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by

J. Ho, B. Kournikakis, P.A. Lockwood and V.L. DiNinno

January 1993

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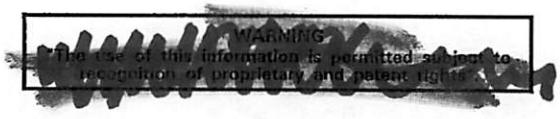

ACKNOWLEDGEMENTS

The authors would like to thank Gary Resnick of Dugway Proving Ground for providing much of the information included in Volume II of this document. The authors would also like to thank Thor Jacobson for his assistance in obtaining some of the references and for his review of the manuscript.

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ABSTRACT

It has been and still is necessary to test biological warfare agent detectors and carry out other research activities (such as studies on individual and collective protection, development of sampling techniques and procedures) in biological defence using microorganisms. To carry out this work, DRES has used *Bacillus globigii* (BG), now renamed *Bacillus subtilis*, for a number of years. Recently it became desirable to use a viral simulant as well and after a detailed search, Newcastle Disease virus La Sota strain, a vaccine strain was chosen. Safety and the lack of environmental risk of these two organisms were the main criteria in their selection. This document summarises information on the safety and environmental effects of these two organisms. Based on this review the use of BG and NDV in the field presents no hazard to either personnel or the environment.

Volume II of this document contains supporting information referred to in the text.

RÉSUMÉ

Il a été, et il est toujours, nécessaire de tester les détecteurs d'agents biologiques et d'effectuer d'autres travaux de recherche (comme des études sur la protection individuelle et collective et l'élaboration de techniques et de méthodes de prélèvement) dans le domaine de la défense contre les agents biologiques. Pour effectuer ces travaux, le CRDS utilise depuis quelques années *Bacillus globigii* (BG), qui a maintenant été renommée *Bacillus subtilis*. Récemment, il est devenu souhaitable d'utiliser également un simulant viral; après une recherche détaillée, on a choisi la souche La Sota du virus de la maladie de Newcastle, qui est une souche vaccinale. Ces deux organismes ont été choisis principalement parce qu'ils sont sécuritaires et qu'ils ne posent aucun risque pour l'environnement. Dans ce document, on résume l'information existante sur l'aspect sécuritaire de ces deux organismes et sur leurs effets sur l'environnement. Selon cette étude, l'utilisation sur le terrain d'essais de BG et du virus de la maladie de Newcastle ne comporte aucun risque pour le personnel ou l'environnement.

GENERAL INTRODUCTION

Use of biological warfare agents on the DRES Experimental Proving Ground (EPG) is prohibited. However, it has been and still is necessary to test biological warfare agent detectors and carry out other research activities (such as studies on individual and collective protection, development of sampling techniques and procedures) in biological defence using microorganisms. To carry out this work, DRES has used *Bacillus subtilis* (BG) for a number of years. Recently it became desirable to use a viral simulant as well and after a detailed search, Newcastle Disease virus La Sota strain, a vaccine strain was chosen. Safety and the lack of environmental risk of these two organisms were the main criteria in their selection.

This document summarises information on the safety and environmental effects of these two organisms. The purpose of this document is to provide the record necessary for a risk/benefit analysis of field trials involving these organisms. This document should be reviewed regularly and amended when new information on either of the two organisms becomes available. Volume II of this document contains supporting information referred to in the text below including the full text of most of the references.

PART I: *Bacillus subtilis*

Introduction

Bacillus subtilis var *niger (BG)* has been used safely as a BW simulant in field trials and DRES would like to continue using the spores of this organism to characterize the performance of biological detectors and in other field trials supporting defence research.

Open Literature Evaluation

A strong case for the safety of BG was presented in a recent paper [1]. The evidence was published in a mini-review which is technical in style and may not be easily comprehensible by non-microbiologists. The full text of this reference is presented in Annex A with annotations and explanations to make it more easily understandable. The intent is for the reader to start by following the evidence and arguments set out by the authors and then refer to the comments within the brackets, { }, for occasional help in clarifying potential points of confusion. Also the full texts of cited references marked with an * (see Annex A) from this review are found in Volume II of this document.

Use of BG for Defence Research in Other Countries

US and UK defence scientists at Dugway Proving Ground and Chemical Biological Defence Establishment Porton Down respectively have encountered public scrutiny of their use of this organism in field trials of BW detection systems. Both Dugway and Porton Down have produced internal safety and environmental assessment documents that have been accepted by their Establishments. In an environmental assessment [2] for a test of remote detection equipment at Dugway Proving Ground in 1986, it was concluded that "There is no evidence of human pathogenicity associated with spores of BG. Since BG is a naturally-occurring bacterium, release of BG spores during the proposed action will not cause any environmental impact." The Final Programmatic Environmental Impact Statement for the U.S. Biological Defence Research Program [3] (dated Apr 89) in reference to the biosimulants BG and MS2 (a bacteriophage viral simulant used by the U.S.) concludes that

"controlled outdoor testing with these materials by trained personnel does not present a hazard to workers or the environment. They have been determined to be safe for humans and the environment." The safety of BG spores is also attested to in a formal declaration by Mottice [4]. Mottice notes, for example, that "anyone who leaves their house (or even has plants in dirt inside their house) is already exposed to this organism." Although it refers to a vast number of other topics, the Draft Environmental Assessment for the Baker Test Facility (draft of 17 June 1992), is included in Volume II of this report because of numerous references to the use and safety of BG. Open air testing in both countries has resumed.

Medical Use of BG

BG has been used to treat a number of diseases and has been administered to patients by a variety of routes including intravenous injection, oral, and bronchial instillation [5]. BG tablets are reportedly used in France to treat certain types of diarrhoea [6].

Summary of Findings

To date there is no evidence of human pathogenicity associated with either vegetative cells or spores of BG. In fact, BG has been used to treat a number of medical conditions in humans. Also since BG is a naturally-occurring bacterium, release of BG spores in the open will not cause any environmental impact.

PART II: Newcastle Disease Virus (La Sota strain)

Introduction

Viruses constitute the single largest group of potential BW agents. Although a BW attack would most likely be effected through aerosol dissemination of agent, our knowledge of virus aerosol stability is minimal.

In order to overcome this lack of knowledge, Canada initiated a research program to develop a safe model system which would allow experiments to be conducted in the open air for field testing of virus collection equipment, detection and identification systems and for testing of individual and collective protection systems.

Selection of the Virus

A literature review of several candidate viruses has been published [7]. The primary consideration in this review was that any virus selected must be considered safe for release into the environment. In the final analysis, the authors decided that the La Sota strain of Newcastle Disease Virus (NDV), a commercially available poultry vaccine commonly used in Canada, would be the candidate virus of choice.

Outside Review

A draft of the report [7] was sent to two outside reviewers for comment. The reviewers were Dr. J.C.N. Westwood (Professor and Chairman, Dept. of Microbiology and Immunology, University of Ottawa) and Dr. E.W. Pearson (Director, Clinical and Medical Affairs, Connaught Laboratories, Willowdale, Ont.)

Dr. Westwood indicated that he felt that NDV was the best choice among the candidate viruses. He noted that domestic animals were not at risk from exposure to NDV and that there was no threat to mammalian fauna. He also indicated that any birds exposed to the vaccine would benefit from the exposure. In terms of human safety, Dr. Westwood

noted that the vaccine was widely used by commercial poultry breeders and commonly administered by mass aerosol exposure under conditions which do not include any elaborate safety precautions to avoid human exposure and that human infections reported have been trivial in nature. Dr. Westwood also stated, "It is unlikely that any public relations problems would arise from the use of NDV vaccine in the field." Dr. Westwood's entire review is included as Annex B.

Dr. Pearson also indicated that NDV was the best choice among the candidate viruses. He commented that he felt DRES had taken the right approach to the study and was pleased to see our concern on protecting our personnel. He did express some concern about the hazard of aerosolized NDV to humans, but did state that "it does not generally cause a serious human disease." Dr. Pearson's comments are included as Annex C.

Commercial Use of NDV

The NDV vaccine has been used by the poultry industry in Canada for over 25 years, and is a commercially available agricultural product. The vaccine is distributed in Canada by Salisbury Laboratories Ltd. (Kitchener, Ontario). In their product directions under "Precautions" they state the following: "Newcastle Disease vaccine virus may cause an eye infection in humans. Do not allow the vaccine to contact the eyes. When using spray methods, wear goggles and a face mask." The eye infection refers to a self limiting conjunctivitis which clears up in a few days. A copy of the entire pamphlet is included as Annex D.

