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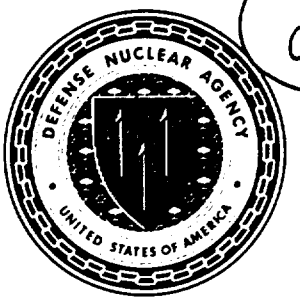

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Evaluation of the Concept of a List for the BWC

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13. ABSTRACT *(Maximum 200 words)*

This paper examines the concept that a list of specific proscribed biologic materials or categories of proscribed materials has utility as a tool for verification of the Biological Weapons Convention. Using the criteria that a useful list has to objective and complete or all-inclusive, we compared existing proposed lists, evaluated current biological, genetic engineering, and natural products research for impact on the ability to prepare a list useful for verification. None of the proposed lists examined were complete, nor was any list of specifically defined materials; new organisms are discovered, known organisms are reclassified and renamed, genetic engineering enables changing trait expression or moving traits between organisms, and natural products research discovers new pharmacologically active materials. Any list that proscribes materials by categories or classes (or any other criterion) is so large that it is unwieldy and impractical to use as a tool for verification. Such a list lacks currency and objectivity; on the cutting edge of research, much information needed to determine whether an organism or strain is proscribed is unavailable. We have not been able to demonstrate the utility of a list as a verification tool; rather, lists have little use as such a tool.

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CONVERSION TABLE

Conversion factors for U.S. customary to metric (SI) units of measurement

To Convert From	To	Multiply
angstrom	meters (m)	1.000 000 X E–10
atmosphere (normal)	kilo pascal (kPa)	1.013 25 X E+2
bar	kilo pascal (kPa)	1.000 000 X E+2
barn	meter^2 (m^2)	1.000 000 X E–28
British Thermal unit (thermochemical)	joule (J)	1.054 350 X E+3
calorie (thermochemical)	joule (J)	4.184 000
cal (thermochemical)/cm^2	mega joule/m^2(MJ/m^2)	4.184 000 X E–2
curie	giga becquerel (GBq)*	3.700 000 X E+1
degree (angle)	radian (rad)	1.745 329 X E–2
degree Fahrenheit	degree kelvin (K)	$t_K=(t^\circ f + 459.67)/1.8$
electron volt	joule (J)	1.602 19 X E–19
erg	joule (J)	1.000 000 X E–7
erg/second	watt (W)	1.000 000 X E–7
foot	meter (m)	3.048 000 X E–1
foot-pound-force	joule (J)	1.355 818
gallon (U.S. liquid)	meter^3 (m^3)	3.785 412 X E–3
inch	meter (m)	2.540 000 X E–2
jerk	joule (J)	1.000 000 X E+9
joule/kilogram (J/Kg) (radiation dose absorbed)	Gray (Gy)	1.000 000
kilotons	terajoules	4.183
kip (1000 lbf)	newton (N)	4.448 222 X E+3
kip/inch^2 (ksi)	kilo pascal (kPa)	6.894 757 X E+3
ktap	newton-second/m^2 (N-s/m^2)	1.000 000 X E+2
micron	meter (m)	1.000 000 X E–6
mil	meter (m)	2.540 000 X E–5
mile (international)	meter (m)	1.609 344 X E+3
ounce	kilogram (kg)	2.834 952 X E–2
pound-force (lbf avoirdupois)	newton (N)	4.448 222
pound-force inch	newton-meter (N·m)	1.129 848 X E–1
pound-force/inch	newton/meter (N/m)	1.751 268 X E+2
pound-force/foot^2	kilo pascal (kPa)	4.788 026 X E–2
pound-force/inch^2 (psi)	kilo pascal (kPa)	6.894 757
pound-mass (lbm avoirdupois)	kilogram (kg)	4.535 924 X E–1
pound-mass-foot^2 (moment of inertia)	kilogram-meter^2 (kg·m^2)	4.214 011 X E–2
pound-mass/foot^3	kilogram/meter^3 (kg/m^3)	1.601 846 X E+1
rad (radiation dose absorbed)	Gray (Gy)**	1.000 000 X E–2
roentgen	coulomb/kilogram (C/kg)	2.579 760 X E–4
shake	second (s)	1.000 000 X E–8
slug	kilogram (kg)	1.459 390 X E+1
torr (mm Hg, 0°C)	kilo pascal (kPa)	1.333 22 X E–1

*The becquerel (Bq) is the SI unit of radioactivity; Bp = 1 event/s.

**The Gray (Gy) is the SI unit of absorbed radiation.

EXECUTIVE SUMMARY

This paper examines the concept that a list, either of specific proscribed biologic materials or of categories of proscribed materials, has utility as a tool for verification of the Biological Weapons Convention. To be useful, a list has to be objective and complete or all-inclusive. Using these criteria, we have compared existing proposed lists. We have evaluated current biological research, genetic engineering research and technologies, and natural products research for their impact on the ability to prepare a list useful for verification.

None of the proposed lists examined were complete. The Russian list did not mention toxins at all; the U.S. list included "Tricothecene toxins" without being specific, while the German list specified two tricothecene toxins by name. None of the lists included two highly pathogenic toxin producing species of *Clostridium*. Neither of the Australian Group's lists included antiplant agents or bioregulators.

Current research has an impact on the ability to prepare a complete list of proscribed materials. The diversity of the bacterial world and the continuing discovery of new genera, species and biologically active compounds make the preparation of a complete list impossible. Reclassification and renaming of existing organisms, selective modification of existing strains and development of new strains have a severe impact on the ability to prepare an all-inclusive list that is useful as a verification tool because the resulting list becomes too large to be useful.

