INDEPENDENT REVIEW OF THE POSSIBLE HEALTH HAZARDS OF THE LARGE-SCALE RELEASE OF BACTERIA DURING THE DORSET DEFENCE TRIALS

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This report discusses the nature of the bacteria that were released during the Dorset Defence Trials, and an opinion of their capacity to cause disease, together with a summary of each of the Microbiological Research Establishment (MRE) Field Trial Reports and the other relevant Ministry of Defence (MOD) documents that I examined. As far as I am aware, I have obtained the reports of all experiments carried out in the 1960's and 1970's that released bacteria over populated areas, including those that are still classified. I also obtained a copy of an internal review (still classified) of all biological warfare/defence experiments carried out by the MOD between 1940 and 1979. I have been assured that no further releases of bacteria have occurred outside of MOD land since the 1970's, that the releases in the 1980's or 1990's have been small scale, such that no significant exposure to bacteria would have occurred outside MOD land, and that they have involved bacteria which are considered by the MOD to be non-pathogenic.

The main report is in two parts, the first of which deals with the types of experiments that were carried out, the bacteria involved and their potential to cause disease, and my conclusions about the potential threats to health. The second part describes and comments on the technical reports of each of the experiments in the 1960's and 1970's that were part of the Dorset Defence Trials.

PART I: THE CONSEQUENCES FOR HEALTH OF THE DORSET DEFENCE TRIALS

Introduction

Experiments in the 1950's alerted the government to the possibility that an aeroplane travelling along the British coastline, outside of territorial waters, might be able to cover much of the country with a lethal dose of a germ warfare agent. These studies (the fluorescent particle trials) used particles of a fluorescent chemical - zinc cadmium sulphide - as a simulant of a germ warfare agent (e.g. a pathogenic bacterium or virus). The demonstration that particles of this chemical could be detected over much of the country caused concern that the UK, as a small island, was particularly vulnerable to a germ warfare attack of this kind.

The bacteria that were likely to be used in a germ warfare attack cause disease when inhaled in very small numbers (e.g. less than 50 bacteria). A few minutes exposure to air containing as little as one living bacterium in every litre of air could result in a potentially lethal exposure to such germ warfare agents. The fluorescent particle trials suggested that exposure of the UK population to these numbers of bacteria could be obtained over much of the country. However, chemical particles are not a good model for the behaviour of particles containing living bacteria as these may die during travel through the air. Studies in the laboratory suggested that bacteria survived for long periods in the air within large sealed chambers, but there were indications that they may survive for much shorter periods in the open air. It was therefore considered necessary to carry out experiments to study the ability of bacteria, released as an aerosol from a ship or aeroplane, to spread and survive under the realistic conditions of a germ warfare attack. The Dorset Defence Trials were therefore undertaken to evaluate the extent to which the UK population was at risk of a germ warfare attack, using bacteria that were considered to be non-pathogenic as simulants of real biological warfare agents. Obviously, this information was also useful for offensive purposes.

This review is not concerned with the political issues surrounding the exposure of an uninformed population to aerosols of bacteria and is only concerned with an evaluation of any possible threats to health of the Dorset Defence Trials. The review is also not concerned with the safety of the releases of zinc cadmium sulphide which will be the subject of a separate report by an independent toxicologist.

A. The nature of the bacteria and viruses released into the environment

The great majority of the experimental releases of bacteria in the 1960's and 1970s involved four species - *Escherichia coli, Bacillus globigii, Bacterium aerogenes* and *Serratia marcescens.*

Some of the experiments involved the release of killed bacteria but in most experiments the bacteria were not killed before they were sprayed across the South of England. The health hazards associated with the four bacteria used in the experiments are outlined below. I then describe the properties of the other bacteria (or bacterial viruses) that were used in a few experiments, which in most cases were not part of the Dorset Defence Trials.

A.1. Release of killed bacteria

A.1.i. *Bacterium aerogenes*

The bacterium called *B. aerogenes* in MRE Field Trial Reports 1 and 3 would now almost certainly be called *Klebsiella aerogenes. K. aerogenes* is an opportunistic pathogen that can cause community-acquired pneumonia, particularly in chronic alcoholics, but is much more commonly associated with infections of patients in hospitals with very weak immune systems (immuno-deficient patients).

This bacterium was always killed before release into the environment. Killing of *B. aerogenes* appears to have been carried out by adding formaldehyde to a final concentration of 0.8% for 12 hours, followed by heating at 50-60°C "to pasteurise" for

an unspecified period. There are no indications that the effectiveness of the killing process was tested but it is considered highly unlikely that this was not checked.

A.l.ii. Serratia marcescens

Serratia marcescens was used as a biological warfare simulant up to the 1950's. At that time this bacterium was believed to be harmless, but it is now known to be an opportunistic pathogen that is a significant cause of disease in seriously ill patients in hospitals, particularly those who are immuno-deficient. A well publicised release of live S. *marcescens* from a ship off San Francisco in 1950 has been associated with a number of cases of S. *marcescens* disease, and one death, and live S. *marcescens is* no longer considered to be a safe biological warfare simulant.

Killed *S. marcescens* were released in the experiments described in MRE Field Report Nos. 12 and 18. In MRE Field Report 12 it is stated that killing of the bacteria was obtained with phenol. The effectiveness of this sterilisation procedure is clearly documented by showing that less than one S. *marcescens* bacterium per millilitre survived the phenol treatment (this means that no *S. marcescens* bacteria were detected when 1 millilitre of the sterilised bacterial suspension was spread over bacterial growth medium (agar plates).

Two sets of experiments used living *S. marcescens*. None of these experiments would have resulted in exposure of the public. MRE Field Trial No. 26 involved the release of live S. *marcescens* inside buildings at MRE Porton Down with protection provided by the use of respirators. MRE Development Note No. 66 used live *S. marcescens* attached to microthreads in the open air at MRE Porton Down. The microthread experiments are not believed to pose any threat to health as significant exposure to bacteria does not occur in this type of experiment (see Section B.1.).

A.2. Release of living bacteria

In many of the experiments live bacteria were released. The majority of the experimental trials used a mixture of two different types of bacteria, *Bacillus globigii* and *Escherichia coli*.

A.2.i. Bacillus globigii

Bacillus globigii (now more commonly called *Bacillus subtilis* var *niger)* has been used in biological warfare/defence research for many years by the US and UK military and also in meteorology. There are several reasons why this organism is used. Of major importance is the ability of the bacterium to form tough spores, which are resistant to desiccation, heat, chemicals etc. *B. globigii* spores do not die when spreading through the air and they can be used to track air flows, or the spread of a bacterial aerosol, over long distances. From the military perspective, spores of *B. globigii* were used to simulate the behaviour of another bacterium which forms spores - the potential biological warfare agent, *Bacillus anthracis,* the causative agent of anthrax. The *B. globigii* used in the experimental releases was provided as frozen suspensions of spores by Fort Detrick (the US biological defence/warfare establishment) in the USA. The fact that the *B. globigii* spores were obtained from the USA is not considered to be of any significance.

B. globigii has been widely used as a biological warfare simulant, and for tracking air flow, as it is believed to be entirely safe. The bacterium is common in the environment and in the countryside we are often naturally exposed to this bacterium. There is very little convincing evidence to suggest that *B. globigii is* harmful to man. Several comments have been made in the press that *B. globigii* can cause disease. A search of the primary medical literature uncovered no examples of significant disease that was clearly attributed to *B. globigii*.

