five days (White & Hood, 1931a) respectively and the TMA and yeast and mould count determined. This will serve as a control to compare with the results from the previous parts.

The second part of the investigation will involve setting the pump to disperse the biocide at a particular concentration for a specific length of time on a regular basis. The concentration to be employed in this second part will be determined by ascertaining which of the four concentrations used initially gave the best result in terms of microbial control. The efficacy of the biocide to control environmental microbes at this concentration will be monitored yet again by exposing nutrient agar and potato dextrose agar plates once more for 5 minutes in the mornings and evenings. The growth of microbes will be observed and the TMA and number of yeasts and moulds recorded after incubation of the plates.

Production factors such as quantity of eggs produced and mortality will be recorded. Environmental changes in temperature and wind will be taken note of as well.

1.5 Hypotheses

It was hypothesized that:

- Dioxy MP 14 (a stabilized form of chlorine dioxide) being a very potent biocide will be able to eradicate pathogens in the poultry environment.
- By using a misting system, the biocide can be sprayed into the poultry to reach all corners.
- The liquid in the aerosols makes them denser than air and so the biocide in the form of a mist will be denser than air making it easier for particles attached to settle.
- A chicken pen environment is a good model for evaluating the control of airborne pathogens because of the susceptibility of chickens to airborne pathogens causing respiratory diseases.
- If airborne microbial load is controlled by Dioxy MP 14 applied through a misting system in a chicken pen environment (dirty conditions), then it is most likely to control airborne pathogens in a hospital environment which is considerably cleaner.

Usually, when there is need to use chlorine dioxide gas for disinfection or decontamination purposes, people have had to be evacuated from the environment in question as happened in 2007 in Oxnard, United States (Kisken, 2007). In this study however, a form of chlorine dioxide (Dioxy MP 14) will be investigated as a biocide without people / animals having to be evacuated from the contaminated environment. The concentration employed is lower than that

employed in the case mentioned above. Preparations that contain chlorine dioxide have been employed in post-harvest treatment of fresh and fresh-cut produce to reduce yeasts, moulds and spores (Fu *et al.*, 2007) in foods such as berries (Sy *et al.*, 2005a), apples and peaches (Sy *et al.*, 2005b).

CHAPTER 2

LITERATURE REVIEW

2.1 Disinfection

To control the spread of the TB bacillus in health care settings, it is necessary to investigate other measures different from the ones already being utilized. Disinfection of the environment with the appropriate disinfectant can serve as one of the environmental control measures to prevent TB transmission from patients to health care workers and other people – family and friends – around them.

Disinfection refers to the reduction of viable microorganisms to a level that is considered safe / non-threatening to the specific environment. This basically involves destruction of pathogenic microorganisms (Lerner & Lerner, 2003). High microbial load translating to an increased risk of infection is common in animal husbandry. Cleaning and disinfection of such premises can be used to control pathogen levels in animal facilities (Ruano *et al.*, 2001).

A proper disinfection program usually has a safe and easy outline of disinfectant application, proper use of application equipment and a system in place for monitoring (Ruano *et al.*, 2001).

Disinfection can be by the use of physical means (such as heat, cold, radiation and filtration) or through the use of chemicals (called disinfectants) (McDonnell, 2007).

By minimizing the risk of transfer of microorganisms, disinfectants prevent the spread of infections (Hardy, 2003).

2.2 The ideal disinfectant

The ideal disinfectant for use in a poultry environment should possess the following characteristics: (Cole, 1987; Springthorpe, 2000; Asano *et al.*, 2007).

Safety: The ideal disinfectant should not pose any threat to the safety of the people applying it or the animals housed in the poultry.

Broad spectrum: At a low concentration, it should be effective against a wide variety of infectious organisms in a very short time.

Rapid action: The ideal disinfectant should have a rapid action.

Absence of toxicity: The disinfectant is expected to be toxic to the target organism (the microbes); however, there should be no toxicity (to the handlers / other higher forms of life) due to the disinfectant or any of its by-products. In essence, it should be selectively toxic against microorganisms.

Environmentally friendly: It should not cause or aggravate any environmental problem. In this period when there is concern over the stability of the planet, the importance of this cannot be over-emphasized.

Non-destructibility to utensils and fabrics: Even in the most careful of settings, the possibility of aqueous disinfectant spillage cannot be overruled. In the event that this does happen and gets in contact with clothing, it should not cause any damage to it.

Neither should it react with or corrode the storage container(s) or utensils used to mix or apply it. This would reduce long term maintenance costs.

Non-accumulation: For reasons of safety, the appropriate disinfectant for use in a poultry setting should not accumulate to any harmful level in meat or eggs.

Stability: It should not react with air or other materials, including the storage container.

Not easily inactivated. Most disinfecting agents take several seconds or minutes to reduce the population of microorganisms to safe levels. Ideally, a disinfecting agent should not lose its potency or effectiveness while in action or while in storage for an extended time.

Water solubility: It should dissolve easily in water, even in hard water.

Effectiveness in the presence of organic matter: The efficacy of disinfectants tends to be reduced, to varying degrees, in the presence of organic matter (Scott & Gorman, 1992). The ideal disinfectant should still be able to act against microbes in spite of this.

In addition, it should be free of volatile organic compounds and have no hormone-disrupting components (Springthorpe, 2000).

However, it is imperative to mention that no one disinfectant possesses all these qualities. Decisions as to which disinfectant is to be used are made with consideration taken of the environment in question and the factors desired for it (Cole, 1987).

2.3 Current poultry disinfectants

Common disinfectant chemicals used in poultry operations include quaternary ammonium compounds (quats), iodophors, phenolic derivatives, formaldehyde and glutaraldehyde, and the oxidizing compounds (Gradel *et al.*, 2005).

The quaternary ammonium compounds are derivatives of ammonium compounds in which all four of the hydrogens bonded to nitrogen have been replaced with hydrocarbyl groups (McNaught & Wilkinson, 1997). They are amphoteric surfactants widely used for the control of bacterial growth in clinical and industrial environment (Brannon, 1997). Their activity is believed to be due to inactivation of cell metabolic pathways and denaturition of proteins (Fraise, 1999). As disinfectants, these products are well tolerated and exhibit no toxic effect on the skin and mucous membranes. They are very active against Gram positive bacteria, lethal effect being observed at concentrations as low as 1:200 000. Optimum activity is at neutral to slightly alkaline pH, inactivity setting in below a pH of 3.5. Activity is seriously impaired in the presence of organic matter. Germicidal activity of the quaternary ammonium compounds is limited as they are not useful against spores (Scott & Gorman, 1992).

The compounds classified as iodophors were developed to overcome the shortcomings of iodine such as loss of stability, irritation and staining. They are a complex of iodine and a carrier which acts as a reservoir of the free iodine (Gottardi, 1991). Antimicrobial activity is believed to be due to rapid penetration and

destruction of major protein groups, nucleotides and fatty acids resulting in cell death (Kruse, 1970; Chang, 1971; Apostolov, 1980; Gottardi, 1991). Germicidal activity is maintained in the iodophors, however antifungal and sporicidal activity is believed to be less than that of the tincture (Rutala, 1995).

Depending on the compound, phenolic-type antimicrobial agents have for long been utilized as antiseptics, disinfectants or preservatives (McDonnell & Russell, 1999). The phenolic disinfectants act by causing leakage of intracellular constituents (McDonnell & Russell, 1999). They possess antiviral and antifungal characteristics, the latter probably due to damage to the plasma membrane, leading to a leakage of intracellular constituents (McDonnell & Russell, 1999). The phenolics which find use as disinfectants have good antimicrobial activity (Scott & Gorman, 1992). Germicidal efficacy of this class of compounds is significantly affected by comparably small dilutions because of their high concentration exponents (Okore, 2005). Phenols have a major disadvantage of systemic toxicity and corrosive effect on skin and tissues (Scott & Gorman, 1992).

The aldehyde disinfectants (formaldehyde and glutaraldehyde) come with a broad spectrum of activity against microorganisms, including spores. Activity is minimally impaired in the presence of organic matter (Hanlon & Hodges, 2007). Glutaraldehyde acts by targeting cross-linking of proteins in the cell envelope (cell wall, outer membrane) and formaldehyde also has a similar mechanism, targeting cross-linking of proteins, RNA and DNA in microbial macromolecules (McDonnell & Russell, 1999). Use of the aldehyde disinfectants is not ideal because of concerns

about the possible carcinogenic properties of formaldehyde (Chiappelli & Chiappelli, 2008). Health problems such as dermatitis, conjunctivitis, rhinitis, epistaxis and asthma among endoscopy personnel are also suspected to have a link to glutaraldehyde exposure (BSG Endoscopy Committee Working Party, 1998; Ayliffe, 2000; Hernandez *et al.*, 2008).

The oxidizing agents inactivate microbial cells by oxidizing functional groups of proteins. A wide spectrum of antimicrobial activity is exhibited by this class of antimicrobials, including sporicidal activity (Hanlon & Hodges, 2007).

Chlorine dioxide is an oxidizing agent. Like other members of the group, it has a wide spectrum of activity (including activity against spores) (Weaver-Meyers *et al.*, 1998). It has a rapid killing effect on microorganisms and even has sustained activity in the presence of organic matter (Dawson & Brown, 1987).

2.4 Why chlorine dioxide?

Chlorine dioxide is a versatile biocide (Isomoto *et al*, 2006; Vandekinderen *et al.*, 2009). Because of sustained activity in the presence of organic matter (Dawson & Brown, 1987), it is a good choice for this study set in a poultry house.

Chlorine dioxide has been rated as an excellent choice among disinfectants. In addition to the fact that it does not form significant amount of trihalomethanes, its disinfectant properties are not significantly reduced at higher pH, unlike that of chlorine. This makes it more effective than chlorine at higher pH levels (AWWA & ASCE, 1998).

2.5 Why a mist-delivery system?

The focus of the study was to evaluate the efficacy of the biocide in the control of airborne microorganisms. An aerosol delivery system is a good medium for this as the biocide would get in contact with airborne contaminants (McKenzie *et al.*, 1959). In the study, Dioxy MP 14 was investigated for its efficacy in reduction of microbial contamination under normal undisrupted living conditions. The misting pump delivered the biocide in so fine a form that even though the mist was visible, no wetting occurred, thus ensuring minimum disruption, an advantage over spraying just ordinary liquid. Staff of the chicken farm were able to move around performing their duties even while the biocide was in delivery.

2.6 Chlorine dioxide as a disinfectant

Dioxy MP 14 has reportedly been tested and found to be effective against a 'broad spectrum of microorganisms' (Product Information Fact Sheet: Dioxy MP 14). Its active ingredient, chlorine dioxide, has been researched on and found to be up to seven times more effective than chlorine (Lillard, 1979).

The biocide of choice for use in a poultry environment should have a wide spectrum of activity and be safe for use in the presence of chicken (Temperley & Limper). It should also be effective in the presence of organic matter of which there is a lot in the poultry (Cole, 1987). Chlorine dioxide has been found to be effective in the presence of organic matter (Dawson & Brown, 1987).

Dioxy MP 14 is a stabilized aqueous solution of chlorine dioxide. Chlorine dioxide is a neutral compound of chlorine in the +IV oxidation state (USEPA, 1999). At room temperature, it exists as a greenish yellow to orange gas with a characteristic pungent chlorine-like odour. It is a strong oxidizing agent and can explode if concentrations are in excess of 10% v/v at atmospheric pressure. It is also easily detonated by sunlight or heat (Budavari, 1996). In the absence of light however, chlorine dioxide can remain stable in dilute solution in a closed container (AWWA, 1990).

In the United States, the liquid form of chlorine dioxide was first registered for use in disinfection and sanitization of a variety of sites (e.g., animal farms, bottling plants, food processing, handling and storage plants) by the United States Environmental Protection Agency (USEPA) in 1967, under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (USEPA, 2007).

2.6.1 Mode of action of chlorine dioxide

Chlorine dioxide is known to exert its effect through oxidation (Renberg *et al.*, 1995). Because chlorine dioxide existence in aqueous solution is almost 100% in the molecular state, i.e. it does not hydrolyze to a significant extent (Aieta & Berg, 1986; Junli *et al.*, 1997), and owing to its strong oxidizing capacity, it is relatively easy for this compound to penetrate the cell wall of bacteria. When the permeability of the bacterial membrane has been interfered with, inhibition of bacterial respiration occurs with inactivation of phosphotransferase. Enzyme activity is also lost resulting in bacterial death (Junli *et al.*, 1997). Chlorine dioxide was also found to inactivate

Hepatitis A virus by damaging viral nucleic acid and / or destroying the antigenicity of the virus (Li *et al.*, 2004).

2.6.2 Use and efficacy of chlorine dioxide

Chlorine dioxide has been found effective in the control of microbes in a number of settings. It enjoyed wide acclaim in 2001 as the agent used (in the United States) to decontaminate the Hart Senate Office Building of *Bacillus anthracis* spores during the bio-terrorism anthrax scare (Gugliotta & Warrick, 2001; Wilson *et al.*, 2005).

Dioxy MP 14 is a locally produced form of stabilized chlorine dioxide in an aqueous medium. Chlorine dioxide is not known to hydrolyse to any significant extent in water (Aieta & Berg, 1986; Junli *et al.*, 1997). As such, in aqueous solution, it should have all the sanitizing properties of chlorine dioxide (gas), a very potent biocide employed for microbial control. As earlier mentioned, aqueous chlorine dioxide has a bactericidal effect, the same as a seven times stronger concentration of chlorine (Lillard, 1979).

Since its discovery in the 1800s by Sir Humphrey Davy (Southwell, 2002), there has been a lot of published work on the uses and efficacy of chlorine dioxide. Among its uses are:

Potable Water Disinfection: It has been employed extensively as a biocidal agent in drinking water. About 700 to 900 public water systems employ chlorine dioxide in the treatment of potable water (Hoehn, 1992).

As far back as the late 1970s, a number of waterworks – 495 in Europe, 84 in the United States, 10 in Canada and about 10 in other parts of the world – were reported to have been using chlorine dioxide to disinfect drinking water. The Environmental Protection Agency (EPA) of the United States ranks it first as a replacement for chlorine (Junli *et al.*, 1997). It is better than chlorine as a disinfectant and is also employed to remove iron and manganese ions from raw water (Junli *et al.*, 1997). One of the reasons why chlorine dioxide may be preferred to chlorine in drinking water disinfection is because, unlike the latter, it is unable to react with ammonia, humic acid and other precursors. This way, it does not form chloramines, chlorophenols or trihalomethanes (THM) which are undesirable in drinking water (Sadiq & Rodriguez, 2004). Dietrich *et al.* (1992) reported that most surveyed utilities used chlorine dioxide, ClO_2, primarily to reduce THM levels.

Microbial Control: It has also been used for the destruction of spores, viruses, bacteria, fungi and other pathogenic organisms. Chlorine dioxide is a versatile antimicrobial that can be used in numerous applications as it is effective against viruses, fungi and algae over wide temperature and pH ranges (Winniczuk & Parish, 1997; Han *et al.*, 2001; Isomoto *et al.*, 2006). Chlorine dioxide can also inactivate 90% of *Cryptosporidium* oocysts, a specific pathogen of interest in drinking water supply (Betancourt & Rose, 2004).

Chlorine dioxide has further been employed in the control of *Legionella* species in hospital water supplies. A 17-month evaluation study reported that chlorine dioxide (at low and safe concentration limits) was very effective in the eradication of

Legionella species from a hospital water supply and even suggested that with its use, the problem of *Legionella* contamination of hospital water supplies may soon be a thing of the past (Srinivasan *et al.*, 2003).

The infamous *Escherichia coli* which are acid tolerant bacteria (Cheng *et al.*, 2003) have also been found to be susceptible to chlorine dioxide (Han *et al.*, 2000; Du *et al.*, 2003).

A chlorine dioxide-containing mouth rinse has also been found to reduce oral malodor (Frascella *et al.*, 2000).

Food processing: Chlorine dioxide is employed in post harvest storage of the fig fruit to delay fruit spoilage (Karabulut *et al.*, 2009). Pathogens on various fruits and vegetables have been reduced with aqueous chlorine dioxide solution and chlorine dioxide gas although the efficacy of the aqueous solution was decreased with injured produce (compared to uninjured ones) because microbes can hide in the wounds of such foods (Gómez-López *et al.*, 2009). It is also utilized in the processing of different foods - vegetables (Costilow *et al.*, 1984; Reina *et al.*, 1995), fish (Key *et al.*, 1996; Lin *et al.*, 1996), and meat (Cutter & Dorsa, 1995) including poultry (Villarreal *et al.*, 1990; Tsai *et al.*, 1995).

Bakery industries reportedly used it to whiten flour in the 1990s (Ranken *et al.*, 1997).

Industrial application: Use is made of chlorine dioxide in some other food unrelated industries for such tasks as bleaching of wood pulp in the pulp and paper industry (Young & Akhtar, 1998), removal and prevention of biofilms (Wirthlin & Marshall, 2001; Gagnon *et al.*, 2005), and for the control of zebra mussels in water (Aldridge, 2006).

2.6.3 Advantages of chlorine dioxide as a disinfectant

Chlorine dioxide does not form disinfection by-products, DBPs, because its action is not through chlorination (Aieta & Berg, 1986). These are compounds, suspected to be carcinogenic, which are formed when chlorine reacts with organic materials. The trihalomethanes consist of four chemical components: bromodichloromethane $CHBrCl_2$, dibromochloromethane $CHBr_2Cl$, chloroform $CHCl_3$ and bromoform $CHBr_3$ (Sorlini & Collivignarelli, 2005).

The WHO has recommended chlorine dioxide for use in disinfection (Lin *et al.*, 2007). It has strong antimicrobial properties (Fu *et al.*, 2007) with activity against viruses, bacteria and even spores (Isomoto *et al.*, 2006) and, unlike chlorine, is still active in the pH range found in natural waters (Thurston-Enriquez *et al.*, 2005).

Chlorine dioxide has more oxidizing capacity than chlorine (Benarde *et al.*, 1965) and unlike chlorine; does not react with ammonia or most organic compounds to form trihalomethanes (Sadiq & Rodriguez, 2004).

It is effective as a gas against microbes and still retains this efficacy in water because even though it is very soluble in water, it does not dissociate extensively and so its gaseous disinfecting properties are still retained (Aieta & Berg, 1986; Junli *et al.*, 1997).

