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Technology and Biological Weapons: Future Threats

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I. Introduction

Some biologists have expressed concerns about the potential impact of genetic engineering capabilities on biological warfare possibilities almost from the time that such engineering became possible.¹ Since the mid-1990s there has been an increasing number of such warnings both from official sources² and from practicing microbiologists.³ The medical profession, in particular, has expressed increasing concerns over the kinds of agents that may be developed and used for hostile purposes.^{4,5}

The recent warnings have made it clear that we could well face an increasing range of different biological agents being used for hostile terrorist and warfare purposes in the coming decades. George Poste⁶, for example, has emphasised the need to think “beyond bugs”, and, more generally, Mathew Meselson has argued convincingly that as the century progresses more and more of life’s fundamental processes will become open to both benign and malign manipulation.⁷

Such a diversity of possible threats evolving from the ongoing revolution in biology raises the very real question of whether a tiered peer-review system as proposed by the Fink Committee⁸, or the tighter system proposed by the Maryland group⁹, would be adequate – even if substantially modified to cover a wider range of agents. The intention of this paper is to provide the basis for a discussion of that issue by reviewing a wide range of possibilities in regard to the agents that could arise.

As a starting point the recent paper written by the US Defense Department analysts Petro et al.¹⁰ is used as a framework for thinking about future trends as it is perhaps the most systematic viewpoint available in the open literature. These authors consider the future evolution of biological warfare in three phases:

i./ As there are only a limited number of traditional biological warfare agents suitable for use they suggest that the defense will eventually be able to counter all of these.

ii./ Moreover, as there are only a limited number of ways in which traditional agents may be effectively modified, the defense will also eventually be able to counter all of these.

iii./ However, as the process described by Meselson continues through the century an ever increasing number of targets will become available for which specific Advanced Biological Warfare Agents (ABWs) may be designed. Thus the defense will be confronted with the problem – should this prediction prove correct – of a diffuse and fundamentally unknowable range of potential agents. And some of these, as the recent CIA report noted, could be, “more severe than any known agent in their effect.”¹¹ Using this framework the following section develops analyses of possible futures in regard to research in microbiology; immunology; the nervous systems; animal diseases; and plant diseases. In each section a current cause of concern is used as an introduction, and then possible modification of traditional agents, and finally, possible ABWs are discussed.

It is not intended here to ask the question of whether a modified tiered peer-review system could cope with such a range of possibilities, but rather to set the stage for a realistic discussion of that question.

II. Examples

1. Immunology – Vulnerability of the Immune System to Modulation

1.1 Introduction

The immune system plays a crucial role in protecting against infectious diseases. This is clearly demonstrated in the case of individuals with genetic defects in certain immune mechanisms, which frequently result in a devastating outcome, despite the use of antibiotics or other chemotherapeutic agents. Indeed, the pathogenicity of a microorganism can only rightly be defined within the scope of its interaction with the immune system.

In this age of rapid biomedical and biotechnological advances, far-reaching manipulations of microorganisms are now possible that can change their properties drastically. Experiments to manipulate microorganisms are being carried out daily, with mostly peaceful aims in mind, such as the elucidation of the pathogenic mechanisms of an infectious agent, which could in turn point the way to the development of better prophylactic and therapeutic measures to counter infections more successfully.

However, it has become evident that these experiments can lead to the creation of particularly dangerous microorganisms that can evade the immune responses in devastating ways. A prime example is the inadvertent creation of a killer mousepox virus by researchers trying to develop a contraceptive vaccine to control the rodent population in Australia.^{12, 13} Particularly disturbing is the fact that another scientist, Mark Buller at St. Louis University, has picked up on these experiments and carried them some steps further by increasing the lethality of the mousepox virus and by carrying out similar manipulations with the cowpox virus.^{14, 15, 16}

Up to now, the focus has been mainly on concerns about the possibilities of manipulating the properties of microorganisms to make them more robust and more pathogenic. It becomes evident from the example cited above that the real target is the immune system, and how vulnerable it is to evasion mechanisms, which naturally potentiate the pathogenicity of the infective agents. This represents a change of focus from the microorganism to systems biology and how it might be misused. The situation is accentuated by the fact that the National Institute of Allergy and Infectious Diseases (NIAID) of the US National Institutes of Health (NIH) has expanded its program significantly in order to attract scientists to the area of biodefense research¹⁷. Within this program, immunology as it relates to biodefense is given special attention. NIAID reported that it awarded a multi-component grant to create an “encyclopedia” of innate immunity, a comprehensive and detailed picture of the type of immunity that represents the essential first line of defense against infectious diseases. The stated goal of this undertaking is to gain knowledge that could lead to the development of treatments for infectious diseases. At the same time, however, this information could provide a blueprint for malign attack of the immune system.

In order to appreciate the dilemma of dual use and the possibilities of misuse in this area, a brief description of scientific and technological aspects underlying research activities in this field, including the elements of the innate and the acquired immune systems will be given. Also, mechanisms of immune evasion used by some microorganisms will be outlined. With this background, examples of research in which microorganisms have been created that evade immune defenses will be presented along with an analysis of the dual use aspects involved.

Finally, a look to possible future threats involving the vulnerability of the immune system will be offered.

1.2. Scientific and Technological Background

1.2.1 Mammalian Immune Systems

The hallmark of the immune system is its ability to respond to an invasion of the body by microorganisms or toxic components in ways that afford protection against the detrimental effects that could occur. The responses of the immune system include both specific (adaptive immune system) and non-specific (innate immune system) components. These react in different ways to antigens, which are substances foreign to the host. Components of innate and adaptive immunity are listed in Table 1.

Table 1. Features of Innate and Adaptive (Specific) Immunity¹⁸

Feature	Innate Immunity	Adaptive Immunity
Characteristics		
Specificity for microorganisms	Relatively low (PAMPs) ^a	High (specific antigens)
Diversity	Limited	Large
Specialization	Relatively stereotypic	Highly specialized
Memory	No	Yes
Components		
Physical and chemical barriers	Skin, mucosal epithelia; anti-microbial chemicals e.g. defensins	Cutaneous and mucosal immune systems; secreted antibodies
Blood proteins	Complement	Antibodies
Cells	Phagocytes (macrophages, neutrophils), Natural killer cells	Lymphocytes (B cells that produce antibodies; T cells that carry out cell-mediated reactions)

^aPAMPs: pathogen-associated molecular patterns

The innate immune system includes components that are present and ready for action even before an antigen challenge is encountered. These are cellular and molecular components that are less specific than those of the adaptive system. That is, they are not specific for a particular antigen but react to classes of antigenic substances from microorganisms called pathogen-associated molecular patterns or PAMPs. Several components of the innate immune system must be activated by agonists such as PAMPs, but this activation can occur within minutes or hours rather than days. Therefore, innate responses are quicker, but the immunity they afford may not be as effective over as long a period of time as adaptive immunity. Nevertheless, the innate immune system represents the all-important first line of defense against pathogens and is absolutely essential for keeping an infection in check before adaptive

immunity can be induced. If innate immunity is malignly attacked, the battle against infections is lost from the start.

The specific components form the basis of adaptive immune responses, which involve the actions of B and T lymphocytes. These are the so-called immunocompetent cells of the immune system, because they are able to react to an antigen challenge with a high degree of specificity. Activation of lymphocytes occurs through engagement of receptors to specific antigens on the cell surface. In the case of B cells, these receptors are membrane-bound antibodies. The antigen receptors of T cells are called the T cell receptor (TCR). T cells are further subdivided into T helper cells (Th), which carry the identifying CD4 molecule on the surface and cytotoxic T cells (CTL or Tc), which carry the CD8 identifying molecule. Antigenic signals are transduced from the receptors over signal cascades that are activated in the inner part of the cell, leading in the end to the expression of genes controlling the biosynthesis of products of the cell. Prominent signal cascades operating in cells of the immune system are presented in Figure 1. This activation of lymphocytes to effector cells usually takes five to six days, resulting in the production of antibodies by the B lymphocytes and other effector molecules by the T lymphocytes. In the course of activation, so-called memory cells of both B and T lymphocyte types are developed, which can respond more quickly to antigen during a secondary or later challenge. Thus, adaptive immunity affords a high degree of protection, but it takes time to be induced.

Macrophages occupy a central position in the immune system, being active both in innate and adaptive immune responses. In innate immunity, macrophages are activated through engagement of receptors on the cell surface by substances called agonists. Most prominent among receptors on the macrophage surface are the Toll-like receptors (TLRs). The TLRs derive their name from the similarity with the transmembrane receptor protein Toll in the fruit fly *Drosophila*, which is involved in development and in protecting flies against fungal infections. This has been termed „an ancient system of host defense“.¹⁹

Up to now, 10 different TLRs (TLR1-TLR10) in humans have been described. These molecules contain a characteristic leucine-rich extracellular domain (LLR), which recognizes conserved structures of the microorganisms called pathogen-associated molecular patterns (PAMPs) and leads in the end through a signaling cascade to the activation of genes that control the production of inflammatory cytokines^{20, 21, 22} as depicted in Figure 1.

Macrophages produce type I interferons (α and β), which are essential for a successful defense against many viral infections. They are also potent producers of inflammatory cytokines including interleukin 1 beta (IL-1 β), IL-6 and tumor necrosis factor alpha (TNF α), which mediate reactions designed to fight infections. When these cytokines are produced in moderate amounts, they contribute greatly to defense mechanisms directed against pathogens and to the healing process in general. If they are produced in particularly large amounts or continually during chronic illnesses, this can lead to various disorders such as coronary insufficiency, thrombus formation, hypoglycemia, and in some cases even to shock and death.²³ This makes these activities particularly vulnerable to malign modulation such as by targeting the TLRs to induce hyper-responses, or by inhibiting key components in signaling cascades that would upset the balance. It is interesting in this regard to note that IL-1 was reported to be effective in aerosol form in pulmonary absorption studies carried out by the US Army under its medical research program²⁴ [Rosenberg & Burck, 1990].

1.2.2 Innate Immunity of Plants

Plants also exhibit a type of innate immunity, revealed by their resistance to certain pathogens.^{25, 26} Essentially two kinds of reactions are recognized. One is cultivar-specific, and

involves complementary pairs of pathogen-encoded avirulence genes (*AVR*) and plant-encoded resistance (*R*) genes. The interaction of *AVR* proteins with plant *R* proteins elicits plant defense reactions. The other kind of reaction involves a large variety of microbe-associated products resembling the PAMPs described above for mammalian systems. The vast majority of plant *R* proteins that have been characterized resemble modular structures of the LRR-containing Toll-like receptors or the more recently discovered intracellular nucleotide-binding oligomerization domain (Nod)-LRR proteins also implicated in PAMP recognition in humans.²⁷

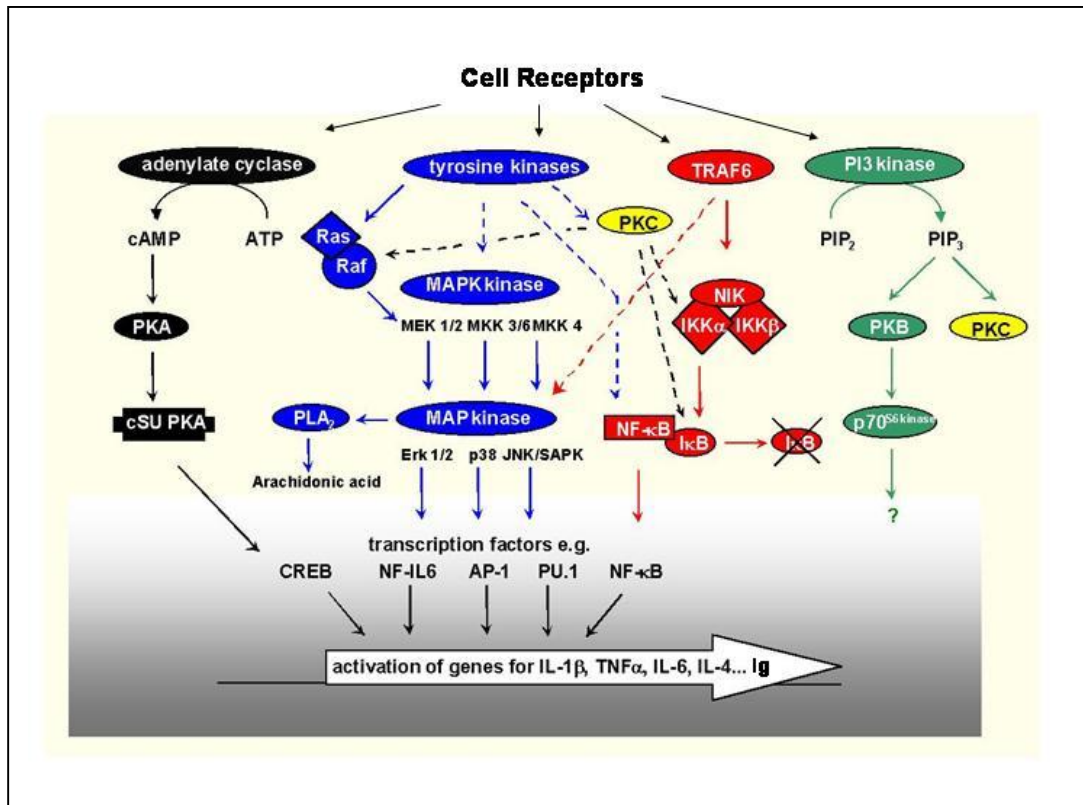


Figure 1. Prominent signal transduction pathways in cells of the immune system. Substances that can activate cells (agonists) bind to receptors for those agonists on the surface of the cells. This induces a reaction that is transferred from the receptors to the inner part of the cells, inducing the activation of kinases, which are enzymes that transduce the signal further. This starts a cascade of subsequent biochemical reactions, leading to the activation of transcription factors (e.g. CREB, NF-IL-6, AP-1, PU.1, NF-κB) that induce expression of genes controlling the synthesis of biologically active substances such as the cytokines interleukin (IL)-1β, IL-6, IL-4, tumor necrosis factor alpha (TNFα) or antibodies (Ig). black, Protein Kinase A signaling pathway; blue, Mitogen Activated Protein (MAP) Kinase pathway; red, Nuclear Factor Kappa B pathway; green, Phosphoinositide 3- Kinase (PI-3K) pathway.

Similar to the macrophages discussed above, plants may be attacked through their innate immune systems, for example by targeting either the receptors of signaling cascades, or by inhibiting or producing an over-reaction in a signaling cascade with the use of inhibitors of key components in that cascade.