B. globigii is now considered to be a variant of *B. subtilis.* The latter bacterium has been very occasionally encountered in hospital patients who are particularly

susceptible to disease, although the association between the presence of this bacterium and disease is often not clear.

A.2.ii. Escherichia coli

Most bacteria that are potential biological warfare agents do not form tough spores and they die as they spread through the air. Experiments were needed to simulate the behaviour of this type of biological warfare agent. In the experiments under review, *Escherichia coli* was used as a simulant of these more fragile biological warfare agents and, in most experiments, *B. globigii* spores were mixed with the *E. coli* bacteria. This was done for technical reasons as it allows an estimate of the rate at which the *E. coli* bacteria die as they spread as an aerosol. This is done by comparing the ratio of live *E. coli* to *B. globigii* spores in the samples of the aerosol obtained at different times after release with that in the original bacterial mixture that was released into the environment.

E. *coli* is present in large amounts in our intestines and is a major component of the normal flora of healthy individuals. Typically, there are between 10 million and a billion *E. coli* bacteria per gram of faeces. The great majority of strains of *E. coli* are harmless and their presence in the intestines is beneficial. *E. coli* isolates recovered from the faeces of healthy individuals are not usually pathogenic and this species has been the most commonly used bacterium for microbiological research and teaching during the last 50 years.

A few strains of *E. coli* do cause disease and these strains contain a number of extra attributes (virulence genes) which allow them to produce diarrhoea. Most of these pathogenic *E. coli* strains cause diarrhoea of varying severity, but infection with the most dangerous strains (e.g. the *E. coli* 0157 H7 strain involved in the well publicised outbreak linked to a Lanarkshire butcher) can lead to acute kidney failure and death.

The *E. coli* strain used in all of the experiments examined in this review (strain MRE 162) was isolated in 1949 by a microbiologist at MRE Porton Down from a

lavatory seat and will have been derived from faeces. In recent years, the extra virulence genes that distinguish the pathogenic strains from the vast majority of non-pathogenic strains of *E. coli* have been identified and, with modern techniques, it is simple to show the presence or absence of these genes in any strain of *E. coli*. *E. coli* strain MRE162 was submitted to the Central Public Health Laboratory in London for analysis. *E. coli* MRE162 did not contain any of the genes associated with pathogenic *E. coli* strains. Dr. Bernard Rowe, Director of the Laboratory of Enteric Pathogens at the Central Public Health Laboratories (the laboratory that specialises in the characterisation of pathogenic *E. coli* strains), has stated that *E. coli* MRE162 is unlikely to be able to cause diarrhoea in humans. I talked at length with Dr. Rowe and he confirmed his view that *E. coli* strain MRE162 was very unlikely to be able to cause diarrhoea following ingestion.

The aerosols produced in the Dorset Defence Trials by spraying bacteria over land contained particles of differing sizes. The fate of bacteria that are inhaled in aerosols depends on the size of the particles containing the bacteria. If the particles are small, the bacteria can penetrate to the inner parts of the lung (the alveoli), whereas, if the particles are larger, they will be trapped in the upper respiratory tract and may be swallowed and enter the stomach. The *E. coli* aerosols released in the Dorset defence Trials contained a range of particle sizes and, following inhalation, some of the bacteria will have penetrated the lungs whereas others will have entered the stomach. It seems very unlikely that the entry of *E. coli* strain MRE162 into the stomach would have health consequences as this strain does not have the extra virulence genes that allow some strains of *E. coli* to cause diarrhoea.

However, as the main exposure to *E. coli* in the Dorset Defence Trials would have been by inhalation of the aerosol, rather than ingestion, we need to consider the potential of *E. coli* MRE162 to cause disease on entering the lungs. *E. coli* entering the lungs may occasionally obtain access to the bloodstream. Entry of small numbers of bacteria into the bloodstream is a relatively common occurrence (for example, this can happen when teeth are brushed) and in most individuals the small numbers of bacteria entering the blood will be destroyed by the natural defences of the body. It is

possible that, in a very small number of people, the bacteria will multiply in the blood to cause septicaemia (infection of the blood), leading to a feverish condition. *E. coli* is quite commonly isolated from blood infections but, with a few exceptions, it is impossible to predict whether any particular strain of *E. coli* would be more likely than any other strain to cause a blood infection in susceptible individuals.

E. coli is also occasionally associated with cases of pneumonia, but these are extremely rare in healthy individuals, and typically occur in artificially ventilated patients in hospital intensive care facilities, or in patients with greatly increased susceptibility to infections. It is not possible to predict whether a particular *E. coli* strain is any more likely to cause pneumonia in a highly susceptible individual than any other *E. coli* strain.

It is therefore considered possible that a strain of *E. coli* such as MRE162, which is unlikely to cause diarrhoea on ingestion, may on inhalation be able to initiate a blood or chest infection in a small number of highly susceptible individuals.

The inhalation of the aerosols of *E. coli* strain MRE162, released during the Dorset Defence Trials, needs to be viewed in the perspective of our normal daily life which often involves exposure to *E. coli* aerosols (e.g. using lavatories and changing dirty nappies). The inhalation of *E. coli* bacteria through these normal activities is not considered to be a risk to health, indicating that exposure to the great majority of *E. coli* strains present in faeces, by inhalation, is harmless to the vast majority of individuals.

A.2.ii.a. The dispute about the serotype of *E. coli* strain MRE162

There has been some comment about the fact that the properties of the *E. coli* MRE162 strain recently sent to Colindale by DERA was different from that in the reports of the experiments carried out in the 1960's and 1970's. In MRE Field Trial Report No. 3, and in some other documents, the strain was said to have the serotype 09K9 (or in more detailed nomenclature, 09K9H9), whereas the two batches of the

strain tested at Colindale were 08H9. There are three possible explanations for this anomaly. Firstly, a typographical error may have occurred at some point, which was perpetuated in later reports. Secondly, the serotyping carried out at MRE Porton may have been incorrect. Thirdly, the wrong strain could have been sent to Colindale.

In the 1960's few laboratories had the expertise to carry out serotyping of *E. coli* strains and Dr. Rowe, Director of the Laboratory for Enteric Pathogens at Colindale, believes that the serotyping carried out by MRE Porton in the 1960's could have been incorrect. Although this is clearly possible, I favour the view that a typographical error occurred in the early 1960's, which was perpetuated in most of the later reports and documents. The main reason for this view is that, among a considerable number of memos and documents I received from the MRE Porton Down archives, there is one document (MRE/2711/9), written by H.M. Darlow on 4th July 1969, which gives the serotype of *E. coli* MRE162 correctly as 08K9H9.

A.3. Other organisms used in experiments during the 1960's and 1970's

A.3.i. *Francisella tularensis*

F. tularensis is the causative agent of tularaemia and is a dangerous bacterial pathogen that is a potential Biological Warfare agent. This is a highly infectious bacterium and inhalation of only tens of bacteria will cause disease. In two experiments live *F. tularensis* were used at MRE Porton Down. In MRE Technical Note No. 14, *F. tularensis* was used under safe conditions in a steel drum within MRE Porton and there would have been no release of organisms from the drum. In Development Note No. 66 the bacteria were attached to microthreads (presumably under safe conditions within MRE Porton Down) and were then exposed in the open air at MRE Porton Down. Very few bacteria should be released from microthreads (see Section B.1.) and it is assumed (but not stated in the very brief report of these experiments) that the microthreads were exposed to the air at MRE Porton Down within a safety enclosure.