Approval from Agriculture Canada

Since the vaccine is an agricultural product, DRES approached Agriculture Canada to determine if there were any objections to DRES' using the vaccine for our studies. In a letter dated Jan 25, 1982 Dr. D.C. Alexander, Chief, Veterinary Biologics at Agriculture Canada indicated that he had no concerns about DRES' planned use of NDV vaccine. DRES contacted Dr. Alexander again in March 1986 to confirm that there was still no objection to our use of the vaccine in the field. His reply dated 7 April 1986, stated that there was no objection to our trial and that it was important to protect DRES personnel

conducting the experiment as we had indicated. Dr. Alexander's letters are included as Annex E.

Experiments on the Environmental Stability of NDV

Laboratory studies on the stability of NDV have shown that its aerosol survival is dependant on temperature and humidity. The virus survives best under conditions of lower humidity and temperature. As either temperature or humidity increases, virus survival decreases significantly [8].

On exposure to open air and light virus survival decreases dramatically. As summarized in Table 1, virus survival in open air when exposed to light is less than 5 minutes [9].

Table I

Survival of NDV Under Various Conditions [9]

Conditions	Halflife (min)
20 °C 20% Relative Humidity No Open Air Factor, Dark	240
20 °C 20% Relative Humidity Open Air Factor, Dark	15-20
20 °C 20% Relative Humidity Open Air Factor, Sunlight	<5

Summary of Findings

NDV vaccine is a commercially available agricultural product used by poultry farmers across Canada. It is essentially non-pathogenic for humans and poses no threat to mammalian fauna and is, in fact, beneficial to birds exposed to it as it affords protection against the virulent strains of NDV present in Canada. Outside reviewers have agreed with

DRES that the virus is suitable as a safe model system to be used in field experiments and Agriculture Canada has stated no objection to field use of the virus. Additionally, DRES studies have shown that the virus survives only a short time when aerosolized in open air.

The use of NDV in open air field studies presents no adverse environmental impact.

GENERAL CONCLUSIONS

There are no identified hazards to personnel or the environment from the use of BG and NDV in the field. Of course, the minimum amount of material necessary to accomplish the goals of the experiments should be used.

This document should be referred to in the environmental impact section of all Field Trial Procedures which involve the use of either BG or NDV. The following paragraph or a similar one should be inserted in all FTPs involving BG or NDV...

> "The DRES Biohazards Committee has determined from a review of the literature to 1992 that there is no significant hazard to personnel or the environment from the release of either *B. subtilis* spores or Newcastle Disease Virus Vaccine in the open air. These findings are detailed in Suffield Special Publication No, 158."

Notwithstanding the conclusions of the present document, all FTPs involving the use of either BG or NDV should be referred to the DRES Biohazards Committee for comment and approval.

REFERENCES

1. De Boer, A.S., and Diderichsen, B., "On the Safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a Review." Appl. Microbiol. Biotechnol. 36, pp.1-4, 1991 [The complete text with annotation of this reference can be found at Annex A.]

2. "Environmental Assessment for 1986 Remote Detection Technology Test" Department of the Army, U.S. Army Dugway Proving Ground, UT 84022. 7 July 1986. [The text of this reference can be found in Volume II of this report.]

3. Biological Defence Research Program. Final Programmatic Environmental Impact Statement dated April 89. [An extract of the text of this reference can be found in Volume II of this report.]

4. Declaration of Susan L. Mottice in the action of DOWNWINDERS Inc vs. Dick Cheney, Secretary of Defence of the United States and Michael P.W. Stone, Secretary of the Army. [The text of this reference can be found in Volume II of this report.]

5. Pham, N.T., Vu, T.C., and Nguyen, T.H. "Le *Bacillus subtilis* en thérapeutique et dans la prophylaxie", Revue d'Immunologie, 32, pp. 53-65,1968. [The text of this reference and a translation can be found in Volume II of this report.]

6. Auwera, R.V., Van der Snoeck, P., Daneau, R.D., and Meunier, F. "Nosocomial Bacteremia Caused by *Bacillus* species", Clin. Microbiol. Infect. Dis., 7, pp. 783-785, 1988. [The text of this reference can be found in Volume II of this report.]

7. Mofford, L., and Fulton, R.E. "Review and Evaluation of Candidate Virus Tracers for Use in Field Trials (U)", SR 313, Defence Research Establishment Suffield, 1982, UNCLASSIFIED.

8. Kournikakis, B., Netolitzky, D., and Fildes, J. "Effects of Temperature and Relative Humidity on the Survival of Newcastle Disease Virus Aerosols in the Rotating Drum (U)" SM 1261, Defence Research Establishment Suffield, 1988, UNCLASSIFIED.

9. Kournikakis, B., Simpson, M., and Netolitzky, D., "Photoinactivation of Newcastle Disease Virus in Aerosol and in Solution (U)" SM 1263, Defence Research Establishment Suffield, July 1990, UNCLASSIFIED.

ANNEX A

On the safety of *Bacillus subtilis* and *B. amyloliquefaciens:* a review

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Introduction

For many years the fermentation industry has used microorganisms to produce antibiotics, amino acids, enzymes and other useful compounds. These microorganisms, which have been isolated from the environment and then mutated to increase yields of the desired product, have proved safe to handle. With the advent of gene technology, it is now possible to transfer genetic properties from one organism to another. It is widely accepted that as long as the recipient microorganism (the host) is harmless and the products of the genes to be transferred are innocuous, the genetically engineered microorganism (the recombinant) is as safe as the host.

An overwhelming majority of recombinant microorganisms to be used by industry are expected to be based on harmless hosts (OECD 1986). Many of these have been proven safe over many years of experience in industrial settings. Furthermore, extensive information on the incapacity to cause disease, i.e. non-pathogenic and non-toxicogenic potential, of some of these organisms can be found in the literature.

We believe that a review of the literature and present experience with some of these host organisms will be useful for assessment of the safety of many recombinant organisms. In particular it may help to classify some of these as GILSP (Good Industrial Large Scale Practice) host organisms as defined by the OECD (1986), thus facilitating the use of recombinant strains by established production procedures. Furthermore, safety reviews on selected host microorganisms may ease the approval process of products produced by recombinant strains derived from these hosts. Thus it is the opinion of qualified experts that the use of genetic engineering per se does not warrant any additional safety assessment. On the contrary, use of a safe and well known host organism may sometimes render superfluous some of the extensive animal testing of a new product. For a more extensive discussion of the safety and regulatory aspects of the use of recombinant organisms see, for example, AMFEP (1990), Diderichsen et al. (1990), IFBC (1990), National Academy of Sciences (1987), and Pariza and Foster (1983).

Taxonomy and ecology

Bacillus subtilis is a Gram-positive, spore-forming bacterium. It is commonly found in soil and on plant material and grows aerobically at intermediate temperatures and pH. As with many other bacilli, *B. subtilis* secretes substantial amounts of protein, especially hydrolytic enzymes such as amylases and proteases. *B. subtilis* is often referred to as a non-pathogenic bacterium and it is even consumed by humans in large quantities in the Japanese food natto (Djien and Hesseltine 1979).

The genetics of *B. subtilis* strain 168 (Burkholder and Giles 1947) has been extensively studied, making it the best characterized Gram-positive bacterium. *B. amyloliquefaciens* was first isolated in 1943 and the suggested distinction from *B. subtilis* (Welker and Campbell 1967) is now well documented. *B. amyloliquefaciens* has been given separate species status and its name has been included on the approved lists of bacterial names (Priest et al. 1987).

Comments: {In this section, the authors provide a brief microbiological description of *B. subtilis* to familiarize the reader with this organism. The Gram-positive characteristic is a taxonomic method used to roughly separate all bacteria into two groups. Biochemical composition in the cell wall confers different dye permeabilities. Those that do not permit dye penetration are Gram-negative, suggesting the presence of an effective barrier for chemicals. Spore formation is a defensive means for an organism to protect its genetic material in hostile environments like low nutrient availability, unfavourable temperatures, harsh chemicals etc. This characteristic is only common to Gram-positive organisms. Due to this property, spore forming bacteria are ubiquitous in most soil environments.}

Industrial uses

Bacilli are widely used by the fermentation industry. Well-known examples are *B. subtilis, B. amyloliquefaciens, B. licheniformis, B. alkalophilus, B. lentus and B. thuringiensis.*For a recent review on the biotechnology of bacilli see Priest (1990).