Genetic engineering research and technologies have already demonstrated that it is possible to affect the expression of a wide variety of genes or move genes between organisms. The recombinant possibilities that are available with this technology are impossible to describe until they are actually prepared. No list of proscribed organisms is adequate because it cannot be complete or all-inclusive; if categories of materials

are proscribed, then the resulting list becomes so large and unwieldy that it is not useful.

Natural products research encompasses a diversity of products with very broad scope. Because of continuing new discoveries, natural products research would ensure that no list could be complete. Any list that attempted to proscribe by categories would result in a list which includes almost everything done in natural products research. That list would be too large to be useful as a verification tool.

In the course of our examination of the utility of lists as a tool for verification of the BWC, we could find no list of specifically defined materials that could meet the completeness criterion; new organisms are being discovered, known organisms are being reclassified and renamed, genetic engineering provides the ability to change the expression of traits, or even move those traits between organisms, and natural products research continues to discover new pharmacologically active materials from the vast world of nature.

A list that attempts to describe proscribed materials by categories or classes (or any other criterion) is so large that it is unwieldy and not practical to use as a tool for verification. Such a list lacks currency and objectivity; especially on the cutting edge of research, much of the information needed to make a determination of whether an organism or strain is in fact proscribed is unavailable. Further, the people in the best position to make that determination are often those with a vested interest in seeing the research completed.

We have not been able to demonstrate the utility of a list as a verification tool; rather, lists have little use as such a tool.

TABLE OF CONTENTS

SECTION 1
BACKGROUND

The Third Review Conference of the Biological Weapons Convention (BWC) considered many proposals for strengthening the BWC. Among them was one to develop a list of specifically prohibited agents of biological origin (ABO); this list would be used as the basis for declaration and treaty verification. This proposal would align the BWC more closely with the Chemical Weapons Convention, which uses lists of prohibited material in three schedules as the basis for declaration, inspection and verification. A list of prohibited agents was proposed as a useful tool to verify compliance with the BWC. States in compliance would possess none of the prohibited agents. States having facilities which produce, process and/or consume a listed agent would need to declare that fact and explain the use as prophylactic, protective or otherwise peaceful (i.e., falls within the dual use category). Furthermore, that state's facilities could be subject to inspection and verification.

SECTION 2
OBJECTIVE

The objective of this task is to examine the usefulness of the concept of a list of prohibited agents of biological origin and its consequent utility as a verification measure.

SECTION 3
APPROACH

The purpose of any list that is made a part of the BWC is to facilitate verification and to provide an objective measure of compliance with the BWC. To do that, any list proposed must be complete or all-inclusive. We will begin our evaluation by examining the concept of lists. The purpose of having a list will be reviewed and various conceptual lists will be considered to determine whether any list can meet the criterion of completeness. We will compare multiple lists which have been proposed for the BWC to determine how well these lists meet the completeness criterion. Current research on pathogens will be reviewed for its impact on lists. The effect of natural products research and the effect of genetic engineering technology on the concept of the completeness of any list will be discussed. Specific examples (from defense, commercial and academic sources) will be provided.

SECTION 4
DISCUSSION

In the sections that follow, we will consider whether any list can be complete and all-inclusive and thus serve as a verification tool by:

a. reviewing the purpose and types of lists possible;
b. comparing existing proposed lists for completeness;
c. evaluating the impact of the current research on pathogenic biologics;
d. evaluating the impact of genetic engineering research;
e. evaluating the impact of natural products research.

4.1 PURPOSE OF LISTS.

The purpose of having a list in the BWC is to facilitate verification and provide an objective basis for evaluating compliance by proscribing specifically *all* those organisms and biologically produced materials which are considered to be warfare threats. A BW threat is defined to be an organism or compound of biological origin that has the capability to kill or severely incapacitate humans or animals, or severely damage crops. Facilities producing, using or storing agents on the list then must be open for inspection and verification.

4.2 TYPES OF LISTS.

There are two general ways to describe the biologics that need to be controlled. First, an "all-inclusive" list can be generated and updated on a regular basis. Such a list describes in a clear, precise manner the biologics which could serve as BW agents and provides a clear definition of those biologics that are controlled, simplifying compliance verification. That is, if a given facility such as a research institute or commercial establishment works with, e.g., botulinum toxin in significant quantities, then the institute must declare its toxin use. However, such a list can never be complete, in part, because of the nature of biological research. New discoveries are continually

being made. Illnesses such as AIDS, Lymes disease and Legionnaires disease and their causal microbes have only been recognized in the very recent past. Natural products research is being actively conducted to identify materials of commercial value. Much of this research is proprietary and would not initially be made public. Biological materials lacking commercial value would not necessarily ever be brought to the public's attention. Any list created for treaty verification purposes would have to be continually updated to include pathogens or other biological materials which are newly discovered. Further, this updating will always be after the fact and reactive, rather than proactive and thus preventing work with potential ABOs.

A difficulty in generating a list of specific organisms is in defining the organisms placed on the list. In contrast to chemicals which can be defined specifically by composition and structure, organisms are very complex. One species of bacteria, for example, can be very closely related to a second species. *B. anthracis* and *B. thuringiensis* are very closely related (to be expanded). (See below: Classification/Taxonomy.)