A.3.ii. Bacterial viruses

In a few experiments bacterial viruses (bacteriophage) were released (e.g. bacteriophage T7 was used in MRE Field Trial Report No. 10 and bacteriophage T1 in the second experiment carried out in the London Underground in 1964 (MRE Report V.T.2). These bacterial viruses are only able to infect *E. coli* bacteria and have no effect on other bacteria, let alone humans, other animals or plants. There is no possible risk to human health posed by the release of these *E. coli* viruses.

B. The types of experiments carried out during the Dorset Defence Trials

B.1. Microthread experiments

Several types of experiments were performed as part of the Dorset Defence Trials. The least controversial of these are the microthread experiments. In this technique a small spider is used to produce a fine thread that is wound onto a small frame. The frames are then exposed to a small aerosol of bacteria produced by a spray and some bacteria stick to the spider threads. A number of identical frames can then be placed at the location of interest. The frames are removed at intervals, the bacteria are washed off, and the numbers of surviving bacteria can be counted by standard microbiological procedures. The decrease in the number of living bacteria with increasing time of exposure to the environment provides a simple and convenient measure of the rate at which the bacteria die in different environments. Apart from the initial small-scale spraying of the bacteria that is needed to apply the bacteria to the spider's threads, the microthread technique results in no significant release of bacteria into the environment. Indeed, the technique requires that the bacteria remain stuck to the spider's threads until they are washed off in the laboratory. In most microthread experiments the initial spraying appears to have been done at MRE

Porton Down and the frames were transported to the locations at which they were to be exposed to the environment. In a few instances the bacteria appear to have been sprayed onto the microthreads outside of Porton Down. The numbers of bacteria that were sprayed onto the microthreads were small and would have resulted in no significant numbers of bacteria outside of the spraying area.

B.2. The large-scale releases of bacteria

As far as I can ascertain, large scale releases of bacteria over land in the 1960's and 1970's were only carried out from a ship (the Icewhale) in Lyme Bay and Weymouth Bay, at MRE Porton Down, and from the air at Tarrant Rushton Airfield (Near Blandford Forum) and Odiham Airfield (50 miles from Porton Down). The majority of the large-scale releases were from the ship, and most of the resulting aerosols passed over land between Bridport and Portland Bill, with a small number passing over land between Torquay and Lyme Regis. Other large-scale releases occurred from ships at sea but these were not intended to spread bacteria over land and probably did not do so (see my synopses of MRE Field Trial Report Nos. 11, 14 and 24).

Most of the experiments were carried out at night since bacteria are known to survive poorly in sunshine and any biological warfare attack would be expected to occur at night.

The initial large-scale releases used killed bacteria, which were stained with a dye, allowing them to be distinguished from other bacteria in the environment. However, killed bacteria are not realistic biological warfare simulants and the use of living bacteria was required to understand the distance bacteria could travel over land without losing viability.

C. Safety testing before the release of the bacteria

Tests were carried out on the *E. coli* bacterial suspensions to see if they were contaminated with other bacteria. Toxicity tests were carried out using mice. In one experiment mice were injected with 10 million bacteria and in another experiment the mice inhaled 100,000 bacteria. The mice were examined for any signs of illness and any batches of *E. coli* that caused illness in mice were discarded. These tests established that the batches of *E. coli* that were released were harmless to mice. This was assumed to imply that they would also be harmless to humans. The extrapolation from "safe in mice" to "safe in humans" is reasonable, although it is certainly possible for a bacterial strain to be harmless to mice and yet pathogenic to man. There is, however, no reason to believe that the MRE162 strain of E. *coli* is pathogenic to man.

Table 5-1 of MRE Field Trial Report No. 3 shows the results of the toxicity tests on the *E. coli* used in the 12 experimental releases that involved this bacterium. Some contamination was found in the *E. coli* suspension used in experiments 9-12. The level of contamination in the *E. coli* used in experiments 9 and 10 was insignificant (1 contaminating bacterium for every 6,600 *E. coli*). The *E. coli* used in experiments 11 and 12 was higher (1 contaminant for every 360 *E. coli*). The nature of the contaminating organism(s) is unknown. These contaminated batches were both used and Table 5.1 of the Field Trials Report indicates that they resulted in no toxicity in mice.

The maximum inhaled dose of *E. coli* at the sampling stations in experimental releases 11 and 12 was 157,000 bacteria, which would correspond to an inhaled dose of the contaminating organism(s) of 440 bacteria. It is possible that some individuals (e.g. those involved in spraying the bacteria from the Icewhale) inhaled substantially higher doses of the bacterial aerosols and of the contaminating bacteria. In the absence of any knowledge of the nature of the contaminating bacteria it is impossible to judge whether the inhalation of these numbers of bacteria would have posed any threat to health. It is, however, surprising that suspensions with this level of contamination with uncharacterised bacteria were sprayed across populated areas, as there was a possible risk that the contaminating bacteria had a significant ability to

cause disease in humans, even though they apparently caused no toxicity in the safety tests in mice.

D. Calculations of total inhaled doses of bacteria

The numbers of bacteria inhaled by a person in the open air at a sampling station can in most cases be estimated approximately from the Tables given in the Field Trials Reports. The key numbers are those labelled NT (or Nt) or "average numbers of bacteria". These are the numbers of bacteria collected by the sampling devices as the cloud of bacteria passed. The sampling devices suck in many litres of air per minute and these values have been adjusted to give the numbers of bacteria that would have been collected if the air had been sucked into the sampling device at a rate of 1 litre per minute. This is the NT or Nt number. A person at rest takes about 12 litres of air per minute into their lungs and the Nt values have to be multiplied by 12 to give the total number of bacteria inhaled by a person at rest as the cloud passed (the total inhaled dose). The inhaled doses I have used in this report are those for individuals at rest and should be doubled for individuals undertaking strenuous exercise, who take about 24 litre of air per minute into their lungs.

The highest doses should occur at the sampling stations closest to the point of release of the bacterial aerosol with decreasing doses at points further away. For most of the releases from offshore the closest sampling stations were those at the coast. It should be noted that relatively few sampling stations were employed and, in some experiments, it is possible that none of these were positioned in the middle of the passing bacterial aerosol. Higher doses than those indicated by the Nt values at the sampling stations may therefore have been inhaled by some individuals. Similarly, local weather conditions, or geographic features, could have resulted in higher local

concentrations of bacteria than those implied by the Nt values at the sampling stations.

Certain individuals may have been exposed to inhaled doses of bacteria substantially greater than those detected by the general public during the Dorset Defence Trials. These include individuals carrying out the releases of the bacterial aerosols, individuals within MRE Porton Down who were sometimes as close as 80 metres downwind of the point of release of the bacterial aerosols, and personnel within some parts of naval ships that were exposed to bacterial aerosols. These individuals appear to have been exposed to as many as 8 million *E. coli* MRE162 or *B. globigii* spores.

E. Opinions on the threat to health from the inhalation of bacteria released during the Dorset Defence Trials

My initial opinions on the risks of inhalation of the bacteria were based on current views on the capacity of each bacterial species to cause disease, the information that was available about the strains of bacteria that were used, and estimates of the doses that would have been inhaled. These initial opinions were tested by consulting five senior Medical Microbiologists, three in the UK and two in the USA. These individuals were given the available information about the strains of bacteria that were used, and they were asked to consider the threats to health from the inhalation of 1 million bacteria, or bacterial spores, a dose that is about ten times higher than that inhaled by the vast majority of the public.