Comments: {Of special interest is the Japanese use *B. subtilis* in fermenting Natto, a soybean product which is eaten in large quantities, 6×10^6 kg per year in 1972 (last available statistics). Other uses include production of industrial enzymes like ß-glucanase, proteases, and the starch hydrolyzing enzyme, amylase.}

Safety aspects

In general, *B. subtilis* is considered an opportunistic microorganism with no pathogenic potential to humans. However, *B. subtilis* is virtually ubiquitous and it is therefore inevitable that it sometimes may be found in association with other microorganisms in infected humans, but only patients treated with immunosuppressive drugs appear to be susceptible to infection with this otherwise harmless microorganism (Doyle et al. 1985). We have attempted to collect all pertinent references reporting such cases and to analyse whether *B. subtilis* can cause human disease. We mainly refer to cases described after 1970 as confusion between *B. cereus and B. subtilis* existed in diagnostic laboratories before that time (Gordon 1973).

B. subtilis, as well as other *Bacillus* species, is an important occupant of most environments. A survey by Finch et al. (1978) of the bacterial flora at different sites in 21 homes showed that *Bacillus* species were present at all of 17 sites in the kitchen and all of 16 sites in the bathroom. Together with *Micrococceae, Bacillus spp.* were the most frequent organisms isolated. This is probably due to the common occurrence in soil of bacilli combined with their ability to produce spores.

Comments: {An opportunistic organism is one which grows where nutrients are available such as food, decaying organic matter, or as the authors indicated, associated with other microorganisms in immunosupressed subjects. An important

point is that prior to 1970, clinical laboratories were taxonomically confused between *B. subtilis* and *B. cereus*, a known food pathogen. As a result, many reports implicating *B. subtilis* as a pathogen before this time may well be cases of mistaken identity.}

Infections

Several authors have noted an increased frequency of registration of infections with *Bacillus* species (Logan 1988; Kramer and Gilbert 1989). As stated by Logan (1988), this might be associated with improved bacteriological techniques and the increasing number of severely debilitated patients, for example those who are immunologically compromised.

The literature describing human infections with B. *subtilis* has been collected from database searches and from our collection of references on *Bacillus* pathogenicity. The search resulted in less than ten relevant articles describing approximately 50 cases of putative *B. subtilis* infections. Note that this figure is extremely low considering the total number of reports on bacterial infections. Almost all cases were related to drug abuse or occurred in severely debilitated patients.

Drug abuse. In drug abusers Tuazon et al. (1979) described four incidents of endocarditis (i.e. inflammations of the heart). *B. cereus* was isolated in all cases. Reller (1973) describes one case of endocarditis caused by *B. subtilis* in a drug abuser.

Infections of drug abusers by bacilli are related to the fact that narcotics are often contaminated by bacilli. Thus, the presence of *Bacillus* species in narcotics for intravenous administration has been examined. Shamsuddin et al. (1982) investigated 49 heroin samples and found 20 to be contaminated. Of these 13 were contaminated by *Bacillus* spp. In a separate study, 47% of the injection utensils and 32% of heroin samples were found to be contaminated by *Bacillus* species (Weller and Nicholson 1979).

Debilitated patients. lhde and Armstrong (1973) reported on 12 cases of *Bacillus* spp. infections during a 5-year period from 1966 to 1971. The patients suffered from malignant cancer diseases. Ten of the cases were described as *B. subtilis* infections, but as the data were collected before *B. cereus* and *B. subtilis* were clearly distinguished from each other, the diagnosis may well be erroneous. Pennington et al. (1976) described two cases of *B. subtilis* infection in two patients suffering from blood cancer. *B. subtilis* was isolated from lung and brain tissue.

In a retrospective examination of cases of *Bacillus* spp. isolated from blood samples at a hospital with a large proportion of immunosuppressed patients, Cotton et al. (1987) analysed 17 cases from a 9.5-year period. Fourteen of the patients had chronic venous catheters and *B. subtilis* was not found in any of the blood samples.

Kiss et al. (1988) reported on 21 *B. subtilis* bacteremias in patients all suffering from debilitating diseases. The treatment of the primary disease in all patients included insertion of intravenous catheters, lumbar puncture or other interventions, which may have introduced the organism to sensitive tissue. Richard et al. (1988) described 11 cases of *Bacillus* bacteremias of which *B. subtilis* was isolated in eight patients. Four of these suffered from cancer diseases and four others had head trauma, stroke or had undergone surgery. A routine of using *B. subtilis* culture as a non-specific support for a stable gastrointestinal flora was suspected of being responsible for the infections.

Comments: {It is interesting to note that drug abusers tend to suffer from *Bacillus* infections due to contaminated needles. This also reflects the ubiquitous nature of these organisms. In the only case where *B. subtilis* was implicated in drug abuse related endocarditis, the author (Reller, 1973) cautioned that the condition could have been caused by intravenously injected talc filler.

In the paper by Ihde and Armstrong (1973) where *B. subtilis* was cultured from severely ill cancer patients, only two cases had speciation confirmed by the Centers for Disease Control. More significantly it was stated that the presence of this organism did not influence the patient's clinical course. Perhaps the most intriguing aspect of the Richard et al. (1988) paper is reference to a "parapharmaceutical" drug widely prescribed by doctors in France and Belgium called "Bactisubtil". Surprisingly, this substance was made of *B. subtilis* spores (10^9 per tablet, similar to what DRES has proposed for field trials), marketed by Wellcome in Belgium. Patients take up to 8 tablets per day for 6-13 days to suppress diarrhea or other digestive dysfunctions related to nasal tube feeding.}

Local infection. Infections of the eye by *B. cereus* has caused irreversible loss of sight (Shamsuddin et al. 1982). According to literature after 1970, however, B. *subtilis* seems not to be the agent of infections of the internal eye. Donzis et al. (1988) reported on a case of *B. subtilis* eye infection related to contamination of contact lenses. Jonas et al. (1981) reported on one case of infection in the shin-bone of a 1-year-old child caused by a splinter in the growth plate of the bone.

Comments: {Donzis et al. (1988) reported on one case of *B. subtilis* eye infection related to contamination of contact lenses and their carrying cases. These authors concluded that this organism, being a non-toxin producer, was not a serious problem. In contrast, *B. cereus,* was considered the one that caused the most concern, partly due to its toxin production and heat resistance. However, cleansing of contact lens equipment with 3% hydrogen peroxide was recommended.}

Food poisoning. B. cereus is well-established as a cause of food poisoning accounting for 1-23% of the reported foodborne illness in humans (Kramer and Gilbert 1989). *B. subtilis* has been isolated in some cases of food poisoning, but the number of episodes is low. Thus, Kramer and Gilbert (1989) reported on only 49 episodes in the UK in the period 1975-1986. Exact and reliable figures are difficult to obtain, since *B. cereus* sometimes may have been classified as *B. subtilis.* As a consequence, there are very few examples of *B. subtilis* as the confirmed cause of food poisoning.

B. amyloliquefaciens. B. amyloliquefaciens has not appeared in any of the cited papers dealing with *Bacillus* sp. as infectious organisms. A search in databases for references on *B. amyloliquefaciens* infections or intoxications revealed no such cases, probably because Gordon et al. (1973) considered *B. subtilis and B. amyloliquefaciens* synonymous.

Comments: {Kramer and Gilbert (1989) summarized 49 episodes of alleged *B. subtilis* related food poisoning in the UK between 1975 and 1986. In each case, large numbers of the organism were isolated from the food items but no toxins were looked for. In the light of a previously cited paper (Richards et al. 1988) where patients were prescribed large doses of this organism without ill effects, it is unlikely that *B. subtilis* alone could have caused the food poisoning. We offer here an

alternate interpretation. Improperly prepared food was contaminated by a variety of bacteria including conventional food pathogens that produce toxins. With time, after these organisms have proliferated and toxins produced, Bacillus species, relatively slow growers, multiplied producing antibiotics which killed all other bacterial types. Thus when health officials later examined the offending food after a poisoning outbreak, only Bacillus species were found.

It is well known that Bacillus species are capable of producing antibiotics (Shoji, J. 1978. Recent chemical studies on peptide antibiotics from the genus Bacillus. Adv. Appl. Microbiol. 24:187-214.). Four classes of antibiotic produced are: (1) cyclic oligopeptides such as bacitracin that inhibit cell wall synthesis; (2) linear or cyclic oligopeptides such as gramicidins and polymyxin that interfere with membrane functions; (3) basic peptides such as edeines that inhibit formation of initiation complex on the small ribosome subunit; and (4) aminoglycoside antibiotics that affect ribosome function.}

Recombinant strains

Since the discovery of plasmids that are able to replicate in *B. subtilis* (Ehrlich 1977), *B. subtilis* 168 has been used as a host for cloning DNA of both prokaryotic and eukaryotic origin. Considering *B. subtilis* harmless, the National Institute of Health (US) has exempted sporulation-deficient strains from its Guide-lines for Research Involving Recombinant Molecules (May 7, 1986). On August 24,1987, the NIH modified the Guide-lines (Appendix C-IV) such that the physical containment of large-scale fermentation experiments involving sporulation-deficient recombinant *B. subtilis* does not need to be greater than for the unmodified host.