Genetic engineering technology has a major impact on the list concept: It is possible to manipulate an organism or a molecule to alter its properties. Some parties to the BWC could manipulate organisms not appearing on the list due to their relatively low pathogenicity to construct more virulent strains. For example, the movement of a *Yersinia pestis* plasmid into *Y. enterocolitica* does not convert *Y. enterocolitica* into *Y. pestis* but it could alter its virulence. Using genetic engineering techniques, a gene from *Yersinia pseudotuberculosis* which encodes a protein involved in mediating *Y. pseudotuberculosis* invasion of eucaryotic cells (part of the pathology of the disease) was cloned and transferred into a common laboratory strain of *Escherichia coli*. In an *in vitro* tissue system, this gene allowed this normally non-invasive *E. coli* strain to invade cells (Miller, 1992). A second group used the same experimental methodology to identify and transfer two genes from *Y. enterocolitica* which are involved in cell invasion into *E. coli* (Miller, 1992). Again the genes allowed a normally non-invasive *E. coli* to invade eucaryotic cells in culture. While it is not known whether the

recombinant *E. coli* produced in this work actually could invade cells in a human host, this example illustrates the type of manipulation that can be used to create new agents or to evade treaty restraints. Expanding a list to include all of these possible threats results in a list that is so large that it may be essentially unpolicable.

A second approach to preparing a list is to provide descriptions of the categories of controlled biologics (with examples) but without attempting to specifically list the potential ABOs. This approach has the advantage of not requiring a complete listing of every potential agent. Also, this approach makes it more difficult to cheat because it does not provide specifics. However, it does require a set of criteria or generic descriptors which will clearly delineate proscribed biologics. Such standards can include "all pathogens and toxins of any kind whose nature or pathogenicity is not fully described in international publications"[1] and "all derivatives of pathogens that have decreased pathogenicity".[1] Or a level of effectiveness can be specified: "all toxins and other biologically produced chemicals affecting vertebrates that have an LD50 (or ED50 where relevant) of 1 microgram/kilogram or less in the most sensitive species known".[1]

This type of list, by attempting to be all-inclusive, can easily become so large that it is not useful for verification. It is not practical to attempt to define or verify all research relating to pathogenicity or toxicity, nor is it practical to develop a BW list which could include common bacteria and viruses used in genetic engineering research. Further, all the newly discovered biologics regardless of their pathogenicity would fall under this criterion, since their nature or pathogenicity is not completely described. (See below.) A further difficulty with this approach is that this determination process can be highly subjective, and colored by potential profits or even scientific prestige. Disputes can

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[1] Taken from *Proposals for the Third Review Conference for the Biological Weapons Convention*, Report of the Federation of American Scientists Working Group on Biological and Toxin Weapons Verification, October 1990.

arise over whether a strain of organism is or is not intended to be included in the BWC and this may put a state technically in a non-compliance status.
Finally, this type of list does nothing for the ease of verification because it adds nothing to the BWC that the definition of a biological warfare agent does not already say in a much simpler, more straightforward and all-inclusive way.

4.3 LIST COMPARISON.

Several lists were obtained for the purpose of comparing included and excluded organisms. The BW lists compared include two from the Australian group (German and U.S.), and a Russian Federation list. When the German, U.S. and Russian BW lists are compared, differences between the lists are apparent. For example, *Pseudomonas pseudomallei*, *Brucella abortus*, *Salmonella typhi*, *Rickettsia rickettsii*, rabies, dengue fever virus, influenza virus and Rinderpest do not appear on all three lists. Toxins are listed in different levels of detail. The U.S. list, for example, lists "Trichothecene toxins"; there are over 60 different toxins in this category. The Russian list has omitted this category completely. The German list has a rather abbreviated listing of trichothecene toxins; only T-2 toxin and satratoxin toxin are mentioned. Omitted from all three of these lists are *Clostridium perfringens* and *Clostridium tetani* which are human pathogens and which, like *Clostridium botulinum*, produce toxins. Plant pathogens, although a potential BW threat, are not mentioned on these lists. Bioregulators are also conspicuous by their absence from the Australian Group's list.

No list examined thus far was complete, nor, as subsequent sections will demonstrate, can any list be complete because current research and the techniques of genetic engineering both contribute to the discovery of new organisms, modified organisms and redefined organisms. This makes it impossible to develop a comprehensive list that can be used as a tool for verification.

4.4 CURRENT RESEARCH.

In this section we will discuss how innate characteristics of biological organisms affect the completeness of any list. We will review research which leads to the discovery of new organisms or new toxins from organisms, research on the characteristics or classification of organisms which leads to renaming or reclassifying organisms, and research on mutation and genetic selection. The diversity of microbes and the continuing discovery of new microbes and toxins makes the construction of an inclusive but manageable list impossible. The reclassification and renaming of organisms complicates the construction of an inclusive list of organisms, as does the selective modification and development of existing strains.

4.4.1 "New" Microorganisms.

Bacteria are an extremely diverse group of organisms. New families of organisms are being found; new members of already catalogued species are being discovered; and pathogenic characteristics of some here-to-fore non-pathogens are being identified.

According to Dr. Norman Pace (University of Indiana), there are more types of bacteria than there are types of insects (American Society for Microbiology 1992 Annual Meeting, Keynote Address). Bacteria can be found in almost any environment, including hot springs (e.g., extremely thermophilic bacteria have been isolated from hot springs; optimal growth occurred in one case at 105 °C [Jannasch and Taylor, 1984]) and Antarctic soil (temperatures from 6 °C to - 6 °C)[2]. Discovery of novel organisms occurs with regularity but historically has been limited by difficulties in culturing some organisms in a laboratory. That is, it is not always possible to identify and recreate

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[2] The isolates from Antarctica reported by Shivaji, et al. (1989), appeared to be various *Pseudomonas* species. These isolates did possess characteristics which were somewhat different than the typical mesophilic strains. The authors conclude, based upon this work and other studies, that the differences in characteristics probably are the result of adaptation to the particular environment they in which they are found.

the conditions a particular organism needs to survive and propagate. Organisms having a parasitic lifestyle can be particularly difficult to grow. Molecular biology techniques now allow study of previously unculturable organisms.