E.1. Threats to health from the release of killed B. *aerogenes* and *S.marcescens*

The inhalation of killed *B. aerogenes* or *S. marcescens,* in the doses estimated in the Field Trial Reports, is not considered to constitute any significant risk to health. Dead bacteria are not able to cause the types of infections that are occasionally associated with living *B. aerogenes* of **S**. *marcescens.* This view was supported by all of the five senior Medical Microbiologists. One of the Medical Microbiologists considered

there was a very slight possibility that inhaling dead bacteria could result in a short-lived mild allergic pneumonitis in susceptible individuals (coughing, wheezing or breathlessness).

E.2. Threats to health from the inhalation of spores of *B. globigii*

The unanimous view of the Medical Microbiologists that I consulted was that *B. globigii is* non-pathogenic, and that inhalation of *B. globigii* spores does not constitute a significant risk to health. *B. globigii is* now considered to be a pigmented variant of *B. subtilis*. *I* therefore asked the five senior Medical Microbiologists to assume that the *B. globigii* spores that was released in the Dorset Defence Trials were in fact spores of *B. subtilis*. None of them believed that the inhalation of one million *B. subtilis* spores would be a significant threat to health.

E.3. Threats to health from the inhalation of *E. coli* strain MRE162

E. coli undoubtedly has the ability to cause serious disease in healthy individuals and is usually associated with ingesting food contaminated with *E. coli* strains that have a special ability to cause food poisoning. *E. coli* strain MRE162 is completely different from these strains and is considered highly unlikely to be able to cause diarrhoea on ingestion or inhalation. All five Senior Medical Microbiologists supported this view.

The Medical Microbiologists I consulted also agreed that the inhalation of *E. coli* MRE162 was likely to be of no consequence for the vast majority of exposed individuals. The suggestion that the strain could cause a blood infection in a few highly susceptible individuals was considered possible, but unlikely. Two of the Medical Microbiologists considered chest infections to be more plausible than blood infections. Both suggested that inhalation of *E. coli* MRE162 was highly unlikely to cause a chest infection in the great majority of exposed individuals, but that it might possibly exacerbate a pre-existing chest infection, or cause a chest infection (e.g.

pneumonia), in some individuals that had serious underlying chest disease (e.g. individuals with cystic fibrosis).

F. Health problems in Dorset

Several individuals and groups who believe they suffered health problems that may be related to the Dorset Defence Trials have met, or corresponded, with me. None of the health problems that were raised during my visits to Dorset, or in correspondence, concerned acute bacterial infections which are the most likely consequence of the release of aerosols of living bacteria across a populated area. However, fevers and pneumonia are relatively common and it is likely that small clusters of such diseases following the bacterial releases would have gone unnoticed.

If there were cases of disease these would almost certainly be restricted to highly susceptible individuals (e.g., those with cystic fibrosis or severe immuno-deficiency) and would have occurred within a few days of the inhalation of the bacteria. It is not possible to estimate how many, if any, cases of infection were caused by the Dorset Defence Trials. The numbers of individuals who were highly susceptible to infection in the 1960's and early 1970's is not known with any precision. The number would certainly be considerably less than today, as a consequence of the increase in the numbers of severely immuno-deficient patients resulting from HIV infection, aggressive cancer chemotherapy, and the use of drugs to prevent rejection following organ transplantation. More importantly, even if the numbers of highly-susceptible individuals in the 1960's were estimated, their risk of infection with the "non-pathogenic" bacteria that were released during the Dorset Defence Trials is completely unknown.

F. 1. Individual health concerns

One microbiologist working at MRE Porton Down on the Dorset Defence Trials died aged 33 in hospital during the period under review. His family are convinced that the death was the result of his work at Porton Down. This person suffered from

diverticulitis and, following surgery, complications led to peritonitis and death. Diverticulitis, like appendicitis, is not a communicable infectious disease and is not believed to be due to the presence of pathogenic bacteria introduced into the gut. Faecal peritonitis secondary to perforation of the bowel is a well-known and serious complication of diverticulitis, response to antibiotics can be poor, and not infrequently leads to septic death. Peritonitis is an infection caused by the leakage of the normal bacterial contents of the gut and is not associated with acquiring new pathogenic bacteria in the gut. The non-pathogenic bacteria that are universally present within the gut (e.g. non-pathogenic strains of *E. coli*) are harmless, indeed beneficial, in their normal location, but can cause serious problems if they leak into, and multiply within, the normally sterile areas surrounding the gut (peritonitis).

According to the death certificate, the individual died from "pulmonary oedema and collapse as a consequence of paralytic ileus as a consequence of peritonitis from diverticulitis". I discussed with an infectious disease consultant at Oxford the information about the course of disease that I received from the family, and the information on the death certificate. The consultant suggested that the cause of death would probably now be called acute respiratory distress syndrome (ARDS), rather than pulmonary oedema, but that the sequence of events from diverticulitis to peritonitis, paralysis of the intestines (paralytic ileus), respiratory distress and death, are well known complications of faecal peritonitis secondary to diverticulitis. The consultant considered that even if this microbiologist had accidentally swallowed *E. coli* strain MRE162, it is highly unlikely that this would have initiated the diverticulitis (which has a structural/physiological rather than an infectious origin).

Another individual who was believed to have been a member of the crew of the Icewhale during some of the bacterial releases suffered breathing problems in the late 1960's (aged 65) which led to chronic poor health. These problems were apparently attributed to smoking by his physician. This individual is believed to have been in good health before the onset of the breathing problems and, although it is possible that the inhalation of particularly high doses of bacteria on the Icewhale might have

caused temporary breathing problems, it is unlikely that exposure to the bacteria released from the Icewhale would have led to chronic poor health.

One individual reported a burning sensation in his eyes while fishing off Portland Bill in the early 1960's which was followed by many years of ill health. The burning sensation could conceivably be attributed to traces of the formaldehyde used to kill the S. *marcescens* released in experiments 1 or 2 of Field Trial Report No. 3. Even if the burning sensation was caused by residual formaldehyde in the bacterial aerosols, this would have been a transient irritation and it is unlikely that the exposure to trace amounts of this chemical would have been the cause of his subsequent long bout of ill health.

The majority of the health concerns that were raised by members of the public were of a general nature - chronic ill health since living in the area of the bacterial releases - or were miscarriages, learning disabilities or, in a few cases, birth defects. The senior Medical Microbiologists that I consulted were unanimous and vigorous in their view that these sorts of health problems are not caused by any of the bacteria that were released in the Dorset Defence Trials.

F.2. Health concerns in East Lulworth

There has been considerable concern from families that lived in East Lulworth during the period of the Dorset Defence Trials. This concern has centred mainly on the health problems of children born to mothers who were growing up in the village during the period of the experimental releases, and an alleged high rate of miscarriages in this group of women.

With a few exceptions, pathogenic bacteria cause infections shortly after an individual is exposed to them. Thus, any health problems in East Lulworth resulting from the Dorset Defence Trials would have occurred in the period 1963-1971 when some of the bacterial releases from Lyme Bay spread bacteria in the direction of East

Lulworth. These problems, if they occurred, would almost certainly have been limited to infections in highly susceptible individuals, as outlined in Section E.