Permission to produce enzymes from recombinant B. *subtilis* strains have been given in the US, Japan, and Denmark and the Danish Ministry of Health has issued an environmental certification stating that a recombinant *B. subtilis* production strain comply with the OECD recommendations on Good Industrial Large Scale Practice organisms (OECD 1986).

Bielecki et al. (1990), described cloning of the structural gene for *Listeria monocytogenes haemolysin,* hlyA, into an asporogenic *B. subtilis* strain. The recombinant, in contrast to the host strain, was able to grow in vitro in the cytoplasm of macrophage-like cells after being internalized. However, the recombinant was absolutely avirulent after intravenous injection in mice and thus did not display any pathogenic properties in vivo. This is in accordance with the general belief that pathogenicity is a multifactorial property.

The FDA (Food and Drug Administration of the US) may grant products the status of being "Generally Recognized As Safe" (GRAS). Evidence, which in FDA's opinion may lead to this conclusion is published for public comment as a GRAS petition, which eventually may lead to clearance as GRAS.

In a GRAS petition by CPC International (1986), the company reviewed the pathogenicity and toxicogenicity of *B. subtilis.* A search covering the period 1907-1983 failed to disclose a single report demonstrating that *B. subtilis* can be the etiological agent of diseases in man or animals. In the GRAS petition it is noted that although *B. subtilis* strains have sometimes been reported to be implicated in food poisoning, the reports are speculative and in no cases were confirmatory toxicological studies conducted. In the same GRAS petition, specific toxicological studies showed that an a-amylase from *B. stearothermophilus* produced by a recombinant *B. subtilis* is safe for use in food. In another GRAS petition Enzyme Bio-Systems (1988) demonstrated the safety of a *B.*

megaterium amylase produced by a recombinant *B. subtilis.* Finally, a GRAS petition from Novo Laboratories (1990) included safety data on a maltogenic amylase produced by a recombinant *B. subtilis.* Andersen et al. 1987 published a safety study on the toxicological and mutagenic potential of the same enzyme.

Conclusion

No case demonstrating invasive properties of *Bacillus subtilis or B. amyloliquefaciens* has been described but in a few cases, *B. subtilis* has been found associated with drug abusers or severely debilitated patients. Thus there is no evidence of any pathogenic potential of *B. subtilis* to humans in general. *B. subtilis* has been associated with some cases of food poisoning which in part may be due to misclassification of *B. cereus.* Thus there are very few examples of *B. subtilis* strains as confirmed causes of food poisoning. We conclude that *B. subtilis* is a safe host for the production of harmless products.

Acknowledgement. We are grateful to Dr. Fergus Priest, Edinburgh, for critical comments on the manuscript.

References

AMFEP (Association of Microbial Food Enzyme Producers) (1990) Regulatory aspects of food enzymes produced by recombinant microorganisms. Association of Microbial Food Enzyme Producers, Bruxelles.

Andersen JR, Diderichsen BK, Hjortkjaer RK, Boer AS de, Bootman J, West H, Ashby R (1987) Determining the safety of maltogenic amylase produced by rDNA technology. J Food Protect 50:521-526

* Bielecki J, Youngmans P, Connelly P, Portnoy DA (1990) *Bacillus subtilis* expressing a haemolysin gene from *Listeria monocytogenes* can grow in mammalian cells. Nature 345:175-176

Burkholder PR, Giles NH (1947) Induced biochemical mutants in *Bacillus subtilis.* Am J Bot 34:345 348

* Cotton DJ, Gill VJ, Marshall DJ, Gress J, Thaler M, Pizzo PA (1987) Clinical features and therapeutic interventions in 17 cases of *Bacillus* bacteriemia in an immunosuppressed patient population. J Clin Microbiol 25:672-674

CPC International (1986) GRAS Petition 4G0293 proposing that alpha-amylase from a strain of *Bacillus subtilis* (ATCC 39 705) containing the gene for alpha-amylase from *B. stearothermophilus* inserted by recombinant DNA techniques be affirmed as GRAS as a direct human food ingredient. Notice of Filing. Federal Register 51:10571

Diderichsen B et al. (1990) The conditions for contained use of genetically engineered microorganisms and cells for industrial production in Europe. Biotechnol Forum Eur 7:484-485

* Djien KS, Hesseltine CW (1979) Tempe and related foods. In: Rose AH (ed) Economic microbiology, vol 4. Academic Press, London, pp 116-140

* Donzis PB, Mondino BJ, Wiessman BA (1988) *Bacillus* keratitis associated with contaminated contact lens care systems. Am J Ophthalmol 105:195-197

* Doyle RJ, Keller KF, Ezzell JW (1985) In: Lennette EH, Ballows A, Hausler WJ, Shadomy HJ (eds) Manual of clinical microbiology, 4th edn. American Society for Microbiology, Washington, D. C., pp 211-215

Ehrlich SD (1977) Replication and expression of plasmids from *Staphylococcus aureus in Bacillus subtilis.* Proc Natl Acad Sci USA 74:1680-82

Enzyme Bio-Systems (1988) GRAS Petition 7G0328 proposing that alpha-amylase from a strain of *Bacillus subtilis* containing *a Bacillus megaterium* alpha-amylase gene be affirmed as GRAS as a direct human food ingredient. Notice of Filing. Federal Register 53:16 191

* Finch JE, Prince J, Hawksworth M (1978) A bacteriological survey of the domestic environment. J Appl Bacteriol 45:375-364

Gordon RE, Haynes WC, Pang CH-N (1973) The genus *Bacillus.* In: Gordon RE, Haynes WC, Pang CH-N (eds) Agricultural Handbook no. 427. US Department of Agriculture, Washington, D. C., pp 23-25

IFBC (international Food Biotechnology Council) (1990) Biotechnologies and food: assuring the safety of foods produced by genetic modification. Regul Toxicol Pharmacol 12:Special Issue

* lhde DC, Armstrong D (1973) Clinical spectrum of infection due to *Bacillus* species. Am J Med 55:839-845

* Jonas M, Cunha BA, Greensher J (1981) *Bacillus subtilis* osteomycelitis. South Med J 74:1421-1422

* Kiss T, Gratwohl A, Frei R, Osterwalder B, Tichelli A, Speck B (1988) *Bacillus subtilis* Infectionen. Schweiz Rundsch Med 77:1219-1223

* Kramer JM, Gilbert RJ (1989) *Bacillus cereus* and other *Bacillus* species. In: Doyle MP (ed) Foodborne bacterial pathogens. Dekker, New York, pp 21-70

Logan NA (1988) *Bacillus* species of medical and veterinary importance. J Med Microbiol 25:157-165

National Academy of Sciences (1987) Introduction of recombinant DNA-engineered organisms into the environment: key issues. National Academy Press, Washington, DC

Novo Laboratories (1990) GRAS Petition 7G0326 proposing that maltogenic amylase enzyme preparation derived from a genetically modified *Bacillus subtilis* be affirmed as GRAS as a direct human food ingredient. Federal Register 55:9772-9773

OECD (1986) Recombinant DNA safety considerations. Paris

* Pariza MW, Foster EM (1983) Determining the safety of enzymes used in food processing. J Food Prot 46:453-468

* Pennington JE, Gibbons ND, Strobeck JE, Simpson GL, Myerowitz RL (1976) *Bacillus* species infections in patients with haematologic infections. J Am Med Assoc 235:1473-1474

* Priest FG (1990) Products and applications. In: Harwood CR (ed) *Bacillus.*Plenum, New York, pp 293-320

Priest FG, Goodfellow M, Shute LA, Berkeley RCW (1987) *Bacillus amyloliquefaciens* sp. nov., nom. rev. Int J Syst Bacteriol 37:69-71

* Reller LB (1973) Endocarditis caused by *Bacillus subtilis.* Am J Clin Pathol 60:714-718

* Richard V, Auwera P van der, Snoeck R, Daneau D, Meunier F (1988) Nosocomial bacteremia caused by *Bacillus* species. Clin Microbiol Infect Dis 7:783-785

* Shamsuddin D, Tuason CU, Levy C, Curtin J (1982) *Bacillus cer*eus phanophthalmitis: source of the organism. Rev Infect Dis 4:97-103

* Tuazon CV, Murray HW, Levy C, Solny MN, Curtin JA, Sheagren JN (1979) Serious infections from *Bacillus* sp. J Am Med Assoc 241:1137-1140

Welker NE, Campbell LL (1967) Unrelatedness of *Bacillus amyloliquefaciens* and *Bacillus subtilis.* J Bacteriol 94:1124-30

* Weller PF, Nicholson A (1979) The spectrum of *Bacillus* bacteremias in heroin addicts. Arch Internal Med 139:293-294

[The full text of references designated with a * can be found in Volume II of this report.]