Fungi and viruses are also diverse groups of organisms and culturing can be a serious obstacle to isolation and study. As more environments are sampled and as microbiological techniques expand and improve, more microbes will be isolated and identified. A summary of some of the most recent "new organism" research follows.

In order to examine the isolation and identification of "new" microbes, a literature search of the BIOSIS database was conducted. The search terms "new species" and "new genus (or genera)" were combined with "bacteria", "fungus", "fungi" and "virus(es)". A limited number of abstracts were collected (generally 1991 and 1992) and the following conclusions were drawn. Many new bacterial species and few new genera were reported. A portion of the new species was reclassifications, as described in the following section for *F. tularensis*. Most of the new bacteria species were environmental isolates; few had clinical importance. Of the "new" bacterial pathogens, many were already known organisms that were newly found to have pathogenic potential. For example, *Leuconostoc* species have not in the past been considered clinically significant but they have now been isolated from clinical samples (Dr. Marcon, Childrens Hospital, Columbus, Ohio, personal communication). Some examples of new microbes are listed below:

1. Two new bacteria species from Ace Lake, Antarctica, *Carnobacterium funditum* and *Carnobacterium alterfunditum*.
2. A new genus and species of bacteria, *Roseococcus thiosulfatophilus.*
3. New species, *Rochalimaea henselae*, proposed; isolated from a patient infected with human immuno-deficiency virus (HIV). This bacteria may be an etiologic agent for bacillary angiomatosis.
4. New food-borne pathogen (isolated in China), *Pseudomonas cocovenenans* subsp. *farinofermentans* which secretes a toxin called Bongkerkic acid.

This toxin is reported to be very different from other bacterial toxins because it is a fatty acid derivative.

5. New fungal species, *Cercospora osirisae*, causes spot disease on emersed leaves of *Echinodorus osiris*.
6. New Bunyavirus from birds; this probable new species is designated Sedlec virus. It is pathogenic to suckling and adult mice when inoculated intracerebrally but not intraperitoneally.

Reports of several new toxins were discovered in the literature search. Often they represented variations/modifications of existing classes of toxins. A few examples of these toxins are listed below:

1. Ponteratoxin - isolated from ant venom
2. New neurotoxin - isolated from two gastropod mollusks from the genera *Zeuxis*. Tetrodotoxin was also isolated from the specimens examined.
3. Two new toxins - isolated from venom of the scorpion *Centruroides noxius* Hoffman. The toxins were very similar to previously isolated *Centruroides* toxins.
4. Toxin - isolated from *Vibrio cholera*. The primary toxin of *Vibrio cholera* is well characterized; apparently this is a second toxin.
5. *Legionella* toxin - produced by *Legionella pneumophila*; this toxin lyses human erythrocytes.

4.4.2 Classification/Taxonomy.

The reclassification and renaming of organisms will complicate the construction of an inclusive list and could allow states to evade the list by manipulating taxonomic groupings. Unlike a chemical, an organism can not be defined in terms of a formula or single structure. Chemicals usually have molecular weights in the hundreds. For reference, a large protein molecule with a molecular weight of 100,000 would be about 0.01 micron in size. Viruses range in size from approximately 0.003 to 0.05

microns. Bacteria range in size from about 0.3 to over 30 microns. An organism minimally consists of genetic material and protein (as in the case of simple viruses). Bacteria, viruses and fungi are complex assemblages of nucleic acids, protein, carbohydrates and lipids. They contain components such as cell walls, membranes and ribosomes. Strains of a single bacterial species can express different physiological traits based upon how they were grown. For example, *Bacillus* spores can have different resistances to heat when grown on different media. It is difficult, therefore, using the data currently available, to simply define a particular microbe as one could define a chemical.

Classical bacterial taxonomy is based upon both morphology and physiology; the taxonomic schemes developed using these characteristics have not been found to be completely sufficient for determining species relatedness. New molecular techniques which exploit DNA and RNA sequences (such as 16s RNA analysis) are proving to be very useful for taxonomic studies and may help resolve this difficulty.

One impact of research using molecular techniques to measure genetic relationships is the continuing redefinition of taxonomic units. For example, *Francisella tularensis* is divided into several biovars (subspecies). Biovar *tularensis* (Type A) was distinguished from biovar *palaearctica* (Type B) on the basis of the ability of Type A strains to ferment glycerol and the presence of the enzyme citrulline ureidase. Type A strains are more virulent than Type B and have been thought to occur naturally only in N. America. 16s RNA analysis has revealed that the two biovars are distinct (Forsman, et al., 1990). Therefore, one might focus verification activities on tracking the more virulent Type A strain outside of N. America. However, the analysis of two additional biovars, *mediaasiatica* and *palaearctica japonica* (found in Asia and Japan, respectively) indicated that these biovars are more closely related to the Type A strain than to the Type B strain based upon the 16S rRNA analysis. However, these two biovars do not have the virulence level associated with N. American Type A strains. In addition, in some type A and B strains the traits of glycerol utilization and the presence of citrulline ureidase do not always follow the archetypal definitions. Therefore,

tracking BW activities outside of the U.S. based upon the use of Type A (non-indigenous) strains is complicated by taxonomic redefinitions and reclassifications. Extending this example on *Francisella* taxonomy, it has been proposed that *F. novicida* be reclassified from a separate species to a biovar of *F. tularensis*. It was also proposed that a strain formerly classified as *Yersinia philomiragia* be reclassified as *F. philomiragia* comb. nov. (Hollis, et al., 1989)[3]. All of these organisms appear to be human pathogens.