Many of the health problems uncovered by a survey of residents of the village (organised by Mr. D. Orman) occurred many years after the releases of bacteria. As mentioned above, none of the bacteria released in the Defence Trials are associated with the types of health problems uncovered by this survey (birth defects, learning disabilities and miscarriages), and they would certainly not cause health problems many years after exposure. There is absolutely no reason to believe that exposure of individuals to these bacteria as children would have any effects on their own children.

All of the senior Medical Microbiologists that I consulted totally rejected the possibility that the bacteria released in the Dorset Defence Trials could be linked in any way to the birth defects, miscarriages, or learning disabilities, that are of concern to the families in East Lulworth.

The analysis of the allegedly high rate of health problems in East Lulworth following the Dorset Defence Trials will not be dealt with in this report since it is the subject of a separate report from the Dorset Health Authority. If the report from the Health Authority finds that there was an increased rate of health problems in East Lulworth families, it is highly unlikely to be anything to do with the release of bacteria in the Dorset Defence Trials.

G. Conclusions

- The releases of bacteria during the Dorset Defence Trials were very unlikely to have had health consequences for the overwhelming majority of individuals that were exposed. Of the four bacteria that were released, the main concern centres on *E. coli*. However, even in this case, there is little reason to believe that the strain of *E. coli* that was released (MRE162) has any significant potential to cause disease in healthy individuals at the doses inhaled in the Dorset Defence Trials.

- The experimental releases of bacteria may have put a small number of individuals at risk of infection. Any infections would probably have been restricted to individuals who were particularly susceptible to disease, and are most likely to have been chest infections (or possibly blood infections) caused by the inhalation of *E. coli* strain MRE162. It is impossible to estimate the numbers of individuals (if any) who might have suffered ill health as a consequence of inhalation of bacteria released during the trials, since the risk of infection of highly susceptible individuals (e.g. those with cystic fibrosis or severe immuno-deficiency) is not known.

- If the released bacteria had been very exotic, it might have been possible to recognise any resulting clusters of infections as they would have been due to a very unusual bacterial species. However, we are commonly exposed to the two species of live bacteria that were released during the Dorset Defence Trials - *E. coli* and *B. globigii* - and, at a distance of 30 years, it is highly unlikely that a clear association between the release of these commonly encountered bacteria and any subsequent infection could be made. Furthermore, lung and blood infections are relatively common, particularly in individuals who are highly susceptible to disease. Therefore, if any infections were caused by the bacterial releases, it is very unlikely that they would have been considered to be anything unusual by GPs, who were not made aware of the Dorset Defence Trials.

- None of the bacteria that were released are known to cause miscarriages, chronic ill health, learning disabilities or birth defects. These types of health problem cannot be attributed to the release of bacteria during the Dorset Defence Trials.

PART II

DESCRIPTION AND COMMENTS ON THE MRE FIELD TRIAL REPORTS AND RELATED TECHNICAL REPORTS

A summary of each of the experiments carried out in Dorset, East Devon and at MRE Porton Down as part of the Dorset Defence Trials, and related experiments that may have exposed members of the public to bacterial aerosols, are given below.

Many of these experiments would have resulted in no significant release of bacteria into the community. The large-scale experiments that did expose the public to significant doses of bacteria are marked with an asterisk. The places where individuals were exposed to bacteria are underlined.

*MRE Field Trial Report No. 1

Between March 1961 and June 1962 there were 12 experiments at MRE Porton Down that released aerosols of primulin-stained killed *B. aerogenes* (now called *Klebsiella aerogenes)* plus *B. globigii* spores. About 10^{14} bacteria were released in each of these experiments. The numbers given on pages 11-22 are the bacteria collected in one litre of air while the cloud of bacteria passed the sampling station (the numbers in parentheses can be ignored). As described in Section D, the total inhaled dose received by a resting person at the sampling station can be calculated from these Nt values by multiplying by 12.

For example, in the experiment on 9th May 1962, the inhaled dose of *B. globigii* at 2 miles from the point of release would have been 12,000 bacterial spores, decreasing to about 2000 spores at 5 miles, and 276 spores at 9 miles. Similarly, the numbers of killed *K. aerogenes* bacteria was 19,000 at 2 miles and 360 at 9 miles. Higher values were obtained in some experiments. For example, in the experiment of 11th September the inhaled dose of *B. globigii* spores would have been 9000 at 5 miles and the dose of killed *K. aerogenes* would have been 21,000 at the same distance. The

highest dose at 5 miles was in the experiment of 11th January, where about 41,000 *B. globigii* spores and 22,000 killed *K. aerogenes* would have been inhaled as the bacterial cloud passed. These variations in the doses at 5 miles in different experiments are probably due to two main factors - the local weather conditions and whether the sampling station was in the middle of the cloud as it passed. The inhaled dose would have dropped off with increasing distance from the point of spraying and in two of the experiments inhaled doses of only 400 and 600 bacteria were obtained at a distance of 15 miles.

These experiments resulted in significant spread of bacteria outside of MOD land at MRE Porton Down. The maximum doses inhaled by individuals outside of MRE Porton Down were probably about 50,000 dead *K. aerogenes* and *100,000 B. globigii* spores.

The possible threats to health from the inhalation of dead K. *aerogenes* and *B. globigii* spores are discussed in Part A of this report.

MRE Field Trial Report No. 2.

This report involves the evaluation of two new devices for detecting and counting the number of bacteria in the air (June/July 1964). These experiments were carried out using a mixture of *E. coli* strain MRE162 and spores of *B. globigii* within MRE Porton Down and, although the amount of bacteria released is not indicated, they appear not to have been very great. Ten trials occurred and sampling of the air took place at distances of 300-2,100 yards. At 2,100 yards the maximum inhaled dose would have been about 1000 *B. globigii* spores and 670 *E. coli* MRE162 bacteria. Outside of MRE Porton Down the dose would probably have been insignificant.

***MRE Field Trial Report No. 3**

The first of the large-scale releases of bacteria from the ship Icewhale off the Dorset coast. Fourteen experiments were carried out between April 1963 and April 1964.

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The first two experiments involved the release of dead *K aerogenes* bacteria. The other twelve used a mixture of *E. coli* strain MRE162 and spores of *B. globigii*. Five of the experiments released bacteria that travelled over the Weymouth and Portland Bill area, the other eight released bacteria that would have passed between Weymouth and Bridport.

The numbers of *E. coli* released (the number of *B. globigii* was always less than the number of *E. coli*) in the latter trials increased from 1.3 x 10^{15} in the first experiment (Serial 3) to 10^{16} in the last two experiments (serials 13 and 14). These differences are not very significant as the bacteria were released over a longer distance in the later experiments, and from further offshore. A more important measure is the number of bacteria released from the ship per metre travelled and the distance offshore that the bacteria were released. On this basis, ignoring differences in weather conditions, the highest concentration of bacteria reaching the shore should have been in experiments 5-8 and 12.

Estimates of the maximum numbers of bacteria inhaled are inevitably imprecise. The highest doses would be expected nearest the coast and many of the sampling stations were at the coast. For example, an estimated 1,836 viable *E. coli* MRE162 would have been inhaled by an individual in the open air at sampling station 9 in experiment 12. The largest number of viable *E. coli* MRE 162 bacteria inhaled may have been about 4,560 - at collection station 10 in experiment 9 off Lyme Bay. The inhaled dose may have been substantially higher in experiment 7 in Weymouth Bay, as the release was close to land, and winds were very low, such that the bacterial cloud drifted for an unusually long time over the sampling stations. The numbers of *E. coli* detected in this experiment cannot be estimated from Table 5, but the numbers of *B.* globigii spores (column 8 in Table 5) collected at the sampling stations on the coast were twice as high as those obtained in any of the other experiments, indicating that the numbers of viable *E. coli* MRE162 was also probably more than twice that in the other experiments. A total inhaled dose of about 10,000 viable E. *coli* MRE162 at the coast would be a reasonable estimate. The maximum inhaled dose of *B.* globigii was about 55,000 spores.