1.3 Immune Evasion by Microorganisms

1.3.1 Antigenic Variation

There are numerous documentations of microorganisms that frequently vary their antigenic composition and are thus able to escape mechanisms of immune defense. The mutation rate of antigen genes in several microorganisms is much higher than normal

Apart from antigenic variation due to intrinsic high mutation rates, variants may be selected due to pressures exerted by the immune system. Those antigens that elicit the strongest immune response will be subject to the greatest immune selection pressures, which will effect the emergence of sets of discrete, non-overlapping antigenic variants. On the other hand, if antigens do not elicit immune responses strong enough to select for discrete strain variants, a set of strains might emerge that exhibit cyclical or chaotic fluctuations in frequency over time.²⁸

1.3.2 Additional Immune Evasion Mechanisms

In addition to antigenic variation, viruses in particular have devised a whole array of mechanisms enabling them to evade immune defenses. The large DNA viruses are most successful in this respect.²⁹

One of the most important mechanisms in innate immunity is the complement system. This is a group of serum proteins consisting of around 30 factors that circulate in the serum in an inactive state. Complement can be activated by a variety of specific and non-specific immunologic mechanisms.³⁰ The vital role of complement in immune defense can be seen in individuals with a genetic defect in component C3, a central protein in the complement cascade. This condition has been termed virtually „incompatible with life“.³¹ However, unrestrained complement activation would cause severe damage to bystander cells, so that complement activity is held in check by a variety of membrane-bound and soluble regulatory factors, designated regulators of complement activation (RCA). Members of the poxvirus, herpesvirus and retrovirus families produce homologues that mimic RCA proteins and are thus able to escape complement attack.^{32, 33}

Cytokines and chemokines are soluble substances of relatively small molecular weight produced by cells of the immune system, which act as messengers to regulate and direct a variety of essential steps in immune responses. The activities of the inflammatory cytokines IL-1 β , TNF α and IL-6 have been referred to above. Other cytokines such as IL-10, IL-12, IL-4 and IL-2 are essential in directing the activities of different arms of the immune system, such as humoral vs. cell-mediated responses. One of the most interesting mechanisms identified in recent years is the mimicry of cytokines and cytokine receptors by large DNA viruses (herpesviruses and poxviruses).³⁴ Chemokines are small proteins that play a key role in the recruitment of immune defense cells into areas of injury or infection during an inflammatory response. Poxviruses use essentially three strategies to modulate chemokine functions: (1) through the production of virus-encoded chemokine-receptor homologs, (2) through the production of virus-encoded chemokine homologs and (3) through the production of virus-encoded chemokine-binding proteins.³⁵

A further immune evasion strategy includes the production of a variety of viral inhibitors of apoptosis, the so-called programmed cell death. In addition, cytotoxic T cells or Tc cells recognize a cell that has been infected by a virus through the presentation by that cell of fragments of viral proteins bound to major histocompatibility complex (MHC) molecules of class I on the surface of the infected cell. This recognition leads to the activation of Tc lymphocytes which attack and kill the cell through the induction of apoptosis. Among other things, viruses can interfere with antigen fragment processing or cause the downregulation of MHC I molecules, which would protect the cell from cytotoxic T lymphocyte destruction.³⁶

Alternatively, viruses such as cytomegalovirus upregulate the expression of a non-classical MHC-I molecule that can bind an inhibitory receptor on the surface of natural killer cells, inhibiting this innate response to infection.³⁷

1.4 Dual-use Aspects of Biomedical Research

There are four categories of manipulations or modifications of microorganisms and their products that have been the subject of discussion since the onset of the development of genetic engineering: (1) the transfer of antibiotic resistance to microorganisms, (2) modification of the antigenic properties of microorganisms, (3) modification of the stability of the microorganism toward the environment and (4) the transfer of pathogenic properties to microorganisms.^{38, 39} All four types of manipulations are being carried out daily in research programs that have legitimate and basically peaceful aims, such as the elucidation of the mechanisms of microbial pathogenesis. This research is essential for developing better means of combatting infectious diseases. At the same time, these techniques can be misused to produce new types of biological agents that could be used as weapons. In order to focus more directly on the dangers involved, two specific examples of work from the recent literature that have produced dangerous microorganisms which are able to evade vital immune mechanisms will be examined.

1.4.1 Accidental creation of a „killer“ mousepox virus

The potential dangers that may be associated with biological research are particularly evident in recent studies in the area of immunology. The headlines in the journal *New Scientist* proclaimed „Disaster in the making. An engineered mouse virus leaves us one step away from the ultimate bioweapon“.⁴⁰ The report was about experiments carried out by Australian researchers who tried to make mice infertile, as a model for controlling rodent populations.^{41, 42} The experimental strategy was to incorporate a gene for the production of a protein that is found on the surface of egg cells of the mouse into the genome of a mousepox virus, against which the mice used in the experiment were resistant. When the mice were infected with the recombinant virus, the egg cell protein was over-produced, and an antibody response to that protein was mounted, which was supposed to cause infertility in the mice. Indeed, the expected antibody response occurred, but it was short-lived.⁴³ In order to boost these antibody responses and prolong their effects, another gene was introduced into the mousepox virus genome. This gene was to direct the production of a substance called interleukin 4 (IL-4), which is known to enhance antibody-type immune responses. However, IL-4 also suppresses the activation and expansion of another type of T-lymphocyte (Th1) that provides essential help to cytotoxic T-lymphocytes (CTL or Tc) needed to fight viral infections. When mice were infected with the recombinant virus, the IL-4 produced did boost antibody responses to the mouse egg protein, but at the same time it also suppressed the activation of CTLs. As a result, most of the mice (60 %) died, even though they were supposed to be resistant to the virus.⁴⁴

The mousepox virus is not infective for humans. However, there is some concern that the same manipulations might be performed on a pox virus that does infect humans, with devastating results.

This work has been continued by Mark Buller, a professor at St. Louis University. Buller constructed a recombinant mousepox virus containing the IL-4 gene that was even more deadly than the one made by Jackson and co-workers. By placing the IL-4 gene in a region of the virus genome that was dispensable and by optimizing its expression, 100 % mortality of

the resistant mice was achieved with the recombinant virus construct. Buller's stated motivation was to explore possible prophylactic and therapeutic defenses against such an agent. However, vaccination or treatment of mice with the antiviral substance cidofovir plus antibodies to IL-4 still did not protect them adequately against a challenge with the highly virulent mousepox constructs.⁴⁵

Now he has apparently gone one step further to alter the cowpox virus, which can infect humans, in a similar way⁴⁶ Buller asserted, however, that this virus would only be lethal in mice and not in humans, because he used the mouse IL-4 gene, which is specific for only the mouse immune system. The head of the Australian research team, Ian Ramshaw, maintains that there was no reason to do the cowpox experiments. He further cautions that while viruses containing the mouse IL-4 gene should not be lethal in humans, recombinant viruses can have unexpected effects. Indeed, it has been pointed out⁴⁷ that these experiments fall into several categories listed by a recent National Research Council Report⁴⁸ as being research of particular concern.

1.4.2 Potentiation of the Virulence of Vaccinia Virus

The smallpox virus *Variola major* causes a serious, virulent infection in humans, while the virus that is used for vaccination against smallpox, vaccinia virus, usually causes only a very mild or even inapparent infection, at least in individuals with an intact immune system. A probable virulence factor for the smallpox virus is the smallpox inhibitor of complement enzymes (SPICE). This component has the ability to inactivate human C3b, one of the key complement components that serve to induce phagocytosis, thus attacking innate immunity in a vital area. Vaccinia virus also has a complement regulatory protein called vaccinia virus complement control protein (VCP), which is, however, much less effective (100-fold less) than SPICE. In the work described,⁴⁹ researchers mutated the VCP gene of vaccinia virus to have the same nucleotide sequence as SPICE. The recombinant mutant VCP proved to be much more efficient than normal VCP in inactivating complement in vitro. Although the researchers did not actually outfit vaccinia virus with this mutated gene, the work was only one step away from this manipulation. Presumably, vaccinia virus with the mutated gene would be much more pathogenic.

These experiments illustrate the absolute dual use dilemma of research in the biotech sector. While such experiments create microorganisms that pose a greater risk than do normal ones and the advisability of their undertaking is certainly open to question, there may at the same time be benefits from such research. In this respect, the National Research Council report states that "even experiments that have the greatest potential for diversion to offensive applications or terrorist purposes may also have potentially beneficial uses for public health promotion and defense".⁵⁰ This points up the difficulty in imposing blanket prohibitions on certain research activities from the start, but clearly emphasizes the need for oversight of research of BW relevance. These aspects will be discussed further in the last chapter.

1.5 Future Threats

1.5.1 Targeted Delivery Systems: Gene Vectors and Immunotoxins

Targeted delivery systems are components that allow an activity to be targeted to a particular site in the body where its activity is desired. An example of such a system are viruses that are used as vectors to transfect a foreign gene into cells for the purpose of immunization or for gene therapy. The gene would become active in infected cells, leading to the production of the

gene product. Vaccinia virus has been investigated for these purposes because of its large genome, which can carry several foreign genes at once, and its effectiveness as a vaccine.^{51, 52} There has been a great deal of work in recent years on the possibility of using adenoviruses as gene vectors. These viruses can be produced at high titers (up to 10^{10} per milliliter) and they also have a carrying capacity of up to 40 kb of insert DNA.^{53, 54} Alternatively, the development of adeno-associated viruses as vectors for gene delivery seems promising, as these viruses are defective by nature and have thus never been shown to have any pathogenic effects in humans.⁵⁵ However, latest investigations have shown that these viruses do indeed integrate into the host genome more frequently than presumed, which might lead to detrimental mutations including the induction of cancerous states,⁵⁶ so that there are still serious safety concerns about the use of these vectors.

It is conceivable that the immune system could be attacked by outfitting viruses with specificities for immune cells (e.g. the specificity of the AIDS virus) and toxin genes.

Another prime example of a targeted delivery system are immunotoxins. These are molecules that contain the antigen binding specificity portion of an antibody molecule coupled to a toxin molecule. The aim is to target the toxin activity to specified cells, such as tumor cells; in this case, the antibody specificity is directed against tumor cell antigens.⁵⁷ An example of an immunotoxin using ricin as the toxic component is presented in Figure 2.⁵⁸

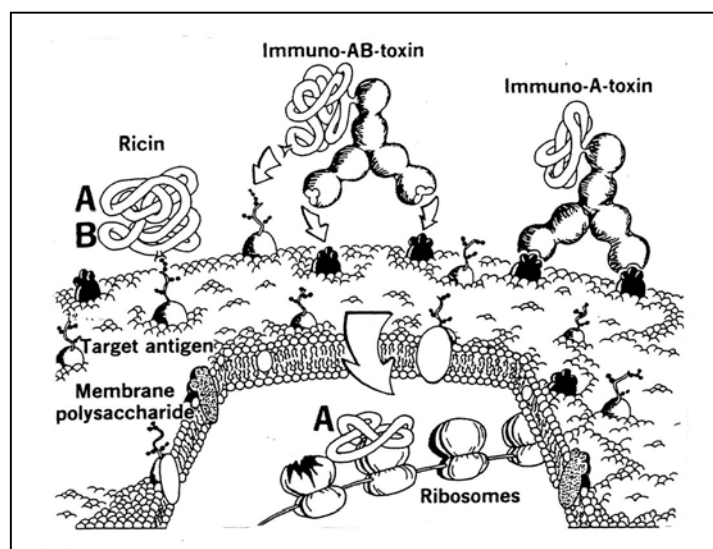


Figure 2. Schematic representation of ricin immunotoxins. Ricin is composed of an A chain that is the toxic part of the molecule and a B chain that binds to a specific receptor on the cell surface (left part of the figure). An immunotoxin consisting of the AB-toxin bound to antibody is shown in the middle of the figure. The antibody binds specifically with particular receptors on the cell surface (dark structures). The A chain alone is non-toxic, because it cannot bind to the cell. When coupled to the antibody by a chemical reaction (Immuno-A-toxin), the A chain can be targeted to the cell surface via the antibody and taken up by the cell. It can subsequently interact with ribosomes, the protein synthesizing factories of the cell (bottom part of the figure) to inhibit protein synthesis. Source: Poncelet et al., 1984, used with kind permission from Hoechst AG.

Alternatively, a fusion protein could be produced that consists of an antigen (ligand) for a receptor fused to the toxic fragment of a toxin molecule. New strategies to reduce immune reactions against the immunotoxins have been developed.⁵⁹

It should be mentioned at this point that aerolization of vectors carrying foreign genes could represent an effective delivery system, especially if the vector is a virulent microorganism, as most infections begin at the mucosa. If the vector is not a microorganism,

such as in the case of fusion proteins or immunotoxins, successful delivery by the aerosol route would depend greatly upon the physical-chemical properties of that vector. In its medical research work on endogenous bioregulators the US Army has for example reported that the hormone insulin and the cytokine interleukin-1 were effective in aerosol form in basic pulmonary absorption studies.⁶⁰

Targeted delivery systems have to be characterized as being strongly dual-purpose. While they may be potentially very useful in vaccine and gene therapy, they can also serve as delivery vehicles for toxins or bioregulators in a negative way.

1.5.2 Immunization with plant foods

There is at present a great deal of interest in developing vaccines as plant foods. This involves the transfer of a gene encoding the antigen of interest into the genome of plants, with subsequent expression of that gene and biosynthesis of the antigen in the plant tissues. Eating the plant tissues would then deliver the antigen to the gut, where it would be taken up by special epithelial cells of the small intestine (M cells) and transferred to the underlying lymphoid tissues, resulting in an immune response to that antigen. There would be several advantages of inducing an immune response in this way, including increased safety, economy and stability of the vaccine, as well as the prospect of inducing mucosal immunity (to localize immunity at mucous membrane sites, where most infections begin).^{61, 62}

There are, however, numerous technical and immunological hurdles that have to be overcome in order for plant vaccines to be practical. One of the first is the avoidance of degradation of the antigen in the digestive tract. Even if the antigen would survive this degradation, oral tolerance mechanisms would have to be overcome which prevent immune responses to the microorganisms residing in the intestine or to protein antigens acquired continually in food. Furthermore, oral immunization usually requires multiple doses in larger amounts than antigen administered over parenteral routes; responses are weak, unreliable and also shorter lived.^{63, 64} Indeed, results to date show that immunization with plant foods is in some cases possible, but the responses are usually modest and appear only after more than one dose.

This discussion serves to illustrate that immunization with plant foods is by no means readily achievable. In this regard, it is unlikely that these techniques can be used successfully in the very near future in malign ways, e.g. for vaccination of unaware populations, thus forcing upon them an involuntary immunity or marking them as possible targets. Nevertheless, there is great interest in developing such vaccines for peaceful use and improvements are actively being sought,^{65, 66} so that developments in this area should be closely monitored.

1.5.3 Vulnerability of the Immune System to Modulation after Immunization

Activation of the immune system in response to an infection is a vital step in countering the threat posed by the causative agent. Nevertheless, activation of components of the immune system is invariably associated with the enhanced production or exposition of predictable markers, that could serve as targets for the delivery of a biological weapon to those sites.

B and T lymphocytes are produced during development and prior to encountering antigens by rearrangement of genes to yield an enormous number of clones, each expressing a unique receptor (membrane-bound antibodies in the case of B cells and the TCR in the case of T cells) recognizing a particular antigen epitope and thus being able to respond to that antigen.⁶⁷ Initially, only a small subset of these clones (estimated at around 0.1 percent) are able to

recognize any one particular antigen.⁶⁸ To generate effective immunity, these naive or resting B cells and T cells must undergo clonal expansion in response to an antigen challenge in order to amass the numbers required to counter an infection. This represents a considerable expansion of antigen-specific lymphocytes in response to immunization, especially when a vaccine is given in several doses over a period of time.

These expanded clones of B and T lymphocytes have an enhanced vulnerability, for example, to being targeted with constructed toxins as discussed earlier (targeted delivery systems). For delivery to B cells, a delivery system might be a fusion protein consisting of the specific antigen (against which the B cells are directed) fused to the toxic chain of a toxin molecule (such as the A chain of ricin or diphtheria toxin). However, since B cells release antibodies to the antigen, the construct might be neutralized and cleared by these antibodies before it could do much damage. T cells might be a more vulnerable target, as they do not secrete their antigen receptors. However, the delivery system containing the toxin would have to be constructed in a way as to include the specific antigen fragment bound to MHC molecule epitopes in order for it to be recognized and engaged by the T cell. This would be a tall order at present, but new studies are providing greater insight into the fine points of the recognition of antigen presented by MHC molecules to T cells⁶⁹ that could make this approach more cause for concern in the future.

In addition to the expansion of specific antigen receptors, immunization also up-regulates the exposition of an array of molecules on the surface of lymphocytes and macrophages. Prominent ones include MHC molecules on lymphocytes and macrophages, CD40 on B cells and macrophages, or CD28 and CD40L on T cells. All of these would be vulnerable to attack for example with immunotoxins consisting of antibodies to these surface components bound to the toxic chain of a toxin.

Whereas most protein antigens are recognized only by a small fraction of lymphocytes, a number of natural proteins have been described that can react with a significant proportion of T cells (up to 5 % of the T cell population)⁷⁰ and some that can bind with up to 50 % of the B cell population.⁷¹ Through binding of these so-called superantigens, the cells undergo an increased rate of apoptosis or cell death. The possibilities for misuse here are intricately involved with dual use aspects of targeted delivery systems.