Because chlorine dioxide is effective over a wide pH range; it can act in considerably more settings than some other disinfectants (White, 1992; AWWA & ASCE, 1998). At low concentrations, it can serve as an alternative to 2% glutaraldehyde. It has the potential to offer rapid high-level disinfection in endoscopy units. Chlorine dioxide solutions have been proven to have faster microbiocidal effects on *B. subtilis* and *M. avium-intracellulare* compared with glutaraldehyde. Indeed, endoscopes contaminated after upper gastrointestinal examination were successfully disinfected by low-level chlorine dioxide solution either manually or by using the automated reprocessor (Isomoto *et al.*, 2006).

2.6.4 Risks associated with chlorine dioxide

The following information on the risks and toxicities associated with chlorine dioxide are available from the Agency for Toxic Substances and Disease Registry, ATSDR (2007), of the United States:

The most serious and risky waste sites in the United States are identified by the Environmental Protection Agency, and placed on the National Priorities List (NPL, a target for the Federal government long-term clean-up activities). Neither chlorine

dioxide nor chlorite (a by-product of chlorine dioxide) has been found in any of the 1,647 current or former NPL sites.

In water and moist body tissues, chlorine dioxide and chlorite are quite reactive. Chlorine dioxide gas has been known to cause irritation to the nose, eyes, throat and lungs if inhaled and to the mouth, oesophagus and stomach if orally ingested. A number of factors such as dose of biocide, duration of exposure, and mode of contact as well as effect of any other co-exposed chemical determine if the victim is at risk and the extent of damage he /she has been exposed to.

Exposure to amounts in air large enough to cause body damage is rare (this is because on exposure to air, chlorine dioxide is quickly broken down to chlorine gas and oxygen); symptoms of such rare exposure include shortness of breath and other respiratory problems. On entrance into the body, chlorine dioxide is quickly broken down to chlorite and subsequently, chloride ions, needed by the body for normal functioning. Most of the unmetabolized chlorite and some of the chloride ions leave the body in a matter of hours or days.

Exposure to very high amount of chlorine dioxide and chlorite results in similar effects in both animals and human beings. Children and adults exposed to high levels of these chemicals would probably be similarly affected. However, reduction in oxygen carrying capacity of the blood (leading to difficulty in breathing) may manifest more quickly in children. High exposure prenatally and in the course of early development (postnatally) has also been noticed to delay brain development.

Although this has been noticed in young animals, no such effect has actually been noticed in humans.

A study carried out on a chlorine dioxide-based disinfectant powder in the Huaxi School of Public Health, Sichuan University in China reports an absence of toxicity and irritation in addition to an absence of mutagenic effect on mouse marrow polychromatic erythrocytes (PCE) (Zhu *et al.*, 2008).

2.7 Study setting: an open system

An open system is one that exchanges matter freely with its surrounding environment. A closed system, on the other hand, has no interaction with its environment. Activities in closed systems can be controlled because of the lack of interference from the larger environment. The characteristics of an open system however, cannot be controlled. A microorganism-containing system responds quickly to environmental changes and is an example of an open system (Gitelson *et al.*, 1997; Borodina *et al.*, 2003). This was the setting in which the study was carried out. Based on the results obtained in such a setting, it has been projected that even more favourable results will be obtained in a closed system or a system which at least possesses some degree of closure such as the strategic future setting of the study, a TB infection ward.

CHAPTER 3

RESEARCH DESIGN AND PROCEDURES

3.1 Materials and Methods

3.1.1 Equipment

The following equipment were employed in the course of the study:

Water distiller *(Analyst HP, Purite Ltd, Oxon, England).*

Electric heater *(IKA-WERKE, Germany).*

37°C incubator *(Memmert incubator, Western Germany).*

25°C incubator *(Labotec, Serial Number 1136).*

Weighing balance *(OHAUS, Model SPU402, OHAUS Corporation, USA).*

Autoclave *(Almor autoclave, Model HA1744, Omron Tateisi Electronics).*

Colony counter *(Suntex, Model CC-560, Taiwan).*

2°C cold room.

3.1.2 Materials

Materials used were as follows:

Dioxy MP 14 – stabilized aqueous chlorine dioxide solution of approx. 2000 ppm *(Med-Pride (Pty) Ltd, Panorama, South Africa).*

Bioscrub™ – 4% chlorhexidine gluconate *(Dismed Pharma (Pty) Ltd, Randjespark, South Africa).*

Residual disinfectant – quaternary ammonium compound / Tributyl tin oxide blend (*SteriTech, South Africa*).

Absolute ethanol *(Saarchem, South Africa).*

Nutrient agar *(Merck, South Africa).*

Potato dextrose agar *(Merck, South Africa).*

Soluble starch *(Merck, South Africa).*

Concentrated sulphuric acid *(Merck, South Africa).*

Sodium thiosulphate pentahydrate *(Riedel-de Haen AG, Germany).*

Sodium hydroxide pellets AR *(B & M Scientific, South Africa).*

Sterile agar plates *(B & M Scientific, South Africa).*

Concentrated hydrochloric acid *(SP Scientific, South Africa).*

Potassium iodide *(Merck, South Africa).*

Potassium bromide *(B & M Scientific, South Africa).*

Appropriate glassware.

Chemical reagents

The following reagents were freshly prepared thus:

Sodium thiosulphate 0.1N solution

To prepare 1L (1000 ml) solution;

1N ≡ 1M

Mass = molar mass x molarity x volume (ml) / 1000

= 248.18 x 0.1 x 1000 / 1000

= 24.818 g in 1000 ml of solution

24.818 g of sodium thiosulphate was weighed out and dissolved in distilled water and the volume of the solution made up to 1000 ml.

Starch 0.5 % solution

This is 0.5 g starch in 100 ml of solution. To prepare this, about 10 ml of water was withdrawn from a beaker containing 100 ml of water. The remainder was put on the heater to boil. 0.5 g of soluble starch was triturated with the water withdrawn and added, with continuous stirring, to the boiling water.

30% caustic soda (NaOH solution)

To prepare 200 ml of solution;

30% caustic soda solution ≡ 30 g NaOH in 100 ml of solution

x NaOH will be in 200 ml of solution

x = 200 x 30 / 100

= 60 g.

60 g of NaOH was dissolved in distilled water and the volume of the solution made up to 200 ml.

10% potassium bromide (KBr) solution

To prepare 250 ml of solution;

10% KBr solution ≡ 10 g KBr in 100 ml of solution

x KBr in 250 ml of solution

x = 250 x 10 / 100 = 25 g.

25 g KBr was dissolved in distilled water and the volume of the solution made up to 250 ml.

70% ethanol

This was prepared from absolute (100%) ethanol. 30 ml of water was measured into a 100 ml measuring cylinder, absolute ethanol was subsequently added by running it gently down the side of the cylinder up to the 100 ml mark to make a 70% ethanol solution.

3.1.3 Rigging of the pen

The pen was rigged with pipes, pressure nozzles and a dosing pump. The pipes (fitted with the pressure nozzles) were 2.8 m above the floor of the chicken house. The nozzles had an orifice size of 0.2 mm and were made of cleanable brass with a removable stainless impeller plate which could be taken apart for cleaning purposes. A distance of 1 metre was maintained between subsequent nozzles. Spray projection ranged from 1.5 to 3 metres depending on a number of factors namely pressure, humidity, orientation, and air movement. The pump was programmed to operate at an appropriate pressure of 60 Bar. Such high pressure was necessary in order to ensure that a fine dry mist with a very small droplet size was formed. The pump system had two functions namely to supply high pressure water flow through the nozzles as well as dose the water with an appropriate concentration of biocide.

The pump also had safety features to bring it to a stop in the case of an interruption of the water supply. The whole fixture in the experimental pen had a total of 73 nozzles and at a pressure of 60 Bar, pumped 6.13 liters of water per minute (flow rate at 60 Bars = 84 ml / minute from each 0.2 mm orifice / nozzle) (C. Pieterse, personal communication, January 23, 2009).

3.1.4 Placement and distribution of the birds

The chicks were purchased at the age of 16 weeks. They were randomly selected and placed in cages in different chicken pens. The experimental and control pen each had an approximate measurement of 25 m by 13 m. Chicks were housed in two horizontal lines of cages adjacent to each other and placed about 2 m from another horizontal line of cages. The height of the lowest cage above the ground was 80 cm. Each cage measured 46 cm x 46 cm x 46 cm and housed four chicks. At the commencement of the study, each pen housed between 2000 and 2400 chicks. The chicks were fed on the formula shown in Table 3.1; feed composition was per ton of feed given to the chickens.

Table 3.1: Chicken feed formula

Lay 105	kg/mix
Maize	641.000
Soya	170.000
Semels	85.000
MCP	5.000
Limestone	92.000
Salt	3.000
Methionine	1.200
Lysine	0.500
PX Rono	2.000
	999.700

Lay 110	kg/mix
Maize	606.000
Soya	147.000
Semels	141.000
MCP	3.500
Limestone	95.000
Salt	3.500
Methionine	1.100
Lysine	0.600
PX Rono	2.000
	999.7

KEY
Lay 105 was for the young and still growing chicks up to the age of 40 weeks
Lay 110 was for mature chicks 40 weeks and above
MCP – Manganese, Calcium and Phosphorus
Semels – Bran
PX Rono – Composition of the essential nutrient requirement of a laying chick in the proportion shown in Table 3.2.

Table 3.2:
Composition of the essential nutrient requirement of a laying chick:

Compound	Unit	Amount
Vitamin A	IU	8000
Vitamin D	IU	2500
Vitamin E	mg	15
Vitamin K	mg	2
Vitamin B1	mg	2
Vitamin B2	mg	4
Vitamin B6	mg	3
Vitamin B12	mg	0.02
Folic acid	mg	0.75
Niacin	mg	30
Pantothenic acid	mg	7
Chlorine (Cl 60%)	mg	250
Biotin	mg	0.05
Vitamin C	mg	0
Manganese	mg	100
Zinc	mg	80
Copper	mg	8
Iron	mg	35

Compound	Unit	Amount
Iodine	mg	1
Selenium	mg	0.25
Cobalt	mg	0.25

* Table 3.1, 3.2 and information on placement and distribution of the birds supplied by Management of Bellevue Farms, Paarl.

3.1.5 Determination of stock concentration of Dioxy MP 14

Dioxy MP 14 used in the study is a locally developed solution of stabilized chlorine dioxide in an aqueous medium (manufactured by Med-Pride (Pty) Ltd). Chlorine dioxide gas was dissolved and stabilized in this product by a metal katalisator at a concentration of approximately 2000 ppm (parts per million). The concentration was verified by the following procedures (Kepinski & Trzeszcynski, 1964):

3.1.5.1 Procedure 1:

5 ml of 10% potassium bromide (KBr) solution and 25 ml of concentrated hydrochloric acid (HCl) solution were placed into a 50 ml Erlenmeyer flask. 20 ml of the chlorine dioxide solution (diluted 1 part biocide solution to 9 parts water to make a 1:10 solution) under examination was added and the flask closed. The flask was then exposed to light for twenty (20) minutes. 1 kg of potassium iodide (KI) was added and the flask placed in the dark for five (5) minutes. The solution was transferred into a 500 ml Erlenmeyer flask containing 30 ml of 30% caustic soda

(NaOH) solution and 100 ml distilled water. This was titrated with sodium thiosulphate ($Na_2S_2O_3$) 0.1N, with the addition of 3 ml of starch as an indicator (volume of $Na_2S_2O_3$ used in titration = D1).

The same procedure was repeated with 20 ml water in place of the biocide sample, as a blank test (volume of sodium thiosulphate, $Na_2S_2O_3$ used in titration = D2). Titre volume was the difference between D1 and D2.

3.1.5.2 Procedure 2:

2 kg potassium iodide (KI) was introduced into a 250 ml glass stoppered flask. 50 ml water, 25 ml of 25% sulphuric acid and 20 ml biocide (diluted 1 part biocide solution in 9 parts water to make a 1:10 solution) were added and the flask contents left in the dark for five (5) minutes. Five drops of starch indicator was added and the solution titrated with 0.1 N sodium thiosulphate solution. The titre volume was noted.

Either of procedure 1 or 2 could be used to determine titre volume. The two procedures were employed in this case and both gave an average titre volume of 2.95 ml.

Stock biocide concentration was then calculated using the formula:

Chlorine dioxide, ClO_2 (mg/ml) = (Titration fig) x 0.1 x 13.49 / 20

to obtain an approximate maximum stock concentration of 2000 ppm (1989.78 ppm).

3.1.6 Preparation and assay of settle plates

The growth media for the microorganisms in this study was solidified with agar. Agar is a seaweed extract which at concentrations between 1% and 2% sets to form a firm gel below 45°C. The medium remains firm after setting because bacteria cannot utilize the agar as a source of nutrient (Hanlon, 2007).

Two types of agar media were used: nutrient agar for the growth of bacteria and potato dextrose agar for the growth of yeasts and moulds. Some tartaric acid was added to the potato dextrose agar to reduce its pH to around 4; this served to inhibit the growth of bacterial colonies (White and Hood, 1931b).

3.1.6.1 Preparation of nutrient agar plates

The following was carried out under aseptic conditions. Nutrient agar was weighed according to the instruction on the container and dispersed in an appropriate amount of distilled water, boiled whilst stirring till the powder was thoroughly dispersed. It was then poured into bottles, loosely corked and sterilized in the autoclave at 121°C for 15 minutes. The agar was allowed to cool (45°C to 50°C) and then poured into plates where the molten agar solidified. The plates were stored in a cold room, stacked upside down from where they were retrieved as needed and exposed for sample collection.

3.1.6.2 Preparation of potato dextrose agar plates

The following was carried out under aseptic conditions. Potato dextrose agar was weighed according to the instruction on the container and dispersed in an appropriate

amount of distilled water, boiled whilst stirring till the powder was completely dispersed. It was then poured into bottles, loosely corked and sterilized in the autoclave. The agar was allowed to cool (45°C to 50°C) and tartaric acid was added to reduce the pH to between 4.0 and 4.1. It was then poured into plates where the molten agar solidified.

In line with basic microbiological procedure, the plates were stored in a cold room, stacked upside down from where they were retrieved as needed and exposed for sample collection.

3.1.6.3 Assay of settle plates

Prior to the retrieval of the settle plates for sample collection, a few of the plates were randomly selected and put in the incubator set at the appropriate temperature. This served as a quality control test of the prepared plates. The nutrient agar plates were placed in the incubator at 37°C for 24 hours while the potato dextrose agar plates were placed in another incubator at 25°C for 5 days. The plates in storage were only used if no microbial growth was observed in the incubated sample plates.

3.1.7 Protocol for environmental micro contamination measurement

3.1.7.1 Settle plate sampling technique (Working group of the SQASIG, 2004)

For an assessment of environmental microbial contamination, the settle plate sampling technique was utilized. In the settle plate sampling technique, an assessment is made of the probable number of microorganisms depositing on the surface of a solid growth medium over a given time interval. Microorganisms can be

found present in air and can settle over time. An average sized microbial particle will deposit, propelled by gravity, at an approximate rate of 1cm per second (Working Group of the SQASIG, 2004).

The settle plate sampling technique involves the opening and exposure of a plate containing solidified growth medium for microbe bearing particles to be deposited. Plates employed are usually of 90 mm diameter size (approximate internal surface area 64 cm^2). To confirm the number of microbe bearing particles that have settled, the plates are incubated, after exposure, at the proper temperature and for the appropriate length of time. Afterwards, the microbial colonies, more commonly known as the colony forming units (CFUs) are counted.

3.1.7.2 Placement of settle plates in the chicken pen

To measure environmental contamination from microbe bearing particles in the pen, nutrient agar and potato dextrose agar plates were utilized. The plates were placed on top of the metal cages housing the birds. The top of the cages were about 1.8 m above the ground and about 1 m from the overhead pipes that supplied the biocide in solution.

A piece of rigid plastic sheet (A4 paper size) was wiped with a solution of 70% alcohol to remove any contaminating microbes suspected to be present. It was placed on top of the cage. The settle plates, clearly marked and labeled, were placed on it with a gloved hand. The cover was removed and placed face down on the sheet to ensure that no extra microbes were introduced through it. At the end of the exposure

period (5 minutes), the cover was replaced. The plates were neatly stacked and secured in a plastic bag that was put into an insulated container for transportation to the microbiology laboratory.

3.1.7.3 Microbiological laboratory procedures

3.1.7.3.1 Incubation of settle plates

The 37°C and 25°C incubators contained flat tray-like surface(s) for placing of the plates. The surface of the trays was cleaned with a soap solution and then wiped with a solution of 70% ethanol. A residual disinfectant (quaternary ammonium compound) solution was sprayed on the tray surfaces. This was not wiped – it was allowed to dry. The settle plates containing the microbe bearing particles were placed upside down on the trays.

The nutrient agar plates were for the growth of bacteria. These were placed in the 37°C incubator which was then closed and the temperature set at 37°C. At the end of 24 hours, the nutrient agar plates were removed from the incubator and taken to the colony counter – an instrument with a magnifying lens, which makes microbial colonies bigger and more visible and therefore easy to count.

The 25°C incubator was also cleaned with soap and 70% ethanol solution. A solution of the residual disinfectant was sprayed and allowed to dry. The potato dextrose agar plates for the growth of yeasts and moulds were placed in the 25°C incubator. The incubator was closed and the temperature set at 25°C. At the end of 5 days, the plates were removed and taken to the colony counter for microbial enumeration.

3.1.7.3.2 Colony count

The settle plates (with already visible colonies) were placed upright in the space provided in the instrument (colony counter). The lens was adjusted to give an appropriate magnification of the colonies, which were then counted. Microbial activity was assessed by checking the nutrient agar plates for the growth of bacteria (Total Microbial Activity) and the potato dextrose agar plates for yeast and mould counts.

The used plates were taped together in groups of three or four to prevent the contents from spilling and then disposed of in the BIOHARZADOUS refuse bin.

3.1.8 Measurement of environmental parameters

Other environmental factors observed at the farm were recorded throughout the duration of the study. The minimum and maximum temperatures were taken for each day; the intensity of the wind was also observed and classified as no (NO), small (S), medium (M) or strong (STRONG); "no" was for very still days when no wind movement was observed while "strong" was for very windy days (see Appendix IV). This was carried out by staff of the chicken farm.

3.1.9 Dosing of Dioxy MP 14 into water system

The Tekna DPZ pump was used for the dispersal of the biocide in the study. It is a programmable multifunctional dry mist dispersal pump. Equipped with liquid handling materials that guarantee a very wide chemical compatibility, the pump head

was made of polypropylene reinforced with glass fiber, a PTFE (polytetrafluoroethylene) diaphragm and unique double ball valve design. No elastomers were in contact with the dosed media, which allowed it to work with almost all chemicals (C. Pieterse, personal communication, January 29, 2009). According to the manufacturer's instructions, it was installed in an area with a relative humidity of less than 90% and ambient temperature of not more than 40°C away from direct sunlight.