The above figures are estimates of the highest doses of viable *E. coli* MRE162 inhaled by individuals in the open air at the coastal sampling stations over which the cloud of bacteria passed. The maximum numbers of viable *E. coli* bacteria inhaled in any of the experiments, by individuals at the coast, would probably have been less than 10,000, and would have been lower for individuals living inland.

The possible threats to health from the inhalation of *E. coli* strain MRE162 *and B. globigii* are discussed in Part A of this report.

***MRE Field Trial Report No. 4**

These 13 experiments, carried out between October 1964 and May 1965, are essentially identical to those described in MRE Field Trial Report No. 3 and were carried out at distances from 5-20 miles off the Dorset coast. The amounts of bacteria released were very similar to those in the earlier experiments (up to 8 x 10^{15} *E. coli*).

Two of the experiments released bacteria in Weymouth Bay with the bacteria passing between Weymouth and Portland Bill. Six experiments released bacteria in Lyme Bay with the bacteria travelling over the coast between Weymouth and Bridport. In the other five experiments the bacterial clouds would have passed over the Devon coastline between Lyme Regis and Torquay.

The inhaled doses of bacteria at the coast appear to be similar to those in the earlier trials. The maximum dose appears to have been in experiments 16 and 24, which were carried out close to shore. An individual at the coast would have inhaled about 3,600 - 3,800 bacteria in these experiments. Experiment 19 was also carried out close to land but the data from this experiment were lost due to the overheating of the incubator used to grow the bacteria. The maximum inhaled dose of *B. globigii* spores was about 14,000 (experiment 23).

***MRE Field Trial Report No. 5**

Four more releases of bacteria were carried out between February 1966 and April 1966 in Lyme Bay between 2 and 15 miles offshore; all of these produced clouds of bacteria that passed over the coast between Bridport and Weymouth. The experiments were essentially identical to those in MRE Field Trial Reports 3 and 4 and used the same mixture of bacteria in similar amounts. The highest total inhaled doses of *E. coli* MRE162 were detected at sampling station 37 in experiment 4 and sampling station 66 in experiment 2 (21,000 - 24,000 bacteria). Both of these sampling stations were on the coast.

Experiments using small numbers of *E. coli* attached to very fine threads spun by a spider (microthread experiments) were carried out at Fleet. The numbers of bacteria involved in these experiments were small and the bacteria should have remained attached to the threads during the experiments. The release of bacteria in these microthread experiments would be trivial and of no possible threat to health (see Section B.1.).

***MRE Field Trial Report No. 6**

Five further massive releases of viable *E. coli* MRE162 plus *B. globigii* spores were carried out as close as 1 mile off the Dorset coast between November 1967 and January 1968. Each experiment involved two releases of bacteria, separated by about 2 hours, over the same part of the coast. The amount of *E. coli* in each of the two releases was about half of that in the previous releases; the total amount of *E. coli* released in the two arms of each experiment was therefore similar to that in the earlier releases. One experiment released bacteria over Lyme Bay, between Abbotsbury and Weymouth, and the other four released bacteria in Weymouth Bay between Weymouth and Portland Bill.

This experiment was intended to test whether coating the bacteria with a protectant chemical would allow them to survive better as they travelled overland. One of the

releases in each experiment therefore used bacteria mixed in a 0.5% solution of the protectant (an ethylene glycol ether of a fatty acid).

The maximum total inhaled dose of *E.coli* MRE162 in these experiments (25,000 bacteria) occurred in experiment 4 at the coastal sampling station 3. The inhaled doses of *B. globigii* spores were in most cases considerably lower.

Microthread experiments were also carried out but they are not considered to be a threat to health.

MRE Field Trial Report No. 7

Between January 1967 and March 1967, microthreads carrying small numbers of *E. coli* MRE162 and *B. globigii* were exposed to the air at a number of locations, from Taunton in the West, to Southampton in the East, and North as far as Shepton Mallet. None of the microthread experiments would have released any significant number of bacteria into the air and they posed no threat to health.

MRE Field Trial Report No. 8

In these experiments the survival of bacteria within a naval ship was examined. Initial experiments were carried out in September 1967 aboard HMS Cleopatra off Portland and used *E. coli* MRE162 attached to microthreads. Each of the second set of six experiments (August 1967) involved the spraying of 1 litre of *B. globigii* spores into the air intakes of HMS Sirius in Portsmouth Dockyard. The total amount of *B. globigii* released was 2×10^{13} bacteria. Most of these bacteria would have been sucked into the air intakes with insignificant release onto land. The third set of four experiments took place in HMS Sirius at sea between Portsmouth and Falmouth in September 1968, and were similar to the second set of experiments, except that they involved the spraying of a mixture of *E. coli* MRE162 and *B. globigii* into the air intakes. The fourth series of experiments were carried out at MRE Porton Down

Using *E.Coli* MRE162 and *B. globigii* attached to microthreads. None of these experiments would have resulted in the spread of any significant number of bacteria on land.

MRE Field Trial Report No. 9

These experiments were carried out on HMS Fearless at sea in December 1968. The first experiments looked at whether there is a build up of naturally occurring bacteria when a ship is in the citadel condition, which is designed to protect the ship against a biological warfare attack. The other experiments use *E. Coli* MRE162 on microthreads to look at bacterial survival within a ship under citadel conditions. None of these experiments are of any concern.

MRE Field Trial Report No. 10

Experiments to test the survival of *E. Coli* MRE162 bacteria and bacteriophage T7 (a virus that is completely harmless as it only attacks and kills *E.Coli* bacteria) on microthreads within the engine room and boiler room of HMS Phoebe. These experiments are of no concern.

MRE Field Trial Report No. 11

These experiments were carried out in the English Channel about 8-12 miles off Portland Bill in December 1970 and were designed to test the penetration of bacteria into a modern frigate (HMS Andromeda) when it passes through a cloud of bacteria. *E. Coli* MRE162 and *B. globigii* spores were used and the doses inhaled by individuals on the ship were estimated to be between 700 – 2000 bacteria in the engine room in the three experiments. Elsewhere on the ship the inhaled doses were 200 bacteria or less. In all three experiments the winds were offshore and bacteria would not have passed over land.

***MRE Field Trial Report No. 12**

This report describes experiments carried out in November and December 1971 by the UK and US defence establishments to compare the efficiency of equipment for the detection of bacteria released into the air. Twelve experimental releases of bacteria were carried out 3-12 miles off Portland Bill. A further 29 releases occurred within MRE Porton Down. Unlike the majority of the previous experiments, these trials used a mixture of killed *S. marcescens* and spores of *B. globigii.*

Inactivation of *S. marcescens* (provided by Fort Detrick, USA) was carried out at MRE Porton Down using phenol and the bacteria were tested on multiple occasions for the presence of surviving bacteria. In all cases, less than one living *S. marcescens* bacterium was found per millilitre of the phenol-treated S. *marcescens* suspensions, indicating that complete killing by phenol was obtained. There appears to have been some contamination of the frozen *S. marcescens* bacteria provided by Fort Detrick, but after phenol treatment these contaminating bacteria should have been killed. The report states that bacteria of the *Bacillus subtilis* group occasionally appeared to survive phenol treatment. The numbers of such contaminating bacteria that survived phenol sterilisation are not given, but would almost certainly be very low. *B. subtilis* group is a slightly vague description, but these bacteria are not generally considered to be pathogens, although (as with many bacteria that are nominally non-pathogenic) they may occasionally be associated with hospital infections in patients with serious underlying disease.