The utility of any list proposed for verification then is greatly diminished by the inability to define and classify specific organisms and species by name.

4.4.3 Effect of Mutation and Genetic Selection.

In BW research and development, it is likely that significant effort will be made to develop strains with altered characteristics relevant to their intended use. Modifications could include increased or decreased virulence or toxicity, better aerosolization characteristics, increased persistence or environmental stability, addition of antibiotic resistance traits, or higher production levels.

Strain development can proceed in several different ways, such as using classical genetic mutagenesis and selection and/or using recombinant DNA techniques. Different strains of a specific organism can be collected from the environment and examined[4]. The strain with the closest match to the desired characteristics can be

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[3] The work described in this report was initiated when the research group received several cultures of "Philomiragia" like organisms that had been isolated from humans. The cultures had been collected over an eleven year period. The name *Y. philomiragia* had been first proposed in 1969 for a bacteria first isolated in 1959. It has been known for some time that *Yersinia* was not the correct genus for this organism.

[4] The diversity of microorganisms is extreme. Even within a single species, multiple strains exist, with different characteristics. The number of strains within any

selected and further developed. Further, as described below, organisms can be manipulated genetically to generate a strain possessing the desired characteristics.

Fungi, bacteria and viruses all can be manipulated genetically. For example, strains of antibiotic producing bacteria have been selected over time to generate "improved" strains which overproduce antibiotics or which produce slightly altered forms of antibiotics with improved efficacy. For many years, improved industrial microorganisms were made by mutagenizing (e.g., by use of chemical mutagens) the strains and screening for mutants with the desired characteristics. More recently, because of the development of genetic engineering technology, specific controlled alterations to the genetic material can be made (see genetic engineering section).

This capability to selectively develop new strains or to carry out genetic manipulations on existing strains to change their expressed characteristics has a major impact on any list developed for verification purposes. It is not possible to prepare an all-inclusive list which completely describes all proscribed organisms and strains when new or modified strains can be readily developed as described above and in the following section.

4.5 GENETIC ENGINEERING.

The technology of genetic engineering makes it possible to perform two types of genetic manipulation which are relevant to the subject of this report. First, genes can be moved from one type of organism to another: for example, from one bacteria to another, or from an animal to a virus. Second, it is generally possible to alter the expression of any particular gene to express more of it or to control its expression.

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one species tends to reflect the level of scrutiny that species has undergone and its medical significance. For example, the American Type Culture Collection lists over 17 pages of *E. coli* strains (with about 30 to 38 strains per page), while for some other species there is only one strain listed.

More complex, but proven techniques can be used to alter the function of genes themselves. For example, it may be possible, with some effort, to increase the toxicity of a particular product by manipulating the gene which encodes it.

In either case, it becomes very difficult to describe conceptually all the recombinant possibilities and prepare a list which is useful for verification without at the same time inhibiting or prohibiting genetic engineering research. Until some of these recombinant organisms are actually prepared by expressing the genes, the characteristics of the organisms are unknown.

The complete design of totally new organisms, particularly ones which might have to function efficiently outside the controlled environment of the laboratory today lies in the realm of science fiction. At this time, the science of genetic engineering still has a trial and error element which limits the chances of succeeding at very complex schemes.

Nonetheless, genetic engineering makes it possible to create organisms that have no natural counterpart by moving genes into organisms. This can result in increased production of a desired material or production from another organism, make non-pathogenic organisms pathogenic, alter drug resistance or alter specific protein expression. Each of these has an impact on the ability to prepare a comprehensive list of specifically defined, proscribed organisms, or of classes and types of organisms. Each will be discussed in the following sections.

4.5.1 Increased Production of Biologics.

Two general classes of biologics can be produced by cell culture techniques: proteins (which can be enzymes) and secondary metabolites. In general, most proteins can be produced by bacterial, yeast or tissue culture, and powerful methods exist for efficiently producing proteins in all these innocuous and standard systems. A good example of a protein that could be overproduced in this way is botulinum toxin.

Secondary metabolites are fairly elaborate molecules produced by a plants, animals or microorganisms. They can function within the organism (for example, as a hormone) or externally (such as an antibiotic directed against the organism's competitors or predators). These compounds are produced within an organism by combining and modifying simpler molecules common to most cells. A good example of a secondary metabolite that acts as a toxin is the tetrodotoxin found in blowfish. The pathway to produce secondary metabolites may be very complex (e.g., the pathway for the production of the fungal antibiotic, penicillin), yet increased production can be achieved.

Generally several genes are required to encode the complete enzymatic pathway for production of an elaborate secondary metabolite. Identification of the key enzymes in the pathway and controlled expression of the genes encoding these enzymes can improve yields. Production of penicillin in industrial strains has been improved orders of magnitude by years of concentrated effort on strain improvement and by altering the internal metabolism of the native fungus *Penicillium notatum*.

4.5.2 New Bacteria Hosts for Virulence Genes.

When bacteria infect a host animal or plant, they have to defeat a formidable set of defenses to cause disease. Pathologically dangerous bacteria have a variety of traits which help them defeat the immune defense of their host. These traits are ultimately controlled by genes within bacteria. These genes are referred to here as virulence factors. They are frequently carried in small circular pieces of DNA (called plasmids) which replicate independently of the bacterial cell chromosome. Virulence factors have been observed to move naturally from one type of bacteria to another, increasing the pathogenicity of the resulting recombinant bacteria.