The pump had provisions for working in both constant and proportional mode. It could be programmed to disperse mist from solutions at different flow rates according to the adjustments made on the flow meter.

The pump and fittings were installed by Magic Mist™, a South African based company that specializes in the use of dry misting and fogging for sanitation among other uses. When approved sanitizers are properly dosed into the water supply of the Dry Mist™ humidification system, it can inactivate airborne microbes. At a very high working pressure, the billions of micro-droplets produced average 10 – 20μ (micron) in diameter and are able to coat 'all surfaces, nooks and crannies with a microfilm of DryMist™'.

The biocide / sanitizing solution was introduced into the water flow system (of the Dry Mist™ humidification system) in the farm through the **connecting pipe**. The whole solution left the pump station through a **bigger pipe** for onward introduction into the test chicken pen (Figure 3.1).

Figure 3.1: Photograph of the dosing pump

In the test chicken pen, the solution was made to go through pipes of smaller diameter (Figure 3.2) which increased the already increased pressure that started from the pump station.

Figure 3.2: Photograph of the poultry set-up:
The pipes for biocide dispersal can be seen above the pens.

The solution was forced through yet smaller nozzles and the reduced diameter of the nozzle orifice (compared to that of the pipes) also served to heighten the pressure. At this very high pressure, the biocide solution was released into the test pen in the form of mist (Figure 3.3).

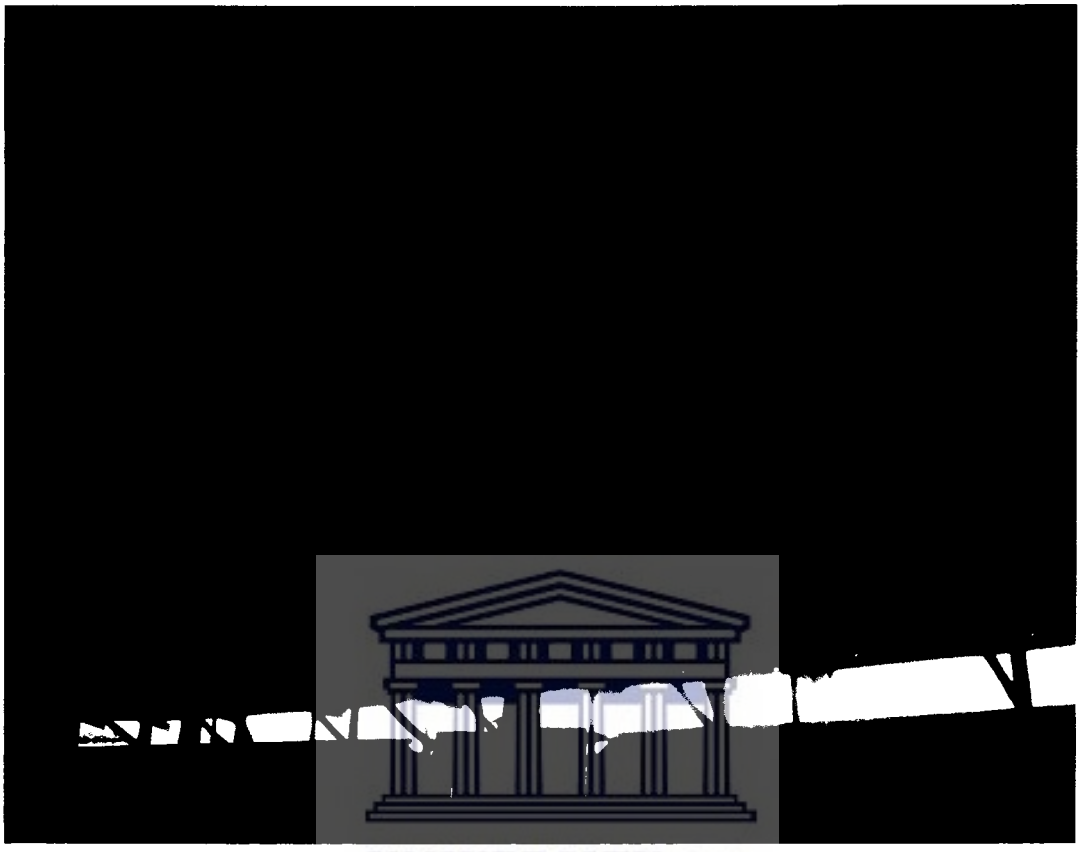


Figure 3.3: Photograph of biocide sanitizing solution leaving nozzles

3.1.10 Calculation of maximum biocide concentration

The misting pump delivered 1 impulse for every 4 litres of water passing through where an impulse refers to the quantity of biocide dosed from the pump settings. When the pump operated at 100 strokes per minute, it was dosing 100 strokes of the stock biocide solution into 4 litres of water. The residual concentration of the biocide at the nozzles was calculated thus:

1 stroke ≡ 0.15 ml

100 strokes ≡ 0.15 ml * 100 = 15 ml

15 ml of biocide in 4 l of water

1 ml biocide ⟶ 4 * 1000 * 1 / 15 = 266.67 ml of water

A stock solution of 1989.78 ppm will give 1989.78 / 266.67 = 7.46 ppm.

This was the maximum concentration employed. Other concentrations were 25%, 50% and 75% of the maximum stock solution translating to 1.865, 3.730 and 5.595 ppm respectively.

3.1.11 Verification of biocide concentration at end-point nozzles

A test was also carried out to see if the concentration of the biocide at the nozzles matched the calculated concentration. A testing sample with colours to match chlorine dioxide concentrations of 3 ppm and 8 ppm was used (see Figure 3.4).

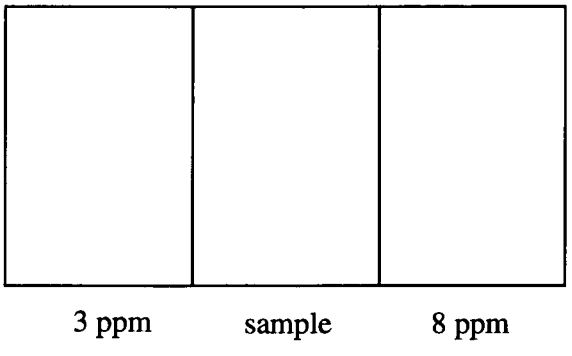


Figure 3.4: Schematic diagram of biocide sample tester:
The sample to be tested can be seen in the middle

The colour of the sample obtained from the nozzles was found to be between those representing 3 ppm and 8 ppm, showing that the maximum concentration employed was between 3 ppm and 8ppm, the same as the result from the calculation.

The standardized test and colour solutions were supplied by the manufacturers of Dioxy MP 14.

3.1.12 Biocide efficacy determination

3.1.12.1 Selection of optimum biocide concentration

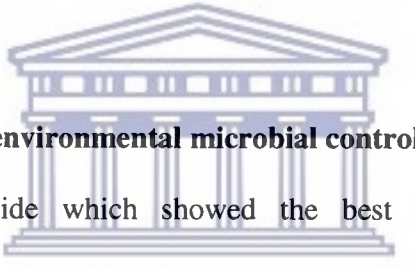
In the first part of the study, the aqueous biocide solution (at four different concentrations) was released into the pen by a dry mist system. The first pen (Pen A) received the biocide while Pen B (which served as the control) was not equipped with a misting system and therefore not treated.

Nutrient agar and potato dextrose agar plates were exposed in both pens for 5 minutes before spraying with the biocide in order to obtain the microbial count before Dioxy MP 14 application. The biocide (Dioxy MP 14) was then applied in the form of a spray for 10 minutes and the settle plates exposed after biocide application. From this, the effect of the biocide on microbial contamination was assessed. Biocide solution was not sprayed again but the plates were subsequently exposed for 5 minutes every hour for up to 6 hours. This was to observe if there was an increase or decrease in microbial contamination and to assess the extent of such increase or decrease. From this, the extent of change in microbial contamination percentage was observed in order to note where an increase (in microbial contamination)

commenced again. By so doing, the duration of action of each concentration of the biocide in the poultry setting was estimated.

3.1.12.2 Control experiment

In the second part of the study, water (the solvent for the biocide solution) was sprayed at regular intervals instead of the biocide. The nutrient agar and potato dextrose agar plates were exposed before and after spraying with water and again for 5 minutes every hour for six hours. They were incubated and bacterial growth assessed.



3.1.13 Long term (8-week) environmental microbial control studies

The concentration of biocide which showed the best control of microbial contamination (for both bacteria and yeasts / moulds) was employed in this part of the study. This concentration was 7.46 ppm. The DryMist™ pump was programmed to disperse the biocide at a concentration of 7.46 ppm for 5 minutes every 2 hours. Settle plates were exposed for 5 minutes each day (in the morning and evening) and then incubated and assessed for microbial growth. The rationale was to see if a constant reduction in contamination was maintained throughout the day while the pump dosed at regular intervals.

3.1.14 Evaluation of chicken performance

Record was taken of bird mortality on a daily basis. From this, the mortality record for each month was calculated (see Section 3.1.14.1).

The performance of the chickens in the experimental and control pens was measured by calculating the production percentage (number of eggs laid by the chicks) (see Section 3.1.14.2). This is a general method of assessing production performance of egg-laying chicks by farmers in the study setting. The quantity of eggs (in trays) laid by the chicks was recorded on each day. A tray contains thirty (30) eggs. From this, the production performance results shown in Appendix IV were obtained.

3.1.14.1 Mortality records

The mortality over the various months (in percentage) was calculated thus:

Mortality percentage = ttl. mort. x 100 / o.stock

where ttl. mort. refers to the total number of chicken deaths for the month

and o.stock refers to the number of chicks at the beginning of the month.

3.1.14.2 Production performance

The production performance (in percentage) was calculated thus:

Production percentage = trays x 30 / c.stock

where trays refers to the number of trays of eggs produced by the chicks

and c.stock refers to the current stock (the population of the chick at the time of egg collection).

3.2 Ethical considerations

Chlorine dioxide is an environmentally friendly biocide (Young and Akhtar, 1998). It has been used in the treatment of drinking water in the United States since the 1940s (Gates, 1998).

The objective of the study was to reduce microbial contamination in the poultry house environment. The concentration of biocide that came in contact with the chicken was within the range of concentrations that have been employed in fresh product transportation (Sozzi & Gorini, 1992) and the processing of foods such as chicken (Caffaro monograph, 1997). A dosing pump and the biocide sample tester ensured that the fine mist delivered through the nozzles was within safe dosing concentrations.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Environmental microbial control at different biocide concentrations

For the four concentrations employed, at time 0, bacterial contamination was assumed to be 100%. This was in comparison with the control thus: the number of microbial colonies at time 0 was taken to be 100%, both for the experimental and the control pens. The number of microbial colonies at different time concentrations was obtained and then made a percentage of this value obtained at time 0. Each percentage value obtained at different times in the experimental pen was also made a percentage of the percentage value obtained in the control pen at that same time (see Appendix I).

As an example, suppose the following raw values (see Table I below) were obtained for bacterial contamination in the experimental pen at different times:

Table I:

Time (hours)	**0 hr before**	**0 hr after**	**1**	**2**	**3**	**4**	**5**	**6**
Microbial count	727	96	112	148	173	139	132	160

'0 hr before' refers to the time just before disinfection with the biocide while '0 hr after' refers to the time immediately after disinfection with the biocide.

Table I shows that just before the biocide was sprayed, 727 microbe bearing particles were counted on the agar plate exposed. Immediately after spraying with the biocide for 10 minutes, microbial contamination was reduced to 96 microbe bearing particles or colony forming units (CFUs). This increased to 112 CFUs in the first hour after disinfection with the biocide, 148 CFUs in the second hour and 173 CFUs in the third hour. In the fourth hour, contamination was 139 CFUs, 132 CFUs in the fifth hour and 160 CFUs in the sixth hour.

The value at 0 time before spraying with biocide (727) was taken to be a 100% contamination. After disinfection, the CFUs obtained (96) was made a percentage of this to give a contamination of 13.20% (i.e. 96 / 727 * 100). The same was done for the values at subsequent time intervals to give the following percentage contamination values (see Table II):

Table II:

Time (hours)	**0 hr before**	**0 hr after**	**1**	**2**	**3**	**4**	**5**	**6**
Microbial count	100.00	13.20	15.41	20.36	23.80	19.12	18.16	22.01

Again, suppose microbial contamination in the control pen at different time intervals was as follows (see Table III):

Table III:

Time (hours)	**0 hr before**	**0 hr after**	**1**	**2**	**3**	**4**	**5**	**6**
Microbial count	819	819	825	838	853	896	978	1035

The same procedure was followed to give the following percentage contamination values (see Table IV):

Table IV:

Time (hours)	0 hr before	0 hr after	1	2	3	4	5	6
Microbial count	100.00	100.00	100.73	102.32	104.15	109.40	119.41	126.37

All the percentage values in the control pen were then taken as 100% contamination levels. The percentage contamination in the experimental pen at different time intervals was made a fraction of the percentage contamination in the control pen at the same time interval thus (see Table V):

Table V:

Time (hours)	0 hr before	0 hr after	1	2	3	4	5	6
% cont (expt)	100.00	13.20	15.41	20.36	23.80	19.12	18.16	22.01
% cont (ctrl)	100.00	100	100.73	102.32	104.15	109.40	119.41	126.37
% cont (overall)	100.00	13.20	15.30	19.90	22.85	17.48	15.21	17.42

where % cont (expt) refers to the percentage contamination in the experimental pen,

% cont (ctrl) refers to the percentage contamination in the control pen and

% cont (overall) refers to the contamination of the experimental pen (as a percentage of the percentage contamination in the control pen); i.e. % cont expt / % cont ctrl * 100 (highlighted above). The highlighted percentage values in Table V were the values plotted against in the graphs.

Record of environmental parameters

These are shown in Appendix IV. The period from September to October saw some very wide fluctuations between minimum and maximum daily temperatures, maintained more or less within the same range up till November. From December to April, the temperature measurements were very high on the maximum side, sometimes above 35°C.

The months of September to October saw little or no wind for the most part, strong wind movements were noticed for a couple of days in November and then for a few more days in January, March and April.

4.1.1: Microbial control at biocide concentration of 7.46 ppm

At time 0, bacterial contamination was 100%. Disinfection was carried out at 7.46 ppm for 10 minutes and plates exposed before and after this. The result obtained showed that at a working concentration of 7.46 ppm, microbial (bacterial) load was reduced to between 10% and 20% and this reduction in microbial contamination was

maintained within this range for up to 6 hours (Figure 4.1).

Immediately after the biocide was sprayed, i.e. after 10 minutes, bacterial contamination reduced to 13.43%. Microbial control was maintained within a range of 13% and 20% with values of 15.07%, 19.86%, 19.37%, 18.48%, 15.59% and 16.39% at 1, 2, 3, 4, 5 and 6 hours after disinfection respectively.

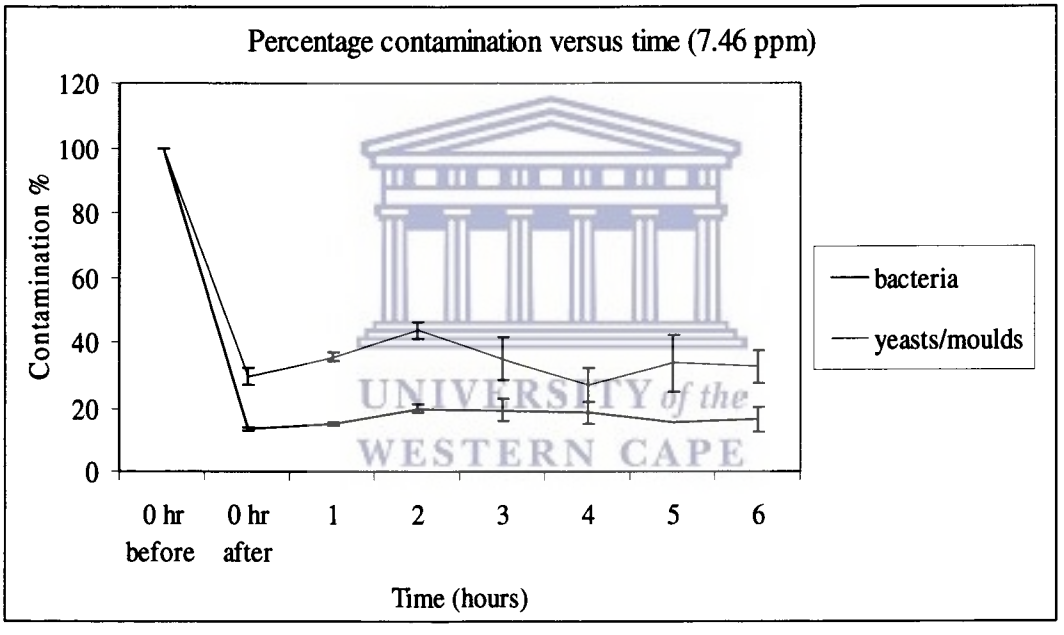


Figure 4.1: Graph of microbial control at biocide concentration of 7.46 ppm

Yeast and mould contamination reduced from 100% to 29.56% immediately after disinfection with Dioxy MP 14 at a concentration of 7.46 ppm. At 1, 2, 3, 4, 5 and 6 hours after disinfection, contamination was at 35.39%, 43.69%, 34.91%, 26.96%, 33.59% and 32.48%.

Wider deviations were observed for yeasts / moulds compared to bacteria. Fungal pathogens can be dispersed by air (Carroll & Wicklow, 1992) and could therefore have been blown in or out at time intervals before consecutive plate exposures resulting in such deviations.

4.1.2: Microbial control at biocide concentration of 5.60 ppm

Disinfection at a biocide concentration of 5.60 ppm reduced bacterial load to between 15% and 45% for up to 6 hours. Immediately after spraying, bacterial contamination reduced from 100% to 18.53%. There was an increase in contamination to a peak value of 40.38% at 1 hour.