The inhaled doses of *B. globigii* spores at the coast (Portland Bill) - were relatively low - the maximal dose was about 11,000 spores and 30,000 dead *S. marcescens*. In the experiments at MRE Porton Down the number of bacteria detected were about 10 times greater as the sampling stations were very close to where the bacteria were released. In one experiment at MRE Porton Down (Challenge 21) the wind dropped following the release and the bacterial cloud hung over the sampling station for about 90 minutes, resulting in large numbers of bacteria being detected. An individual at this sampling station would have inhaled about 640,000 *B. globigii* spores and about 1 million dead *S. marcescens*. Otherwise, the maximal inhaled doses at the sampling

stations in the experiments at MRE Porton Down were about 110,000 *B. globigii* spores plus 175,000 dead *S. marcescens*. These high numbers are a consequence of the sampling stations being only about 800-1300 metres from the point of bacterial release. The inhaled doses outside of MRE Porton Down are difficult to estimate but would probably have been much less than 50,000 bacteria.

MRE Field Trial Report No. 13

These experiments were carried out at MRE Porton Down and involved the passage of armoured vehicles through small clouds of *B. globigii* spores to test the penetration of bacteria into the vehicles. These experiments would have resulted in no significant spread of the bacteria outside of MRE Porton Down.

MRE Field Trial Report No. 14

These experiments were carried out in January 1973 to test further the vulnerability of naval ships to a germ warfare attack and involved the passage of HMS Achilles through a cloud of *B. globigii* spores released from a nearby ship. The releases were off Portland Bill but the distance from land is not reported. It appears, however, that in all seven releases of bacteria the wind was from the North or East and would have been unlikely to spread the bacteria over land. The doses of *B. globigii* inside the ship were extremely low, except in the engine room, where total inhaled doses of 300,000 to 600,000 spores were obtained in 5 of the 7 experimental releases.

MRE Field Trial Report No. 15

These experiments were carried out at MRE Porton Down during 1972 or 1973 and involved the release of *B. globigii* spores to test the levels of contamination on different types of military clothing when exposed to a bacterial cloud. These appear to have been small releases of bacteria and there is unlikely to have been any significant spread of the bacterial spores outside of MRE Porton Down.

MRE Field Trial Report No. 16

This report describes experiments carried out at MRE Porton Down in March 1974 to investigate the penetration of a small bacterial cloud into a Portacabin, to see the extent of protection from a biological warfare attack provided by a simple shelter. *B. globigii* spores were used and the release was unlikely to have spread significant numbers of bacteria outside of MRE Porton Down.

MRE Field Trial Report No. 17

No bacteria were used in these experiments.

MRE Field Trial Report No. 18

The experiments were carried out at MRE Porton Down to evaluate a new type of instrument for detecting bacteria released into the environment. The small releases of bacteria occurred between February and June 1974 and used various combinations of phenol-killed *E. coli* MRE162, phenol-killed *Serratia marcescens,* and living spores of *B. globigii*. The spread of bacteria outside of MRE Porton Down is considered to have been insignificant.

MRE Field Trial Report No. 19

Experiments to test the entry of *B. globigii* spores into missile launchers were carried out at MRE Porton Down during 1975. These involved the passage of the missile launcher through small clouds of *B. globigii* spores and there was unlikely to be any significant spread of the bacteria outside of MRE Porton Down.

MRE Field Trial Report No. 20

Tests to examine whether Portacabins can be modified to provide protection from a biological warfare attack. The experiments were carried out in 1975 and involved small releases of *B. globigii* spores outside a Portcabin at MRE Porton Down. No significant spread of bacteria outside of MRE Porton Down was likely.

MRE Field Trial Report No. 21

Tests to evaluate methods for decontaminating clothing exposed to a bacterial cloud. These experiments were carried out in May 1975 at the Protection Training Unit at Phoenix Naval School, Portsmouth. Military personnel were exposed to a small cloud of *B. globigii* spores in the open air at the Naval School and went inside to test decontamination procedures. Less than one litre of bacterial suspension was used in the seven experiments. Exposure of the military personnel was high (up to 1.3 million spores, as they were only 80 metres downwind of the released bacterial spores. The inhaled doses more than a few hundred metres from the site of release are likely to have been less than 100,000 spores.

MRE Field Trial Report No. 22

This report is still secret and describes experiments using a new bacterial detection system. The experiments were carried out inside MRE Porton Down, in a totally enclosed vessel from which bacteria could not have escaped into the environment.

MRE Field Trial Report No. 23

Experiments carried out in Spring/Summer 1976 to evaluate the bacterial clouds produced by various portable spray guns. The experiments used *B. globigii* and were carried out at MRE Porton Down. The amounts of bacteria released were small and there would not have been significant spread of the bacteria outside of MRE Porton Down.

MRE Field Trial Report No. 24

These experiments were carried out in HMS Galatea at sea off Plymouth during October 1976 and were designed to test decontamination procedures after a biological warfare attack. HMS Galatea passed through a cloud of *B. globigii* spores released from a second ship and sailors exposed on the deck followed decontamination procedures before re-entering the ships citadel. The distance offshore of Plymouth that these experiments were performed is not stated. Six releases of bacteria were carried out and in four cases the wind would have blown the bacterial cloud away from the Devon coast. In the other two experiments the winds were very low and the path of the bacterial cloud is unclear. About 8 x 10^{14} bacterial spores were released in each of the experiments. The bacterial spores were released very close to HMS Galatea and the total inhaled dose of the sailors on the deck of HMS Galatea would have been high (**about 8 million spores**).

MRE Field Trial Report No. 25

This report is still secret but it describes tests of new equipment inside MRE Porton Down. Bacteria were released within an enclosed vessel from which they cannot escape into the environment.

MRE Field Trial Report No. 26

These experiments were designed to establish whether evidence of a recent biological warfare attack could be obtained by sampling for bacteria from individuals and objects believed to have been exposed to the attack. They were carried out at MRE Porton Down in 1977 and used *B. globigii* spores, *E. coli* strain MRE162, and live *S. marcescens*. This is the only time that live *S. marcescens* appears to have been used.

In most experiments (all of which only used *B. globigii* spores) MRE Porton Down personnel were exposed at a distance of about 80-200 metres from the source of the bacterial spray. The amount of bacteria released was relatively small and there would have been no significant spread of the bacteria outside of MRE Porton Down.

One experiment was carried out with *B. globigii* spores mixed with live *E. coli* MRE162, and one experiment with *B. globigii* mixed with live *S. marcescens.* These two experiments were carried out indoors using a small Humbrol paint spray to produce an aerosol. There would have been no release of these bacteria outside of MRE Porton Down and the MRE personnel were protected from the *S. marcescens* with respirators.