1.5.4 Vulnerability of the Immune System in Interaction with the Neuroendocrine Systems

It is being recognized more and more that the the immune system interacts intricately and extensively with the nervous and the endocrine systems. This topic will be dealt with more thoroughly in the next section, but suffice it to say here in the present context that there is a fine network of checks and balances exerted on the operation of all three systems by the elements within these systems. The perturbation of one system will invariably affect the operation of the others. The immune system is particularly vulnerable to modulations of the nervous/endocrine systems by bioregulators which include substances active in the nervous system affecting behaviour.⁷² All three systems are interconnected through the hypothalamus-pituitary-adrenal (HPA) axis via cytokines, hormones, neurotransmitters, peptides and their receptors, and also through hardwiring of neural and lymphoid organs and even cells of the immune system themselves.⁷³

Some recent work illustrates the fact that these systems have apparently evolved together over time during the evolution of complex multicellular organisms. Anti-microbial peptides represent an ancient form of defense, involved in innate immunity as discussed earlier.

Similarities between pathogen recognition, signalling pathways and effector mechanisms in innate immunity of insects, mammals and plants have been referred to above. In this regard, newly characterized neuropeptides that exert anti-bacterial and anti-fungal activities have been reported recently.⁷⁴ These peptides are therefore active not only in the neuroendocrine system, but also in innate immunity and most likely play a role in inflammatory processes.

2. Neuroscience - The Threat from Incapacitating Biochemical Agents

2.1 Introduction

In October 2002 a group of Chechen separatists took control of a Moscow theatre and held 800 people hostage. After three days the Russian authorities ended the siege by pumping an aerosolised chemical incapacitating agent into the auditorium through the ventilation system. Allowing at least 30 minutes for the agent to take effect on hostages and hostage-takers alike, troops then stormed the building and shot and killed the majority of the hostage-takers.⁷⁵

A number of months prior to this incident, in response to concerns over commercial airline security following the events of 11 September 2001, the US National Institute of Justice (NIJ) completed a report entitled *Less-Than-Lethal Weaponry for Aircraft Security*.⁷⁶ The Director of NIJ summarized the conclusions of the report in a statement to the House of Representatives. In the section covering the potential for use of chemical incapacitating agents she stated that:

“Anesthetics or calmativive chemicals could, in principle, be developed into a system whereby they could be remotely released into the cabin in order to incapacitate all passengers, and the hijackers, until the plane can be landed safely.”⁷⁷

Unfortunately there was not a safe outcome in Moscow. Over 120 hostages died as a result of exposure to the incapacitating agent and many survivors needed hospital treatment.⁷⁸ This incident emphasized the danger of devising a discrete ‘non-lethal/less-lethal’ category for chemical incapacitants that would separate them from other toxic chemicals with the potential to cause lethal effects.⁷⁹ Also, you only have to alter the aircraft scenario slightly to see the problems that could arise if the hijackers rather than the airline were armed with chemicals that could incapacitate everyone on the plane. Experts have warned of this ‘double-edged sword’⁸⁰.

After an inexplicable delay of several days after the Moscow siege the Russian Health minister finally released the identity of the agent used, stating that it was a ‘fentanyl-based’ compound.⁸¹ Although there is some debate as to whether it was a mixture of compounds or perhaps a novel agent⁸², a number of experts believe that carfentanyl, an analogue that is 30 times more potent than fentanyl, was most likely a major constituent.⁸³ Fentanyl and analogues are synthetic opioid analgesics that exert their major effects through action on μ opioid receptors in the *central nervous system (CNS)*. The main side effect of fentanyl, which is commonly used in clinical anaesthesiology having been introduced in the 1960’s⁸⁴, is respiratory depression. This is thought to have been a major factor in the death of so many in Moscow. The effect of opioid agonists such as the fentanyls can be reversed by the non-selective opioid antagonist, naloxone. One recent paper discussing the implications of events in Moscow commented:

“In the United States, naloxone, for a long time a critical antidote to treat heroin overdose and iatrogenic opioid toxicity, has now become a crucial component of our chemical warfare antidote repository.”⁸⁵

Before looking at some of the potential agents we could face now and in the not-too-distant future, it is worth emphasising the overlap of chemistry and biology in this area. Substances that can influence CNS functions by action on specific receptor sites can have either a synthetic chemical origin or a natural biological origin. Wheelis has termed these substances potential *biochemical weapons*.⁸⁶

2.2 Possible Modification of Traditional Agents

Military interest in incapacitants has a long history.⁸⁷ Fentanyl was being investigated as a potential weapon by the US military in the 1960's.⁸⁸ Other agents under consideration by the UK and US at this time were a group of psychoactive compounds called the glycolates⁸⁹ that interfere with acetylcholine metabolism. One of these, BZ (3-quinuclidinyl benzilate), was subsequently weaponized by the US.⁹⁰ There are also reports that the Former Soviet Union developed a derivative of BZ as a weapon⁹¹ and Iraq's chemical weapons program may have incorporated a related glycolate compound known as Agent 15.⁹² BZ was eventually rejected by the US as a suitable weapon due to its non-specific and unpredictable effects.⁹³

Since then there have been significant developments in neuroscience. The 1980's saw the identification of numerous peptide neurotransmitters that mediate chemical transmission in the nervous system alongside 'classical' neurotransmitters such as acetylcholine. It is work during the past 10-15 years that has revolutionized the field however. The impact of genomics has led to a greater understanding of receptor systems and the elucidation of the structure and function of certain receptor sub-types that have now become targets for therapeutic drugs. Concurrently another enabling technology, combinatorial chemistry, has allowed the screening of large numbers of compounds to find those affecting these specific receptor targets.⁹⁴ As well as offering the opportunity to develop more effective new drugs to treat a variety of mental illnesses, as is a priority of the global pharmaceutical industry, this knowledge is of course dual use.⁹⁵

Might the cholinergic system in the CNS now be targeted more specifically by weaponizers? The muscarinic acetylcholine receptors (there are 5 sub-types), which are thought to have a CNS role in motor control, temperature regulation, cardiovascular regulation, and memory, are potential targets.⁹⁶ The M2 inhibitory autoreceptor regulates levels of acetylcholine release at muscarinic synapses and it has been suggested that a specific and potent agonist for this receptor could impact these fundamental acetylcholine-mediated processes in the body.⁹⁷

Military interest in incapacitants never receded⁹⁸ but it has gained new impetus through these scientific advances. Events in Moscow are likely, if anything, to have heightened this attention.⁹⁹ One of the main recommendations of the 2003 report on non-lethal weapons (NLWs) science and technology, compiled by the Naval Studies Board of the US National Academy of Sciences (NAS), was for increased research on incapacitating chemicals, or 'calmatives' as they are termed by the US military, and their delivery systems.¹⁰⁰ The report indicated that calmatives are now being studied at the US Army Edgewood Chemical Biological Center (ECBC) after a "...lull in R&D for 10 years". One project is a sponge projectile designed to deliver a 'dose' of a fentanyl derivative.

In October 2000, two years before the Moscow siege, The Applied Research Laboratory at Pennsylvania State University, whose scientists have worked closely with the Joint Non-Lethal Weapons Directorate (JNLWD) of the US military for a number of years, published a report entitled *The Advantages and Limitations of Calmatives for Use as a Non-Lethal Technique*.¹⁰¹ It points out that potential calmatives are "...compounds known to depress or inhibit the function of the central nervous system", including "...sedative-hypnotic agents,

anesthetic agents, skeletal muscle relaxants, opioid analgesics, anxiolytics, antipsychotics, antidepressants and selected drugs of abuse.” Their analysis of the available literature identified several classes of compound they considered to have high potential for use as 'non-lethal' calmatives. These, along with their sites of action in the nervous system, can be seen in Table 2.

Table 2. Selected calmatives¹⁰²

Drug Class	Site of Action
Benzodiazepines	GABA receptors
Alpha ₂ Adrenergic Receptor Agonists	Alpha ₂ -adrenergic receptors
Dopamine D3 Receptor Agonists	D3 receptors
Selective Serotonin Reuptake	5-HT transporter
Serotonin 5-HT _{1A} Receptor Agonists	5-HT _{1A} receptor
Opioid Receptors and Mu Agonists	Mu opioid receptors
Neurolept Anesthetics	GABA receptors
Corticotrophin-Releasing Factor	CRF receptor
Cholecystinin B receptor antagonists	CCKB receptor

Many of these classes of compounds could clearly be used for harmful purposes. We have already discussed the effects of μ opioid agonists in the context of fentanyl (and analogues). The report examines a drug called dexmedetomidine, which is a selective agonist of the α_{2A} adrenergic receptor, the sub-type that plays an important role in sedation.¹⁰⁵ Work by the US military during the 1990's to develop α_2 adrenergic agonists as weapons for the non-lethal weapons program has been documented.¹⁰⁴ Neuropeptide transmitter systems are also discussed in the report. CCK-B receptor agonists can induce panic attacks in humans and the authors suggest the use of CCK-B antagonists as potential anxiolytic calmative agents. Another neuropeptide, Substance P (not mentioned in the report), is thought to be involved in depression and anxiety and it has been suggested that, since receptor antagonists reduce these systems, agonists may induce them.¹⁰⁵ Clearly there may be opportunities for misuse of potent selective agonists affecting these two receptor systems.

One 'classical' neurotransmitter that receives attention in the Pennsylvania State report is serotonin (5-HT). Serotonin is widely distributed in the nervous system and has been implicated as having a role in many types of human behaviour.¹⁰⁶ Of interest to those developing incapacitants is its role in sleep, mood, and aggression. One document that has recently come to light is a research proposal from 1994 from the US Army Edgewood Research, Development and Engineering Center (ERDEC) (now Edgewood Chemical Biological Center (ECBC)) that sets out an idea for a potential calmative.¹⁰⁷ In the proposal, a calmative is defined as:

“... an antipersonnel chemical that leaves the victim awake and mobile but without the will or ability to meet military objectives or carry out criminal activity.”¹⁰⁸

It goes on to report the observations of a University Professor of Anaesthesiology on the “profound calming effect” of a serotonin antagonist, structurally similar to ketanserin, in wild

elk that are normally unapproachable.¹⁰⁹ It is suggested in the proposal that this chemical or a related compound "... should be an ideal candidate calmativ agent." The first part of the feasibility study proposed was to carry out a literature search:

"...to correlate chemical structure of serotonin antagonists to serotonin receptor subtypes" and to "... determine receptor subtype connected with both desired and undesired pharmacological effects."

The exact mechanisms by which serotonin affects certain behaviours such as aggression is not fully understood. However, human and animal studies have shown that increased serotonergic function is associated with decreased aggressive behaviour and vice-versa.¹¹⁰ Studies in animals have provided other insights; in monkeys:

"It is clear that serotonin does not simply inhibit aggression; rather, it exerts a controlling influence on risky behavior, which includes aggression."¹¹¹

Having reviewed the literature in this area the authors of the 2000 Pennsylvania State study point out that:

"It is hypothesised that the increase in the amount of serotonin leads to improved control of behaviours linked to this transmitter system, which include aggression, agitation, anxiety, general affect (mood), and sleep, among others."¹¹²

One potential calmativ technique they suggest is the use of a selective 5-HT_{1A}receptor antagonist, which "... would reduce symptoms of anxiety in an individual or individuals and promote a calmer and more compliant behavioral state."¹¹³ One such compound, buspirone is used clinically to treat anxiety, and they note that numerous others are under development in the pharmaceutical industry.

As for the two military proposals in 1994 to develop specific incapacitant weapons including those acting on the 5-HT system (the other was to develop synthetic opioid agonists), their fate is unclear.¹¹⁴ However, the author of those proposals subsequently worked as a senior researcher at Optometrics Inc., which won a contract with the Department of Defense in early 2000 to carry out the first phase of study to assess incapacitants for use in military and law-enforcement applications.¹¹⁵ This phase, which is now complete,¹¹⁶ is described in the contract solicitation as follows:

"Phase I studies will consist of a Front End Analysis comprising the following elements: review existing data on the candidate agents; define scenarios of use and operational parameters; conduct range finding toxicological animal tests, and correlate results with those from previous studies."¹¹⁷

Meanwhile, objectives listed in the JNLWD's Technology Investment Project for 'Front End Analysis of Non-Lethal Chemicals' for the fiscal year 2001/02 included:¹¹⁸

- Identify advances in the pharmaceutical industry and elsewhere for potential non-lethal applications
- Conduct military user workshops to identify range of desired operational effects
- Create a searchable database of potential candidates
- Provide a list of promising candidates to Judge Advocate General's office for preliminary legal review

Writing in early 2003, the University Professor who had contributed to the 1994 proposal to explore serotonin antagonists as incapacitants reflected on events in Moscow. Recognizing the dangers of employing fentanyl and other opioids he goes on to say:

“However, remarkable progress has been made in the techniques to deliver immobilizing agents and in the development of safer, faster-acting potent compounds of extremely short duration in the last decade. Much of this work is either privileged or currently not available to the public and therefore unpublished.”

2.3 Future Threats - Possible Advanced Biological Warfare Agents (ABWs)

A 2001 review of bioregulators with potential for use in bioterrorism emphasized the varied nature of these compounds:

“Bioregulators are structurally diverse compounds that are capable of regulating a wide range of physiologic activities, such as bronchial and vascular tone, muscle contraction, blood pressure, heart rate, temperature, and immune responses.”¹¹⁹

Those reviewed included cytokines, eicosanoids, plasma proteases, neurotransmitters and hormones. It is important to place this discussion in historical perspective. The Soviet biological weapons effort, ostensibly halted in 1992, included programs, championed by the most influential biomedical scientist of the time, to develop bioregulators as weapons:^{120, 121}

“He [Yuri Ovchinnikov] saw a way around arms control treaties and weapons conventions by using microbes to produce biologically active substances that would replace classic chemical weapons; their production could then be concealed in the biotechnology or pharmaceutical industry.”¹²²

But what of the systems biology approach to agent design of which Petro et al. warn, that may enable targeting of certain biological processes to produce a variety of effects including “...death, incapacitation, or neurological impairment.”¹²³

Some examples can be seen from consideration of the interconnectivity between the nervous, immune, and endocrine systems. In the past 25 years it has emerged that immune regulation is influenced by the brain and that neural and endocrine functions are influenced by the immune system.¹²⁴ These systems also share the same means of communication through hormones, neurotransmitters, cytokines, and their respective receptors.¹²⁵ One well-known communication ‘route’ between these systems is the hypothalamic-pituitary-adrenal (HPA) axis, which is the major system co-ordinating the body’s response to stress.¹²⁶ Under conditions of stress the hypothalamus region of the brain releases corticotrophin-releasing factor (CRF), which in turn causes the release of adrenocorticotrophin hormone (ACTH) from the pituitary gland. ACTH in the blood results in release of glucocorticoid hormones that regulate metabolism and immune function. Glucocorticoids have a negative feedback effect on CRF and ACTH release. Other neurotransmitters are also involved in regulating the HPA axis. It is known that disturbances in this system have significant effects: over stimulation of the HPA axis and excessive production of glucocorticoids leads to immune suppression and increased susceptibility to infection while under stimulation resulting in lower glucocorticoid levels can lead to inflammation and autoimmune conditions.¹²⁷ Clearly, this system is open to influence at several levels and could be a target of weapons designers. In addition, a dual-acting weapon could combine a substance that suppresses immunity with a pathogenic microorganism for increased effect, or a non-pathogenic bacterium with a plasmid expressing a gene for say CRF production might cause immune suppression in the target person(s). The Pennsylvania State report looked at the actions of CRF in the brain alone rather than within the HPA axis. They propose that CRF antagonists might be used to produce “...a calm behavioral state” because of the role of CRF1 and CRF2 receptors in the brain in anxiety and stress.¹²⁸

Another example of potential interference at the systems level relates to cytokines. One recent review of psychoneuroimmunology pointed out that chronic inflammation, marked by increased production of proinflammatory cytokines, "...had been suggested as one key biological mechanism that may fuel declines in physical function leading to frailty, disability, and, ultimately, death."¹²⁹ Interleukin-6 (IL-6) is especially implicated in this process since "...the age associated rise in IL-6 has been linked to lymphoproliferative disorders, multiple myeloma, osteoporosis, and Alzheimer's disease."¹³⁰ Furthermore, it has been suggested that IL-6 may be a general marker of health deterioration.¹³¹ Interestingly depression and stress have also been seen to increase production of IL-6.¹³² Might a weapon agent that enhances IL-6 production and thereby increases chronic inflammation actually contribute to a 'speeding up' of the ageing process?