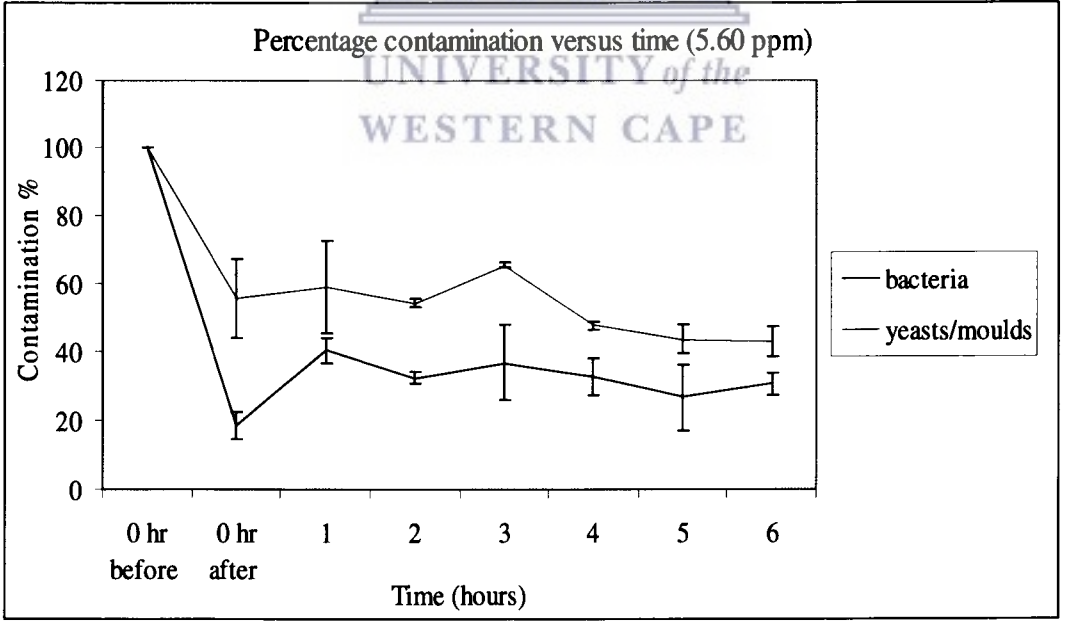


Figure 4.2: Graph of microbial control at biocide concentration of 5.60 ppm

Contamination reduced again at 2 hours to 32.36% and increased slightly to 36.76% at 3 hours. There was another slight decrease to 32.82% and 26.66% at 4 and 5 hours respectively and a contamination of 30.64% at 6 hours (Figure 4.2).

Yeasts and moulds contamination reduced to 55.40% from the initial contamination value of 100% immediately after spraying with Dioxy MP 14 at a concentration of 5.60 ppm. Subsequent measurement of contamination showed a fluctuation within the range of 40% and 70% with values of 59.01%, 54.38%, 65.50%, 47.59%, 43.65% and 43.02% contamination at 1, 2, 3, 4, 5 and 6 hours respectively.

Deviations in percentage contamination values appeared to be a bit wider for bacteria compared to the yeasts and moulds. Just as the percentage contamination with fungal pathogens could fluctuate with environmental changes in air (Carroll & Wicklow, 1992), the same could perhaps be applicable for bacterial cells also (Polanczyk *et al.*, 2009).

4.1.3: Microbial control at biocide concentration of 3.73 ppm

Disinfection at 3.73 ppm reduced bacterial contamination levels to between 30% and 65% with variations in value between 30 and 65%. At 0, 1, 2, 3, 4, 5 and 6 hours after disinfection, bacterial contamination was at 60.48%, 42.39%, 32.34%, 43.08%, 43.29%, 45.52% and 41.36% respectively (Figure 4.3).

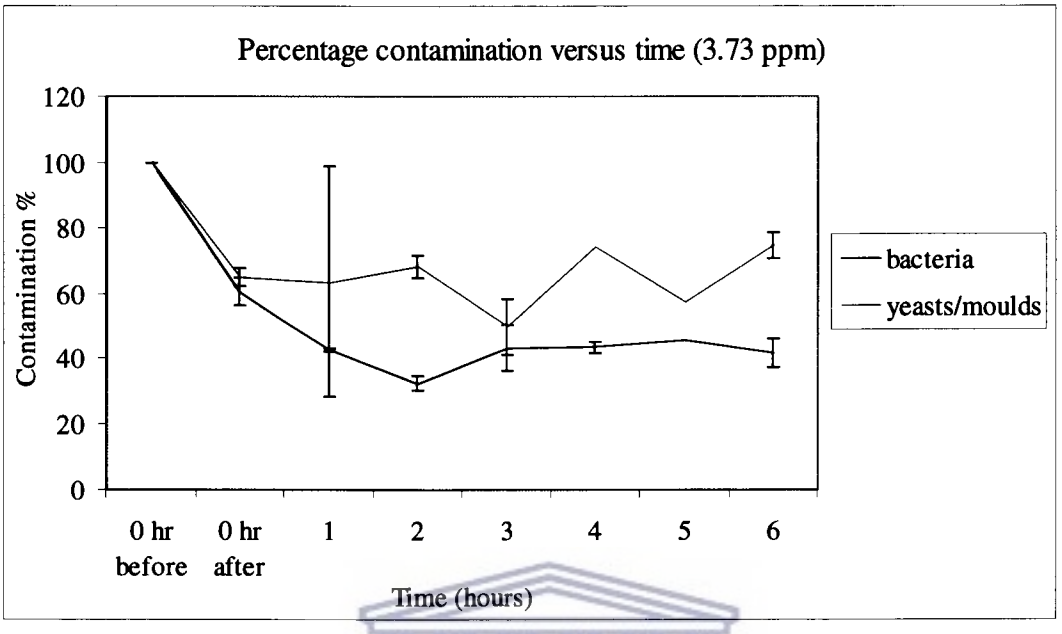


Figure 4.3: Graph of microbial control at biocide concentration of 3.73 ppm

Yeasts and moulds contamination at this concentration was reduced from 100% to 64.93% immediately after disinfection. Subsequent contamination reductions and increases gave values of 63.45%, 68.22%, 49.66%, 74.28%, 57.45% and 74.54% at 1, 2, 3, 4, 5 and 6 hours after disinfection respectively. Efficacy was probably reduced due to the decrease in concentration of the biocide.

There was a wide deviation for yeasts and moulds in the second hour when compared to deviations at other times.

4.1.4: Microbial control at biocide concentration of 1.87 ppm

On disinfection with Dioxy MP 14 at biocide concentration of 1.87 ppm, there was a reduction in contamination in the experimental pen when compared to the control

pen immediately after disinfection. However, this reduction was not as much as that obtained at the other higher concentrations. Recontamination also occurred quicker than at higher concentrations.

For bacteria, contamination was at 83.64% immediately after disinfection. Values obtained at other times were 104.83%, 71.87%, 134.65%, 129.08%, 121.07% and 82.68% at 1, 2, 3, 4, 5 and 6 hours after disinfection respectively.

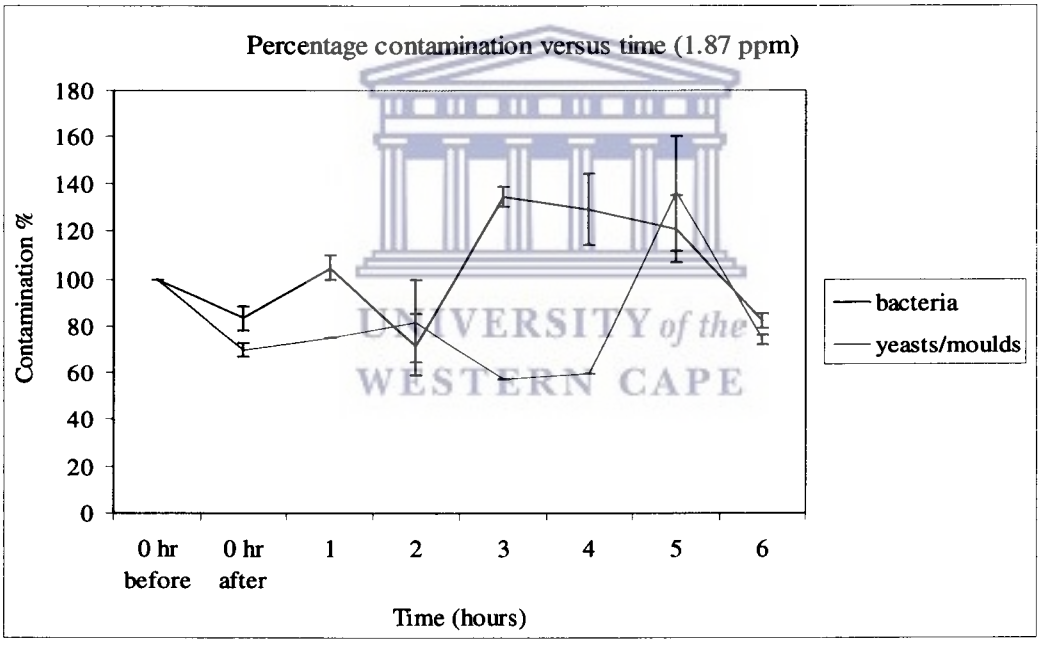


Figure 4.4: Graph of microbial control at biocide concentration of 1.87 ppm

For the yeasts and moulds, contamination was at 69.89% immediately after disinfection. The following values were obtained at 1, 2, 3, 4, 5 and 6 hours: 75.59%, 82.10%, 56.66%, 58.98%, 136.15% and 74.63% respectively (Figure 4.4).

Environmental disinfection of the poultry house at this concentration did not show any particular trend when microbial colony forming units were counted. For the other concentrations of the biocide, bacterial contamination after disinfection was always lower compared to yeast and mould contamination. Immediately after disinfection at this concentration (1.87 ppm), bacterial and yeast/mould contamination was reduced. However, this reduction did not last. Both bacterial and yeast /mould contamination increased and decreased again at different time intervals with bacterial contamination going higher than yeast and mould contamination in some instances (see Figure 4.4).

This has been put down to the concentration, which was perhaps not sufficient to make an impact and so re-contamination occurred quickly. Environmental factors could also have influenced microbial cells to be dispersed from the outside into the pen or from one side of the chicken house to another, leading to sustenance of contamination (Carroll & Wicklow, 1992; Polanczyk *et al.*, 2009).

4.1.5 Microbial control at zero concentration of biocide - water only

In the control experiment, water alone instead of the biocide solution was sprayed in the pen for 10 minutes (concentration of biocide in this case was equivalent to 0 ppm) and then plates exposed before and after spraying just like in the experimental set up. Water was the solvent for the delivery of the biocide. Investigation with water alone was necessary in order to see what effect (if any) this solvent had on the results obtained. Microbial contamination increased after spraying with water from an initial

value of 100% to 228.57% for bacteria and 185.41 for yeasts and moulds. There was a reduction in contamination to 139.23% and 81.11% for bacteria and yeasts and moulds respectively at 1 hour after disinfection but this reduction was temporary, peak contamination value was 234.97% for bacteria and 290.57% for the yeasts and moulds (see Appendix II for values plotted in Figure 4.5).

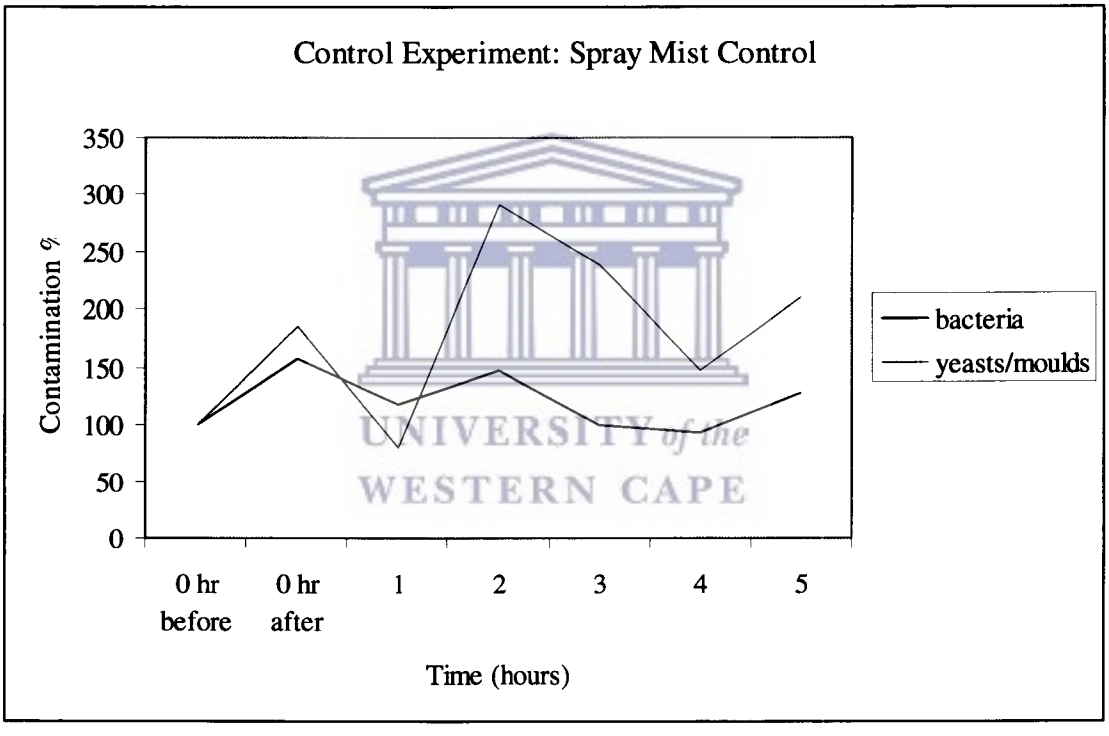


Figure 4.5: Graph of microbial control at zero biocide concentration

Even though it was hypothesized that water had no effect on the activity of the biocide, a reduction in microbial contamination immediately after spraying with water was expected. This was because it was thought that water would cause a settling of microbe-bearing particles and so an assessment immediately after

spraying with water only was expected to reveal a reduction in contamination though this reduction was not expected to last.

However, this expected initial reduction in microbial contamination was not observed; instead, there was an increase in microbial contamination at 0 hr after spraying with water. Both bacterial and yeasts and moulds count increased beyond the original value. This could have been from the water itself which was not sterile. The increase in contamination after spraying with water also confirmed the assumption that water did not contribute to the decrease in microbial contamination observed when the aqueous biocide solution was sprayed in the pen. The decrease in microbial activity observed at different biocide concentrations can therefore be said to be due to the effect of the biocide in solution and not the dispersal phase / vehicle, i.e. water.

4.2 Summary of microbial control at different biocide concentrations

Control of microbial contamination was best at biocide concentration of 7.46 ppm, both for bacteria and yeasts / moulds. At all the working frequencies, contamination fluctuated within a particular percentage range, with bacterial contamination values confined to a narrower range after disinfection compared to the yeasts and moulds, at disinfectant concentrations of 7.46 ppm and 5.60 ppm. Contamination fluctuation range also widened as the biocide concentration decreased. There was some reduction though, noticed at 0 hour, i.e. immediately after disinfection, for all the working frequencies. This proves that the biocide was able to reduce both bacterial

and yeast and mould cells; at lower concentrations though, this reduction was not sustained for as long as that obtained from higher concentrations, hence the increase in contamination over time at certain biocide concentrations.

The experimental set up was an open system where environmental factors such as wind could not be controlled. Microbial cells could have been dispersed, borne by wind or rain, in various directions in the environment under study (Polanczyk *et al.*, 2009).

It is thought that at the times when contamination was more than the value obtained at the start of the experiment (for each concentration), more microbial cells had been blown into the study area. Environmental factors are known to influence microbial movement from one place to another (Polanczyk *et al.*, 2009). This could have been the reason for the sizeable deviations from the mean obtained at certain times.

Because disinfection at a working concentration of 7.46 ppm was most effective in the control of bacterial and yeast / mould spores, it was chosen as the frequency at which regular dosing would be carried out to monitor the efficacy of a specific dose of the biocide against microbes and in relation to production parameters. At this concentration, no negative effect was noticed in the chicks in terms of appearance, mortality and productivity.

4.3 Environmental microbial control over 8-week period (pump dosed a regular concentration of 7.46 ppm)

As shown from the previous graphs, Dioxy MP 14 at a biocide (chlorine dioxide) concentration of 7.46 ppm showed a better reduction in bacterial and yeasts and moulds contamination than the other concentrations also investigated. This was not surprising because this concentration was higher than the others. This concentration was used for regular disinfection of the pen for 5 minutes every 2 hours. A spacing of 2 hours was observed between successive dosing in order to accommodate for a reduction in both bacterial and yeasts and moulds contamination.

4.3.1 Bacterial Contamination

Initial contamination at week 0, i.e. before regular dosing commenced was 100%. On dosing at 7.46 ppm every two hours, bacterial contamination in the mornings was reduced to a record low of 12.44% in the third week. An increase to 49.46% was noticed in the fourth week which culminated in a 77.46% contamination in the fifth week. This was because in the fourth week, a blockade was noticed in some of the nozzles. By the fifth week, most of the nozzles were blocked hence the increase in contamination. The blocked nozzles were resolved by the sixth week when contamination reduced to 34.23% (Figure 4.6) (see Appendix III for contamination values).

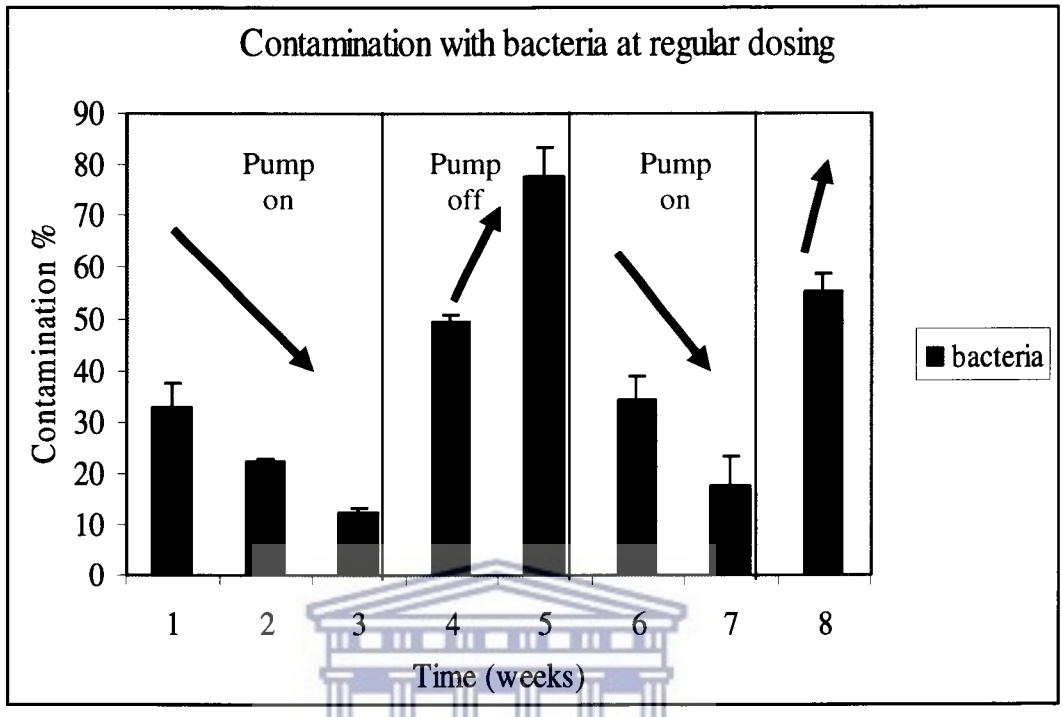


Figure 4.6: Percentage bacterial contamination during regular dosing at 7.5 ppm: A comparison of experimental pen to control pen

Wider deviations in mean contamination values were noticed in the fifth week (compared to the second, third and fourth weeks). At this time, the pump was not functioning optimally; some areas were disinfected while the other areas that should have been covered by the blocked nozzles were not, hence this deviation.