Genetic engineering techniques could be used to move these traits with relative ease from one strain to another, even moving traits between two organisms which normally

would not be capable of communicating genetic material.[5] In addition, the expression of virulence factors could be altered by genetic means to alter the virulence of the resulting strain. While it is not likely that the introduction of a single virulence factor from an unrelated strain of bacteria can transform a safe bacteria into a virulent, pathogenic one, moving several factors could accomplish this. The net effect would be the creation of a new pathogen not found on a list of proscribed biologics.

4.5.3 Altered Drug Resistances of Hosts.

It is comparatively easy to take a virulent bacterial strain and increase its resistance to common antibiotics. A very large number of drug resistance genes have been cloned from naturally drug resistant bacteria. Many of these genes will function efficiently in a broad range of bacterial hosts. Infection by an multi-antibiotic resistant strain of virulent *Y. pestis* would be very difficult to treat.

4.5.4 Alteration of Specific Proteins or Introduction of New Genes into a Viral System.

The interaction between an host plant or animal with viruses, bacteria and other microorganisms is complex and usually does not lead to a disease state. Some viruses, such as HIV, which cause disease are difficult to transmit, or have long periods of latency. These pathogens have evolved to persist for generations in populations of host animals or plants. It is a poor parasite that rapidly kills or disables its host so efficiently that it destroys its own life support system. Genetic engineering could be used to overcome the built-in limitations evolved in a chronically infectious virus, which does not meet the criteria for inclusion on a list, and the resulting virulent strain could be maintained by propagation and storage in a laboratory environment.

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[5] It is important to note that not all organisms can be manipulated genetically at this time.

One system currently under development by F.M.C. Corporation involves introducing new genes into a viral system. Baculoviruses are being developed as insect control agents. Insect-specific Baculoviruses are used as a delivery vehicle for a recombinant spider venom gene. When a caterpillar is infected by the Baculoviruses, the toxin gene is expressed, and the toxin produced rapidly kills the caterpillar. The speed of caterpillar death is over twice as fast as killing with the Baculoviruses alone. Neither biologic by itself would be expected to be on a list of proscribed materials.

4.6 NATURAL PRODUCTS RESEARCH OVERVIEW.

The scope of natural product research is extremely broad. The idea that plant or animal extracts could have medicinal properties has been a part of disease treatment since the prehistoric ages. The sheer diversity of natural products without synthetic predecessors makes them very attractive research targets. As a consequence, researchers are continuously investigating samples and collections from around the world. These materials are new and thus are not included on any list of proscribed biologics. Yet, because their nature and pathogenicity are not described (or known), they could be proscribed on that basis. The result is a very large and heterogeneous list which would essentially include everything done in natural products research. Such a list would not be useful for verification. The following sections include a discussion of how natural products research progresses and describe the diversity of pharmaceutical and agricultural research in natural products in more detail; they illustrate the difficulties of applying any form of a list for verification of compliance to these activities.

4.7 NATURAL PRODUCTS RESEARCH.

Natural products research activities can be grouped into several areas:

- Sample collection
- Development of efficient screening systems for detecting biological activity
- Characterization of the active substances
- Applications of natural products.

4.7.1 Sample Collection.

Four types of samples constitute the bulk of natural products collections:

- Plant extracts and specimens
- Marine organism collections
- Microbial samples, especially soil samples, and clinical isolates, and pure cultures
- Animal specimens, especially venoms (including invertebrate species)

In modern jargon, these collections select and preserve examples of "biological diversity" from around the planet. Efforts have always been made to include samples from exotic locations and environments in these collections, including samples from rain forests and from extreme environments like deep sea hydrovents and hot springs. Some collections are of preserved (dead) plant or animal material. Living material is deposited in culture, tissue or seed collections.

Many and varied types of national and international collections of plant, microorganism and animal material are available for routine research activities around the world. In addition, numerous private collections exist; for example, some pharmaceutical companies keep large collections of soil samples as part of their efforts to identify new classes of antibiotics. While collections of extreme viral and microbial pathogens are recognized as dangerous, there are no real limitations on the distribution of pathogens. Most of these materials have not yet been very well characterized or described; they

either would not appear on a list of proscribed biologics (i.e., the list could not have been complete) or could all be included as of unknown nature and thus be presumed to be proscribed. In the latter case, the list would be so large (see below) as to be unwieldy and not useful as a verification tool.

4.7.2 Screening of Natural Products.

Once a researcher has access to a useful sample collection, the next task is to identify from all of the samples which ones might be useful for a particular task. In order to do this, thousands of samples may have to be screened for activity and winnowed to manageable numbers, so efficient methods of testing individual samples must be devised. For example, soil sample microbes may be grown up on petri plates and then screened for the production of compounds which inhibit the growth of some particular kind of bacteria.

Modern research is constantly developing new biological techniques for screening natural products. Screening for active compounds can be quite sophisticated, including tests which involve using tissue cultures of human cells to screen for compounds with specific activities. For example, human T cell cultures may be used to find compounds that alter the activation of the immune system, or nerve cells may be used to screen for compounds which alter synaptic function; these tests are frequently highly automated in order to increase the number of samples which can be screened. A detailed knowledge of the target activity can be very helpful; modern screening systems are specific for compounds which inhibit specific enzymes or cell receptors. For example, a large number of compounds have been found which inhibit a specific protease (angiotensin converting factor) involved in control of blood pressure. Some of these compounds or closely related chemical derivatives are being developed as pharmaceuticals for the control of blood pressure.

Samples found to contain biologically active substances could potentially meet the definition of a B.W. agent. But these biologically active materials may not be on any

list either because they are so new (the list is incomplete), or because none is sufficiently well characterized so that all of them would be proscribed as undefined. In any event, it would be difficult to routinely monitor these screening efforts using any sort of verification list.