3 Anti-Animal Threats

3.1 Introduction

This section of the paper will address the threat of anti-animal biological warfare. The 2001 epidemic of foot-and-mouth disease (FMD) in the UK provides a contemporary example of the continuing impact of animal disease outbreaks. A brief history of anti-animal biological warfare (AABW) is provided to establish the precedent of the deliberate instigation of animal diseases. General themes, drawn from this history, enable a discussion of the impact of advances in the biological sciences and their implication for the anti-animal threat. This is highlighted by an examination of the potential role of prions in AABW. The section concludes with an examination of the potential for a future threat from this form of warfare, with particular attention being played to the role of bioinformatics.

3.2 The 2001 FMD Epidemic in the UK

On 20 February 2001, the presence of FMD was confirmed in Essex marking the beginning of the 2001 epidemic in the UK. The disease spread rapidly, the number of confirmed cases rose almost exponentially for the first five weeks. The rate at which new cases occurred then peaked and began to decline (Figure 3). The disease remained prevalent in the UK for an extended period, demonstrating the difficulties in eradicating highly infectious animal diseases.

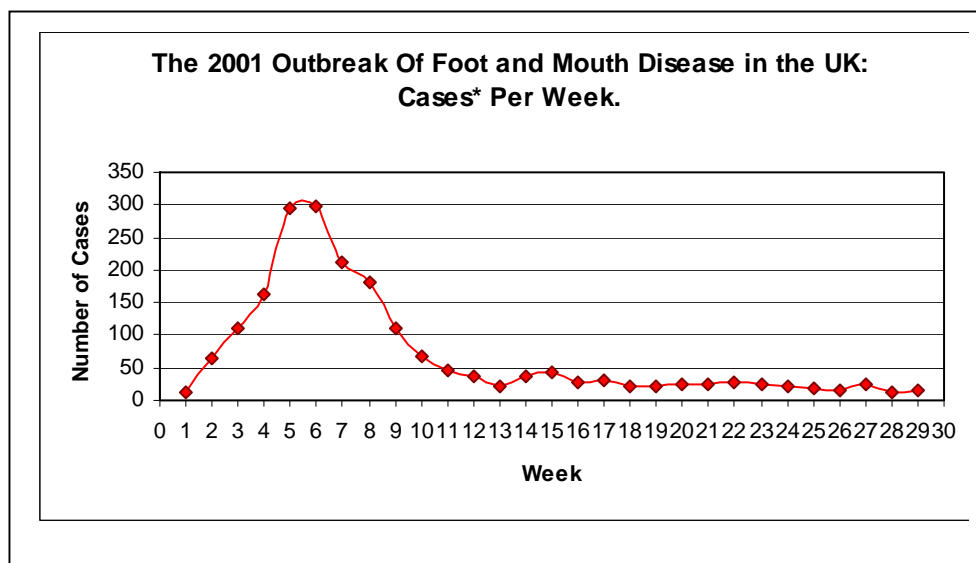


Figure 3. The number of confirmed cases per week during the first 29 weeks of the 2001 outbreak of FMD in the UK.

*A case represents the confirmation of the presence of the virus in at least one animal at a given facility (e.g. farm, slaughter house, market, etc).

33 counties were involved during the first fifteen weeks of the outbreak (Figure 4), which included 1,697 confirmed cases, involving 1,012,242 animals. The epidemic eventually resulted in over 3.5 million animals being slaughtered. 2.3 million of these were culled for preventative purposes - clearly demonstrating that responses to outbreaks of highly infectious animal diseases can prove more costly than the infections themselves. Other indirect effects

worthy of note include the suicides of farmers, the postponement of the General Election, estimates that the cost of the epidemic would surpass £40 billion, fears over air and water pollution through the disposal of carcasses, and the withdrawal of UK forces from NATO exercises to prevent the spread of disease.¹³³

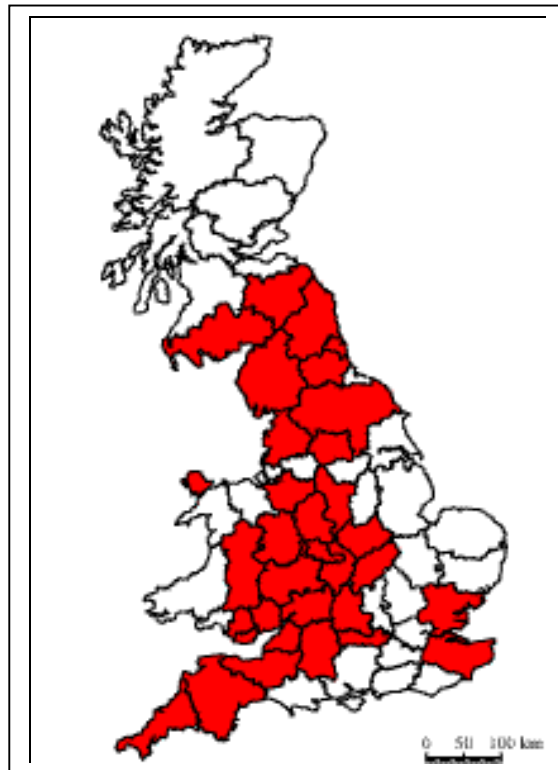


Figure 4. Counties in the UK which suffered from FMD during the 2001.epidemic

2.2 Historical Precedents of the Anti-Animal Threat

Wheelis¹³⁴ established that anti-animal biological sabotage (AABS) operations were carried out by Germany in at least five countries during World War I. It would appear France was also engaged in similar activities and surviving intelligence archives in the UK indicate a number of other European countries, including the Netherlands, Belgium and Eire, had indicated their interest in pursuing this form of biological warfare.¹³⁵

During World War II several other countries became interested in anti-animal biological warfare. Project Vegetarian, involving the construction of anthrax-laced cattle cakes by the UK, has been well documented.¹³⁶ The Japanese developed tactical munitions designed to infect a variety of targets, including animals.¹³⁷ Simultaneously, Unit 100 - the Kwantung Army Anti-Epizootic Protection of Horses Unit, conducted research into “*the mass extermination of animals*”.¹³⁸ It also appears that Germany was close to obtaining an anti-animal capability by 1945.¹³⁹

After World War II and throughout the Cold War, the US engaged, with varying degrees of conviction, in a range of AABW projects which produced strategic, tactical and sabotage weapons. The UK appears to have redirected its efforts to focus on offensive foot-and-mouth disease research.¹⁴⁰ Little is known about the AABW components of the program of the

former Soviet Union, but Alibek has alleged that “A special division was established to research and manufacture anti-livestock and anti-crop weapons”.¹⁴¹

Since the end of the Cold War other programs have come to light, including one run by South Africa. Although there are no details indicating the presence of an AABW component, allegations have been made, and are yet to be disproved, connecting an unusual veterinary anthrax outbreak in the region to this program. It also appears that UNSCOM did not investigate the existence of AABW activities in Iraq.¹⁴² It is unclear whether UNMOVIC or the Iraq Survey Group have examined such a possibility.

3.3 Modern Advances and their Implication for the Anti-Animal Threat

There are a number of general themes which can be drawn out of this history. Elucidating these ‘rules of thumb’ may provide an insight into the nature of the threat posed by these weapons in the future and the possible effect of the revolution in the biological sciences. These include, firstly, two separate methodological approaches to this form of warfare:

- (1) ‘Military’ AABW programs - included the mass production of the agent, delivery devices, and required a degree of control over the resulting disease outbreak. Such programs saw the development of strategic, tactical, and point-source weapons.
- (2) ‘Clandestine’ AABW programs – were designed to induce outbreaks through AABS. These programs relied heavily on the characteristics of the agents, allowing more rudimentary agent production and minimal dispersal technology. They often saw the initiation of epidemics and/or the creation of endemic status as desirable outcomes and more closely resembled a ‘bioterrorist’ threat.

Secondly, AABW has been targeted tactically (to impede military utility), socially (to disrupt food production), and economically (to induce financial burdens).¹⁴³ Situations may exist in the world where each targeting approach is still desirable.

Thirdly, there are desirable characteristics for ‘military’ and ‘clandestine’ biological weapons. Some indicative characteristics are listed in Table 3

Table 3: Some Desirable Characteristics of Military and Clandestine Anti-Animal Biological Weapons

Military Programmes	Clandestine Programmes
An agent should produce a known effect consistently.	An agent should produce a known effect consistently.
The dose needed to produce the effect should be low.	An agent should be highly infectious
There should be a short predictable incubation period.	There should be a long sub-clinical infectious period.
The target population should have little or no immunity.	An agent should pose a significant threat to livestock production (or associated industries)
Treatment for the disease should not be easily available to the target population.	The dose needed to produce the effect should be low.
The user should have means to protect their own animals.	The disease should not be zoonotic.
The disease should not be zoonotic.	The disease should be epidemiologically explainable.
It should be possible to mass-produce the agent.	It should be possible to store the agent for short periods of time.
It should be possible to disseminate the agent efficiently.	
It should be stable in storage and in munitions.	

Finally, the innate properties of the pathogens have been utilized in attempts to achieve the desirable characteristics. This has included:

- (A) Infectivity – in ‘military’ programs minimal lateral transmission facilitated control over the resulting outbreak, and for the ‘clandestine’ programs there were a number of “*transmissible diseases which have the potential for very serious or rapid spread, irrespective of national borders*”.¹⁴⁴
- (B) Lethality – agents were produced representing a spectrum of effect, ranging from the lethal to incapacitants.
- (C) Ability to be disguised as natural events – animal diseases emerge periodically in unusual geographical circumstances and differentiation between natural and unnatural origins may be more complex than with other forms of BW.¹⁴⁵ This is especially true of ‘clandestine’ programs which may intentionally mimic natural events.

The revolution in the biological sciences should facilitate developments which have implications for both the advancement and prevention of AABW. Such developments can be characterized as either offensive or defensive. Possible offensive and defensive developments are listed in Table 4.

Analysis¹⁴⁶ appears to indicate defensive developments designed to counter a ‘military’ threat, such as the development of novel prophylactic and therapeutic protocols or improved epidemiological surveillance, could be manifested in the short-term. Further-reaching defensive developments, such as improved hand-held / transportable sensor technologies and special resource allocation to animal health and its interface with public health architecture designed to counter the clandestine threat may be more realistically achievable in the medium-term.

Offensive developments for military programs, such as enhanced agent stability and environmental resistance or the creation of novel agents, could be attainable in the short to medium-term. As it can be argued that AABW is more likely to be utilized in scenarios rather than between developed countries¹⁴⁷ (which are also those currently possessing the scientific and technological base to take advantage of the revolution in biological sciences) additional time may be required to enhance existing capabilities to a degree sufficient to enable such applications. Hence, these developments may be more likely to occur in the medium-term.

Offensive developments for clandestine programmes are unlikely to be manifested in anything less than the long-term. Although improvements, such as the directed evolution of natural agents or the development of techniques to enhance the characteristics of non-traditional agents, will become scientifically possible, it is likely that these manipulations would be detectable (using foreseeable technology) through thorough investigation. This would reduce the ability to disguise outbreaks as natural events. Identifying such manipulations might also provide clues as to the origin of the outbreak.

One aspect of the revolution in the biological sciences which has been linked to the future threat of BW is proteomics. Although protein-based weapons might be a future threat in the anti-personnel or anti-crop fields, it is more contemporary for AABW, especially following the discovery of prions.

Prions have been described as:

*novel infectious pathogens that cause a group of fatal neurodegenerative disorders termed transmissible spongiform encephalopathies*¹⁴⁸

Prions are protein-based agents which appear to lack any genetic material. Although not experimentally proven, the favoured hypothesis suggests that prions are replicating, altered forms of important neurological proteins.¹⁴⁹ They are already responsible for a number of important animal diseases, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in deer and elk, and exotic ungulate encephalopathy in a number of exotic ungulates.

These prion diseases demonstrate a number of unusual properties, including:

extremely long incubation period from a few months to several years, there are no inflammation and no disease-specific immune responses, and they have three different manifestations that are unlikely related: infectious, inherited and sporadic disorders.

Such properties lend themselves to use as an AABS agent (Table 3), as these agents correspond to the latter six desirable characteristics. Although prions appear to be infectious they may not be naturally 'highly infectious' and the lack of a 'known effect consistently' produced is likely the result of their recent discovery and may be a situation which will be resolved in the future.

3.4 A Future Anti-Animal Threat

Of the biological advances yet to come of age, bioinformatics may prove particularly relevant to AABW. Bioinformatics can be considered as the digitization of biology covering all aspects of the biology / digital technology interface, ranging from electronically stored experimental data to high-throughput laboratory equipment.

Bioinformatics may prove crucial in both promoting and combating the ability to disguise AABS as a natural event. Next generation automated sensors may well not only be able to detect biological agents but also may be able to monitor for tell-tale signs of human intervention in the origin of an outbreak – such as genetic manipulation or highly unusual epidemiological characteristics.¹⁵⁰ It may also prove possible to misuse advances in sensor technology. Increasing levels of automation and reduced levels of human interaction with detection and diagnostic processes may facilitate malign manipulation in at least two ways:

1. Adding a new desirable characteristic to the desirability of AABW agents – its ability to bypass detection equipment; or
2. Increasing the efficiency of hoaxes – allowing the development of agents specifically designed to trigger false alarms (minimizing the risk that an epidemic may spread out of control).

It is to be hoped that the ultimate manifestation of sensor technology would be a system which can analyze genotype, phenotype, and possibly proteotype in real time and one which could use global epidemiological databases to conduct simple analysis of the nature of an outbreak. Many bioinformatic sub-disciplines would be crucial in the development of such sensor technology.

The trend towards electronically storing biological data has implications for biological warfare. Increases in the availability of information and decreases in the time-frame and resources required to access this information would provide a firm foundation for the development of novel technologies to confront the biological threat. A prime example of such a development has occurred in the USA where new biosecurity legislation has been

formulated which should decrease the potential for biological agents or dual-use technology to be diverted from their intended use. If the data are not properly digitally secured, however, it may actually facilitate the efforts of potential proliferators. For example, if the locations and security measures relating to Select Agents gathered in partial fulfilment of obligations under biosecurity legislation were accessed for malign purposes, it could become a 'shopping-list' of raw-parts for a BW program. Although it appears that measures are in place to minimize such an eventuality, it is to be hoped that equal attention will be given to animal-related capabilities and the Department of Agriculture will receive resources proportional to the magnitude of this task.

The potential for the future misuse of bioinformatics has already begun to be manifested. The recent creation of a polio virus¹⁵¹ from its electronically stored genome using high-throughput digital machines demonstrates the need to ensure bioinformatic resources are used in a responsible manner. It is hoped that early attention to such issues can ensure this and other important biological advances are only used for the benefit of mankind.

Table 4. Possible offensive and defensive developments in anti-animal biological warfare.

Note: this table is a generalization. It is possible to envisage military and clandestine scenarios which would require alternative characteristics.