Bacterial contamination reduced further to 17.48% in the seventh week before increasing again to 55.12% in the eighth week at which time the pump had broken down again due to a fault in the pipe fittings.

4.3.2 Yeast / Mould Contamination

Initial contamination at week 0, i.e. before regular dosing commenced was 100%. For the yeasts and moulds, contamination reduced increasingly between 10% and 50% for the first three weeks (minimum of 14.89% in the third week) and then increased to 40.98% in the fourth week and subsequently to 66.13% in the fifth week. After resolving the problems experienced with the pump, contamination was again reduced to 35.48% in the sixth week and 20.18% in the seventh before an increase to 34.68% in the eighth week when the pump became non-operational again (Figure 4.7).

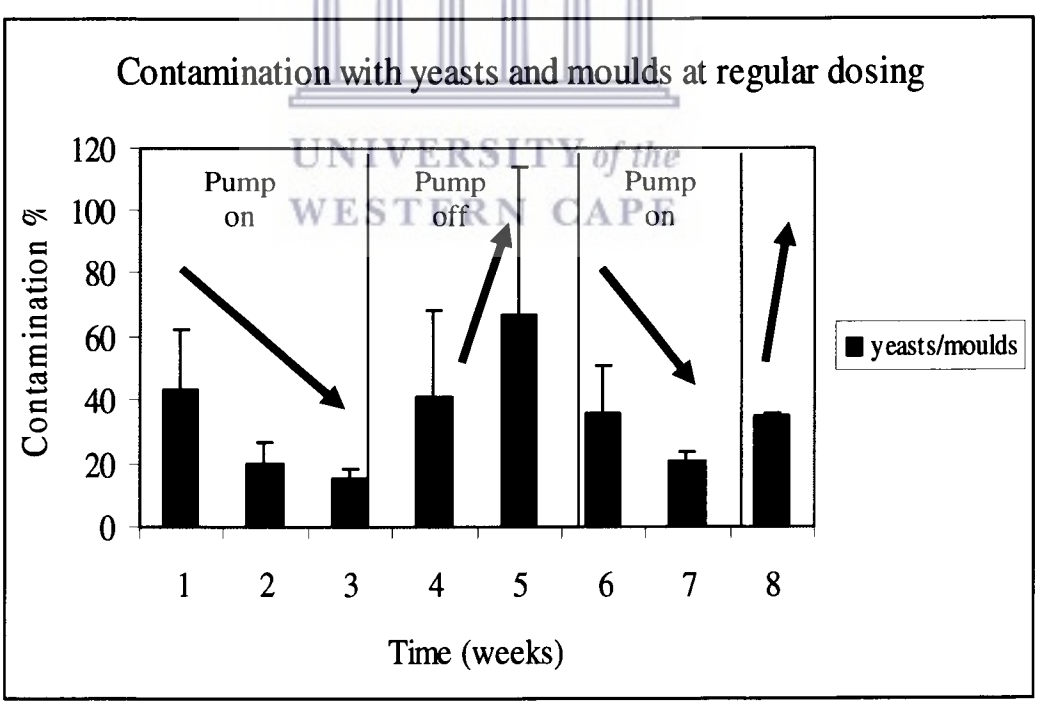


Figure 4.7: Percentage contamination with yeasts / moulds during regular dosing at 7.5 ppm: A comparison of experimental pen to control pen

As with bacterial contamination, a wide deviation in contamination values was noticed for the yeasts and moulds in the fifth week; unlike that for bacteria however, there was also a wide deviation in mean contamination value for the fourth week. At this time, the pump was not functioning optimally; some areas were disinfected at the expense of others.

Generally, for both bacteria and yeasts / moulds, a reduction in contamination was noticed for the weeks when the misting pump was functioning optimally (and dispersing the biocide) whereas there was an increase in contamination for the weeks when the pump was off or not functioning optimally, hence not delivering the biocide (see pointers and the 'pump on' and 'pump off' labels in Figure 4.6 and Figure 4.7).

4.4 Mortality records

Mortality percentage values are shown in Table 4.1. Calculation of mortality percentage has been explained in section 3.1.14.1. Refer to Appendix IV for values used to calculate percentage mortality.

Table 4.1: Mortality records in the experimental and control pens for an 8-month period

Month	Pen A	Pen B
September 2007	1.55 %	1.43 %
October 2007	1.57 %	2.99 %
November 2007	2.03 %	2.26 %
December 2007	2.12 %	2.66 %
January 2008	2.32 %	2.84 %
February 2008	2.17 %	1.98 %
March 2008	2.06 %	1.91 %
April 2008	1.13 %	0.76 %

Figure 4.8 is a graph of the percentage mortality in the two pens under study – the experimental and the control pens. It shows a decrease in mortality in the experimental pen compared to the control pen for the first 5 months. Mortality in the experimental and control pens were almost similar at the start of the study with values of 1.55% and 1.43% for the experimental and control pens respectively. Over the next four months, mortality in the experimental pen (at values of 1.57%, 2.03%, 2.12%, and 2.32% for the months of October, November, December and January respectively) reduced compared to the control pen (at values of 2.99%. 2.26%, 2.66% and 2.84% for the months of October, November, December and January respectively).

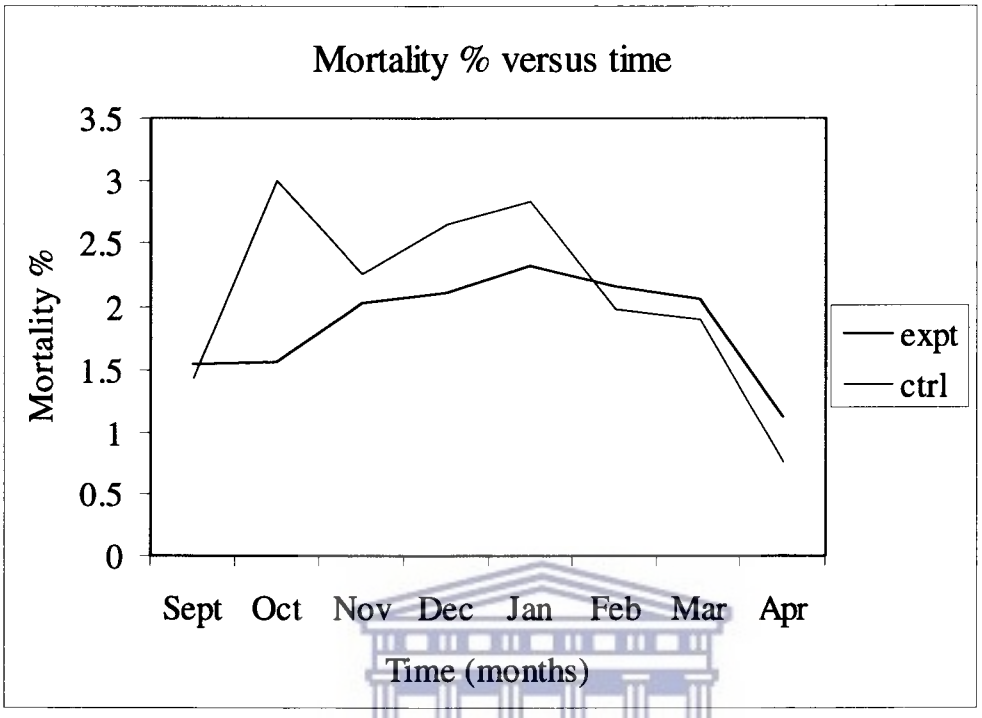


Figure 4.8: Graph of percentage mortality versus time

In the last three months, there was an increase in mortality in the experimental pen (higher than that for the control pen). This could have been due to a number of factors not necessarily sanitation related such as pecking by other birds (El-Lethey *et al.*, 2000) and sudden death syndrome (Ononiwu *et al.*, 1979; Karki & Cabana, 2008) which is a disease of acute cardiac failure in chickens (Ononiwu *et al.*, 1979). The cause of sudden death syndrome is still unclear; rapid growing male chickens appear to be the most affected (Ononiwu *et al.*, 1979). Temperature could also have been a major factor. From the data in appendix IV, it can be seen that the transition from September to October went hand in hand with huge fluctuations between minimum and maximum daily temperatures, while from December some of the highest

temperatures were recorded (> 35°C). It is known that animals such as chicken may find it difficult to adapt to temperature changes (Kendeigh, 1969; Pardue *et al.*, 1985).

4.5 Production performance

Production factors were monitored throughout the duration of the experiment.

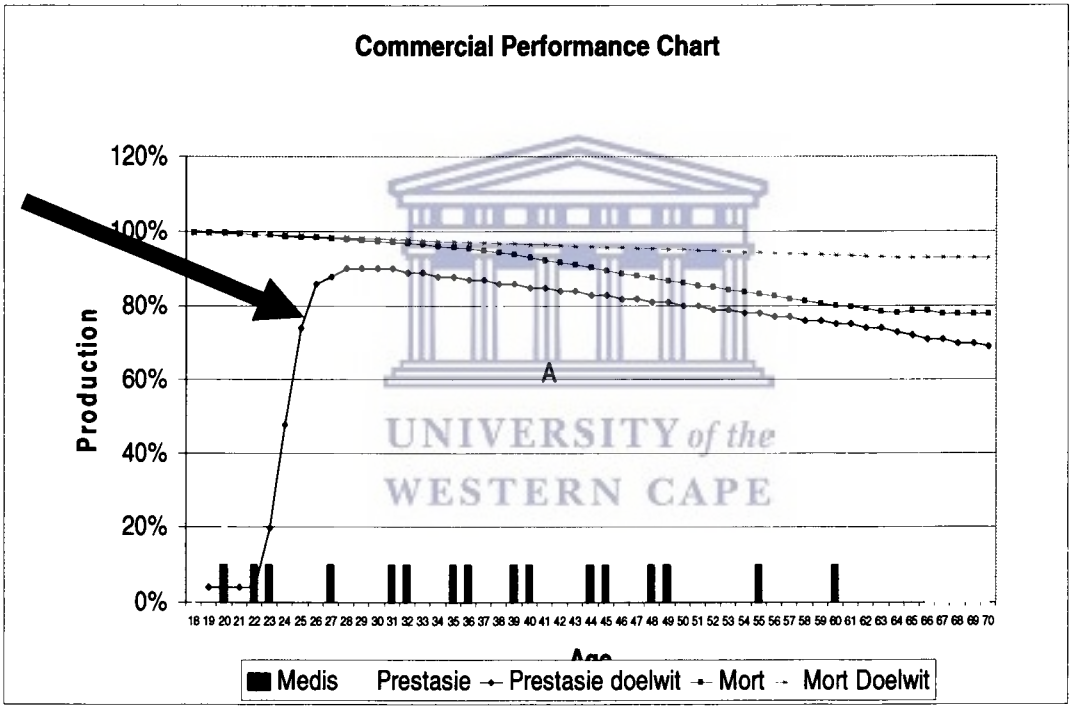


Figure 4.9: Commercial Performance Chart

KEY:	Medis:	Average
	Prestasie:	Performance
	Prestasie doelwit:	Expected performance
	Mort:	Expected mortality

* Chart sourced from Bellevue Farms, Paarl.

The chicks used for the study were purchased at 16 weeks of age. They were housed in the poultry and started laying eggs in the same week. Production records (egg-laying capacity) were taken in both pens and observed to increase steadily from then up to a maximum, was maintained on a plateau at this maximum for a while and then slowly went into a decline. This is normal in production as shown in the performance chart (see pointer, Figure 4.9). The chart is used by poultry farmers and gives an idea of the expected production performance of egg-laying chicks over weeks.

Three pens in a house (three individual chicken pens in a row make up a chicken house) had production factors recorded. Pen A in the house was for the experiment / investigation while pen B served as a control. Records were also taken of all the pens in the house (made up of pen A, pen B and pen C) to give an estimate of the general production trend in the chicken house.

Results from the different pens and from the house as a whole are as shown in Table 4.2:

Table 4.2: Production performance results for an 8-month period

	1	2	3	4
Period	**Production % (Pen A)**	**Production % (Pen B)**	**Production % (Target)**	**Production % (House)**
September 2007	90.63 ± 3.44	87.23 ± 4.54	87.93 ± 0.74	87.13 ± 3.72
October 2007	86.96 ± 2.48	82.22 ± 2.61	85.77 ± 0.72	80.78 ± 1.63
November 2007	86.99 ± 1.78	85.55 ± 2.82	83.60 ± 0.62	81.87 ± 2.31
December 2007	87.40 ± 4.14	84.70 ± 3.54	81.35 ± 0.66	80.29 ± 3.32
January 2008	80.88 ± 3.14	80.13 ± 2.85	79.16 ± 0.73	78.10 ± 2.50
February 2008	80.95 ± 3.03	78.59 ± 2.90	77.03 ± 0.73	77.59 ± 2.62
March 2008	79.58 ± 2.85	76.01 ± 4.05	74.90 ± 0.75	75.78 ± 2.04
April 2008	80.27 ± 2.45	76.52 ± 3.41	72.32 ± 1.07	76.49 ± 2.21

KEY:
Column 1: Performance results from the experimental pen (Pen A)
Column 2: Performance results from the control pen (Pen B)
Column 3: Expected performance results (Theoretical target)
Column 4: Actual production performance results from the chicken house (a combination of Pen A, Pen B and Pen C)

The production performance results are as shown in Table 4.2. For the graph of production performance, the production performance values in the experimental and control pens were compared to and made a percentage of the expected production values.

For instance, the production performance from pen A for the month of September 2007 will be given by (90.63 / 87.93) * 100 to give a value of 103.07% (see Table 4.2 for values used in calculation).

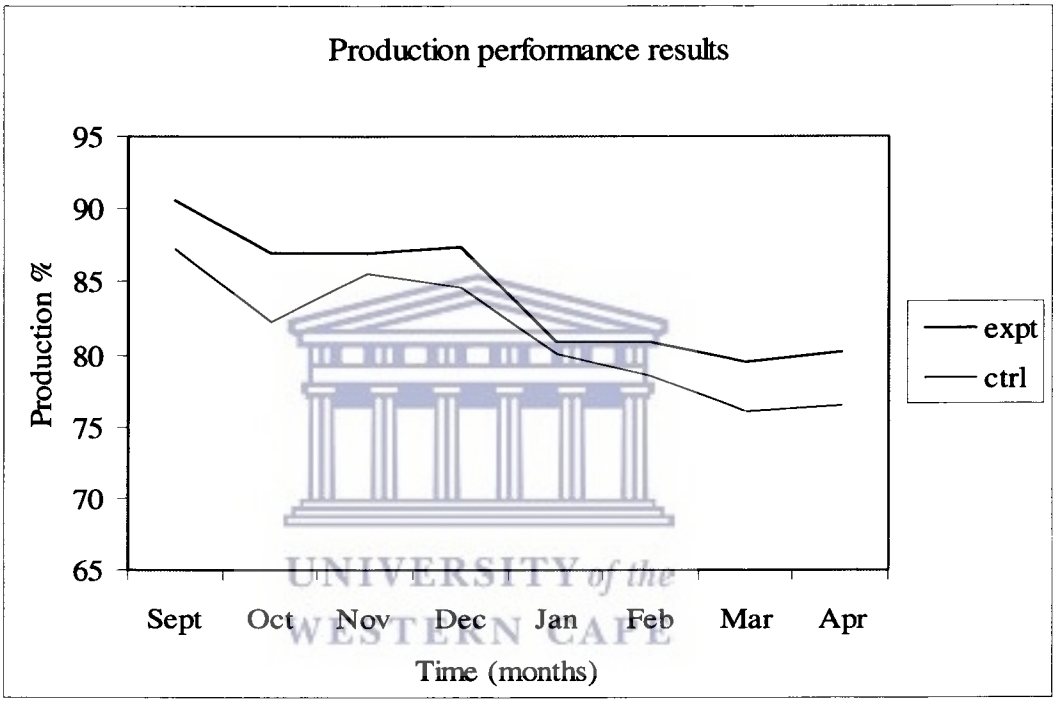


Figure 4.10: Production performance results: A comparison of experimental and control pens to target / expected performance.

The control pen (pen B) did not perform as well as the experimental pen judging from a production percentage of 87.23%, 82.22%, 85.55%, 84.70%, 80.13%, 78.59%, 76.01% and 76.52% (for the control pen) and 90.63%, 86.96%, 86.99%, 87.40%, 80.88%, 80.95%, 79.58% and 80.27% (for the experimental pen) during the months of September, October, November, December, January, February and March respectively.

The experimental pen in terms of production performance remained consistently higher than the control pen and the whole pens in the house (see Table 4.2 and Figure 4.10).

The birds were given medication at intervals in the course of the experiment as part of normal livestock growing procedure. The medication was not specific to chicks in any particular pen; it was administered to all the chicks in the pen. We can therefore infer that the only difference between the experimental pen (pen A) on the one hand and the control pen (pen B) and pen C on the other is the spraying of the biocide in pen A. This being the case, the increase in production noticed in pen A can be said to be due to the introduction of Dioxy MP 14 in the experimental pen.

Comparing each of the experimental (pen A), control (pen B) and the house as a whole (Pens A, B, C) to the expected / theoretical production value showed the following differences:

Table 4.3: Percentage performance differences: A comparison of experimental, control pens and house to target / expected performance

1	2	3	4
Period	**Production %** **(Pen A)**	**Production %** **(Pen B)**	**Production %** **(House)**
September 2007	3.07	- 0.80	- 0.91
October 2007	1.38	- 4.14	- 5.82
November 2007	4.06	2.33	- 2.07
December 2007	7.44	4.12	- 1.30
January 2008	2.17	1.23	- 1.34
February 2008	5.09	2.03	0.73
March 2008	6.25	1.48	1.17
April 2008	10.99	5.81	5.77

To obtain the values in Table 4.3, the performance in each column (see Table 4.2) was compared to the target performance. The difference between the two was made a percentage of the target performance. For instance, to calculate the production performance difference in pen A for the month of September 2007,

(90.63 – 87.93) = 2.7, then (2.7 / 87.93) * 100 = 3.07%.