ANNEX B

UNIVERSITE D'OTTAWA UNIVERSITY OF OTTAWA

March 11, 1981

Dr. Lloyd White,
Defence Research Establishment Suffield,
Ralston, Alta. ,
TOJ 2NO.

Dear Lloyd:

I have now completed the assessment of the viruses which you have suggested for use in the field trials at Suffield, and am enclosing my report with this letter.

I am afraid it has taken considerably longer to complete than I had expected, mainly because the issue of the poliovirus vaccine proved to be a difficult one when I came to look at it in detail. This necessitated a considerable amount of reading which I had not bargained for when I promised you a quick result.

I must say, that the part of your submission which you sent to me is an admirable literature review, very clearly written, and I feel that you will not be greatly surprised by my conclusions, which you will find on the last two pages of my report. You will see there that I support the use of NDV vaccine in the field trials but I am very uneasy about the use of the poliovirus Sabin vaccine. The reasons for this are given in the appropriate part of the body of the report and re-reading your own remarks in the light of my own investigations, I suspect that you were equally uneasy in this respect. This is why I doubt that you will be too surprised at my conclusions, although you may find them disappointing. I will look forward to hearing your reactions.

In view of the amount of work involved in both researching and writing the report which took almost six days, I am enclosing a bill covering four days of work which we agreed to be the maximum allowable. I would be very interested eventually to know the ultimate fate of this project, and will in any case, look forward to hearing your comments with it.

[original signed by]

J.C.N. Westwood, M.B., B. Chir.,
Professor and Chairman

ASSESSMENT OF THE SUITABILITY OF VARIOUS VIRUSES AS TEST AGENTS FOR USE IN FIELD TRIALS

Based on

Part I: "Review and evaluation of candidate virus simulants for use in field trials" of a Programme Submission by Dr. L.A. White, Ph.D., Suffield Experimental Establishment, Medicine Hat, Alberta.

by

J.C.N. Westwood, M.B., B.Chir., Dip. Bact. (Lond.)
Professor and Head, Department of Microbiology and Immunology, School of Medicine, University of Ottawa, Ottawa, Ontario.

Introduction

This assessment is based upon a study of Part I "Review and Evaluation of Candidate Virus Simulants for use in Field Trials" of a programme submission by Dr. Lloyd White, Ph.D. and has been carried out in the light of the Author's personal knowledge and experience in the B.W. field together with a study of relevant literature.

The Author has not seen Parts II & III of the submission covering the suitability and selective recommendation of agents from the group discussed in Part I but has had the benefit of preliminary discussion of the objectives of the study with Dr. White.

In the present assessment, the Author does not intend to re-cover in detail the ground so well and exhaustively covered in Dr. White's excellent review but rather to select the critical features which underly the choice of agents for the purpose intended and to state these with minimum ambiguity in each case.

It must be appreciated that the conclusions reached are based mainly on scientific factors, but that any course of action which may be adopted must also take into consideration political factors arising from normal and social implications and possible medico-legal action. These lie outside the terms of reference of the present assessment and will only be mentioned briefly.

SCIENTIFIC INTRODUCTION

A. Objective

It is the Author's understanding that the objective of the proposed programme is to develop effective and reliable systems for the detection of virus attack and for the identification of the agent(s) used.

B. Requirement

In order to achieve this objective, it is necessary to develop devices and techniques capable of detecting, collecting and identifying viral B.W. agents under operational conditions in the field.

C. Enabling R & D Stages

The development of the necessary devices and techniques involves two stages of Research and Development.

Phase 1. Laboratory development, construction, testing and modification of hardware together with laboratory development of the techniques for its use.

Phase 2. Field testing of hardware and techniques under operational or simulated operational conditions in order to determine:

- Suitability and practicality of the hardware.
- Reliability and sensitivity of the test procedures.
- Reproducibility of results.
- Effects of extraneous substances in the atmosphere.

It is the Author's understanding that the laboratory development of Phase 1 has already been carried out and the Submission is concerned only with Phase 2, the Field Testing of existing systems.

The Author has been asked to give his assessment of the suitability of various viruses for field use in this Phase of the programme.

CRITERIA FOR SELECTION OF VIRAL AGENTS

Selection of a suitable agent or agents for field experiments requires a precise evaluation of the objective of the study and a precise definition of objective limits.

In Para 1, line 8 of the Submission the requirement is stated to be for a "... vertebrate virus model, herein referred to as a virus simulant..."

In the B.W. field, the "Simulant" is used for a bacterial or virus strain of low pathogenicity which, in all other respects, is identical to, or at least closely resembles, a B.W. agent of high pathogenicity.

Thus Bacillus globigiosus may be used as a simulant for Bacillus anthracis, vaccinia virus for smallpox virus, or an avirulent strain of influenza virus for a virulent strain. The use of a simulant virus thus presupposes a precise knowledge of the virus agent, or at least the virus group against which protection, or a detection and identification ability is required. This clearly is not the case in the present programme which is general in scope, although specific simulants may well be needed at a later stage if a third Phase is undertaken in order to develop systems tailor-made for the detection and identification of specific viruses regarded as possible B.W. agents for use against this Country or its Armed Forces.

For the present purpose, however, the requirement is for a non-pathogenic virus strain which may be used to represent all mammalian viruses in field experiments designed

to test detection instrumentation and identification systems. Since no single virus strain can simulate the entire range of viruses, with their great variation of size, structure, stability and chemical composition, it is clear that the selection can, at best, only be for strains which will permit useful results to be obtained. In the first instance, it is probable that a strain of a stable virus capable of acting as a "Tracer" would be the most useful but, when systems have been well calibrated using such a strain, it would clearly be desirable to include a representative of the less stable groups.

Criteria

The choice of a suitable virus strain involves five classes of criteria.

1. Acceptability
2. Physicochemical properties
3. Assay
4. Availability
5. Relevance

Acceptability

The primary issue governing acceptability is that of safety which must be assessed in relation to staff carrying out the experiments, to incidental staff who might be inadvertently exposed, to the general public, to domestic animals and to the ecology.

Physicochemical properties

Of these, the most important is stability which should lie within practical limits, as regards storage, aerosolization, collection and assay.

Assay

A simple, reliable, sensitive and accurate assay procedure should be available.

Availability

A straightforward system for production of high-titre virus on an adequate scale should be available or, alternatively, suitable suspensions should be obtainable from some alternative source.

Relevance

The virus strain(s) chosen should be such that the results obtained from the programme are relevant to the objective inasmuch as they can be confidently interpreted as representing the probable behaviour of human pathogenic virus strains.

Of these criteria, the issue of safety is clearly paramount and is discussed in detail in the Submission. on general grounds, the possible choice has been limited to the short list of viruses requiring no more than category A level of containment (MRC 1979) and of these, the viruses of lower animals, with the single exception of the vaccine strains of Newcastle Disease Virus (NDV), must be excluded because of the uncertain ecological effect of their dissemination amongst the wild-life fauna.

It is further convincingly argued that, of the remaining viruses, only vaccine strains with which there has been a minimum of ten years experience, should be considered for use and of these, only those preparations which have been screened to exclude the presence of known adventitious pathogens.

These valid and sensible restrictions leave a residue of six vaccine strains, five of which:- vaccinia, measles, mumps, rubella and poliomyelitis, have been used in the human population, and one, Newcastle Disease Virus, equally extensively used for mass immunization of chickens.

CANDIDATE VIRUSES

Vaccinia Virus

Despite its extensive use for immunization of the human population against smallpox, vaccinia virus is a virus of relatively high pathogenicity with a long, if sporadic, history of lethal infections and postvaccinial encephalitis. Even more serious than the standard complications listed in the Submission is the prospect of vaccinial pneumonia in a population now almost devoid of the individual or herd immunity resulting from smallpox vaccination.

Vaccinia virus must unquestionably, therefore, be excluded. This is unfortunate in one sense since smallpox must now be regarded as by far the most dangerous biological weapon available for either overt or covert use.

Measles Virus

Measles virus vaccine strains could satisfy many of the criteria and would provide a useful test strain in the range of the less stable viruses. However, no vaccine strain is totally free from the induction of sporadic active illness and the possible induction of neurological complications, including Subacute Sclerosing Pan-Encephalitis (SSPE), preclude its selection.

Mumps Virus.

Mumps virus is probably too unstable for profitable use in field trials and the vaccine strains, like those of measles are not free from the stigma of inducing neurological complications. The virus is, therefore, unacceptable on safety grounds.