Desirable Characteristic	Possible Offensive Developments		Possible Defensive Developments	
	For A Military Threat	For A Clandestine Threat	Against a Military Threat	Against A Clandestine Threat
An agent should be highly infectious	-	Directed evolution of natural agents through advanced systems biology to preferentially increase infectivity.	-	Novel prophylactics, therapies and anti-infectious agents derived from advances from many of the biological sciences and designed to counter the infectivity of the pathogens,
An agent should pose a significant threat to livestock production (or associated industries)	-	Developing techniques to enhance the characteristics of agents on the OIE class A and B pathogen lists that have not previously been considered candidates for anti-animal biological warfare.	-	Improved prophylactics and therapies which could be created from scratch faster, more safely and the development of biochemical protocols designed to minimise the impact of diseases on the animal production industry.
An agent should produce a known effect consistently.	Enhanced manipulation and regulation of the biological pathways of an agent through systems biology, genomics, proteomics and bioinformatics.	Enhanced understanding of factors affecting production of the effect through advanced systems biology.	Development of novel prophylactic and therapeutic protocols which could alter the course of an infection.	Development of novel prophylactic and therapeutic protocols which could alter the course of an infection.
It should be possible to store the agent for short periods of time.	-	Enhanced understanding of factors affecting agent degradation and loss of infectivity / pathogenicity through advanced systems biology.	-	Improved hand-held / transportable sensor technologies, through developments in the field of bioinformatics, genomics, and proteomics, increasing the difficulty of transporting or storing pathogens, even short distances or for short durations.
It should be possible to disseminate the agent efficiently.	Enhanced agent stability and environmental resistance conferred by genetic manipulation.	-	Development of novel biochemical agents to diminish the stability and environmental resistance of pathogenic agents.	-

It should be possible to mass-produce the agent.	Misapplication of advances from the field of biotechnology combined with conferred preferential production characteristics through genetic manipulation.	-	Improved epidemiological surveillance and the development of remote sensing technology by advanced bioinformatics techniques to prohibit the illicit mass-production of biological agents.	-
It should be stable in storage and in munitions.	Induced spore formation or environmentally stable characteristics through genetic manipulation	-	Creation of agents or biochemical agents designed to degrade pathogens without a negative impact on natural biological systems (ensured through increasing capabilities in the field of systems biology).	-
The disease should be epidemiologically explainable.	-	Greater understanding of epidemiology of animal disease outbreaks through bioinformatics increasing the number of available epidemiological explanations.	-	Greater understanding of epidemiology of animal disease outbreaks through bioinformatics and systems biology.
The disease should not be zoonotic.	Altered biochemical structure to prevent infection of human tissue.	Enhanced diseases surveillance and future sensor technology reducing risk of zoonotic outbreak spreading to humans.	Enhanced interaction and information flow across the animal health / public health interface.	Enhanced interaction and information flow across the animal health / public health interface.
The dose needed to produce the effect should be low.	Increased infectivity and pathogenicity through genomic and proteomics manipulation.	Directed evolution of natural agents through advanced systems biology to preferentially increase infectivity and pathogenicity.	Improved disease surveillance and sensor technologies negating some of the advantages of reduced doses combined with enhancements in animal immune technologies which might require larger doses to be overcome.	Improved disease surveillance and sensor technologies negating some of the advantages of reduced doses combined with enhancements in animal immune technologies which might require larger doses to be overcome.
The target population should have little or no immunity.	Creation of novel agents for which no innate immunity exists, or the genomic or proteomic manipulation of traditional agents.	-	Improved specific immunological protocols, the development of broad-spectrum prophylactics and immuno-boosting biochemical treatments.	-
The user should have means to protect their own animals.	Enhanced control over process of infections through systems biology, combined with enhanced capabilities to develop prophylactics and therapies derived from advances made in almost all biological fields.	-	Improved surveillance of disease prevention activities through advances in bioinformatics.	-

There should be a long sub-clinical infectious period.	-	Directed evolution of natural agents through advanced systems biology to preferentially increase infectious period whilst suppressing clinical presentation.	-	Novel biochemical agents designed to interfere with the sub-clinical infectious period of an infectious agent and enhanced detection and diagnostics capabilities allowing earlier intervention into the course of an outbreak.
There should be a short predictable incubation period.	Enhanced control over incubation through systems biology, combined with conferred preferential characteristics through genetic manipulation.	-	Novel biochemical agents designed to interfere with the incubation processes of an infectious agent and enhanced detection and diagnostics capabilities allowing earlier intervention into the course of an outbreak.	-
Treatment for the disease should not be easily available to the target population.	Creation of novel agents for which no treatment currently exists, or the genomic or proteomic manipulation of traditional agents.	-	Improved prophylactics and therapies which could be created from scratch faster and more safely which could be proliferated to a wider groups of consumers through the spread of biotechnology.	-

4 Anti-Plant Threats

4.1 Introduction

Programs devoted to the development and application of agents¹⁵² for use in the intentional destruction of plant life have formed an important component in military programs. Biological agents have been developed for their military utility in bringing about the destruction of a wide variety of plant life including food and cash crops. In the civil sector the large scale production of agents for the biological control of plant pests and weeds is of increasing relevance to a strengthened international legal regime against biological warfare. And technologies closely related to biological warfare and biological control are being developed for use against illicit drug crops. In the age of international terrorism, the obvious challenge that the existence of such technologies throws up is how, in the light of existing and future scientific and technological developments, their hostile use can be prevented without placing regulatory measures on science that stifle scientific progress in the area of plant biology?

In the first section plant diseases are discussed in the context of their development in both military programs and in regard to developments in the civil sector that have military applications. In the second section, current capabilities and concerns are then discussed in the light of relevant scientific developments in these areas. In the final section there is an evaluation of the threat posed by the future development of Advanced Biological Warfare (ABW's) Agents.

4.2 State Programs

The principal intention in military programs has been the development of agents for hostile use against an adversary's food and cash crops. A great number of agents pathogenic to plant life were selected for their disease-producing potential including bacteria, fungi and viruses transmitted to plants via an agent of dissemination such as an insect. While not discussed in any detail here, investigations also involved exploring the potential of the physical destruction of plants by insects.

Regarded as a first generation¹⁵³ program, the now widely-acknowledged WWI campaign of covert sabotage operations by German agents against livestock is also noted as one of the first instances of deliberate disease against crops; as part of this campaign, contamination of quantities of wheat took place.¹⁵⁴ Mid-century programs in France in the later inter-war years, in Germany after the invasion of France, in Japan, and in the UK, Canada and in the US, all benefited from the systematic scientific study of plant pathology for hostile purposes. Such activities represent a second generation in biological warfare programs. Together with anti-crop developments in the former Soviet Union and Iraq, each of the above programs possessed a central characteristic relating to the selection of anti-plant agents. The agents of choice in all of the above programs were fungal plant pathogens – those that cause losses amounting to billions of dollars on an annual basis in some of the world's most important food and cash crops. Characteristically, fungal diseases of wheat and rice (and other cereals) are spread by means of a hardy microscopic spore and show high levels of resistance to environmental degradation. Such pathogens infect the aerial parts of plants and cause diseases that have the capability of spreading rapidly to epidemic proportions throughout the course of a single growing season.

Examples of the role such pathogens have played in the devastation of food crops

include the Irish Potato Famine (1845-6) and the Bengal Famine (1943). While the French, German, Japanese, UK, Canadian and Iraqi programs were restricted to fundamental research and testing with pathogens and insects and were – as far as is known - capable of only modest levels of deployment, the programs in the US and the former Soviet Union involved great investment and the allocation of considerable resources. Both programs resulted in the acquisition of a militarily-significant anti-crop biological warfare capability, with the former resulting in the standardization of munitions and the large-scale stockpiling of agents, and the latter resulting in a large-scale capability to produce huge quantities of such agents on demand.

Anti-crop agents standardized by the US, anti-crop agents listed as under review in 1969, anti-crop agents in the former Soviet Union; and anti-crop agents in Iraq are described below (Table 5).

Table 5. Anti-crop Agents

Agents standardized by the US ¹⁵⁵	Agents listed as under review in 1969 ¹⁵⁶	Anti-crop agents in the former Soviet Union ¹⁵⁷	Anti-crop agents in Iraq ¹⁵⁸
causal agent of stem rust of wheat (<i>Puccinia graminis</i> code named TX)	<i>Puccinia graminis</i> var. <i>tritici</i> Erikss. & Henn., race 56.	causal agents of diseases affecting wheat	causal agent of ‘cover smut’ or stinking smut or bunt of wheat fungus of the genus <i>Tilletia</i>
causal agent of rice blast <i>Piricularia oryzae</i> , code named LX	<i>Piricularia oryzae</i> Cavara, races 11 and 25	causal agents of diseases affecting rice	
causal agent of late blight of potatoes <i>Phytophthora infestans</i> code named LO	causal agent of diseases of wheat and barley, <i>Puccinia striiformis</i> West	causal agents of diseases affecting corn	
causal agent of stem rust of rye code named SX	causal agent of diseases of rice, wheat, corn, barley, rye, sorghum, Hoja blanca virus transmitted by plant hopper, <i>Sogata orizicola</i>	causal agents of diseases affecting rye	
Identity of 5 th agent not available in the public domain	causal agent of diseases of rice <i>Xanthomonas oryzae</i> Uyeda and Ishiyama		
	causal agent of downy mildew of poppy and diseases of papaver and argemone, <i>Peronospora arborescens</i>		

The above listed agents can be regarded as indicative of those ‘classical’ anti-crop biological warfare agents that were under development in second generation biological warfare programs. These include fungal plant pathogens that affect the world’s most economically and socially significant food and cash crops. However, it is important to note that a much greater number than those described above of naturally occurring, unmodified agents pose a significant threat to food and cash crops. For example, if we consider the situation in only one country, as Wheelis and Madden¹⁵⁹ observe, there are “...many thousands of plant diseases in the United

States, and an exact number is probably impossible to determine. Over 13,000 unique fungal plant pathogen species ...and over 75,000 plant-fungus combinations (because a single pathogen species may infect many host plant species) [are listed by one source]. A given crop species such as wheat may be affected by over 200 different diseases worldwide.”

It is also worth noting that although the program in the former Soviet Union is regarded as a third-generation program - due to the existence of secondary-source evidence of the application to pathogens of techniques of genetic modification¹⁶⁰ - the available evidence does not appear to suggest that anti-crop warfare pathogens were subject to manipulation during the course of this program. The latter may also be true of the anti-crop program in Iraq. An explanation as to why a capability was developed in the latter two countries based on a classical anti-crop BW agent is advanced in the final section. The following paragraphs consider developments in the civil sector that have military applications

4.3 Biological Control and Plant Inoculants

A recent initiative has attempted to highlight the importance of strengthening the international legal prohibition against the threat posed by plant pathogens used in the civil sector for peaceful purposes. Raised at the official level, the head of the South African delegation to the Fifth Review Conference of the Biological and Toxin Weapons Convention (BTWC) notes the increasing threat posed by the large scale production of plant inoculants and biocontrol agents used routinely in agriculture in the control of plant pests and weeds. The argument put forward relating to these agents is that, due to their dual-use capabilities, production facilities should be the subject of declarations under a strengthened BTWC. However, our interest here is in developing an appreciation of the plethora of organisms that could be used as possible plant inoculants and biocontrol agents and their possible use for malign purposes.

Plant inoculants are formulations containing living microorganisms, used in the treatment and propagation of seeds and plant propagation materiel for enhancing growth and disease resistance in plants. They are also used for the restoration of the microflora of soil. Unsophisticated technology is required for the production of dry peat-based formulations, and large quantities of this form of plant inoculant can be disseminated over crops. Sophisticated production facilities are required in the large-scale production of liquid formulations and could easily be switched to the production of plant inoculants for malign purposes. Future developments in regard to the delivery methods for plant inoculants in both dried (powder) and liquid (aerosolized) forms may further increase the future malign utility of this technology.

Biocontrol agents are living organisms, such as bacteria, fungi, insects, mites or weeds, or microorganisms that are used in the control of microbes or other organisms. A large number of biocontrol agents are currently available, for example, in the US, where they are marketed as biopesticides and include bacteria such as *Agrobacterium*, the widely-used *Bacillus thuringiensis* that produces a protein toxic to species of insects pests belonging to the orders lepidoptera (caterpillars), diptera (flies), and coleoptera (beetles and weevils), *Pseudomonas*, and *Streptomyces*. Further biopesticides include fungi such as *Ampelomyces*, *Candida*, *Coniothyrium*, and *Trichoderma*.¹⁶¹ Interestingly, the scientific literature¹⁶² on biocontrol agents contains references that are freely available, giving details of fermentation techniques used in the rapid and large-scale production of such agents. Indeed the literature contains references to production methods that require only limited resources, and there is

increasing emphasis in the above areas on research into genetic manipulation in order to increase the effectiveness of such agents. In addition, a number of biocontrol agents with the above properties are awaiting registration (for example, in the US, with the Environmental Protection Agency (EPA)) but are publicly available for purchase as growth promoters and plant strengtheners. Other uses for plant inoculants and biocontrol agents have proved controversial.

4.4 Anti-narcotics

The use of biocontrol agents has been envisaged in connection with the destruction of illicit drug crops. In this connection, *Fusarium* fungi (affecting cannabis and coca) and *Pleospora* fungi (affecting poppy plants) have been developed as potential biocontrol agents. Conducted under the auspices of the United Nations Drug Control Program (UNDCP) the US has financed research into fungal pathogens of coca and cannabis, and US and UK financed research into fungal pathogens of poppy has been conducted in Uzbekistan.¹⁶³ An ongoing debate raises doubts over claims regarding the host specificity of such organisms, and concern remains over the potential implications of the impact of these agents on complex ecosystems. Although no primary source data appears to be available giving the details of above anti-narcotics research programs one author has commented on the extent to which research on anti-narcotics biological control agents has featured genetic manipulation aimed at enhancing the target specificity and the virulence of these organisms. This secondary source features some limited evidence of the way in which advanced techniques may have been applied in the above programs. According to Hogshire,¹⁶⁴ research scientists have conducted experimentation into manipulating the gene responsible for *Fusarium's* destructive effect on coca. This has included isolating, "...a gene for the 24kDa protein from *Fusarium oxysporum* and [developing] a transformation system in *Fusarium oxysporum* to allow alteration of the gene expression." The following section describes developments in the area of civil plant biotechnology.

4.5 Genetic Modification

Negotiations by states parties under the auspices of the Ad Hoc Group to develop a means by which compliance with the BTWC could be verified through the implementation of a legally binding Protocol resulted in the production of a list of plant pathogens of concern. While not definitive in its scope the list – which was designed to assist states parties in filing their respective declarations - assessed agents against criteria where agents of concern were judged as such due to having been: either the subject of research and development in biological warfare programs and developed as weapons, or agents that cause severe socio-economic damage to staple crops. The list is interesting in that it raises official concern over the future prospect that agents with BW potential might be subject to genetic manipulation (see Appendix A). The list includes both bacteria and fungi that affect a broad host spectrum of important food and cash crops as likely candidates for genetic manipulation but no information is available from this source as to how these pathogens might be modified.

While there is little evidence to suggest that applications from genome studies were used in past anti-crop biological warfare programs, given recent advances in genomics, it would be irresponsible to assume that such techniques are not being or will not be applied in current or future third-generation offensive biological warfare

programs. Indeed, a number of major developments impacting on phytopathology appear to support this line of reasoning.

In the last ten years, genome studies have facilitated manipulation of the genetic characteristics of food crops. Some examples are as follows. Crops can now be produced with built in defences against insect predators (such as *Bacillus thuringiensis* as discussed above). Crop varieties can be tailored to confer tolerance to drought or salt or resistance to herbicides. They can also be manipulated to delay ripening, as in the case of the slow-ripening Flavr Savr tomato, which was approved for sale in the US in 1994. Infertility can be conferred on plant seeds, as in the case of the controversial Terminator gene. It has been possible to produce genetically modified strains of rice with increased levels of vitamins and iron. Some 40 genetically modified crops and microorganisms had been approved for sale by US regulatory authorities by 1998, with almost half of US soya production resulting from genetically-modified varieties in 1999.