This value shows that production performance in pen A (the experimental pen) exceeded the target performance by 3.07%. On the other hand, production

performance in pen B (control pen) and in the house decreased by 0.80% and 0.91% respectively when compared to the target.

From Table 4.3, a comparison of the production records from Pen A, Pen B and the house to the target performance shows a reduction in production in the chicken house as a whole for the first five months (Table 4.3, column 4). There was also a percentage reduction in the control pen (pen B) for the first two months. Production in Pen B and in the house later moved from the negative to a positive value but even this was not as much as that obtained from pen A (the experimental pen). Pen A performed better than both the control and the house; exceeding the target production performance in all instances (see Table 4.2).

Table 4.4: Test of significance values for production performance records obtained from the experimental and control pens

Period	p value	Inference
Sept 2007	< 0.0001	Extremely significant
Oct 2007	< 0.0001	Extremely significant
Nov 2007	0.0263	Significant
Dec 2007	0.0016	Very significant
Jan 2008	0.1926	Not significant
Feb 2008	< 0.0001	Extremely significant
Mar 2008	0.0011	Very significant
Apr 2008	< 0.0001	Extremely significant

Statistical analysis of the production performance means using the two-tailed non-parametric test showed for most of the months a significant difference between production performances in the experimental (Pen A) and the control (Pen B) pens.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Improvement in experimental set-up

The experimental set up for the study was chicken house 3A in Bellevue Farms, Paarl. Pen A is beside pen B, the control pen (refer to Figure 5.1). The open windows (AB and CD) which served as ventilation source in the chicken house were covered with a mesh of wire. Chicken house 3B served as the control. The wall separating the two pens had an opening about half a metre wide as shown in the diagram below:

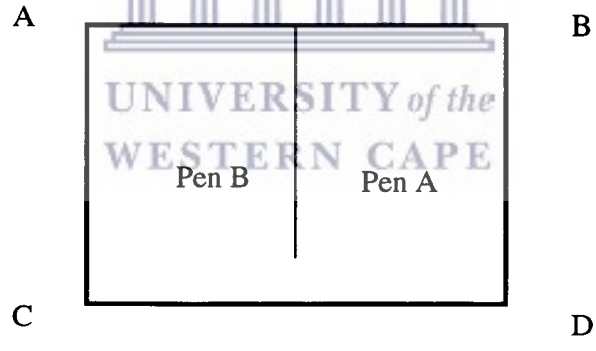


Figure 5.1: Schematic representation of the experimental and control pens

With such an opening, it is possible that pen B (the control pen) was not entirely free of the biocide. Since Dioxy MP 14 was dispersed in the form of an aerosol / spray with the capacity to travel some distance / remain suspended in air over a period of time (McKenzie *et al.*, 1959), it is probable that some amount of the biocide could

have been present in pen B. However, whatever amount of the biocide that got into pen B in the course of the study can be assumed to be of such small magnitude as to be comparable to spraying the pens with water.

Pen A and pen B also had perforated gauze opening on the same side of the house. While this serves to emphasize that both pens had some uniformity, it may also be a cause for discrepancy; if the biocide leaves from pen A through the ventilation opening and goes back into pen B through another section of the ventilation source. Again, it may safely be assumed that this was small enough to be considered insignificant; chlorine dioxide is broken down into chlorine and oxygen on contact with air / oxygen (ATSDR, 2007). If this had happened too, it must have been too small to have had a significant effect on mortality and production in the control pen since this was seen to be quite different from that obtained with the experimental pen.

The experimental set up, being an open system, could also have had some interaction with the surrounding environment hence resulting in fluctuations and discrepancies observed in some of the results. Further testing of this biocide in a closed setting with controlled variables could have given results with less variation.

In addition, temperature measurements were of the minimum and maximum for each day. Measurement (during the day) of temperature as well as wind speed and direction at more points could be done in further studies of this nature; this may be useful in an explanation of certain microbial contamination variations.

A longer period of constant dosing of the biocide (longer than the 8 weeks employed in the study) can also be carried out to give an indication of the long term effects of such a test.

5.2 Improvement in technical set-up

The TEKNA DPZ pump installed by Magic Mist, South Africa, was used for the dispersal of the biocide. It was able to deliver the biocide at the programmed frequencies. However, with prolonged use, some limitations were noticed. The fittings of the pipe were made of brass. They were not resistant to the biocide and corroded which in turn caused the nozzles to block. This resulted in low / no biocide dosing over some days / weeks. Improvements can be made on these fittings by casting them from such material as would not be corroded by the biocide.

Another problem noticed was that in the course of biocide delivery, with an increase in pressure from liquid flow, the width of the pipes increased and fit very tightly against the brass fittings.

In the event of a problem necessitating partial dismantling of the setup so that repair work can be performed, not only is it tasking to separate the joined pipes but very difficult to fit the said pipes back into the former fitting because of the increase in width. This was because of the applied pressure on the pipes. To overcome this, the pipes can be made of some type of material that will not increase in size with increase in the pressure of the liquid flowing through it. Where this is not feasible,

the fittings holding the pipe can be made big and loose enough for the pipes to go through easily, with perhaps smaller fittings and / or screws to help with tightening if necessary.

5.3 Final comment and rationale for further studies

Infection control and basic hygiene are fundamental to good hospital management (HSLC, 1998). Two major reasons can account for this. In the first instance, an unclean hospital environment gives a poor impression of healthcare facilities. Secondly, the risk of infection is increased in such a setting (Evans *et al.*, 1998). Some concern has been expressed over the fact that dirtier health care facilities may be an outcome of attempts to reduce expenses (ICNA/ADM Working Group, 1999). The opportunity cost of not controlling infections in hospitals is likely to be more than the cost of controlling such infections (Wilcox & Dave, 2000). Hospitals are the location for about 47% of general outbreaks of infectious intestinal disease (Evans *et al.*, 1998). The percentage for the transmission of airborne infectious diseases will probably be higher.

According to the WHO (2002), infectious diseases constitute much of the total disease burden in developing countries and are a dominant health care priority for most African countries; the greatest health burden being caused by HIV/AIDS, malaria and tuberculosis.

Hospital acquired infections are possible in TB facilities (Dooley *et al.*, 1992). Greater virulence and greater resistance to commonly used antimicrobials is associated with pathogens responsible for nosocomial infections than with other pathogens (Auriti *et al.*, 2003; Kieninger & Lipsett, 2009; Tanaka *et al.*, 2009).

Occurrence of nosocomial infections prolongs hospital stay thereby increasing health care expenses. These infections are also usually life threatening when they occur among hospitalized patients (Mylotte *et al.*, 2001). The health care workers who look after such patients are at risk of contacting infection from the hospitalized patients (Dooley *et al.*, 1992; Joshi *et al.*, 2006), so also are other patients (Dooley *et al.*, 1992; Laing, 1999). Research reports that more than 50% of all health care workers in developing parts of the world are infected with latent tuberculosis (Joshi *et al.*, 2006). This can ultimately lead to loss of skilled health care workers (Joshi *et al.*, 2006), further compounding the problem.

Tuberculosis is still on rampage in different parts of the world. The challenge of achieving the United Nations Millennium Development Goals (MDGs) with regard to TB control will be greatest in Africa and Eastern Europe (Dye *et al.*, 2005). The WHO in its 2008 report estimates that 9.2 million new global cases occurred in 2006 (139 per 100 000), including 4.1 million (62 per 100 000) new smear-positive cases. Twelve of the fifteen countries with the highest estimated TB incidence rates are in Africa (WHO, 2008b).

Since TB is mainly transmitted through infected airborne droplets (Dye *et al.*, 2006), it follows that the risk of transmission will be increased in a congested environment. Similarly, infectious diseases like tuberculosis have the potential to spread rapidly in highly populated poultry houses (close confinement) (WRAI, 1988) with disastrous consequences. Currently, there is no treatment for the disease in animal husbandry; focus is on destruction of infected animals (WRAI, 1998). Disinfection therefore is an important and necessary part of the cleaning routine of such settings (McKenzie *et al.*, 1959). Preparations containing chlorine dioxide have been reported to be effective against *Mycobacterium tuberculosis* among other *Mycobacterium* species (Hernandez *et al.*, 2008).

Disinfection of poultry settings in most cases is not easy and so disinfection by aerosol mist may be employed. In aerosol form, the disinfectant particles can be so generated to be small or large particles (ranging from 5 to 125 microns). The smaller the particle size, the longer the disinfectant will remain suspended in the atmosphere (McKenzie *et al.*, 1959), increasing its probability to come in contact with airborne microbes.

It is pertinent though to mention that determining the efficacy of a disinfectant under controlled conditions cannot be said to be the same as assessing its performance in actual use. To be of value, aerosol mists must be effective under conditions obtained in commercial settings, which may not permit disinfection under optimum conditions of temperature and humidity (McKenzie *et al.*, 1959). In line with this, the study

investigated the use of Dioxy MP 14 in the control of airborne microbial contamination in a chicken pen under uncontrolled conditions. In such a condition, there was the possibility of a wide deviation in values obtained at the same time and fluctuations with or against a trend from one point to another due to the influence of the outside environment. Disconcerting though this may sound, it serves to emphasize how difficult it is to ensure that disinfectant action shall be effective in all circumstances (McKenzie *et al.*, 1959).

In this study, Dioxy MP 14, reportedly effective against a wide range of microorganisms (Information Fact Sheet: Dioxy MP 14) was also found to reduce microbial contamination in the experimental pen. It was hypothesized at the onset of the study that a decrease in microbial contamination would result in healthier chicks that would boost production. From the results obtained, the following conclusions could be drawn:

- Dioxy MP 14 at a maximum chlorine dioxide concentration of 7.46 ppm is safe for use in a poultry environment when employed over the period as in the study. If toxic at this concentration, over the study period, the health of the chicks in the experimental poultry pen would have been negatively affected and increased mortality and decreased production would have been noticed.
- The biocide under investigation (Dioxy MP 14) reduced microbial contamination in a contaminated setting.

- Reduced microbial contamination in the experimental (Dioxy MP 14-treated) pen resulted in a decrease in mortality in this pen.
- Reduction in microbial contamination also improved production in the experimental pen when compared to the control pen.

The result of the performance obtained from the chicks in the experimental versus the control pen showed a significant positive effect in the treated pen.

This study was only exploratory; the dose found to reduce microbial contamination in another contaminated setting or even a different poultry house could vary from that obtained in the study. Such values may in effect depend on the original contamination (before disinfection) in the said setting and the influence of the surrounding environment.

To see if this statistically significant difference will translate into a clinically significant one, more data with inferential basis is required. To generate such data, ethical approval is needed for further studies using this biocide – applied in this manner – in a suitable environment. Such a study will need to be carried out in a setting where airborne microbial contamination is a problem (such as a tuberculosis ward). In this era when rates of multidrug resistant (MDR) and extremely drug resistant (XDR) tuberculosis are at an all time high (WHO, 2008a), the importance of preventive measures, especially in health care facilities cannot be over-emphasized. This is necessary as there is need to quell this scourge (tuberculosis) before it beats us.

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APPENDIX I: TABLE SHOWING RAW DATA AND PERCENTAGE OF CONTAMINATION VALUES

CALCULATION OF PERCENTAGES FROM RAW DATA VALUES

CONTAMINATION WITH BACTERIA

RAW EXPT refers to the raw data obtained in the experimental pen. B1, B2 and B3 beside this are the bacterial colony forming units observed at three different points in the experimental pen.

RAW CTRL refers to the raw data obtained in the control pen where the biocide was not sprayed. B1, B2 and B3 beside this are bacterial colony forming units observed at three different points in the control pen.

Under **% EXPT**, the raw data at different time intervals was made a percentage of the raw data at time 0 (before disinfection) as explained in chapter 4.

% CTRL refers to similar percentage conversions of the raw data from the control pen, taking the value obtained at time 0 (before disinfection) to be 100%.

% EXPT /% CTRL describes the overall percentage contamination in the experimental pen as a percentage of the percentage contamination in the control pen, i.e. **% EXPT /% CTRL * 100.**

CONTAMINATION WITH YEASTS AND MOULDS

RAW EXPT refers to the raw data obtained in the experimental pen. Y1, Y2 and Y3 beside this are the number of yeasts and moulds observed at three different points in the experimental pen.

RAW CTRL refers to the raw data obtained in the control pen where the biocide was not sprayed. Y1, Y2 and Y3 beside this are the number of yeasts and moulds observed at three different points in the control pen.

Under **% EXPT,** the raw data at different time intervals was made a percentage of the raw data at time 0 (befoe disinfection) as explained in chapter 4.

% CTRL refers to similar percentage conversions of the raw data from the control pen, taking the value obtained at time 0 (before disinfection) to be 100%.

% EXPT /% CTRL describes the overall percentage contamination in the experimental pen as a percentage of the percentage contamination in the control pen, i.e. **% EXPT /% CTRL * 100.**

STD DEV refers to standard deviation.

Pump dosing at 7.46 ppm

CONTAMINATION WITH BACTERIA

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**B1**		698	96	104	132		113		120
EXPT	**B2**		727	96	112	148	128	139	132	160
	B3		759	108	143	168	173	158	148	188
RAW	**B1**		819	819	825	838	853	896	978	1035
CTRL	**B2**		698	703	701	712	729	672	813	801
	B3		758			793		759		
%	**B1**		100	13.75	14.9	18.91		16.19		17.19
EXPT	**B2**		100	13.2	15.41	20.36	17.61	19.12	18.16	22.01
	B3		100	14.23	18.84	22.13	22.79	20.82	19.5	24.77
%	**B1**		100	100	100.73	102.32	104.15	109.4	119.41	126.37
CTRL	**B2**		100	100.72	100.43	102.33	104.44	96.28	116.48	114.76
	B3		100			104.62		100.13		
% EXPT /% CTRL		**1**	100	13.75	14.79	18.48	16.91	14.8		13.6
		2	100	13.11	15.34	19.96	21.82	19.86	15.59	19.18
		3	100			21.15		20.79		
AVERAGE			[illegible]	[illegible]	[illegible]	19.86		18.41	[illegible]	[illegible]

Pump dosing at 7.46 ppm

CONTAMINATION WITH YEASTS AND MOULDS

		0 hr before	0 hr after	1	2	3	4	5	6
RAW	**Y1**	39	12	18	26	16	17	17	24
EXPT	**Y2**	41	15	17	27	26	26	23	25
	Y3	41	13	18	23	19	26	30	31
RAW	**Y1**	20	22	27	30	30	42	37	45
CTRL	**Y2**	32	36	36	46	52	67	46	59
	Y3	24	27	30	33	29	51	46	49
%	**Y1**	100	30.76	46.15	66.67	41.03	43.59	43.59	61.53
EXPT	**Y2**	100	36.59	41.46	65.85	63.41	63.41	56.1	60.98
	Y3	100	31.71	43.9	56.1	46.34	63.41	73.17	75.61
%	**Y1**	100	110	135	150	150	210	185	225
CTRL	**Y2**	100	112.5	112.5	143.75	162.5	209.38	143.75	184.38
	Y3	100	112.5	125	137.5	120.83	212.5	191.67	204.17
% EXPT /%									
CTRL	**1**	100	27.96	34.19	44.45	27.35	20.76	23.56	27.35
	2	100	32.52	36.85	45.81	39.02	30.28	39.03	33.07
	3	100	28.19	35.12	40.8	38.35	29.84	38.17	37.03
AVERAGE		100	29.5667	35.3866	43.6866	34.9066	26.96	33.5866	32.4833333

Pump dosing at 5.60 ppm

CONTAMINATION WITH BACTERIA

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**B1**		580	84	212	172	144	180	136	228
EXPT	**B2**		596	128	260	204	296	204	220	228
	B3		721	144	300	240	304	316	240	248
RAW	**B1**		830	824	817	815	854	910	975	992
CTRL	**B2**		856	866	842	852	943	931	947	
	B3		792	804	831	811	813	897		960
%	**B1**		100	19.97	36.55	29.66	24.83	31.03	23.45	39.31
EXPT	**B2**		100	14.48	43.62	33.29	49.66	34.23	36.91	38.26
	B3		100	21.48	41.61	34.23	42.16	43.83	33.29	34.4
%	**B1**		100	99.28	98.43	98.19	102.89	109.64	117.47	119.52
CTRL	**B2**		100	101.17	98.36	99.53	110.16	108.76	110.63	
	B3		100	101.52	104.92	102.4	102.65	113.26		121.21
% EXPT /%					37.1329	30.2067	24.1325	28.3017	19.9625	
CTRL		**1**	100	20.11483	9	4	7	1	4	32.8898929
							45.0798	31.4729	33.3634	
		2	100	14.31254	44.3473	33.4472	8	7	6	#DIV/0!
					39.6587	33.4277		38.6985		28.3804966
		3	100	21.15839	9	3	41.0716	7		6
AVERAGE			100	18.52859	40.1796	32.3605	36.7613	32.8284	26.663	30.6351947

Pump dosing at 5.60 ppm

CONTAMINATION WITH YEASTS AND MOULDS

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**Y1**		30	22	25	18	30	25	22	26
EXPT	**Y2**		50	29	36	34	40	27	28	33
	Y3		22			14			16	
RAW	**Y1**		33	38	40	37	50	57	55	62
CTRL	**Y2**		26	32	38	32	32	30	37	43
	Y3		36		39		41		55	
%	**Y1**		100	73.33	83.33	60	100	83.33	73.33	86.67
EXPT	**Y2**		100	58	72	68	80	54	56	66
	Y3		100			63.63			72.73	
%	**Y1**		100	115.15	121.21	112.12	151.52	172.73	166.67	187.88
CTRL	**Y2**		100	123.08	146.15	123.08	123.08	115.38	142.31	165.38
	Y3		100						152.78	
% EXPT /%										
CTRL		**1**	100	63.68	68.75	53.51	66	48.24	44	46.13
		2	100	47.12	49.26	55.25	65	46.8	39.35	39.91
		3	100						47.6	
AVERAGE			100	55.4	59.005	54.38	65.5	47.585	43.65	43.02