Rubella Virus

The most recently developed strain of rubella vaccine virus provokes a considerably milder clinical response than the earlier strains but still causes some degree of fever, malaise and joint manifestations in up to one out of every four adults vaccinated. More serious, however, is the potential risk of foetal damage during pregnancy. Vaccine strains cause viraemia in vaccinated individuals and are known to be capable of crossing the placental barrier to infect the foetus. So far no instances of foetal damage have been reported even when there has been evidence of foetal infection, but the risk is clearly unacceptable.

Newcastle Disease Virus (NDV)

Newcastle disease is primarily a disease of fowls which suffer a very high case-fatality when infected by strains of high virulence. Many different species of birds, both wild and domestic, are known to be susceptible but these suffer a clinically less severe illness.

The virus is a member of the paramyxovirus group and, being enveloped in a lipoprotein- envelope, is susceptible to lipid solvents and is also readily inactivated by a range of chemical agents. It is, however, considerably more stable under a variety of conditions, including aerosol suspension, than most other paramyxovirus and myxoviruses and could from the physicochemical standpoint provide a good test virus of the more labile end of the stability spectrum.

Acceptability

In nature, the virus exhibits a wide range of strain virulence and from the avirulent strains, a number of live vaccine strains have been developed. These have for many years been widely used in the poultry industry and have been administered by a variety of

methods including mass-vaccination procedures using drinking-water transmission or aerosol exposure.

Under farm conditions, these practices have led to extensive human exposure to vaccine strains and, in time of epidemic spread, human exposure to virulent wild strains has also been frequent. The latter have caused sporadic infections in man resulting in acute conjunctivitis with or without mild respiratory symptoms of short duration, but no serious clinical features have been reported. There is no report of clinically apparent infection following exposure to vaccine strains.

Domestic animals are not at risk from exposure to either virulent or vaccine strains of NDV and there is no ecological threat to the wild mammalian fauna. A range of wild birds are known to be susceptible to infection by wild strains of the virus but do not suffer severe clinical illness. Although indigenous wild birds must be pressured to be infectable by the vaccine strains, such infection should be beneficial rather than the reverse.

It may be concluded that the use of a well-tried vaccine strain of NDV for field trials could be regarded as being as safe as any such trial could be.

Poliovirus

Poliovirus is a small stable virus, easy to assay and easy to produce in high titre. The Sabin vaccine strains have been in wide use throughout the world for over 20 years and their degree of safety is known with some precision. Vaccine strains, tested for safety and screened against the presence of adventitious viruses are available in quantity and much work has already been carried out on their aerosol characteristics and appropriate sampling techniques. These characteristics have made poliovirus vaccine strains, particularly the Sabin Type 1, amongst the most widely used test viruses in the laboratory. There is also no doubt that they are admirably suited for use in the field as virological "Tracers". The only

problem in their use is the need for a judgement decision as to whether their safety justifies their release in aerosol form in the field where there can be no guarantee that uninvolved persons will not be unwittingly exposed to them. The safety issue, therefore, must be discussed in some detail.

Safety

Trivalent Oral Polio-Vaccine (TOPV) has now been administered to many millions of persons of all ages across the entire world and the incidence of serious complications in the form of vaccine-associated paralytic poliomyelitis is known with some precision. The most recently revised figures accepted by the World Health organization are:

Frequency of paralytic poliomyelitis -

In vaccine recipients	1 in 11.5 million
In household contacts of recipients	1 in 3.9 million
In community contacts of recipients	1 in 33.9 million

Despite these very low probabilities vaccine-associated cases have occurred consistently throughout the USA, for which the best figures are available, since a survey of such cases was started in July 1964. At that time, 57 vaccine-associated cases had been reported. In the 12-year period through 1976, the numbers were as follows:

Natural & Vaccine Associated Paralytic Poliomyelitis 1964-1976

	Up to July 1964	July 1964-Dec. 1966	1969	1970	1974-1976
Natural infections	?	76	14	31	15
Vaccine recipients	57	15	0	0	0
Contacts of recipients	?	?	5	1	10
Total paralytic cases	?	>91	19	32	25

The greater frequency of paralytic cases amongst contacts than amongst vaccinees may well be associated with increased neurovirulence of the vaccine strains after multiplication in the gut of the vaccine recipient. Melnick (1978 - Bull. W.H.O. 56/2l, p.32) states: "Vaccine progeny virus after multiplication in the vaccinees, although still attenuated, would no longer pass the safety tests required of the vaccine itself."

As in the U.S.A., so in Canada, poliomyelitis from natural causes is now rare but cases do still occur and the recent occurrence of 5 cases in Ontario, 1 in Alberta and 1 in B.C. all associated with an outbreak in the Netherlands, indicated the presence of unprotected pockets of the population (Furesz, 1979). these individuals had refused vaccination on religious grounds and the virus was imported, but, in recent studies, wild strains of poliovirus have been isolated from sewage and from Ottawa River water (Payment, 1979a, 1979b; Sattar & Westwood, 1977, 1979) indicating that wild virus is still circulating in the community and that sporadic cases of natural paralytic infection could occur.

Under these conditions, there are two separate issues to be considered in relation to the proposed field trials.

1. The danger that vaccine-associated cases might be induced amongst inadvertently exposed individuals and/or their contacts.

2. That one or more natural cases of paralytic poliomyelitis might occur within the radius of possible travel of the released cloud with the possibility of resulting legal action.

1. The danger of vaccine-associated cases

With regard to this first danger, the likelihood of such cases occurring is clearly vanishingly small. By the normal oral route of administration of the vaccine, the incidence of paralytic poliomyelitis in recipients would be expected to be about 1 in 11.5 million. The population of the whole of Alberta, therefore, estimated as 1,899,700 in 1977, would be unlikely to yield a single case even if all were susceptible and all were exposed. In actual fact, the only major population centre within the immediate vicinity of the Suffield Experimental area is Medicine Hat, with a population of under 50,000. Moreover, a recent Canada national survey of immunological status suggested that over 90% of the Alberta population possessed demonstrable antibody levels against the three poliovirus types (Furesz, 1979). Even the most pessimistic surveys have indicated a level higher than 60%. These figures justify the conclusion that the maximum possible number of uninvolved persons lacking antibody who might be inadvertently exposed lies far below the threshold at which vaccine-associated cases might be expected, even amongst the contacts of recipients. If, therefore, it could be assured that all persons living or working in the area of the Experimental Station were protected by prior vaccination, then the danger of vaccine-associated cases from oral exposure to Sabin vaccine could safely be ignored.

Unfortunately exposure would not be by the oral route but would result from the inhalation of artificially generated aerosols. Since all available data have been generated by oral administration of monovalent and trivalent vaccine, there is no means by which their applicability to respiratory exposure may be judged. The fact that respiratory transmission of poliomyelitis has not been documented with certainty means little since it may reflect only the fact that faecal shedding of the virus does not generate significant aerosols. A comparable situation is seen in the cases of Brucellosis, Tularemia, and Anthrax in which respiratory transmission is unusual in nature although the organisms are highly infective

when aerosol challenge is artificially induced in the laboratory. It would in fact be in keeping with experience with other organisms if the paralysis inducing infective dose of poliovirus were found to be smaller by respiratory than by oral challenge. The writer knows of no experimental or epidemiological information by which this particular doubt may be resolved.

2. The possibility and consequences of the coincidental occurrence of naturally acquired poliomyelitis

The likelihood of naturally acquired paralytic poliomyelitis occurring in the general area of Suffield is clearly very small in view of the rarity of the disease in Canada. Nevertheless, sporadic cases do in fact occur and the possibility cannot be entirely ruled out. Should such a case occur due to the same type of virus as that being used in the field experimental work or, alternatively, due to an unidentified virus type in a clinically but not virologically diagnosed case, or even should a clinically suggestive illness occur which could not be proven to have some other aetiology, it could be difficult to prove that there was no causal association. This is due in part to the fact that there is no certain means of identifying the antecedents of a poliovirus isolate. It is known that vaccines strains undergo some degree of reversion in the gut of a recipient to the point that they would fail to pass the safety requirements for vaccine use, and the closest that it is possible to come to a definitive conclusion regarding the origin of such an isolate is that it is 'vaccinelike' or 'non-vaccine-like'. It is highly unlikely that this degree of characterization would be regarded as conclusive should a court challenge develop.