Four major areas of research and development in plant genomics are of relevance. One rapidly developing area of research involves studies into the reaction of plants to pathogen invasion and the development of disease. Related research led to the discovery of a protein called harpin that is used prior to pathogen invasion to activate crop defenses. In order to confer resistance to plant diseases, the genes involved in resistance are being gradually identified. Another promising area of research and development concerns protecting plants from disease through a concept referred to as 'pathogen-derived resistance'. This involves genes that are engineered into plants that are derived from the pathogens themselves. A third area of research concerns investigations into the role of antimicrobial peptides and proteins that confer antimicrobial properties on plants, thus strengthening immunity and resistance to fungal and bacterial plant pathogens. With the objective of conferring a level of immunity or resistance to a pathogen, the fourth area concerns the development genetically engineered plants to express an antibody against a protein that is found to be crucial to the process of pathogenesis.

In addition to the above, there are already a number of plant-derived recombinant human proteins used in pharmaceuticals.¹⁶⁵ Research in plant pathology into bacterial pathogens has also revealed recently a number of previously unknown natural chemical products such as pyrrolnitrin produced by *Pseudomonas* bacteria that is used in the manufacture of a broad-spectrum chemical fungicide. Analysts have already begun thinking through the possibilities of how plant pathogens might be manipulated for malign purposes. A simple scenario, according to Kagan,¹⁶⁶ might be to simply insert noxious DNA material in the form of a bioregulator into a biocontrol agent such as *Bacillus thuringiensis* that would be present in sufficiently large quantities to contaminate the food-supply chain of a country, region, or economic zone.

The complete genome sequence for *Ralstonia solanacearum*,¹⁶⁷ one of the most devastating soil borne plant pathogens affecting an unusually wide host range of plants globally, was published recently. This is likely to advance considerably our future understanding of the molecular determinants that govern an organism's pathogenicity. It is important to note that the above developments open up a range of possibilities for the hostile use of plant pathogens across the biochemical spectrum and it is easy to envisage that genome studies in plants could be used now and in the future for malign purposes. The following section turns briefly to deal with the matter of future Advanced Biological Warfare Agents.

4.6 Advanced Biological Warfare Agents

It is possible to envisage that advanced agents might emerge inadvertently as a result of scientists working with plant pathogens, as appears to have been the case in animal biology where scientists attempting to develop a contraceptive vaccine for mice from a relatively benign strain of mouse pox virus resulted in the development of a lethal agent.¹⁶⁸ It may be possible to construct a plant pathogen from respective component parts as has been achieved recently in the case of the construction of human polio virus.¹⁶⁹ It may also be possible to envisage the production of plant pathogens with novel characteristics or it may be possible to engineer a pathogen in such a way that it becomes lethal to a broad host spectrum of plant life. It is possible to envisage the near eradication of an entire species from world regions as in the case of elm trees destroyed in some parts of the US and parts of Europe by a non-indigenous exotic fungal plant pathogen.¹⁷⁰ It would be naïve to ignore the future possibility that advanced anti-crop biological warfare agents might result in the total extinction of plant species. While such pathogens are easy to envisage, their production would require significant scientific investment and infrastructure.

However, given the potential of naturally occurring and genetically modified organisms against food, cash crops and other plant life, and the inherent vulnerabilities associated with large-scale agricultural practices in advanced industrialised countries, it is hard to envisage the need for advanced anti-crop biological weapons. J.E. van der Plank¹⁷¹ writing in the early 1960 warned of the threat posed by naturally occurring plant pathogens that increase at a rate of 40% per day over several months. In commenting on the threat posed by the spores produced by wheat stem rust fungus van der Plank notes, "...Many types of spores disperse as easily as smoke. Many are tough and durable. They have only to be dispersed in the proper places at the proper times. Nature sees to the explosion....An enemy need only introduce the appropriate races, and resistance will vanish." Wheelis¹⁷² notes that large-scale high-density production and a reliance on monoculture where there is a restricted range of genotypes make agriculture in advanced industrialised countries particularly vulnerable to naturally occurring but exotic pathogens to which crops can offer no resistance.

The number of naturally occurring plant pathogens that pose a risk to plant life is at present unquantifiable. A great deal more work needs to be done regarding the identification of the number pathogens and pathogen-host combinations. Genetically modified plant pathogens would place great strain on plant extension services that struggle to address the problem of pathogens that are naturally occurring in the environment. It is hard to envisage the need for the development of advanced anti-crop biological weapons but if we consider a worst case scenario for plant life, it is possible to envisage the future extinction of the plant species upon which the world's burgeoning populations are increasingly reliant for the production of food.

III. Overall Conclusions

In its report on Biotechnology Research in an Age of Terrorism, the Fink Committee on Research Standards and Practices to Prevent the Destructive Application of Biotechnology recommended that seven categories of experiment should be subject to review. However, it clearly believed that these categories were only the initial stages in a developing system of review, stating that: “The system proposed in this report is intended as a first step in what will be a long and continuously evolving process to maintain an optimal balance of risks and rewards.”

The kinds of threats discussed in the various sections of the present report clearly demonstrate the correctness of this view. The possibility of the production of novel agents is at present and will in the future continue to be of concern, as expressed in all sections of this report. The modern techniques of molecular biology, including genomics and proteomics will promote the elucidation of the mechanisms of pathogenicity and in particular the interaction of agents with cell receptors. These activities are essential in leading a more effective battle against disease, but at the same time they exacerbate the dilemma of dual use, in that the information gained can more easily serve malign intent. The research oversight system proposed by the University of Maryland group¹⁷³ could deal adequately with this type of research that is focussed on pathogens.

The present report tries to view the dual-use dilemma within a broader scope of consequences by focussing on the real target of malign use, biological systems that are interactive. In this respect, the perturbation of one system by malign agents will necessarily affect another. This can be seen most clearly in the interactions of the immune system with the neuroendocrine systems of humans and animals and points up the necessity to come to grips with bioregulators in a heightened arena. However, plants are also acutely affected by systems biology through their own innate immune system as well as other systems involved in interactions with pathogenic agents. Attacks on the neuroendocrine and immune systems are intimately related to developments in targeting technology. When the attacks use modified microorganisms to deliver the modulators, this would also be covered in the University of Maryland oversight system. However, the present report raises questions about activities that are outside of the system in its present form, such as the delivery of modulators using immunotoxins or fusion proteins, which are not microorganisms. The possibility of immune evasion is of particular concern, and any research that would allow a microorganism to evade the immune system must be considered extremely dangerous. Modulation of the immune system using bioregulators would fall into this category. It is therefore suggested that an additional type of research be placed in the “Table: Illustrative Categories of Research Activities” under the category “Extremely Dangerous Activities” to include “work that would allow agents to evade immune mechanisms”. This would take into account modulating the immune system using immunotoxins or fusion proteins as a delivery system, because even though the delivery system is not a microorganism, manipulation of this type would allow a pathogenic microorganism to be even more dangerous.

In all areas discussed in the paper the directed evolution of natural agents as well as developments in bioinformatics are seen as further areas of particular concern for the future.

How proposals designed to “maintain an optimal balance of risks and rewards” can be applied to the threats delineated in the present report is a matter for further discussion.

Appendix A
Appendix 1

Plant Pathogens Important for the BTWC

Name of Pathogen	Disease Caused.	Distribution:	Transmission	Control	Environmental Stability	Ease of production	BW potential
1. <i>Colletotrichum coffeanum</i> var <i>virulans</i> [coffee berry disease];	Causes coffee berry disease. Can be very destructive in terms of yield loss and seedling death of this non-staple food crop but does not kill mature plants. Different races have not yet been recorded.	Central and southern Africa.	Seed borne, rain splash, passive vectors such as man, birds and machinery.	Fungicide sprays are not effective. Chemical seed treatment not yet successfully developed. Resistant varieties are available.	Can survive as latent infection. Conidiospores have a short life but conidia can survive more than a year on plant debris	Can be mass produced on artificial substrate but is notoriously unstable under these conditions and loses its pathogenicity rapidly.	Not a staple food and thus not regarded as important but may cause serious world wide economic problems.
2. <i>Dothistroma pini</i> (Scirrhia pini) (CMI 368) [blight of pines];	Dothistroma blight of pines can be highly destructive depending on the frequency of infection.	Europe, Asia, Africa, North and South America. Different races have not been recorded.	Seed borne, wind, clouds may carry spore inoculum.	Resistant pine species are available. Non-systemic fungicide sprays show some activity but are not practical and economically viable.	Inoculum viability on debris limited to 2-6 months. Mass production of the pathogen is easily done on artificial substrates.		Is good although pine is not a staple food it is of strategic [significance?]
3. <i>Erwinia amylovora</i> (CMI 44) [fire blight of apple, pear, quince and related species];	Fire blight of apple, pear, quince and related species is very destructive. Not yet recorded in South Africa.	North America, Central America, New Zealand, Japan, China, Europe, North Africa.	Water, vegetative material, insects	Eradicate infected material. Chemical and antibiotic sprays not very successful. The bacteria is not stable in the environment outside its host material. This pathogen can easily be produced in commercial fermenters.			Good.
4. <i>Pseudomonas solanacearum</i> (CMI 15) [wilt associated with numerous hosts particularly potato, tomato and tobacco];	Potato, tomato and tobacco wilt; slime disease, Granville wilt; bacterial ring disease, Moko disease of banana are some of the most devastating diseases caused by this bacterium which attack numerous hosts of Solanaceae, Musaceae, Compositae, Fabaceae, etc. Different races of the bacterium occur which combined with its broad host range make breeding for resistance difficult.	Tropical, subtropical and warm temperate parts of: Asia, Africa, Australasia, Europe, West Indies, North and Central America.	Infected material, contaminated soil, water, implements	No effective chemical treatments available. Resistant cultivars of varieties but new races develop continuously. The bacterium is stable in soil and host tissue. Spores are not produced and vegetative unprotected cells have limited life span. Easily produced in relatively simple fermenters.			Excellent.

5. <i>Piricularia</i> [<i>Piricularia</i>] <i>orzae</i> (CMI 169) [rice blast disease];	Blast disease of rice can be very destructive (90%) on this staple food. With its many races (219) and broad host spectrum, breeding for resistance is complex. The fungus needs high temperature and humidity for infection.	Widespread; Africa, Asia, Australasia, Europe, N. America, S. America, C America, W. Indies.	Wind.	Resistant cultivars, sprays of environmental harmful fungicides can be effective.	Stable, overwinters on straw and debris from reinfection takes place. Can easily be mass produced.	Good
6. <i>Ustilago Maydis</i> (CMI 79) [maize smut, blister smut and common smut];	Maize smut, Common smut, Blister smut can cause appreciable losses (10-17%). In addition, the spores can induce allergic reaction in man and may be toxic to animals and man. More than 500 races have been noted complicating the search for resistance.	Worldwide where maize (corn) is grown except New Zealand	Wind, seed surface borne, contaminated soil.	Heat or chemical seed treatment but this is useless where soil is contaminated. Possibly resistant cultivars.	is excellent. Spores remained viable after 8 years in dry soil. Can be mass produced on artificial substrates	Good.
7. <i>Xanthomonas albilineans</i> (CMI 18);	This bacterium causes leaf scald on sugarcane where [it] can become highly destructive. It has a wide host range and can occur on maize and a number of grass species. The large number of races complicates breeding for resistance.	Africa, Central & South America, Asia, Australasia.	Infected sets, Aerial dispersal, Insects, Rodents.	Heat treatment of sets, resistant varieties. No chemical treatment available.	The bacterium does not produce resistant spores. Disease may remain dormant as systemic infection until environmental conditions favours symptom expression. The bacterium can easily be mass produced in simple commercial fermenters.	Good.
8. <i>Xanthomonas campestris</i> pv. <i>oryzae</i> (CMI 239)	The broad host range bacterium causes bacterial blight of rice and Kresiek disease of rice. Kresiek is caused by the systemic infection in the tropics and is extremely destructive. Differences in pathogenicity between isolates have been reported but there are no differential varietal reaction to complicate breeding for resistance.	Asia, Africa, S. America, Mexico, Korea, Taiwan, Indonesia	Wind, Rain, Flood, vegetative material, Seed borne.	Chemical seed treatment, Resistant cultivars, Elimination of volunteers. Chemical spray not successful	Does not produce resistant or hardy spores. Overwinters on volunteers or in weed shizosphere. Survival on debris seem limited. Can be easily mass produced in simple commercial fermenters.	Medium to good. Candidate for genetic manipulation.

<p>9. <i>Tilletia tritici</i> [cover smut, stinking smut and common bunt of wheat]</p>	<p>Cover smut, Stinking smut, Common bunt of wheat is caused by this broad host range fungus pathogen which has a single host life cycle. The fungus attacks the inflorescence [flower] replacing the kernels with bunt balls of black teliospores. The disease is regarded as very important, it suppresses yields and lowers the quality and smelly trimethylamine is produced while the spores may ignite and cause an explosion during harvesting.</p>	<p>Worldwide.</p>	<p>Seed surface borne, Wind, contaminated soil.</p>	<p>Resistant cultivars - they are short lived because new races continuously develop. Chemical seed treatment.</p>	<p>Teliospores can survive up to 2 years in soil. Production of this obligate parasite needs live hosts but as vast numbers of spores can be harvested, mass production is not impossible.</p>	<p>Good. Could possibly be enhanced by genetic manipulation.</p>
<p>10. <i>Sclerotinia Sclerotiorum</i> (CMI 513) [cottony soft rot and white mould of vegetables, beans, sunflower, groundnuts and soya beans].</p>	<p>This plurivorous fungus causes cottony soft rot, white mold, and watery soft rot on a broad host spectrum such as vegetables, beans, sunflower, groundnuts soya bean and many others except cereals and woody plants. The fungus can attack any above ground parts at any development stage and is extremely destructive under cooler moist conditions as found under irrigation.</p>	<p>Worldwide.</p>	<p>Airborne ascospores, Seed infected with mycelium or contaminated with sclerotia (survival structure).</p>	<p>Airborne ascospores, Seed infected with mycelium or contaminated with sclerotia (survival structure).</p>		<p>High. Good candidate for genetic manipulation to broaden its temperature spectrum.</p>