Pump dosing at 3.73 ppm

CONTAMINATION WITH BACTERIA

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**B1**		296	179	144	104		145		166
EXPT	**B2**		345	212	150	109	127	146	161	176
	B3		345	227	151	121	168	155	183	222
RAW	**B1**		340	360	384	395	328	369	442	500
CTRL	**B2**		395	412	409	387	382	398	405	452
	B3		328	330	341	333	332	345		
%	**B1**		100	60.47	48.65	35.13		48.99		56.08
EXPT	**B2**		100	61.45	43.48	31.59	36.81	42.32	46.67	51.01
	B3		100	65.8	43.77	35.07	48.7	44.93	53.04	64.35
%	**B1**		100	105.88	112.94	116.18	96.47	108.53	130	147.06
CTRL	**B2**		100	104.3	103.54	97.97	96.71	100.76	102.53	114.43
	B3		100	100.61	103.96	101.52	101.22	105.18		
% EXPT /%					43.0759	30.2375		45.1395		38.1340949
CTRL		**1**	100	57.11182	7	6		9	0	3
					41.9934	32.2445	38.0622	42.0007	45.5183	44.5774709
		2	100	58.91659	3	6	5	9	8	4
					42.1027	34.5449	48.1130	42.7172		
		3	100	65.40105	3	2	2	5	#DIV/0!	
AVERAGE			100	60.47649	42.3207	32.3423	43.0876	42.2858	45.521	41.3557829

Pump dosing at 3.73 ppm

CONTAMINATION WITH YEASTS AND MOULDS

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**Y1**		21	19			16		17	24
EXPT	**Y2**		30	28	15	27	17	21	26	24
	Y3		35	30	31	30		30		29
RAW	**Y1**		22	32	27	28	30	32	31	32
CTRL	**Y2**		23	33	30	30	30			26
	Y3		26	33	26			30	29	29
%	**Y1**		100	90.48			76.19		80.95	114.28
EXPT	**Y2**		100	93.33	50	90	56.67	70	86.67	80
	Y3		100	85.71	88.57	85.71		85.71		82.86
%	**Y1**		100	145.45	122.73	127.27	136.36	145.45	140.91	145.45
CTRL	**Y2**		100	143.48	130.43	130.43	130.43			113.04
	Y3		100	126.92	100			115.38	111.54	111.54
% EXPT /%										
CTRL		**1**	100	62.21			55.87		57.45	78.57
		2	100	65.05	38.33	70.72	43.45			70.77
		3	100	67.53	88.57	65.71		74.28		74.29
AVERAGE			100	66.95	63.45	68.215	49.66	74.28	57.45	74.54333333

Pump dosing at 1.87 ppm

CONTAMINATION WITH BACTERIA

			0 hr before	0 hr after	1	2	3	4	5	6
RAW	**B1**		216	168	172	120	170	213	243	197
EXPT	**B2**		226	171	223	132	202	268	250	197
	B3									
RAW	**B1**		460	410	362	314	275	325	395	495
CTRL	**B2**		442	418	402	414	287	442	440	478
	B3									
%	**B1**		100	77.78	79.63	55.56	78.7	98.61	112.5	91.2
EXPT	**B2**		100	75.66	98.67	58.4	89.38	118.58	110.62	87.17
	B3									
%	**B1**		100	89.13	78.7	68.26	59.78	70.65	85.87	107.61
CTRL	**B2**		100	94.57	90.95	93.67	64.93	100	99.55	108.14
	B3									
% EXPT /%										
CTRL		**1**	100	87.26579	101.1817	81.39467	131.6494	139.5754	131.012	84.75048787
		2	100	80.00423	108.4882	62.34654	137.6559	118.58	111.12	80.6084705
AVERAGE			100	84.63501	105.3349	71.8706	134.6527	129.0777	121.066	82.67949919

Pump dosing at 1.87 ppm

CONTAMINATION WITH YEASTS AND MOULDS

		0 hr before	0 hr after	1	2	3	4	5	6
RAW	**Y1**	45	33	37	44		53	80	30
EXPT	**Y2**	50	43	43	49	49	55	90	63
	Y3								
RAW	**Y1**	57	58	62	59	61		66	52
CTRL	**Y2**	37	47		52	64	69	56	42
	Y3								
%	**Y1**	100	73.33	82.22	97.78		117.78	177.78	66.67
EXPT	**Y2**	100	86	86	98	98	110	180	86.46
	Y3								
%	**Y1**	100	101.75	108.77	103.51	107.02		115.79	91.23
CTRL	**Y2**	100	127.03		140.54	172.97	186.49	151.35	113.51
	Y3								
% EXPT /%									
CTRL	**1**	100	72.07	75.59	94.46			153.37	73.08
	2	100	67.7		69.73	56.66	58.98	118.93	76.17
	3								
AVERAGE		100	69.885	75.59	82.095	56.66	58.98	136.15	74.625

APPENDIX II:
SPRAY MIST CONTROL: SPRAYING WITH WATER ALONE (NO BIOCIDE)

CONTAMINATION WITH BACTERIA

		0 hr before	0 hr after	1	2	3	4	5
RAW EXPT	**B1**	165	396	244	368	325	172	
	B2	115	243	184		209	141	139
	B3	84		141	90			
RAW CTRL	**B1**	177	169	188	168	207		
	B2	149	153			171	137	140
	B3							
% EXPT	**B1**	100	240	147.88	223.03	196.97	104.24	
	B2	100	211.3	160		181.74	122.61	120.87
	B3	100		122.61	78.26			
% CTRL	**B1**	100	95.48	106.21	94.92	116.95		
	B2	100	102.68			114.77	91.95	93.96
	B3							
% EXPT /% CTRL	**1**	100	251.36	139.23	234.97	168.42		
	2	100	205.78			158.35	133.34	128.64
	3	100						
AVERAGE		100	228.57	139.23	234.975	163.385	133.84	128.64

SPRAY MIST CONTROL: SPRAYING WITH WATER ALONE (NO BIOCIDE)

CONTAMINATION WITH YEASTS AND MOULDS

		0 hr before	0 hr after	1	2	3	4	5
RAW EXPT	**Y1**	32	48	18	57	45	55	59
	Y2	22	40	56	61	49	56	56
	Y3							
RAW CTRL	**Y1**	29	22	19	17	18	30	28
	Y2	18	13	60	18	16	35	20
	Y3							
% EXPT	**Y1**	100	150	56.25	178.13	140.63	171.88	184.38
	Y2	100	125	254.55	277.27	222.73	254.55	254.55
	Y3							
% CTRL	**Y1**	100	75.86	65.52	58.62	62.07	103.45	96.55
	Y2	100	72.22	333.33	100	88.89	194.44	111.11
	Y3							
% EXPT /% CTRL	**1**	100	197.73	85.85	303.87	226.57	166.15	190.96
	1	100	173.08	76.37	277.27	250.57	130.91	229.1
	3							
[illegible]		100	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

APPENDIX III: RAW DATA FOR SPRAYING OF BIOCIDE AT REGULAR INTERVALS

CONTAMINATION WITH BACTERIA

Sample 1

Week	Pen A - Expt	Pen B - Ctrl	Cont %
1	184	510	36.08
2	84	380	22.11
3	38	325	11.69
4	99	204	48.53
5	196	267	73.41
6	84	273	30.77
7	20	149	13.42
8	103	196	52.55

Sample 2

Week	Pen A - Expt	Pen B - Ctrl	Cont %
1	252	846	29.79
2	65	285	22.81
3	119	902	13.19
4	130	258	50.39
5	141	173	81.5
6	52	138	37.68
7	81	376	21.54
8	75	130	57.69

	Week	1	2	3	4	5	6	7	8
	Sample								
Cont %	1	36.08	22.11	11.69	48.53	73.41	30.77	13.42	52.55
	Sample								
Cont %	2	29.79	22.81	13.19	50.39	81.5	37.68	21.54	57.69
AVERAGE		32.935	22.46	12.44	49.46	77.455	34.225	17.48	55.12

* Cont % refers to contamination percentage.
Pen A – Expt refers to the experimental pen while Pen B – Ctrl refers to the control pen.

CONTAMINATION WITH YEASTS AND MOULDS

Sample 1

Week	Pen A - Expt	Pen B - Ctrl	Cont %
1	18	61	29.51
2	9	61	14.75
3	9	72	12.5
4	39	180	21.67
5	30	93	32.26
6	37	148	25
7	23	103	22.33
8	26	76	34.21

Sample 2

Week	Pen A - Expt	Pen B - Ctrl	Cont %
1	79	141	56.03
2	35	145	24.14
3	24	139	17.27
4	41	68	60.29
5	32	32	100
6	51	111	45.95
7	11	61	18.03
8	13	37	35.14

	Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cont %	**Sample 1**	29.51	14.75	12.5	21.67	32.26	25	22.33	34.21
Cont %	**Sample 2**	56.03	24.14	17.27	60.29	100	45.95	18.03	35.14

* Cont % refers to contamination percentage.
Pen A – Expt refers to the experimental pen while Pen B – Ctrl refers to the control pen.

APPENDIX IV: RAW DATA FOR MORTALITY AND PRODUCTION RECORDS

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/09/2007	2132	0	2132	65	91.46	15	29	M
2	2132	1	2131	68	95.73	16	32	NO
3	2131	1	2130	66	92.96	19	32	NO
4	2130	1	2129	66	93	10	37	NO
5	2129	3	2126	68	95.95	10	22	S
6	2126	0	2126	66	93.13	11	22	NO
7	2126	1	2125	67	94.59	11	22	NO
Sat, 08/09/2007	2125	1	2124	67	94.63	11	24	NO
9	2124	1	2123	65	91.85	11	23	NO
10	2123	1	2122	66	93.31	10	22	NO
11	2122	0	2122	67	94.72	10	23	S
12	2122	0	2122	66	93.31	11	23	NO
13	2122	1	2121	66	93.35	10	23	NO
14	2121	3	2118	65	92.07	11	23	NO
Sat, 15/09/2007	2118	0	2118	65	92.07	11	23	NO
16	2118	2	2116	64	90.74	10	22	NO
17	2116	0	2116	60	85.07	11	23	NO
18	2116	1	2115	65	92.2	10	28	NO
19	2115	0	2115	64	90.78	11	30	NO
20	2115	0	2115	63	89.36	12	31	NO
21	2115	1	2114	62	87.98	12	31	NO
Sat, 22/09/2007	2114	1	2113	61	86.61	12	31	NO
23	2113	1	2112	61	86.65	12	31	S
24	2112	1	2111	62	88.11	9	31	S
25	2111	6	2105	61	86.94	10	31	NO
26	2105	1	2104	60	85.55	10	31	NO
27	2104	0	2104	62	88.4	10	31	NO
28	2104	0	2104	60	85.55	10	31	S
Sat, 29/09/2007	2104	4	2100	59	84.29	10	31	S
30	2100	1	2099	62	88.61	10	31	M
				Average prod:	90.63233			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/09/2007	2171	0	2171	65	89.82	15	29	M
2	2170	1	2170	65	89.86	16	32	NO
3	2170	0	2170	63	87.1	19	32	NO
4	2170	0	2170	65	89.86	10	37	NO
5	2170	0	2170	65	89.86	10	22	S
6	2170	0	2170	63	87.1	11	22	NO
7	2167	3	2167	65	89.99	11	22	NO
Sat, 08/09/2007	2166	1	2166	67	92.8	11	24	NO
9	2166	0	2166	66	91.41	11	23	NO
10	2165	1	2165	64	88.68	10	22	NO
11	2163	2	2163	65	90.15	10	23	S
12	2163	0	2163	65	90.15	11	23	NO
13	2161	2	2161	65	90.24	10	23	NO
14	2158	3	2158	66	91.75	11	23	NO
Sat, 15/09/2007	2156	2	2156	63	87.66	11	23	NO
16	2155	1	2155	64	89.1	10	22	NO
17	2155	0	2155	73	101.62	11	23	NO
18	2154	1	2154	60	83.57	10	28	NO
19	2154	0	2154	60	83.57	11	30	NO
20	2154	0	2154	60	83.57	12	31	NO
21	2152	2	2152	60	83.64	12	31	NO
Sat, 22/09/2007	2151	1	2151	59	82.29	12	31	NO
23	2150	1	2150	59	82.33	12	31	S
24	2147	3	2147	60	83.84	9	31	S
25	2146	1	2146	62	86.67	10	31	NO
26	2146	0	2146	59	82.48	10	31	NO
27	2145	1	2145	57	79.72	10	31	NO
28	2143	2	2143	59	82.59	10	31	S
Sat, 29/09/2007	2141	2	2141	59	82.67	10	31	S
30	2140	1	2140	59	82.71	10	31	M
				Average prod:	87.22667			

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Mon, 01/10/2007	2099	1	2098	60	85.8	10	31	NO
2	2098	0	2098	62	88.66	10	31	NO
3	2098	1	2097	60	85.84	9	31	S
4	2097	0	2097	60	85.84	9	31	S
5	2097	1	2096	61	87.31	9	31	NO
Sat, 06/10/2007	2096	1	2095	60	85.92	6	31	S
7	2095	2	2093	55	78.83	6	31	S
8	2093	0	2093	61	87.43	5	31	S
9	2093	1	2092	61	87.48	6	31	NO
10	2092	0	2092	61	87.48	6	31	S
11	2092	0	2092	61	87.48	6	31	NO
12	2092	1	2091	60	86.08	6	31	NO
Sat, 13/10/2007	2091	1	2090	61	87.56	6	31	NO
14	2090	1	2089	61	87.6	6	31	NO
15	2089	1	2088	62	89.08	6	31	NO
16	2088	3	2085	61	87.77	6	31	NO
17	2085	2	2083	60	86.41	6	31	M
18	2083	0	2083	64	92.17	6	31	NO
19	2083	0	2083	60	86.41	6	31	M
Sat, 20/10/2007	2083	1	2082	62	89.34	6	31	NO
21	2082	3	2079	62	89.47	6	31	NO
22	2079	3	2076	60	86.71	6	32	S
23	2076	0	2076	60	86.71	6	33	S
24	2076	0	2076	59	85.26	6	33	M
25	2076	1	2075	61	88.19	6	32	NO
26	2075	2	2073	59	85.38	6	32	S
Sat, 27/10/2007	2073	1	2072	59	85.42	6	32	NO
28	2072	2	2070	60	86.96	6	31	M
29	2070	0	2070	61	88.41	6	31	NO
30	2070	2	2068	63	91.39	6	31	M
31	2068	2	2066	56	81.32	6	31	NO

Average prod: 86.95839

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Mon, 01/10/2007	2139	1	2139	58	81.35	10	31	NO
2	2138	1	2138	60	84.19	10	31	NO
3	2138	0	2138	57	79.78	9	31	S
4	2137	1	2137	58	81.42	9	31	S
5	2136	1	2136	57	80.06	9	31	NO
Sat, 06/10/2007	2135	1	2135	58	81.5	6	31	S
7	2134	1	2134	57	80.13	5	31	NO
8	2132	2	2132	59	83.02	5	31	S
9	2126	6	2126	57	80.43	6	31	NO
10	2123	3	2123	58	81.96	6	31	S
11	2123	0	2123	56	79.13	6	31	NO
12	2121	2	2121	56	79.21	6	31	NO
Sat, 13/10/2007	2121	0	2121	56	79.21	6	31	NO
14	2121	0	2121	59	79.21	6	31	NO
15	2119	2	2119	59	83.53	6	31	NO
16	2117	2	2117	57	80.77	6	31	NO
17	2116	1	2116	58	82.23	6	31	M
18	2113	3	2113	59	83.77	6	31	NO
19	2113	0	2113	58	82.35	6	31	M
Sat, 20/10/2007	2112	1	2112	59	83.81	6	31	NO
21	2109	3	2109	59	83.93	6	31	NO
22	2106	3	2106	59	84.05	6	32	S
23	2103	3	2103	58	82.74	6	33	S
24	2100	3	2100	58	82.86	6	33	M
25	2097	3	2097	59	84.41	6	32	NO
26	2095	2	2095	57	81.62	6	32	S
Sat, 27/10/2007	2089	6	2089	59	84.73	6	32	NO
28	2087	2	2087	61	87.69	6	31	M
29	2082	5	2082	58	83.57	6	31	NO
30	2080	2	2080	62	89.42	6	31	M
31	2076	4	2076	53	76.59	6	31	NO

Average prod: 82.21516

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Thu 01/11/2007	2066	0	2066	59	85.67	6	31	NO
2	2066	1	2065	61	88.62	6	31	S
Sat, 03/11/2007	2065	2	2063	60	87.25	6	31	NO
4	2063	1	2062	60	87.29	6	31	NO
5	2062	0	2062	59	85.84	6	31	S
6	2062	0	2062	60	87.29	6	31	NO
7	2062	3	2059	59	85.96	6	31	S
8	2059	3	2056	59	86.09	6	31	NO
9	2056	2	2054	59	86.17	15	34	M
Sat, 10/11/2007	2054	3	2051	59	86.3	15	34	M
11	2051	2	2049	61	89.31	15	34	S
12	2049	1	2048	59	86.43	6	30	M
13	2048	2	2046	61	89.44	12	31	NO
14	2046	2	2044	59	86.59	15	34	NO
15	2044	1	2043	61	89.57	6	31	STRONG
16	2043	0	2043	60	88.11	6	31	NO
Sat, 17/11/2007	2043	1	2042	60	88.15	6	31	S
18	2042	2	2040	58	85.29	6	31	M
19	2040	3	2037	58	85.42	11	29	NO
20	2037	1	2036	60	88.41	11	29	NO
21	2036	2	2034	59	87.02	13	29	S
22	2034	2	2032	57	84.15	13	29	STRONG
23	2032	1	2031	60	88.63	13	30	M
Sat, 24/11/2007	2031	0	2031	58	85.67	14	29	S
25	2031	1	2030	58	85.71	14	30	NO
26	2030	1	2029	60	88.71	14	31	M
27	2029	0	2029	60	88.71	12	31	NO
28	2029	3	2026	55	81.44	12	31	NO
29	2026	1	2025	60	88.89	12	31	M
30	2025	1	2024	59	87.45	13	29	NO
				Average prod:	86.986			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Thu 01/11/2007	2076	0	2076	59	85.26	6	31	NO
2	2075	1	2075	59	85.3	6	31	S
Sat, 03/11/2007	2074	1	2074	60	86.79	6	31	NO
4	2073	1	2073	57	82.49	6	31	NO
5	2073	0	2073	60	86.83	6	31	S
6	2071	2	2071	61	88.36	6	31	NO
7	2068	3	2068	53	76.89	6	31	S
8	2066	2	2066	55	79.86	6	31	NO
9	2065	1	2065	57	82.81	15	34	M
Sat, 10/11/2007	2062	3	2062	59	85.84	15	34	M
11	2060	2	2060	60	87.38	15	34	S
12	2059	1	2059	59	85.96	6	30	M
13	2056	3	2056	60	87.55	12	31	NO
14	2054	2	2054	57	83.25	15	34	NO
15	2053	1	2053	61	89.14	6	31	STRONG
16	2053	0	2053	60	87.68	6	31	M
Sat, 17/11/2007	2053	0	2053	58	84.75	6	31	S
18	2050	3	2050	59	86.34	6	31	M
19	2048	2	2048	58	84.96	11	29	NO
20	2047	1	2047	60	87.93	11	29	NO
21	2044	3	2044	59	86.59	13	29	S
22	2042	2	2042	60	88.15	13	29	STRONG
23	2041	1	2041	58	85.25	13	30	M
Sat, 24/11/2007	2040	1	2040	59	86.76	14	29	S
25	2039	1	2039	59	86.81	14	30	NO
26	2038	1	2038	60	88.32	14	31	M
27	2038	0	2038	59	86.85	12	31	NO
28	2034	4	2034	56	82.6	12	31	NO
29	2033	1	2033	55	81.16	12	31	M
30	2029	4	2029	60	88.71	13	29	NO