The statistical likelihood of such a challenge is, of course, impossible to assess. However, three major cities lie within 250 miles and could easily be reached by a viral aerosol generated on the Suffield range. The distances could be covered in a matter of hours under moderate breeze conditions. The strains of poliovirus which have been tested

for aerosol stability are rapidly inactivated at relative humidities below 60% but survive well at RH above that figure if aerosolized from suspension in a suitable medium. Although high relative humidities are not the norm under the prairie conditions, the survival of even 1% of virus from a suspension containing 10^8 infective units per ml. would still represent almost one million infective units per ml. and survival of 1% of infective virus in an aerosol at the end of 10-15 hours is possible under field conditions.

The final danger lies in the fact that the nature of the tests and the scale of the population which might theoretically be inadvertently exposed precludes any possibility of obtaining "informed consent". This would almost inevitably result in a strong bias in favour of any complainant.

CONCLUSIONS

In the light of the facts and arguments outlined above, I must draw the following conclusions:

1. Of the various vaccine strains considered in the Submission, all but Newcastle Disease Virus and Sabin poliovirus fail on grounds of safety.

2. Newcastle Disease Virus would satisfy the objectives of the proposed field trials from the technical standpoint as a representative of the less stable enveloped viruses. Despite belonging to this group, its own stability is adequate for useful results to be obtained. From the technical standpoint, a suitable vaccine strain of NDV is easy to obtain in adequate quantity and at adequate concentration. Assay presents no major problems.

In term of safety, NDV vaccine is reported to be widely used amongst commercial poultry breeders in the area and is commonly administered by mass aerosol exposure of poultry flocks under conditions which do not include elaborate safety measures to avoid

human exposure or to prevent escape of aerosolized virus. Such human infections as have been reported are trivial in nature. It is unlikely that any public relations problems would arise from the use of NDV vaccine for field trials.

3. Poliovirus is, under many conditions, a very stable virus which is technically easy to handle, and the vaccine strains are easily available. From the standpoint of safety, live poliovirus vaccine administered by the oral route in the standard manner has proven to be probably the safest live, vaccine in use, and when the statistics of occurrence of vaccine-associated complications are analyzed, it is clear that the probability of vaccine-associated paralytic poliomyelitis arising as a result of its use in field trials is extremely small. However, unlike the trivial nature of NDV vaccine infections in man, vaccine associated poliomyelitis is far from trivial and there is no means of estimating its frequency of onset following respiratory exposure. Even the suspicion of a case amongst the general public which could be remotely connected with the use of the virus in field trials could raise unanswerable questions and could well lead to indefensible court challenge.

While it is not my responsibility to assess the probability or possible outcome of litigation arising from the use of poliovirus for field trials, it is within the province of my virological expertise to state that, in my opinion, it would be very difficult to present an unbreakable defence on virological grounds should its use be challenged in the courts.

[Original signed here by J.C.N. Westwood]

REFERENCES

1. Furesz, J., Armstrong, R.E. & Contreras, G. 1979. Can. Med. Ass. J., 120:905.

2. Payment, P., Larose, Y. & Trudel, M. 1979a. Can. J. Microbiol., 25 (10) -1212-4.

3. Payment, P., Larose, Y. & Trudel, M. 1979b. Can. J. Microbiol., 25(11):1305-9.

4. Sattar, S.A. & Westwood, J.C.N. 1977. Can. Med. Ass. J., 116:25-27.

5. Sattar, S.A. & Westwood, J.C.N. 1979. Bull. W.H.O., 57(1):105-108.

ANNEX C

April 15, 1981

Your Ref: DRES 3616B-1 (PMS)
1770-ETP-4

Dr. Lloyd A. White
Head/ Preventive Medicine Section
Defence Research Establishment Suffield
RALSTON, Alberta TOJ 2NO

Dear Dr. White:

I am sorry I have not got back to you earlier but I have had great difficulty in understanding how these experiments will be carried out.

I realize that it is probably not any of my business, also that you would have a very sophisticated and up to date facility that can cope with this problem. Please understand that you are working towards the type of experiment with aerosols that we take every possible precaution to avoid.

After our last conversation on the phone I was surprised to hear that you had decided on the Newcastle Disease virus, but after having read your documents over and over, I suppose you are really right. In your letter you ask me to give a priority listing and I would establish it as follows:

Newcastle Disease Virus
Polio

None of the others.

Newcastle Disease Virus has much in its favour in that there are several strains to choose from, referring to its virulence. It would also seem to be a relatively hardy virus and capable of maintaining viability for a fairly long period. It is a virus that is easily propagated and, in general, not too pathogenic for man except when you get into the aerosol state. Even then, it does not generally cause a serious disease. Other mammals are really not susceptible, therefore I assume that when you commence to work in the "outdoors" I suppose the local wildlife will be checked for susceptibility to the virus if not physically at least a search of the literature.

In mentioning wildlife I was referring mainly to the mammals but my main concern would be birds. I am totally uninformed as to what birds would be a hazard to the experiment in that they would possibly get infected and transmit the disease to other areas. I do not know whether this is an endemic disease in birds, though I would seriously doubt it. I was surprised at how far the virus can be projected or transmitted in the air and its

viability. This is a problem I am sure you have considered at length and probably have the solution.

It would seem to me from our conversation that you have chosen the best candidate for your study. I certainly would not have considered it but, after all, I do have a bias towards human pathogens.

Poliomyelitis would be my second choice though it would certainly not be anywhere as good a candidate for your experiment as the Newcastle Disease virus. The reasons why I mention polio is that it is relatively easy to grow and therefore easy to find but, of course, if you have an attenuated strain it certainly does not stand up too well in the heat or dispersed in small volumes (aerosols). In this condition I would think that it has no protection and would probably be not viable very quickly. A wild strain would be much more dangerous and would have no greater viability in the form of experiment I think you are trying to devise. When referring to the virus I am thinking of the pure form, and not in any suspension that would give it a protective covering.

I must add that one of my difficulties was to see your experiments in the light of polio virus, and no matter how much I tried I could not envisage the problem at all.

As to the other viruses you mention, I do not think them good candidates at all and I believe you have given good reasons for not using them.

It would appear that you have taken the right approach to the whole study and you are very fortunate to be in the right location to carry out these studies. I was pleased to see that you intended protecting your personnel with appropriate immunization,where possible,and that the correct clothing and meterological conditions would be chosen for the study. if you are going to use Newcastle Disease virus I do not know how you would immunize, so I suppose you would have to rely on protective devices.

Thank you for having the confidence of allowing me to see your documents, which I would really like to keep as they are an excellent summary of the findings in the literature of the virus you researched. If, on the other hand you would like them returned, please feel free to let me know and I will do so. I would like to assure you that I have not discussed or shown this documentation to anyone within Connaught Laboratories or outside. They have been kept totally confidential.

Yours sincerely,

[Original signed by E.W. Pearson, M.D.]

E.W. Pearson, M.D.
Director,
Clinical and Medical Affairs

EWP:as

ANNEX D

READ IN FULL

FOR VETERINARY USE ONLY

These directions may be used for any of the following Salsbury vaccines.

WITH DILUENT: FOR INTRANASAL, INTRAOCULAR, SPRAY OR WATER ADMINISTRATION

WITHOUT DILUENT: FOR WATER ADMINISTRATION ONLY

Available in 10x1000 dose size

1. **Infectious Bronchitis (Mass. Type) Vaccine, LV CEO***
2. **Infectious Bronchitis (Mass. & Conn. Types) Vaccine, LV CEO***
3. **Newcastle (B1 Strain) Vaccine, LV CEO****
4. **Newcastle (LaSota Strain) Vaccine, LV CEO****
5. **Newcastle (B1 Strain) and Infectious Bronchitis (Conn. Type) Vaccine, LV CEO***
6. **Newcastle (B1 Strain) and Infectious Bronchitis (Mass. Type) Vaccine, LV CEO***
7. **Newcastle (B1 Strain) and Infectious Bronchitis (Mass. & Conn. Types) Vaccine, LV CEO***
8. **Newcastle (LaSota Strain) and Infectious Bronchitis (Mass. Type) Vaccine, LV CEO***

* Use for initial vaccination and revaccination of chicks, broilers and replacement birds

** Use for both chickens and turkeys of any age.

STORE THIS VACCINE IN A REFRIGERATOR UNTIL IMMEDIATELY BEFORE USE

Vaccination Programming

This vaccine stimulates susceptible birds to develop immunity. Revaccination requirements depend on age, type of bird and severity of the particular disease in the area. Vaccination schedules should be planned in consideration of these requirements.

Following is a suggested vaccination schedule for growing chickens that has proven satisfactory in most areas.

	1 to 10 Days of Age	4 to 5 Weeks of Age	4 Months of Age
Bronchitis	X	X	X
Newcastle	X	X	X*

* Repeat every 3 months during laying period.