4.7.3 Characterization of the Active Substances.

The technique for identifying a biologically active substance usually only characterizes its activity in a crude way. Once a sample with biological activity has been identified, the next task is to identify and purify the active substance in the sample. The product may be a complex organic molecule produced by the organism's metabolism, an enzyme or an active peptide. If the active substance is a large peptide or enzyme, an effort would probably be made to clone the gene that encodes its sequence. Complex organic molecules are typically analyzed by the techniques of organic chemistry, and a total organic synthesis of the molecule may be attempted if the compound has sufficient merit.

A biologically active extract can be separated into individual compounds by standard chromatographic techniques. Rescreening each of the pure components leads ultimately to the identification of the active compound in the mixture. The mechanism of the substance's action and an assessment of its full range of effects takes a long time to evaluate. Until these materials are sufficiently characterized, they could be included on a list as proscribed because their nature is unknown, only to be removed at some later time when their nature and biological activity is sufficiently defined.

One group of substances of possible concern are Central Nervous System (CNS) active compounds, because of their potential for rapid debilitation of targets. However, most of the compounds identified as active against CNS functions are in fact not candidates for pharmaceutical development and could not be used directly against the CNS because of the difficulty in crossing the blood/brain barrier. The research interest in these compounds stems from the fact that they aid in identifying new cell receptors

and cell functions. As basic tools for research, these natural products are superb. Although they may have a use as potential B.W. agents, they certainly would fall outside any list of specifically proscribed materials. But until their nature and physiological effects are fully reported in the literature, these tools could be included in a list under the category of unknown nature. Identification, characterization and reporting of new, potential biologics from natural products research is likely to be a very slow process. This means that a list of these materials could become very large and difficult to use for verification purposes.

A question for the utility of a list for verification efforts is whether new un-named compounds or organisms with BW significance could be identified covertly. The biologic would remain unknown and unlisted until identified by a researcher with legitimate uses for this material. It is beyond the scope of the this task to predict the relative probability at which this scenario could occur.

4.8 APPLICATIONS OF NATURAL PRODUCTS.

4.8.1 Pharmaceuticals.

Natural products have historically been the most promising source of new pharmacologically active compounds. Most of the antibiotics and other therapeutic drugs currently in use were either first identified as natural products or are related to a family of natural products in some important way.

According to a recent report (Biotechnology News, 12 (20), 1992), 25% of prescription drugs are derived from plants but only 10% of the known plant species have been evaluated for therapeutic potential. Research to discover new pharmaceuticals from plants is ongoing. For example PHYTOpharmaceuticals, Inc. (San Carlos, CA) has established cooperative agreements with two Brazilian biotechnology institutes. Plants are to be collected in Brazil and sample libraries for

screening will be made. This company is also looking for collaborators in China and Eastern Europe.

4.8.1.1. Immunoactive Compounds. Gludapcin is a one example of a microbial secondary metabolite which can act as a macrophage activator and which can stimulate antibody production. It is currently being tested for activity in mice. It is also possible to isolate compounds which suppress immune function, as is the case with FK-506, a compound which suppresses the mixed lymphocyte reaction in mice. This second compound was isolated as a secondary metabolite from a species of *Streptomyces*. Both of the mentioned compounds were first reported by Japanese researchers, an indication that not only United States researchers are interested in this competitive research area.

4.8.1.2 Antibiotics. Most of the traditional antibiotics currently in use were derived, at least in part, from natural product research; these include the penicillins, tetracyclines and aminoglucosides such as kanamycin, as well as a host of others. Screening of soil microorganisms for new antibiotic products is a large, on-going task.

A recent example of a less traditional product is the apparently highly effective peptide Magainin, derived from frog skin. The skin of African frogs of the genus *Xenopus* is protected from bacterial infection by the action of Magainin. Synthetic Magainin is also an effective antibiotic, and the product may be commercialized.

4.8.1.3. Anti-Cancer Agents: Taxol. Bristol Meyers Squibb, a large pharmaceutical manufacturer, is investigating the anti-tumor drug Taxol, which is derived from the leaves and bark of the pacific yew tree. The taxol story illuminates many of the facets of natural product research. First, Taxol is present in low concentration in the bark and needles of a particular species of yew tree. The compound was first recognized and purified because of its effect on cultured cells. Related species of yew produce related compounds, but these do not have the full effect of taxol. Taxol recently achieved some notoriety because of its scarcity and the fact that it is a product of old growth forest of the Pacific Northwest. Because of its scarcity, an effort was made to

produce it synthetically, starting with the more abundant taxol-like compounds available from other yew species. Finally, synthetic compounds related in structure to taxol are being created with the hopes of finding still more effective compounds. Taxol is in the clinical trial stage for action against refractory cases of ovarian cancer.

Cyclosporines are also derived from natural product research. Early work with the cyclosporine compound campothecin has revealed that it is relatively toxic. A second promising cyclosporine drug, topotecan, is currently undergoing development by the Smithkline Beecham corporation.

4.8.1.4. Central Nervous System (CNS) Active Compounds. The compound Argiotensis is a secondary metabolite found in spider venom and is active against a variety of insect as well as mammalian species. It acts to block glutamate receptors in the brain, and may have some use as a pharmaceutical for the treatment of stroke victims. According to Natural Products Sciences, a Utah based company developing spider venoms natural products, about 20% of the spider and scorpion venom compounds active against insects are also active against the CNS of mammals; they have BW potential as well as therapeutic value.