Notes

- ¹ Geissler, E. (1986) A new generation of biological weapons. In E. Geissler (ed.) *Biological and Toxin Weapons Today*. Oxford: Oxford University Press (for SIPRI).
- ² Cohen, W. (1997) *Proliferation: Threat and Response*. Washington D.C.: Department of Defense.
- ³ Nixdorff, K., Brauburger, J. and Hahlbohm, D. (1997) The biotechnology revolution: the science and applications. In M. Dando, G. Pearson and T. Toth (Eds) *Verification of the Biological and Toxin Weapons Convention*. Dordrecht: Kluwer Academic Publishers.
- ⁴ Nathanson, V., Darvell, M. and Dando, M.R. (1999) *Biotechnology, Weapons and Humanity*. London: Harwood Academic Publishers (For the British Medical Association).
- ⁵ International Committee of the Red Cross (2002) *Biotechnology, weapons and humanity: summary report of an informal meeting of government and independent experts. Montreux, Switzerland, 23-24 September*, Geneva: ICRC.
- ⁶ Poste, G. (2002) *Advances in biotechnology: promise or peril*. Available at <www.hopkins-defense.org/sympos/trans-crypts/trans/post.html>
- ⁷ Meselson, M. (1999) *The problem of biological weapons*. Cambridge Mass: Presentation given to the 1818th Slated Meeting of the American Academy of Arts and Sciences, 13th January.
- ⁸ Committee of Research Standards and Practices to Prevent the Destructive Application of Biology (2003) *Biotechnology research in an age of terrorism: confronting the dual use dilemma*. Washington D.C. The National Academies Press.
- ⁹ Steinbruner, J.D. and Harris, E.D. (2003) Controlling dangerous pathogens. *Issues in Science and Technology*, 19, 47-54. [Hwww.nap.edu/issues/19.3/steinbruner.htm](http://www.nap.edu/issues/19.3/steinbruner.htm)
- ¹⁰ Petro, J. B., Plasse, T.R. and McNulty, J. A. (2003) Biotechnology: impact on biological warfare and biodefense. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice and Science*, 2, 161-8.
- ¹¹ Office of Transnational Issues (2003) *The darker bioweapons future*. OTISF 003-108, 3 November. Washington D. C.: Central Intelligence Agency.
- ¹² Nowak, R. (2001) Disaster in the making. An engineered mouse virus leaves us one step away from the ultimate bioweapon. *New Scientist* 13 January, 4-5.
- ¹³ Jackson, R. J., Ramsay, A. J., Christensen, C., Beaton, S., Hall, D. F. R. and Ramshaw, I. A. (2001) Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *Journal of Virology* 75, 1205-1210.
- ¹⁴ Buller, M. (2003) The potential use of genetic engineering to enhance orthopoxviruses as bioweapons. Presentation at the International Conference "Smallpox Biosecurity. Preventing the Unthinkable", October 21-22, 2003, Geneva, Switzerland.
- ¹⁵ Steinbruner, J.D. and Harris, E.D. (2003) When science breeds nightmares. *International Herald Tribune*, December 3, , p. 8.
- ¹⁶ MacKenzie, D. (2003) US develops lethal new viruses. *New Scientist* 180, 6.
- ¹⁷ NIH (2003) NAID biodefense research agenda for CDC category A agents. Progress Report, August 2003. [Hhttp://www.niaid.nih.gov/biodefense/research/bioresearchagenda.pdf](http://www.niaid.nih.gov/biodefense/research/bioresearchagenda.pdf)
- ¹⁸ Abbas, A.K., Lichtman, A.H. and Pober, J.S. (1997) *Cellular and Molecular Immunology*, Third Edition, W.B. Saunders Company, Philadelphia.
- ¹⁹ Medzhitov, R. and Janeway, C.A., Jr. (1998) An ancient system of host defense. *Current Opinion in Immunology* 10, 12-15.
- ²⁰ Medzhitov, R., Preston-Hurlburt, P. and Janeway, C.A., Jr. (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388, 394-397.
- ²¹ Akira, S. (2003) Mammalian Toll-like receptors. *Current Opinion in Immunology* 15, 5-11.
- ²² Triantafilou, M. and Triantafilou, K. (2002) Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends in Immunology* 23, 301-304.
- ²³ Rietschel, E.T. and Brade, H. (1992) Bacterial endotoxins. *Scientific American* 267, 54-61.
- ²⁴ Rosenberg, B. and Burck, G. (1990) Verification of compliance with the Biological Weapons Convention, in S. Wright (ed.), *The MIT Press*, Cambridge, Massachusetts, pp. 301-329.
- ²⁵ Parker, J.E. (2002) Plant recognition of microbial patterns. *Trends in Plant Science* 8 (6), 245-247.
- ²⁶ Nürnberger, T. and Brunner, F. (2002) Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. *Current Opinion in Plant Biology* 5, 318-324.
- ²⁷ Inohara, N. and Nunez, G. (2003) Nods: intracellular proteins involved in inflammation and apoptosis. *Nature Reviews Immunology* 3, 371-382.

-
- ²⁸ Gupta, S., Ferguson, N., and Anderson, R. (1998) Chaos, persistence, and evolution of strain structure in antigenically diverse infectious agents. *Science* 280, 912-915.
- ²⁹ Alcami, A. and Koszinowski, U.H. (2000) Viral mechanisms of immune evasion. *Trends in Microbiology* 8, 410-418.
- ³⁰ Turner, M.W. (1996) Mannose binding lectin: the pluripotent molecule of the innate immune system. *Immunology Today* 17, 532-536.
- ³¹ Unanue, E.R. (2002) Innate immunity in bacterial infections. *Immunology of Infectious Diseases* ASM Press, Washington, D.C., pp. 93-103.
- ³² Alcami and Koszinowski (2000), *op. cit.*
- ³³ Tortorella, D., Gewurz, B.E., Furman, M.H., Schust, D.J. and Ploegh, H. (2000) Viral subversion of the immune system. *Annual Review of Immunology* 18, 861-926.
- ³⁴ Alcami and Koszinowski (2000), *op. cit.*
- ³⁵ Mahalingam, S. and Karupiah, G. (2000) Modulation of chemokines by poxvirus infections. *Current Opinion in Immunology* 12, 409-412.
- ³⁶ Alcami and Koszinowski (2000), *op. cit.*
- ³⁷ Carayannopoulos, L.N. and Yokoyama, W.M. (2004) Recognition of infected cells by natural killer cells. *Current Opinion in Immunology* 16, 26-33.
- ³⁸ Nathanson, Darvell and Dando (1999), *op. cit.* Chapter 3, pp. 33-51.
- ³⁹ Nixdorff, K., Hotz, M., Schilling, D. and Dando, M. (2003): *Biotechnology and the Biological Weapons Convention*. Agenda Verlag, Münster.
- ⁴⁰ Nowak, 2001, *op. cit.*
- ⁴¹ Jackson, R. J., Maguire, D. J., Hinds, L. A. and Ramshaw, I. A. (1998) Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein. *Biology of Reproduction* 58, 152-159.
- ⁴² Jackson et al. (2001), *op. cit.*
- ⁴³ Jackson et al. (1998), *op. cit.*
- ⁴⁴ Jackson et al. (2001), *op. cit.*
- ⁴⁵ Buller (2003), *op. cit.*
- ⁴⁶ MacKenzie (2003), *op. cit.*
- ⁴⁷ Steinbruner and Harris (2003), *op. cit.*
- ⁴⁸ National Research Council of the National Academies (2003) *Biotechnology Research in an Age of Terrorism: Confronting the Dual Use Dilemma*, The National Academies Press, Washington, D.C.
- ⁴⁹ Rosengard, A. M., Liu, Y., Nie, Z. and Jimenez, R. (2002) Variola virus immune evasion design: expression of a highly efficient inhibitor of human complement. *Proceedings of the National Academy of Sciences USA* 99, 8808-8813.
- ⁵⁰ National Research Council (2003), *op. cit.*
- ⁵¹ Moss, B. (1985) Vaccinia virus expression vector: a new tool for immunologists. *Immunology Today* 6, 243-245.
- ⁵² Carter, B.J. (1996) The promise of adeno-associated virus vectors. *Nature Biotechnology* 14, 1725-725.
- ⁵³ Morsy, M.A., Caskey, C.T. (1997) Safe gene vectors made simpler. *Nature Biotechnology* 15, 17.
- ⁵⁴ Kochanek, S., Clemens, P.R., Mitani, K., Chen, H.-H., Chan, S., Caskey, C.T. (1996) A new adenoviral vector: Replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and β -galactosidase. *Proceedings of the National Academy of Sciences USA* 93, 5731-5736.
- ⁵⁵ Carter, B.J. (1996) The promise of adeno-associated virus vectors. *Nature Biotechnology* 14, 1725-725.
- ⁵⁶ Check, E. (2003) Harmful potential of viral vectors fuels doubts over gene therapy. *Nature* 423, 573-574.
- ⁵⁷ Kreitman, R.J. (1999) Immunotoxins in cancer therapy. *Current Opinion in Immunology*. 11, 570-578.
- ⁵⁸ Poncelet, P., Blythman, H.E., Carrier, D. Casellas, P. Dussossoy, D., Gros, O., Gros, P., Jansen, F.K., Laurent, J.C., Liance, M.C., Vidal, H. and Voisin, G.A. (1984) Present potential of immunotoxins. *Behring Institute Mitteilungen* 74, 94-100.
- ⁵⁹ Hayden, M.S., Gilliland, L.K. and Ledbetter, J.A. (1997) Antibody engineering. *Current Opinion in Immunology* 9, 201-212.
- ⁶⁰ Rosenberg, B. and Burck, G. (1990), *op. cit.*
- ⁶¹ Nossal, G.J.V. (2003) Vaccines, in W.E. Paul (ed.), *Fundamental Immunology*, Fifth Edition, Lippencott Williams & Wilkins, Philadelphia, pp. 1319-1369.

- ⁶² Streatfield, S.J., Jilka, J.M., Hood, E.E., Turner, D.D., Bailey, M.R., Mayor, J.M., Woodard, S.L., Beifuss, K.K., Horn, M.E., Delaney, D.E., Tizard, I.R. and Howard, J.A. (2001) Plant-based vaccines: unique advantages. *Vaccine* 19, 2742-2748.
- ⁶³ Nossal (2003), *op. cit.*
- ⁶⁴ Marquet-Blouin, E., Bouche, F.B., Steinmetz, A. and Muller, C.P. (2003) Neutralizing immunogenicity of transgenic carrot (*Daucus carota* L.)-derived measles virus hemagglutinin. *Plant Molecular Biology* 51, 459-469.
- ⁶⁵ *Ibid*
- ⁶⁶ Streatfield, S.J., Lane, J.R., Brooks, C.A., Barker, D.K., Poage, M.L., Mayor, J.M., Lamphear, B.J., Drees, C.F., Jilka, J.M., Hood, E.E. and Howard, J.A. (2003) Corn as a production system for human and animal vaccines. *Vaccine* 21, 812-815.
- ⁶⁷ Carayannopoulos and Yokoyama (2004), *op. cit.*
- ⁶⁸ Silverman, G.J., Nayak, J.V., Warnatz, K., Jajjar, F.F., Cary, S., Tighe, H. and Curtiss, V.E. (1998) The dual phases of the response to a neonatal exposure to a V_H family-restricted staphylococcal B cell superantigen. *Journal of Immunology* 161, 5720-5732.
- ⁶⁹ Stewart-Jones, G.B.E., McMichael, A.J., Bell, J.I., Stewart, D.I. and Jones, E.Y. (2003) A structural basis for immunodominant human T cell receptor recognition. *Nature Immunology* 4, 657-663.
- ⁷⁰ Goldsby, R.A., Kindt, T.J., Osborne, B.A. and Kuby J. (2003) *Immunology*, Fifth Edition, W.H. Freeman and Company, New York.
- ⁷¹ Goodyear, C.S. and Silverman, G.J. (2003) Death by a B cell superantigen: in vivo V_H-targeted apoptotic supraclonal B cell deletion by a staphylococcal toxin. *Journal of Experimental Medicine* 197, 1125-1139.
- ⁷² Dando, M. (2001): Genomics, bioregulators, cell receptors and potential biological weapons. *Defense Analysis*, 17, 239-258.
- ⁷³ Blalock, J. (1994) The syntax of immune-neuroendocrine communication. *Immunology Today* 15, 504-11
- ⁷⁴ Metz-Boutigue, M.H., Kieffer, A.E., Goumon, Y. and Aunis, D. (2003) Innate immunity: involvement of new neuropeptides. *Trends in Microbiology* 11, 585-592.
- ⁷⁵ BBC News (2002) How special forces ended siege. *BBC News*, 29 October 2002. Available, February 2004, from: [Hhttp://news.bbc.co.uk/1/hi/world/europe/2363601.stm](http://news.bbc.co.uk/1/hi/world/europe/2363601.stm)H
- ⁷⁶ Hart, S. (2002) *Statement Before The Subcommittee on Aviation, Committee on Transportation And Infrastructure, U.S. House Of Representatives*. Washington D.C.: U.S. House Of Representatives. Available, February 2004, from: [Hhttp://www.house.gov/transportation/aviation/05-02-02/hart.html](http://www.house.gov/transportation/aviation/05-02-02/hart.html)H
- ⁷⁷ *Ibid.*
- ⁷⁸ This figure may have been higher, see Walsh, P. (2003) Families claim death toll from gas in Moscow siege kept secret. *The Guardian*, 18 October 2003. Available, February 2004, from: [Hhttp://www.guardian.co.uk/international/story/0,3604,1065611,00.html](http://www.guardian.co.uk/international/story/0,3604,1065611,00.html)H
- ⁷⁹ See: Klotz, L., Furmanski, M., Wheelis, M. (2003) *Beware the Siren's Song: Why "Non-Lethal" Incapacitating Chemical Agents are Lethal*. Washington D.C.: Federation of American Scientists (FAS). Available, February 2004, from [Hhttp://www.fas.org/bwc/papers/sirens_song.pdf](http://www.fas.org/bwc/papers/sirens_song.pdf)H & Federation of American Scientists Working Group on Biological Weapons (2003) *Position Paper: Chemical Incapacitating Weapons Are Not Non-Lethal*. Washington D.C.: Federation of American Scientists (FAS). Available, February 2004, from: [Hhttp://www.fas.org/bwc/papers/pp_chemical_incapacitants.pdf](http://www.fas.org/bwc/papers/pp_chemical_incapacitants.pdf)H
- ⁸⁰ See for example: Coupland, R. M. (2003) Incapacitating chemical weapons: a year after the Moscow theatre siege. *The Lancet*, Vol. 362, p. 1346 & Wheelis, M. (2003) "Nonlethal" Chemical Weapons: A Faustian Bargain. *Issues in Science and Technology*. Spring 2003. Available, February 2004, from: [Hhttp://www.nap.edu/issues/19.3/wheelis.htm](http://www.nap.edu/issues/19.3/wheelis.htm)H
- ⁸¹ BBC News (2002) Russia names Moscow siege gas. *BBC News*, 31 October 2002. Available, February 2004, from: [Hhttp://news.bbc.co.uk/1/hi/world/europe/2377563.stm](http://news.bbc.co.uk/1/hi/world/europe/2377563.stm)H
- ⁸² BBC Television (2003) *Horizon: The Moscow Theatre Siege*. Transcript available, February 2004, from [Hhttp://www.bbc.co.uk/science/horizon/2004/moscowtheatretrans.shtml](http://www.bbc.co.uk/science/horizon/2004/moscowtheatretrans.shtml)H
- ⁸³ Stanley (2003) Human immobilization: is the experience in Moscow just the beginning? *European Journal of Anaesthesiology*. 20, pp. 427-8.
- ⁸⁴ US Drug Enforcement Administration (DEA) (2004) Fentanyl. Available, February 2004, from [Hhttp://www.usdoj.gov/dea/concern/fentanyl.html](http://www.usdoj.gov/dea/concern/fentanyl.html)H
- ⁸⁵ Wax, P., Becker, C. and Curry, S. (2003) Unexpected "Gas" Casualties in Moscow: A Medical Toxicology Perspective. *Annals of Emergency Medicine*. 41:5, pp. 700-5.