Birds should be free of all diseases—including chronic respiratory disease (CRD), coccidiosis, blackhead, parasites, etc. Although disease may not be evident, the flock owner must assume risks entailed by vaccination. Stress factors can affect vaccination reaction.

To Reconstitute the Vaccine

1. Remove rubber stopper, add diluent to half-fill bottle. Replace stopper, shake so all material is dissolved.
2. Pour dissolved vaccine into remaining diluent, shake again.
3. If the vaccine has been purchased without diluent, remove the rubber stopper and half-fill the vial with clean, cool non-chlorinated tap water. Replace the rubber stopper and shake until vaccine is in solution.

This vaccine may be used by any of four vaccination methods. Follow directions carefully.

Intranasal Administration

For birds of any age, usually under 10 days.

1. Reconstitute the vaccine, as directed.
2. Fit drop dispenser on bottle. (Extra droppers supplied upon request)
3. Place finger over one nostril of bird. Allow one drop of the vaccine to fall into the other nostril.
4. Vaccination is completed when vaccine is inhaled into the nasal cavity. Don't release the bird until this occurs.

Intraocular Administration

For use in chickens as early as one day of age

1. Reconstitute the vaccine, as directed
2. Fit drop dispenser on bottle. (Extra droppers supplied upon request).
3. Hold the bird so one eye is upward, and allow one drop of vaccine to fall into the eye

Drinking Water Administration

For any age bird 3-4 days old or older

1. Never use less than one dose per bird.
2. Discontinue all medication and sanitizers in water 24 hours before and 24 hours following vaccination
3. Withhold water for 2 hours before vaccinating to stimulate thirst.
4. Provide enough waterers so two-thirds of birds may drink at one time. Scrub them with fresh, clean non-chlorinated water, without a disinfectant; then drain. Turn off automatic waterers, so only vaccine water is consumed. Do not administer through medication tanks or medicators.
5. Reconstitute the vaccine, as directed. If the vaccine has been purchased without diluent follow Step #3 only.
6. Use a clean container partially filled with cool fresh, clean, non-chlorinated water. Add 30 g of dried milk powder if final volume of water per 1000 doses of vaccine is to be 10 litres; 60 g if final volume of water per 1000 doses is to be 20 litres, etc. Shake mixture until the dried milk powder is in solution
7. Be certain to add the dried milk powder first, then the rehydrated vaccine from the vial. Shake until mixed.
8. Add the mixture to the final volume of water as follows:

Add This Amount of Vaccine	To This Amount of Water for Birds 4 days to 8 Weeks Old	To This Amount of Water for Birds Over 8 Weeks Old
1000 doses (1 litre = 0.22 Imp. gal)	10 to 20 litres	20 to 40 litres

9 Distribute evenly in the clean waterers. Do not place in sunlight. Return to regular watering only after vaccine-water-milk mixture is consumed.

Spray Administration

Use only for revaccinating chickens 4 weeks of age or older. Reduce light intensity to avoid exciting birds. When spraying, employ a power sprayer.

1. Reconstitute the vaccine, as directed, immediately before spraying.
2 Further dilute reconstituted vaccine with non-chlorinated tap water or distilled water in accordance with the output of the power sprayer to be used.
3 Use at least one dose per bird.
4. Close doors, windows, ventilators and shut off fans immediately before spraying. Do not spray in drafts. Leave buildings closed and fans off for 20-30 minutes after spraying, unless birds show discomfort.
5 Walk slowly through house, spraying vaccine well above the birds
6 Immediately after spraying, flush sprayer with clean water

Precautions

1 If possible vaccinate all susceptible birds on the premises at the same time
2 For 10 to 14 days after vaccinating don't transport vaccine particles on shoes, clothing, etc. into areas containing unvaccinated birds
3 Newcastle Disease vaccine virus may cause an eye infection in humans. Do not allow the vaccine to contact the eyes. When using spray methods, wear goggles and a face mask. Those persons not essential to flock care should not enter building during spraying or for 24 hours after.
4. Birds transported by parcel post should be vaccinated at the destination—not prior to shipment—to avoid possibility of violating postal regulations.
5. Dispose of unused vaccine by placing open virus vials and diluent bottles in burning material in a trash burner.
6. Do not vaccinate within 21 days before slaughter

Notice: This vaccine has undergone rigid potency, safety and purity tests and meets Company and Government requirements. Since we, as the manufacturer, have no control over field conditions of transporting, handling and administration neither we nor our agents and representatives can express or imply a warranty in connection with the use of this vaccine.

Records: Keep a record of vaccine serial number, expiration date, date of receipt, date of vaccination and any reactions that were observed.

Manufactured by Salsbury Laboratories Inc
Charles City, Iowa, U.S.A. for Salsbury Laboratories Ltd
Establishment No. 29 (licensed by Agriculture Canada)

SALSBURY LABORATORIES LTD.
Kitchener, Ontario N2C 1L4
Member of the SOLVAY GROUP

ANNEX E

Agriculture
Canada

801 Fallowfield Road
Box 11300, Station H
Nepean, Ont. K2H 8P9

Food Production and
Inspection Branch

Direction générale,
Production et inspection des aliments

January 25, 1982

Dr. L.A. White
Head/Preventive Medicine Section
National Defence
Defence Research Establishment Suffield
Ralston, Alberta
TOJ 2NO

Dear Dr. White:

Re: Use of NDV in Canadian laboratories

I am replying to your letter of January 13, 1982 written to Dr. Langer.

We are not concerned with laboratory use of licenced vaccine strains in your studies. We do require a permit to import all strains and would be prepared to consider importation of other than vaccine strains under special circumstances. It is our understanding that you do not presently hold cultures other than vaccine strains.

Certainly escape of virulent strains is our concern, whether to the atmosphere by carcasses or other agents or by fomites.

We had hoped to visit your laboratory this past year, because of the presence of vesicular stomatis virus (VSV). We will be in contact prior to any visit planed.

Sincerely,

D.C. Alexander
Chief, Veterinary Biologics
Animal Health Division

DCA:ls

National Défense
Defence nationale

DRES 3616B-1 (PMS)

26 March 1986

Dr. D.C- Alexander
Chiefs Veterinary Biologics
Animal Health Division
Agriculture Canada 801 Fallowfield Road
Box 113009 Station H Nepean, Ontario K2H OP9

Dear Dr.,Alexander:

Reference: Your letter of January 25, 1982, concerning our use of NDV.

We are planning, after final Departmental approval, to begin experiments this summer involving the release of live LaSota strain NDV vaccine (obtained from Salsbury Laboratories Ltd., Kitchener, Ontario) into the atmosphere. Personnel involved in these experiments will be required to wear protective clothing and NBC respirators, and a safety template will be set up so that no unprotected person will be able to approach within a 2 km distance downwind.

At this time we would appreciate a confirmation of your earlier letter (attached) which we interpret to imply that you have no objection to our use of this licenced vaccine strain in this manner. If for some reason there are some objections, we would appreciate some guidelines on how these studies could be performed in a manner acceptable to your department.

Thank you very much for your cooperation.

Sincerely yours,

[Original signed by BVE Kournikakis]

B. V . E . Kournikakis
Preventive Medicine Section
for Chief/DRES

Attachment

Agriculture
Canada

Food Production and Inspection Branch
Direction générale, Production et inspection des aliments

801 Fallowfield Road
Box 11300, Station H
Nepean, Ont. K2H 8P9

April 7th, 1986

Mr. B.V.E. Kournikakis
Preventive Medicine Section
for Chief/DRES
National Defense
Defence Research Establishment Suffield
Ralston; Alberta.
TOJ 2NO

Dear Sir:

In response to your letter of March 26, 1986 I want to confirm that there is no objection to your proposed trial with the licenced Newcastle vaccine.

It is important that you protect the operators as you have outlined in your letter.

Sincerely,

[Original signed by D.C. Alexander]

D.C. Alexander
Chief, Veterinary Biologics
Animal Health Division

DCA/dl

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It has been and still is necessary to use test biological warfare agent detectors and carry out research activities (such as studies on individual and collective protection, development of sampling techniques and procedures) in biological defence using microorganisms. To carry out this work, DRES has used *Bacillus subtilis* (BG) for a number of years. Recently it became desirable to use a viral simulant as well and after a detailed search, Newcastle Disease virus La Sota strain, a vaccine strain was chosen. Safety and the lack of environmental risk of these two organisms were the main criteria in their selection. This document summarises all available information on the safety and environmental effects of these two organisms. This review identified no hazards to personnel or the environment from the use of BG and NDV in the field.

Volume II of this document contains supporting information referred to in the text.

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Bacillus subtilis

Newcastle Disease Virus

Biological Simulants

Safety

Environmental Assessment