MRE Field Trial Report No. 27

These experiments were carried out in 1977 at MRE Porton Down and at the Underwater Weapons Establishment at Portland Bill. They were designed to test the efficiency of a ship's prewetting system in removing bacteria from the external surfaces of a ship after a biological warfare attack. Steel plates painted to naval standards were exposed in the open air to a spray of *B. globigii* spores released at a distance of 40 metres. The amount of bacteria released was relatively small and it is unlikely that there was any significant spread of the bacteria outside of the military establishments.

MRE Report No. 31

This report is the 1963/1964 progress report for activities at MRE Porton Down and it gives a useful summary of the activities carried out in the early 1960's.

It mentions "tests in the London Underground system that began in July 1963 and which are expected to continue at infrequent intervals for some time". One experiment is described in which a face powder carton containing *B. globigii* spores was dropped from a window somewhere on the Northern line "producing an aerosol of high concentration that persisted for a considerable time". These are available as MRE Reports Nos. V.T. 1 and V.T.2 and are discussed briefly below.

It also mentions the plan to make larger scale releases than those from offshore (i.e. those described in MRE Field Trial Report Nos. 3 and 4) from an aircraft to allow detection of bacteria at up to 100 miles from the point of release. Preliminary bacterial releases from an aircraft are described in MRE Development Notes Nos. 67 and 71 (see below).

There is a discussion of the need for another bacterium that can be safely used for large-scale release experiments and describes the possible use of *B. aerogenes*. Safety tests are reported which suggested that mice challenged by the respiratory or intra-peritoneal route showed no ill effects or abnormal lung histopathology. There is no evidence that live *B. aerogenes (Klebsiella aerogenes)* were subsequently used.

MRE Report No. 35

The four parts of this report (produced between 1965 and 1967) describes in detail the experiments with microthreads.

Part 1 describes how small spiders are used to produce the microthreads.

Part 2 describes experiments with *E. coli* MRE162 and *B. globigii* spores attached to microthreads. These were exposed at a number of sites (Portland Bill, near Maiden Castle, and in Central London) and the survival of the bacteria was assessed.

Part 3 provides further evaluation of the microthread method, with microthreads exposed indoors and near Weymouth.

Part 4 is a summary of microthread experiments carried out to understand why bacteria die at different rates in air that has passed over countryside or cities.

MRE Report No. 36.

This report summarises experiments that compare the survival of *E. coli* strain MRE162 bacteria on microthreads. with bacteria in aerosols released from offshore,

and bacteria within an enclosed vessel. The large-scale releases from offshore, carried out between November 1964 and May 1965, are those described in MRE Field Trial Report No. 4 and need not be further commented upon here.

MRE Report No. 39.

This describes microthread experiments carried out with *B. globigii* and *E. coli* strain MRE 162.

MRE Report No. 42.

A number of experiments with microthreads containing E. *coli* strain MRE162 and *B. globigii* are described. The microthreads were placed at a number of different sites, mostly around Southampton and Swindon, to examine the loss of viability of bacteria in air passing over cities.

MRE Development Note No. 57

This is a theoretical note discussing some of the problems in detecting bacteria at low concentrations within aerosols.

MRE Development Note No. 62

A description of a new piece of apparatus for sampling bacteria in aerosols.

***MRE Development Notes Nos. 67 and 71**

In 1959/1960 there were preliminary experiments to test the possibility of producing bacterial aerosols from a low-flying aeroplane. Several experiments were carried out at Odiham airport (about 50 miles from MRE Porton Down), which was then a non-operational RAF station, using killed *K. aerogenes*. Additional releases from an aircraft were made over MRE Porton Down using *K. aerogenes* and *B. globigii*

spores. High doses of bacteria were detected at the sampling tower which was only 150 yards down wind of the point of release. It is not possible to estimate the doses outside of the airport.

Subsequently, a Canberra bomber was converted to allow the spraying of large amounts of bacteria from the air and was delivered to the Aircraft and Armament Experimental Establishment at Boscombe Down in December 1966. The total capacity of bacterial suspension that could be carried by the aircraft was 917 gallons and the intention was to have the capacity to spray larger amounts of bacteria than could be delivered from offshore by the Icewhale. The modified Canberra bomber was capable of delivering about 8 x 10^{16} bacteria - nearly ten times the greatest amount sprayed from the Icewhale. Development note 67 describes the testing of this aircraft and the release in April 1967 of about 200 gallons of *E. coli* MRE162. Although not clearly stated, the *E. coli* was probably mixed with *B. globigii* spores. The location of the release is not shown in the report but was Tarrant Rushton Airfield, Dorset (near Blandford Forum). This corresponds to a release of about 9 x 10^{15} E. *coli* MRE162, an amount close to the maximum used in the large-scale releases from Icewhale off the Dorset coast.

Note 71 describes two further releases from the Canberra bomber in June 1967, and two in October 1967, at Tarrant Rushton Airfield. The amount of bacteria released appears to have been about 12 gallons (a rate of release of 70 gallons per minute for 10-12 seconds) which is estimated to correspond to a release of about 10^{15} *E. coli* bacteria and a slightly smaller number of *B. globigii* spores.

In neither of these development notes is it possible to estimate the numbers of bacteria present in the bacterial cloud and it is therefore impossible to estimate the inhaled doses of *E. coli* and *B. globigii* around the points of release. The much faster speed of the Canberra bomber compared to the Icewhale should have resulted in a greater dilution of the cloud and it is reasonable to assume that the release of 9 x 10^{15} bacteria from the Canberra in the experiment described in Note 67 would have given inhaled

doses of E. *coli* MRE162 outside of the airport that are no greater than those obtained at the coast in the trials from the Icewhale (e.g. MRE Field Trial Report No. 3).

MRE Technical Note No. 14.

This note describes experiments with the pathogen *Pasteurella* (now *Francisella) tularensis* carried out in a sealed steel drum within a building at MIZE Porton Down. Although this organism is a serious pathogen, the bacteria were completely contained within the drum and there would have been no escape of bacteria into the environment.

MRE Development Note No. 66.

This note outlines experiments carried out around 1966 which examined the survival of the pathogen *Pasteurella* (now *Francisella) tularensis* on microthreads. The details of the experiments are vague but it appears that the organism was sprayed onto the microthreads (presumably in a safety cabinet within MRE Porton Down) which were then exposed to the atmosphere on the roof of MRE Porton Down. The microthread technique requires that the bacteria are not released from the spider's threads but it possible that a very small number of bacteria are released in this type of experiment. Given the very low dose of *F. tularensis* required to initiate disease, it is almost certain that the microthreads were exposed within a safety enclosure positioned on the roof of Porton Down.

***London Underground Trials (MRE Reports V.T.I. and V.T.2.)**

B. globigii spores were used in the first trial carried out in 1963 on the Southern part of the Northern line (release of spores between Colliers Wood and Tooting Broadway). *B. globigii* plus bacteriophage TI were used in the second trial in 1964. Bacteriophage T1 is a bacterial virus that only infects *E. coli* and is of no possible consequence to human health.

Both trials gave *B. globigii* doses of about 1- 3.6 x 10^4 spores per litre of air at Tooting Broadway Station soon after release and the levels dropped by a factor of about 30 after 30 minutes. The inhaled dose in the station closest to the release (Tooting Broadway) would have been about 5 million spores during the 30 minutes following the arrival of the aerosol. The dose of spores at the neighbouring station would have been about 100-fold less with the numbers dropping sharply at more distance points. Only a few hundred individuals are likely to have been exposed to the highest doses of *B. globigii* and, given the non-pathogenic nature of this bacterium, it is unlikely that these releases caused any disease.