-
- ⁸⁶ Wheelis, M. (2002) Biotechnology and Biochemical Weapons. *The Nonproliferation Review*. Volume 9, Number 1. Available, February 2004, from: [Hhttp://cns.miis.edu/pubs/npr/vol09/91/91whee.htm](http://cns.miis.edu/pubs/npr/vol09/91/91whee.htm)H
- ⁸⁷ Ketchum, J. and Sidell, F. (1997) Incapacitants. *In: Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare*. Washington D.C.: Office of the Surgeon General, Department of the Army.
- ⁸⁸ Meselson, M. and Perry Robinson, J. (2003) 'Non-Lethal' Weapons and Implementation of the Chemical and Biological Weapons Convention. *20th Pugwash Workshop Study Group on the Implementation of the CBW Conventions: The BWC Intersessional Process towards the Sixth Review Conference and Beyond*. Geneva, Switzerland, 8-9 November 2003. Available, February 2004, from [Hhttp://www.pugwash.org/reports/cbw/cbw20/cbw20-meselson-robinson.htm](http://www.pugwash.org/reports/cbw/cbw20/cbw20-meselson-robinson.htm)H
- ⁸⁹ Dando, M. (2002) Future Incapacitating Chemical Agents: The Impact of Genomics. *In: Lewer, N (Ed) (2002) The Future of Non-Lethal Weapons. Technologies, Operations, Ethics and Law*. London: Frank Cass.
- ⁹⁰ Ketchum, J and Sidell, F. (1997), *op. cit.*
- ⁹¹ MacKenzie, D. (2002) Russian gas may be secret crowd-control weapon. *New Scientist*, 28 October 2002. Available, February 2004, from [Hhttp://www.newscientist.com/news/news.jsp?id=ns99992974](http://www.newscientist.com/news/news.jsp?id=ns99992974)H
- ⁹² Dando, M. (2002), *op. cit.*
- ⁹³ Dando, M. (2003) Dando, M. (2003) The Danger to the Chemical Weapons Convention from Incapacitating Chemicals. *CWC Review Conference Paper No.4*, Department of Peace Studies, University of Bradford.
- ⁹⁴ Wheelis, M. (2002), *op. cit.*
- ⁹⁵ Dando, M. (2002) Scientific and technological change and the future of the CWC: the problem of non-lethal weapons. *Disarmament Forum*. 4, pp. 33-44.
- ⁹⁶ Dando, M (2003), *op. cit.*
- ⁹⁷ Dando, M (2003), *op. cit.*
- ⁹⁸ Dando, M. (1996) *A New Form of Warfare: The Rise of Non-Lethal Weapons*. London: Brassey's.
- ⁹⁹ Dando, M (2003), *op. cit.*
- ¹⁰⁰ National Academy of Sciences (2003) *An Assessment of Non-lethal Weapons Science and Technology*. Washington D.C.: National Academies Press. Available, February 2004, from: [Hhttp://www.nap.edu/books/0309082889/html](http://www.nap.edu/books/0309082889/html)H
- NAS report
- ¹⁰¹ Lakoski, J., Bosseau Murray, W., and Kenny, J. (2000) *The Advantages and Limitations of Calmatives for Use as a Non-Lethal Technique*. College of Medicine, Applied Research Laboratory, The Pennsylvania State University. Available, February 2004, from: [Hhttp://www.nldt.org/documents/calmativereport.pdf](http://www.nldt.org/documents/calmativereport.pdf)H
- ¹⁰² *Ibid.*
- ¹⁰³ Lakoski et al. (2000), *op. cit.*
- ¹⁰⁴ Dando, M. (2002) Future Incapacitating Chemical Agents: The Impact of Genomics. *In: Lewer, N (Ed) (2002) The Future of Non-Lethal Weapons. Technologies, Operations, Ethics and Law*. London: Frank Cass.
- ¹⁰⁵ Dando, M. (2002) Scientific and technological change and the future of the CWC: the problem of non-lethal weapons. *Disarmament Forum*. 4, pp. 33-44.
- ¹⁰⁶ Feldman, R., Meyer, J, and Quenzer, L. (1997) Serotonin. *In: Principles of Neuropsychopharmacology*. Sunderland, MA: Sinauer Associates. pp. 345-89.
- ¹⁰⁷ Ferguson, P. (1994) Antipersonnel Calmative Agents. US Army Edgewood Research, Development & Engineering Center. Available online, February 2004, from: [Hhttp://www.sunshine-project.org/incapacitants/jnlwdpdf/H](http://www.sunshine-project.org/incapacitants/jnlwdpdf/H)
- ¹⁰⁸ *Ibid.*
- ¹⁰⁹ For example of related experiments with elk see: Stanley, T., Port, D., van der Maaten, J., Kimball, J. (1986) Treatment of Stress Hyperthermia in Elk with Ketanserin, a Serotonin Receptor Blocker. *Veterinary Surgery*. 15, 2, pp. 214-217.
- ¹¹⁰ Feldman, R. et al. (1997), *op. cit.*
- ¹¹¹ Carlson, N. (2001) Emotion. *In: Physiology of Behavior*, 7th Edition. Boston: Allyn and Bacon. pp. 339-70.
- ¹¹² Lakoski et al. (2000), *op. cit.*
- ¹¹³ Lakoski et al. (2000), *op. cit.*

-
- ¹¹⁴ Sunshine Project (2004) The Return of ARCAD. The Sunshine Project News Release, 6 January 2004. Available, February 2004, from [Hhttp://www.sunshine-project.org/publications/pr/pr060104.html](http://www.sunshine-project.org/publications/pr/pr060104.html)H
- ¹¹⁵ *Ibid.*
- ¹¹⁶ Ruppe, D. (2002) United States: U.S. Military Studying Nonlethal Chemicals. *Global Security Newswire*, 4 November 2002. Available, February 2004, from [Hhttp://www.nti.org/d_newswire/issues/2002/11/4/7s.html](http://www.nti.org/d_newswire/issues/2002/11/4/7s.html)H
- ¹¹⁷ Department of Defense (2000) *CBD 26 Phase I Selections from the 00.1 Solicitation*, Department of Defense SBIR Awards 2000. Available, February 2004, from [Hhttp://www.nttc.edu/resources/funding/awards/dod/2000sbir/001cbd.asp](http://www.nttc.edu/resources/funding/awards/dod/2000sbir/001cbd.asp)H
- ¹¹⁸ US Joint Non-Lethal Weapons Directorate (2003) Front End Analysis for Non-Lethal Chemicals. Available, February 2004, from [Hhttp://www.sunshine-project.org/incapacitants/jnlwdpdf/feachemical.pdf](http://www.sunshine-project.org/incapacitants/jnlwdpdf/feachemical.pdf)H
- ¹¹⁹ Kagan, E. (2001) Bioregulators as Instruments of Terror. *Clinics in Laboratory Medicine*. Vol. 21, No. 3. pp. 607-18
- ¹²⁰ Alibek, K. with Handelman, S. (1999) *Biohazard*. New York: Random House.
- ¹²¹ Davis, C. (1999) Nuclear Blindness: An Overview of the Biological Weapons Programs of the Former Soviet Union and Iraq. *Emerging Infectious Diseases*. Vol. 5, No. 4. pp. 509-12. Available, February 2004, from [Hhttp://www.cdc.gov/ncidod/EID/vol5no4/pdf/davis.pdf](http://www.cdc.gov/ncidod/EID/vol5no4/pdf/davis.pdf)H
- ¹²² *Ibid.*
- ¹²³ Petro, J., Plasse, T., and McNulty, J. (2003) Biotechnology: Impact on Biological Warfare and Biodefense. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*. Vol. 1, No. 3. pp. 161-8.
- ¹²⁴ Dunzendorfer, S. and Wiedermann, C. (2001) Neuropeptides and the Immune System: Focus in Dendritic Cells. *Critical Reviews in Immunology*. 21(6). pp. 523-57.
- ¹²⁵ Blalock, J. (1994) The syntax of immune-neuroendocrine communication. *Immunology Today*. Vol. 15, No. 11. pp. 504-11.
- ¹²⁶ *Ibid.*
- ¹²⁷ Webster, J., Tonelli, L., Sternberg, E. (2002) Neuroendocrine Regulation of Immunity. *Annual Reviews in Immunology*. 20. pp. 125-63.
- ¹²⁸ Lakoski et al. (2000), *op. cit.*
- ¹²⁹ Glaser, J. and McGuire, L. (2002) Psychoneuroimmunology: Psychological Influences on Immune Function and Health. *Journal of Consulting and Clinical Psychology*. Vol. 70, No. 3. pp. 537-547.
- ¹³⁰ Ershler, W. and Keller, E. (2000) Age-Associated Increased Interleukin-6 Gene Expression, Late-Life Diseases, and Frailty. *Annual Reviews in Medicine*. 51. pp. 245-270.
- ¹³¹ Glaser, J. and McGuire, L. (2002), *op. cit.*
- ¹³² *Ibid.*
- ¹³³ These events were reported in broadsheet newspapers at the time of the outbreak in the UK and are now in an archive held by the author.
- ¹³⁴ Wheelis, M., *Biological sabotage in World War I*, in Geissler, E & van Courtland Moon, J.E., *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*, SIPRI No 18, Oxford University Press, UK, 1999.
- ¹³⁵ JIC, *Present State of Progress in BW in Foreign Countries*, Chiefs of Staff Committee, Joint Intelligence Sub-Committee, JIC (47) 22 (0), Public Records Office, London, UK, DEFE 55/135, 25th April 1947.
- ¹³⁶ Balmer, B., *Britain and BW: Expert Advice and Science Policy, 1930-65*, Palgrave, London, 2001. Or, Carter, G.B., *Chemical and Biological Defence at Porton Down*, The Stationary Office, London, 2000.
- ¹³⁷ Unknown, *Notes on Japanese Photostats 57 and 97*, Rev. Moule, Public Records Office, WO188/690, London, UK, 26th March 1945.
- ¹³⁸ IB Dong, Z., Y., *Kwantung Army Number 100*, in Historical Material on Jilin History, Peoples Press, Changchun, 1987. From: Harris, S., *The Japanese BW programme: an overview*, in Geissler, E., and van Courtland Moon, J.E., *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*, SIPRI 18, Oxford University Press, UK, 1999, p149.
- ¹³⁹ Millett, P., *Anti-Animal Biological Warfare: Past, Present and Future, and the Revolution in the Biological Sciences*, Doctoral Thesis, University of Bradford, UK, *Forthcoming*.
- ¹⁴⁰ Millett, P., *Anti-Animal Bioweapons*, in Dando, M., Wheelis, M., (Eds.) *Bioweapons Research, Development and Use from 1945 to the Present*, Harvard University Press, *Forthcoming*.
- ¹⁴¹ Alibek, K., *Biohazard*, Arrow Books, London, 2000, pp37-38.

-
- ¹⁴² Interview by the author with a former UNSCOM Chief Weapon's Inspector.
- ¹⁴³ Millett, P., *Anti-Animal Bioweapons*, in Dando, M., Wheelis, M., (Eds.) *Bioweapons Research, Development and Use from 1945 to the Present*, Harvard University Press, *Forthcoming*.
- ¹⁴⁴ The OIE definition of such diseases continues "...which are of socio-economic or public health consequence and which are of major importance in the international trade of animals and animal products". OIE, *OIE Classification of Diseases*, [Online] http://www.oie.int/eng/maladies/en_classification.htm [Last Accessed 11/12/03]
- ¹⁴⁵ Hugh-Jones, M., *Distinguishing Natural and Unnatural Outbreaks of Animal Diseases*, in Dando, M., Preason, G., Kriz, B., *Scientific and Technical Means of Distinguishing Between Natural and Other Outbreaks of Disease*, NATO Science Series, Disarmament Technologies Vol. 35, Kluwer Academic Publishers, London, 1998, pp63-73.
- ¹⁴⁶ Millett, P., *Anti-Animal Biological Warfare: Past, Present and Future, and the Revolution in the Biological Sciences*, Doctoral Thesis, University of Bradford, UK, *Forthcoming*.
- ¹⁴⁷ Millett, P., *Anti-Animal Bioweapons*, in Dando, M., Wheelis, M., (Eds.) *Bioweapons Research, Development and Use from 1945 to the Present*, Harvard University Press, *Forthcoming*.
- ¹⁴⁸ Hur, K., Kim, J.I., Choi, S.I., Eun-Kyoung, C., Carp, R.I., Yong-Sun, K., *The Pathogenic Mechanisms of Prion Diseases, Mechanisms of Aging and Development*, Vol. 123, 2002, pp1637-1647.
- ¹⁴⁹ Prusiner, S.B., *Molecular Biology of Prion Diseases, Science*, Vol. 252, 1991, pp1515-1522.
- ¹⁵⁰ This might include disease events involving agents connected to specific seasonal vectors which present at periods when natural transmission mechanisms are not present.
- ¹⁵¹ Cello, J., Paul, A.V., Wimmer, E., *Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template, Science*, Vol. 297, No. 5583, 9 August 2002.
- ¹⁵² Methods of dissemination are not discussed in this paper.
- ¹⁵³ First generation programs are those regarded to lack systematic scientific basis?.
- ¹⁵⁴ Wheelis, M. Biological Sabotage in World War I, *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*, SIPRI Chemical & Biological Warfare Studies, no. 18,
- ¹⁵⁵ Annex to Appendix VI, Capabilities for Production of Anti-Crop Agents in Operational Effectiveness of Biological Warfare, Volume 14, Operational Research Group Study, Number 21, p. 27, (AD identifier not available)..
- ¹⁵⁶ Thomas G. Roetzel, Miscellaneous Publication 34, *Biological Agents and Munitions Data Book*, Department of the Army, Fort Detrick, Frederick, Maryland, AD 505971.
- ¹⁵⁷ Primary sources not available at time of writing for anti-crop agents in the former Soviet Union. Table 1 entries based on A. Rimmington, Invisible Weapons of Mass Destruction: The Soviet Union's BW Programme and its Implications for Contemporary Arms Control, *Journal of Slavic Military Studies*, Vol 13, No. 3, September 2000, p. 7. Alibek, K. The Soviet Union's Anti-Agricultural Biological Weapons, *Annals of the New York Academy of Sciences*, 894, (1999), pp. 18-19, and Alibek, K. *Biohazard, Biohazard, Random House; 1999, ISBN: 0375502319*.
- ¹⁵⁸ UNSCOM, *United Nations*, S/1995/864, 11 October 1995.
- ¹⁵⁹ Wheelis, M., and Madden, L. The Threat of Plant Pathogens as Weapons Against US Crops, *Annual Review of Phytopathology*, 2003.
- ¹⁶⁰ Dennis, C. The Bugs of War, *Nature*, 411, pp.232-235, 17 May 2001. According to Dennis research in the former Soviet biological weapons program on *Yersinia pestis*, the causal agent of plague, resulted in a form of the organism that was resistant to 16 different antibiotics. See also Alibek, K.. *Biohazard, Random House; 1999, ISBN: 0375502319*.
- ¹⁶¹ McSpadden Gardener, B. B., and Fravel, D. R. 2002. Biological control of plant pathogens: Research, commercialisation, and application in the USA. Online, *Plant Health Progress* doi:10.1094/PHP-2002-0510-01-RV.
- ¹⁶² Jackson, M.A., Cliquet, S., and Iten, L.B., 2003. Media and Fermentation Processes for the Rapid Production of High Concentrations of Stable Blastospores of the Bioinsecticidal Fungus *Paecilomyces fumosoroseus*, *Biocontrol Science and Technology*, Volume 13, pp. 23-33.
- ¹⁶³ Rufford, N. Britain Funds Biological Warfare Against Heroine, *Sunday Times*, 28 June 1998.
- ¹⁶⁴ Hogshire, J. 1998. Biological Roulette: The Drug War's Final Solution? *Covert Action Quarterly*, No. 64, Spring, pp. 41-44. The information to which this story relates is no longer available of the website of the US Agricultural Research Service (correct at time of writing).
- ¹⁶⁵ Gruber, V. and Theisen, M. 2000. Genetically Modified Crops as a Source of Pharmaceuticals, *Annual Reports in Medicinal Chemistry*, Vol 35, pp. 357-364.

¹⁶⁶ Kagan, E. (2001). Bioregulators as Instruments of Terror. Laboratory Aspects of Biowarfare. *Clinics in Laboratory Medicine*, Volume 21, Number 3, September, pp. 607-618.

¹⁶⁷ Salanoubat M, et al. Genome sequence of the plant pathogen *Ralstonia Solanacearum*. *Nature* 2002; 415.

¹⁶⁸ Jackson et al. (2001), *op. cit.*

¹⁶⁹ Jeronimo Cello, Aniko V. Paul, and Eckard Wimmer. Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template, *Science* 297: 1016-1018.

¹⁷⁰ Fry, W. E. *ASM News*, Volume 62, No. 11, 1996, pp. 595-597.

¹⁷¹ van der Plank, J.E. *Plant Diseases: Epidemics and Control*, Academic Press, London, p. 212.

¹⁷² Wheelis, M. Agricultural Biowarfare & Bioterrorism: An Analytical Framework & Recommendations for the Fifth Review Conference, Pugwash Meeting No. 258, *Proceedings of 14th Workshop of the Pugwash Study Group on the Implementation of the Chemical and Biological Weapons Convention: Key Issues for the Fifth BWC Review Conference 2001*. Geneva, 18-19 November 2000.

¹⁷³ Steinbruner, J., Harris, E.D., Gallagher, N., and Gunther, S. (2003) Controlling dangerous pathogens: a prototype protective oversight system. This paper represents a work in progress and is periodically being revised. Please check [Hwww.puaf.umd.edu/cissm/projects/amcs/amcs.html](http://www.puaf.umd.edu/cissm/projects/amcs/amcs.html) for the most recent version before quotation or citation. Comments should be sent to: Hpuaf-pathproj@umail.umd.edu.