4.8.2. Agricultural Chemicals.

As plant molecular biology becomes more sophisticated, various collections of potential natural product sources are being screened for all types of pesticides, as well as for compounds with more general effects on plant or insect physiology. The most well known natural product currently under development as a commercial agrochemical is BT toxin. BT toxin was originally isolated from *Bacillus thuringiensis* because of its ability to kill insects selectively. Its chief advantage over chemical pesticides is that it is not toxic to mammals and is extremely specific in the types of insects it kills.

The new commercial agricultural chemicals developed from compounds first identified as natural products would not have been included on a list of specifically proscribed materials during their development, but because their nature and pathogenicity were undescribed, they could have been included on a list that proscribed by that category. Because the development of these materials required and will require very long term programs, large numbers of these ultimately low toxicity materials could be included on a list, making it very unwieldy as a verification tool.

SECTION 5
CONCLUSIONS

This paper has examined the concept that a list, either of specific proscribed ABOs or of categories of proscribed materials, has utility as a tool for verification of the BWC. To be useful, a list has to be objective and complete or all-inclusive. Using these criteria, we have compared existing proposed lists. We have evaluated current biological research, genetic engineering research and technologies, and natural products research for their impact on the ability to prepare a list useful for verification.

None of the proposed lists examined were complete. The Russian list did not mention toxins at all; the U.S. list included "Tricothecene toxins" without being specific, while the German list specified two tricothecene toxins by name. None of the lists included two highly pathogenic toxin producing species of *Clostridium*. Neither of the Australian Group's lists included antiplant agents or bioregulators.

Current research has an impact on the ability to prepare a complete list of proscribed materials. The diversity of the bacterial world and the continuing discovery of new genera, species and biologically active compounds make the preparation of a complete list impossible. Reclassification and renaming of existing organisms, selective modification of existing strains and development of new strains have a severe impact on the ability to prepare an all-inclusive list that is useful as a verification tool because the resulting list becomes too large to be useful.

Genetic engineering research and technologies have already demonstrated that it is possible to affect the expression of a wide variety of genes or move genes between organisms. The recombinant possibilities that are available with this technology are impossible to describe until they are actually prepared. No list of proscribed organisms is adequate because it cannot be complete or all-inclusive; if categories of materials

are proscribed, then the resulting list becomes so large and unwieldy that it is not useful.

Natural products research encompasses a diversity of products with very broad scope. Because of continuing new discoveries, natural products research would ensure that no list could be complete. Any list that attempted to proscribe by categories would result in a list which includes almost everything done in natural products research. That list would be too large to be useful as a verification tool.

In the course of our examination of the utility of lists as a tool for verification of the BWC, we could find no list of specifically defined materials that could meet the completeness criterion; new organisms are being discovered, known organisms are being reclassified and renamed, genetic engineering provides the ability to change the expression of traits, or even move those traits between organisms, and natural products research continues to discover new pharmacologically active materials from the vast world of nature.

A list that attempts to describe proscribed materials by categories or classes (or any other criterion) is so large that it is unwieldy and not practical to use as a tool for verification. Such a list lacks currency and objectivity; especially on the cutting edge of research, much of the information needed to make a determination of whether an organism or strain is in fact proscribed is unavailable. Further, the people in the best position to make that determination are often those with a vested interest in seeing the research completed.

We have not been able to demonstrate the utility of a list as a verification tool; rather, lists have little use as such a tool.

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TDANP-TRC August 1, 2001

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ATTN: OCQ/MR LARRY DOWNING

SUBJECT: DOCUMENT CHANGES

The Defense Threat Reduction Agency Security Office reviewed the following documents in accordance with the Deputy Secretary of Defense Memorandum entitled, "Department of Defense Initiatives on Persian Gulf War Veterans' Illnesses" dated 22 March 1995, and determined that the documents were unclassified and cleared for public release:

DNA-TR-93-84, AD-B244408, Acoustic Resonance Spectroscopy in CW Verification Tooele Field Trial (August 1992).
DNA-TR-93-129-V1, AD-B192045, Global Proliferation – Dynamics, Acquisition Strategies and Responses, Volume 1 – Overview.
DNA-TR-93-129-V2, AD-B192046, Global Proliferation – Dynamics, Acquisition Strategies and Responses, Volume 2 – Nuclear Proliferation.
DNA-TR-91-216, AD-B163637, Harmonizing the Chemical Weapons Convention with the United States Constitution.
DNA-TR-92-180, AD-B175230, Evaluation of the Concept of a List for the BWC.
DNA-TR-92-61, AD-B167663, Basic State Party Functions and Skills Under CWC.
DNA-TR-92-66, AD-B167357, Domestic Reporting Requirements for Chemical Industry.
DNA-TR-91-213, AD-B163260, Analysis of the Interactions Between Treaties.
DNA-TR-93-70, AD-B177262, Chemical Weapons Convention Inspections of Private Facilities Application of United States Environmental and Safety Laws.
DNA-TR-92-182, AD-B173450, Commercial Products from Demilitarization Operations.
DNA-TR-91-217-V3, AD-B169350, Chemical Weapons Process Parameters, Volume 3 – Users' Guide.
DNA-TR-92-116-SUP, AD-B175292, Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC), Supplement.
DNA-TR-92-128, AD-B175452, Task 1 Report Target Vapor Identification and Database Development.
DNA-TR-92-196, AD-B174940, Task 2 Report Algorithm Development and Performance Analysis.
DNA-TR-93-68, AD-B178109, CW Detection Instrument R&D Design Evaluation.

Enclosed is a copy of the referenced memorandum. If you have any questions, please call me at 703-325-1034.

Ardith Jarrett

ARDITH JARRETT
Chief, Technical Resource Center