Average prod: 85.55233

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/12/2007	2024	2	2022	59	87.54	12	30	S
2	2022	2	2020	57	84.65	12	30	NO
3	2020	2	2018	60	89.2	11	30	NO
4	2018	0	2018	60	89.2	11	30	S
5	2018	1	2017	59	87.75	12	31	S
6	2017	1	2016	57	84.82	12	30	S
7	2016	1	2015	60	89.33	12	30	NO
Sat, 08/12/2007	2015	1	2014	61	90.86	12	32	S
9	2014	2	2012	63	93.94	12	30	S
10	2012	3	2009	61	91.09	11	32	NO
11	2009	1	2008	62	92.63	11	32	NO
12	2008	0	2008	60	89.64	12	30	NO
13	2008	1	2007	59	88.19	12	34	S
14	2007	1	2006	62	92.72	12	35	NO
Sat, 15/12/2007	2006	2	2004	59	88.32	12	35	NO
16	2004	2	2002	60	89.91	12	32	NO
17	2002	3	1999	61	91.55	12	31	NO
18	1999	3	1996	58	87.17	12	32	NO
19	1996	0	1996	55	82.67	12	30	NO
20	1996	0	1996	60	90.18	10	36	NO
21	1996	2	1994	59	88.77	12	36	NO
Sat, 22/12/2007	1994	2	1992	54	81.33	12	36	NO
23	1992	0	1992	56	84.34	12	36	NO
24	1992	1	1991	58	87.39	12	37	NO
25	1991	1	1990	60	90.45	12	37	NO
26	1990	0	1990	60	90.45	12	37	NO
27	1990	3	1987	55	83.04	12	36	NO
28	1987	1	1986	56	84.59	12	37	NO
Sat, 29/12/2007	1986	2	1984	54	81.65	11	32	S
30	1984	3	1981	52	78.75	10	32	NO
31	1981	0	1981	51	77.23	10	32	NO
				Average prod:	87.39839			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/12/2007	2028	1	2028	58	85.8	12	30	S
2	2026	2	2026	57	84.4	12	30	NO
3	2023	3	2023	56	83.04	11	30	NO
4	2016	7	2016	55	81.85	11	30	S
5	2016	0	2016	62	92.26	12	31	S
6	2016	0	2016	55	81.85	12	30	S
7	2015	1	2015	56	83.37	12	30	NO
Sat, 08/12/2007	2014	1	2014	57	84.91	12	32	S
9	2012	2	2012	57	84.99	12	30	S
10	2008	4	2008	59	88.15	11	32	NO
11	2006	2	2006	59	88.24	11	32	NO
12	2004	2	2004	58	86.83	12	30	NO
13	2003	1	2003	57	85.37	12	34	S
14	2001	2	2001	59	88.46	12	35	NO
Sat, 15/12/2007	1998	3	1998	57	85.59	12	35	NO
16	1996	2	1996	56	84.17	12	32	NO
17	1992	4	1992	57	85.84	12	31	NO
18	1989	3	1989	59	88.98	12	32	NO
19	1989	0	1989	55	82.96	12	30	S
20	1989	0	1989	58	87.48	10	36	NO
21	1987	2	1987	59	89.08	12	36	NO
Sat, 22/12/2007	1986	1	1986	55	83.08	12	36	NO
23	1985	1	1985	54	81.61	12	36	NO
24	1985	0	1985	54	81.61	12	37	NO
25	1984	1	1984	50	75.6	12	37	NO
26	1983	1	1983	58	87.75	12	37	NO
27	1981	2	1981	59	89.35	12	36	NO
28	1981	0	1981	54	81.78	12	37	NO
Sat, 29/12/2007	1978	3	1978	54	81.9	11	32	S
30	1976	2	1976	53	80.47	10	32	NO
31	1975	1	1975	52	78.99	10	32	NO
				Average prod:	84.70194			

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Tue 01/01/2008	1981	2	1979	50	75.8	12	36	NO
2	1979	1	1978	58	87.97	12	36	NO
3	1978	1	1977	50	75.87	12	37	STRONG
4	1977	2	1975	50	75.95	12	37	NO
Sat, 05/01/2008	1975	2	1973	50	76.03	9	41	NO
6	1973	2	1971	52	79.15	8	41	NO
7	1971	2	1969	53	80.75	8	41	S
8	1969	2	1967	54	82.36	6	41	STRONG
9	1967	1	1966	56	85.45	6	41	NO
10	1966	2	1964	54	85.54	16	34	NO
11	1964	0	1964	51	77.9	16	35	NO
Sat, 12/01/2008	1964	2	1962	52	79.51	14	30	NO
13	1962	2	1960	53	81.12	14	30	NO
14	1960	1	1959	51	78.1	14	30	NO
15	1959	2	1957	53	81.25	16	36	STRONG
16	1957	2	1955	55	84.4	16	36	S
17	1955	0	1955	53	81.33	16	36	M
18	1955	2	1953	54	82.95	16	35	STRONG
Sat, 19/01/2008	1953	1	1952	55	84.53	16	35	NO
20	1952	2	1950	54	83.08	16	35	NO
21	1950	3	1947	52	80.12	16	35	NO
22	1947	1	1946	52	80.16	16	35	S
23	1946	0	1946	52	80.16	16	35	M
24	1946	0	1946	52	80.16	15	35	S
25	1946	0	1946	52	80.16	15	35	NO
Sat, 26/01/2008	1946	3	1943	53	81.83	16	36	NO
27	1943	2	1941	54	83.46	16	36	NO
28	1941	3	1938	52	80.5	16	36	S
29	1938	1	1937	50	77.44	16	36	NO
30	1937	0	1937	51	78.99	16	36	NO
31	1937	2	1935	55	85.27	17	28	NO
				Average prod:	80.88032			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Tue 01/01/2008	1975	0	1975	50	75.95	12	36	NO
2	1974	1	1974	58	88.15	12	36	NO
3	1972	2	1972	53	80.63	12	37	STRONG
4	1968	4	1968	52	79.27	12	37	NO
Sat, 05/01/2008	1965	3	1965	50	76.34	9	41	NO
6	1964	1	1964	50	76.37	8	41	NO
7	1959	5	1959	53	81.16	8	41	S
8	1957	2	1957	52	79.71	6	41	STRONG
9	1955	2	1955	52	79.8	6	41	NO
10	1953	2	1953	51	78.34	16	34	NO
11	1952	1	1952	51	78.38	16	35	NO
Sat, 12/01/2008	1950	2	1950	52	80	14	30	NO
13	1947	3	1947	50	77.04	14	30	NO
14	1945	2	1945	51	78.66	14	30	NO
15	1943	2	1943	56	86.46	16	36	STRONG
16	1941	2	1941	54	83.46	16	36	S
17	1941	0	1941	55	85.01	16	36	M
18	1941	0	1941	53	81.92	16	35	STRONG
Sat, 19/01/2008	1940	1	1940	53	81.96	16	35	NO
20	1938	2	1938	50	77.4	16	35	NO
21	1934	4	1934	52	80.66	16	35	NO
22	1934	0	1934	52	80.66	16	35	S
23	1933	1	1933	52	80.7	16	35	M
24	1931	2	1931	51	79.23	15	35	S
25	1929	2	1929	50	77.76	15	35	NO
Sat, 26/01/2008	1927	2	1927	52	80.95	16	36	NO
27	1924	3	1924	51	79.52	16	36	NO
28	1922	2	1922	53	82.73	16	36	S
29	1921	1	1921	50	78.08	16	36	NO
30	1920	1	1920	50	78.13	16	36	NO
31	1919	1	1919	51	79.73	17	28	NO
				Average prod:	80.13419			

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Fri, 01/02/2008	1935	1	1934	52	80.66	16	35	S
Sat, 02/02/2008	1934	2	1932	51	79.19	17	35	NO
3	1932	2	1930	50	77.72	15	35	M
4	1930	1	1929	52	80.87	15	35	S
5	1929	2	1927	50	77.84	15	36	NO
6	1927	2	1925	52	81.04	15	36	NO
7	1925	1	1924	52	81.08	16	35	S
8	1924	1	1923	52	81.12	16	35	S
Sat, 09/02/2008	1923	1	1922	53	82.73	16	35	NO
10	1922	2	1920	52	81.25	16	35	NO
11	1920	2	1918	59	92.28	16	36	NO
12	1918	3	1915	54	84.6	16	36	NO
13	1915	2	1913	53	83.12	16	36	NO
14	1913	1	1912	50	78.45	16	36	NO
15	1912	2	1910	50	78.53	16	36	NO
Sat, 16/02/2008	1910	2	1908	48	75.47	16	36	NO
17	1908	0	1908	53	83.33	16	36	S
18	1908	1	1907	52	81.8	16	36	NO
19	1907	1	1906	51	80.27	15	36	M
20	1906	2	1904	53	83.51	16	36	NO
21	1904	2	1902	50	78.86	16	36	NO
22	1902	1	1901	50	78.91	16	36	S
Sat, 23/02/2008	1901	1	1900	51	80.53	16	36	NO
24	1900	3	1897	50	79.07	16	36	NO
25	1897	1	1896	52	82.28	16	36	NO
26	1896	1	1895	51	80.74	16	36	NO
27	1895	1	1894	50	79.2	16	36	NO
28	1894	0	1894	53	83.95	16	36	NO
29	1894	1	1893	50	79.24	15	36	NO
				Average prod:	80.9531			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Fri, 01/02/2008	1918	1	1918	51	79.77	16	35	S
Sat, 02/02/2008	1917	1	1917	50	78.25	17	35	NO
3	1917	0	1917	49	76.68	15	35	M
4	1917	0	1917	50	78.25	15	35	S
5	1916	1	1916	50	78.29	15	36	NO
6	1915	1	1915	50	78.33	15	36	NO
7	1915	0	1915	52	81.46	16	35	S
8	1915	0	1915	51	79.9	16	35	S
Sat, 09/02/2008	1914	1	1914	52	81.5	16	35	NO
10	1912	2	1912	51	80.02	16	35	NO
11	1909	3	1909	51	80.15	16	36	NO
12	1906	3	1906	50	78.7	16	36	NO
13	1905	1	1905	51	80.31	16	36	NO
14	1904	1	1904	45	70.9	16	36	NO
15	1902	2	1902	48	75.71	16	36	NO
Sat, 16/02/2008	1900	2	1900	48	75.79	16	36	NO
17	1897	3	1897	50	79.07	16	36	S
18	1896	1	1896	53	83.86	16	36	NO
19	1895	1	1895	51	80.74	15	36	M
20	1893	2	1893	51	80.82	16	36	NO
21	1889	4	1889	44	69.88	16	36	NO
22	1887	2	1887	48	76.31	16	36	S
Sat, 23/02/2008	1886	1	1886	50	79.53	16	36	NO
24	1884	2	1884	50	79.62	16	36	NO
25	1883	1	1883	50	79.66	16	36	NO
26	1882	1	1882	50	79.7	16	36	NO
27	1882	0	1882	50	79.7	16	36	NO
28	1882	0	1882	50	79.7	16	36	NO
29	1881	1	1881	48	76.56	15	36	NO
				Average prod:	78.59172			

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/03/2008	1893	1	1892	54	85.62	15	36	NO
2	1892	0	1892	55	87.21	15	36	NO
3	1892	1	1891	51	80.91	15	36	NO
4	1891	1	1890	47	74.6	15	36	NO
5	1890	2	1888	50	79.45	15	36	NO
6	1888	3	1885	46	73.21	15	36	NO
7	1885	1	1884	48	76.43	16	36	NO
Sat, 08/03/2008	1884	2	1882	48	76.51	15	36	NO
9	1882	3	1979	49	74.28	15	36	NO
10	1979	1	1878	50	79.87	15	36	NO
11	1878	1	1877	50	79.91	15	36	S
12	1877	1	1876	50	79.96	15	36	S
13	1876	1	1875	50	80	15	36	M
14	1875	2	1874	50	80.04	15	36	S
Sat, 15/03/2008	1874	1	1872	50	80.13	15	36	STRONG
16	1872	2	1870	50	80.21	15	36	NO
17	1870	3	1867	50	80.34	15	36	NO
18	1867	1	1866	50	80.39	15	36	NO
19	1866	0	1866	50	80.39	15	36	NO
20	1866	2	1864	50	80.47	15	36	STRONG
21	1864	0	1864	51	82.08	15	36	NO
Sat, 22/03/2008	1864	0	1864	50	80.47	15	36	NO
23	1864	0	1864	50	80.47	15	36	NO
24	1864	1	1863	51	82.13	15	36	NO
25	1863	0	1863	50	80.52	15	36	NO
26	1863	1	1862	49	78.95	15	36	S
27	1862	1	1861	50	80.6	15	36	NO
28	1861	1	1860	48	77.42	15	36	NO
Sat, 29/03/2008	1860	1	1859	48	77.46	15	36	NO
30	1859	2	1857	48	77.54	15	36	NO
31	1857	3	1854	49	79.29	15	36	M
				Average prod:	79.57613			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Sat, 01/03/2008	1880	1	1880	45	71.81	15	36	NO
2	1880	0	1880	43	68.62	15	36	NO
3	1878	2	1878	49	78.27	15	36	NO
4	1874	4	1874	54	86.45	15	36	NO
5	1873	1	1873	52	83.29	15	36	NO
6	1872	1	1872	44	70.51	15	36	NO
7	1862	10	1862	47	75.73	16	36	NO
Sat, 08/03/2008	1860	2	1860	50	80.65	15	36	NO
9	1860	0	1860	53	85.48	15	36	NO
10	1860	0	1860	47	75.81	15	36	NO
11	1860	0	1860	47	75.81	15	36	S
12	1860	0	1860	47	75.81	15	36	S
13	1857	3	1857	47	75.93	15	36	M
14	1856	1	1856	46	74.35	15	36	S
Sat, 15/03/2008	1856	0	1856	45	72.74	15	36	STRONG
16	1856	0	1856	47	75.97	15	36	NO
17	1856	0	1856	48	77.59	15	36	NO
18	1856	0	1856	46	74.35	15	36	NO
19	1856	0	1856	47	75.97	15	36	NO
20	1856	0	1856	46	74.35	15	36	STRONG
21	1856	0	1856	49	79.2	15	36	NO
Sat, 22/03/2008	1856	0	1856	46	74.35	15	36	NO
23	1853	3	1853	49	79.33	15	36	NO
23	1852	1	1852	45	72.89	15	36	NO
25	1850	2	1850	48	77.84	15	36	NO
26	1849	1	1849	45	73.01	15	36	S
27	1849	0	1849	46	74.63	15	36	NO
28	1847	2	1847	47	76.34	15	36	NO
Sat, 29/03/2008	1847	0	1847	43	69.84	15	36	NO
30	1847	0	1847	46	74.72	15	36	NO
31	1845	2	1845	46	74.8	15	36	M
				Average prod:	76.01419			

CHICKEN PEN A

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Tue 01/04/2008	1854	1	1853	48	77.71	15	36	NO
2	1853	2	1851	48	77.8	15	36	S
3	1851	1	1850	50	81.08	15	36	NO
4	1850	2	1848	48	77.92	15	36	S
Sat, 05/04/2008	1848	1	1847	52	84.46	15	36	M
6	1847	0	1847	48	77.96	15	36	M
7	1847	0	1847	47	76.34	15	36	S
8	1847	2	1845	51	82.93	11	36	NO
9	1845	2	1843	52	84.64	11	36	NO
10	1843	1	1842	48	78.18	11	36	NO
11	1842	1	1841	48	78.22	11	36	NO
Sat, 12/04/2008	1841	1	1840	47	76.63	11	36	S
13	1840	0	1840	49	79.89	11	35	S
14	1840	1	1839	51	83.2	11	36	NO
15	1839	2	1837	50	81.65	11	36	S
16	1837	2	1835	50	81.74	15	30	NO
17	1835	0	1835	50	81.74	12	30	S
18	1835	2	1833	50	81.83	13	30	NO
Sat, 19/04/2008	1833	1	1832	50	81.88	11	30	STRONG
20	1832	2	1830	50	81.97	11	30	STRONG
21	1830	1	1829	47	77.09	12	30	STRONG
22	1829	2	1827	49	80.46	12	30	NO
23	1827	1	1826	48	78.86	12	30	NO
24	1826	0	1826	49	80.5	12	30	NO
25	1826	0	1826	50	82.15	12	30	NO
				Average prod:	80.2732			

CHICKEN PEN B

DATE	O.STOCK	MORT	C.STOCK	EGGS(TRAYS)	PROD %	T° MIN	T° MAX	WIND
Tue 01/04/2008	1843	2	1843	47	76.51	15	36	NO
2	1840	3	1840	46	75	15	36	S
3	1840	0	1840	46	75	15	36	NO
4	1839	1	1839	44	71.78	15	36	S
Sat, 05/04/2008	1839	0	1839	47	76.67	15	36	M
6	1837	2	1837	43	70.22	15	36	M
7	1836	1	1836	45	73.53	15	36	S
8	1836	0	1836	49	80.07	11	36	NO
9	1835	1	1835	50	81.74	11	36	NO
10	1834	1	1834	47	76.88	11	36	NO
11	1833	1	1833	50	81.83	11	36	NO
Sat, 12/04/2008	1832	1	1832	45	73.69	11	36	S
13	1832	0	1832	49	80.24	11	35	S
14	1832	0	1832	49	80.24	11	36	NO
15	1832	0	1832	48	78.6	11	36	S
16	1832	0	1832	48	78.6	15	30	NO
17	1832	0	1832	48	78.6	12	30	S
18	1831	1	1831	49	80.28	13	30	NO
Sat, 19/04/2008	1831	0	1831	48	78.65	11	30	STRONG
20	1831	0	1831	45	73.73	11	30	STRONG
21	1831	2	1829	48	78.73	12	30	STRONG
22	1829	1	1828	44	72.21	12	30	NO
23	1828	0	1828	44	72.21	12	30	NO
24	1828	2	1826	46	75.58	12	30	NO
25	1826	2	1824	44	72.37	12	30	NO

Average prod: 76.5184