
3.4 Biological Weapons Convention (BWC)

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1. The problem with the Biological Weapons Convention

Efforts to prevent the use of biological weapons (BW) began with discussions about international controls towards the end of the nineteenth century, just at the time when microbiology was becoming established as a science. Following the large-scale use of chemical weapons in World War I, a milestone in the control of chemical and biological weapons (CBW) agents was reached with the signing of the Geneva Protocol in June 1925. This treaty, which subsequently entered into force in February 1928, prohibits the “...use in war of asphyxiating, poisonous or other gases and of bacteriological methods of warfare”, thus banning the use of both chemical and biological weapons. Although the treaty allows states to have reservations on retaliation which renders the protocol a no-first-use agreement only, it has now become accepted as customary international law.

For quite some time thereafter, it was extremely difficult to strengthen controls by moving towards a ban not only on use but also on possession of chemical and biological weapons. After World War II, negotiations that considered both chemical and biological weapons together were contentious and reached an impasse. There was an indication that the US was concerned that CBW disarmament might set a precedent for nuclear weapons. It was subsequently reasoned that it might be easier to agree a treaty on biological weapons if it were handled separately. This was no doubt due to the reluctance of some states to give up completely an established and proven chemical weapons capability, which could be used for deterrence or retaliation. Biological weapons on the other hand were not as extensively developed and regarded by many to be of uncertain utility.

In the end, a British proposal to handle biological weapons separately was followed. Negotiations over a Biological Weapons Convention received a decided boost when Richard Nixon announced on February 25, 1969 that the United States was unilaterally renouncing its offensive BW programme and would from that time on engage solely in defensive BW research. Several explanations for this startling announcement have been offered. One reason was that extensive US analysis of BW capabilities concluded that these weapons had limited tactical utility and were not a reliable strategic deterrent. The military nevertheless preferred to retain an offensive capability because of the realization that biological weapons could have equivalent lethality to nuclear weapons, but they yielded to the argument that it was important to discourage other countries from acquiring them. Indeed, it has been suggested that the US had actually become convinced about how devastating the use of biological weapons could be. At the same time, Nixon could deflect criticism of the US over the war in Vietnam, in which it had used chemical riot control agents and herbicides.

During the negotiations over the BWC, one very contentious issue from the start was that over the verification of compliance. The US view was that a verification regime would have to be totally intrusive if it were going to be effective, and it demanded tough verification measures accordingly. The
Soviet Union on the other hand was stubbornly unwilling to accept on-site inspections. The reasons why the Soviet Union did not want an effective verification system became clear in later years, when it became known in 1992 that it had engaged in a massive offensive BW programme all along, even after signing the convention. Although the US insisted throughout negotiations upon tough verification measures, it actually held the view that the types of measures it was calling for still could not guarantee a fool-proof system of verification. Possibly for this reason, the US finally gave up its insistence upon a tough verification regime.

The BWC, referred to as the Biological and Toxin Weapons Convention, was subsequently agreed in 1972, and it entered into force in 1975. To date, some 154 countries are states parties to this convention. Agreement was primarily achieved after the introduction of the General Purpose Criterion, which formed the basis of the prohibitions laid out in Article I of the Convention, which states, in part, that:

"Each State Party to this Convention undertakes never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:

1. Microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes". (emphasis added)

Clearly, the General Purpose Criterion (italicised above) allows the peaceful uses of biological agents but prohibits any use that is not for peaceful purposes. The Convention thus applies to all possible agents and future developments and is not a prisoner of the technology of the 1970s. This is the real strength of the Convention; its weakness lies in its ineffective implementation.

1.1. Verification mechanisms within the BWC

As a result of non-resolvable disagreement over verification measures, and the fact that few states were willing to accept intrusive investigation procedures, only rudimentary verification provisions were written into the Convention in the form of Articles V and VI.

1.1.1. Article V mechanisms: Consultations

Article V of the BWC calls upon states parties to „undertake to consult one another and to cooperate in solving any problems which may arise in relation to the objective of, or in the application of the provisions of, the Convention.“ It does not, however, specify how these consultations are to proceed. It merely states an obligation to consult in a cooperative manner when there is a question of non-compliance, and that these consultations can take place either on a bilateral or multilateral basis, in response to a reasonable request. This mechanism has been invoked in two instances, but the results in both cases were inconclusive.

One of these attempts employed a bilateral procedure under Article V. The US tried on three separate occasions to obtain an official clarification from the Soviet Ministry of Foreign Affairs about the outbreak of human anthrax in the Soviet city of Sverdlovsk in April, 1979. Each time, Moscow denied any wrongdoing and claimed the outbreak was of natural origin, occurring from the ingestion of infected meat. However, many questions remained unanswered, so the question of compliance remained unresolved. Years later, a forensic investigation of the Sverdlovsk incident reached the conclusion that the outbreak was the result of accidental release of anthrax spores from a military facility.

Article V also provides for multilateral consultations. This mechanism was invoked for the first time in answer to an allegation by Cuba that the US government had deliberately released a crop-destroying insect pest over the island from an aircraft in order to disrupt its agricultural system. Again, the issue could not be resolved bilaterally, so Cuba turned to the Russian Federation (which was one of the BWC depositary states to be approached in the event of a request for a multilateral consultation procedure) in 1997 and requested a meeting of the states parties to consider the issue. The consultations were subsequently held in Geneva August 25-27. The evidence was reviewed and thirteen states parties...
submitted their written comments on the case. Most of these said they were not convinced of a causal link between the flight of the US aircraft over the island and the infestation, and three states argued that the lack of detailed information and the technical complexity of the issue made it impossible to reach a clear verdict. As a result, it was concluded that it “has not proved possible to reach a definitive conclusion with regard to the concerns raised by the Government of Cuba”.

In both cases outlined above, the US and Cuba chose not to invoke the mechanisms available in Article VI.

1.1.2. Article VI mechanisms

Article VI provides that any party “which finds that any other State Party is acting in breach of obligations deriving from the provisions of the Convention may lodge a complaint with the Security Council of the United Nations. Such a complaint should include all possible evidence confirming its validity...”. Article VI also requires each State Party “to cooperate in carrying out any investigation which the Security Council may initiate...”. Although some attempts were made by the UK during negotiations to structure Article VI so that a permanent member of the Security Council could not veto an investigation (i.e. that investigations of alleged use be carried out under the auspices of the UN Secretary General), the final language of Article VI retained the right of veto by a permanent member of the Security Council. The implicit threat of a Soviet veto was no doubt why the UK and the US refrained from requesting an investigation into the Sverdlovsk incident.

1.2. Efforts to strengthen the BWC

The Second Review Conference of the BWC in 1986 coincided with the initial stages of the ending of the east-west Cold War, and there was some optimism about the possibility of introducing confidence building measures (CBMs) into the Convention. Indeed, a series of CBMs (mainly annual data exchanges) were agreed in 1986 and were expanded and developed in 1991 at the Third Review Conference. Unfortunately, it was not possible to add these CBMs as legally binding measures to the BWC, so they remain only politically binding. It is clear from numerous analyses that these merely voluntary CBMs have not been treated seriously by most States Parties and have done little to increase transparency and trust.

The Third Review Conference of the BWC took place just after the 1991 Gulf War amid great concern about the possible use of biological weapons by Iraq. Thus, a group of governmental experts under the name of VEREX was mandated to examine potential verification measures and to consider their technical feasibility. This series of VERTEX meetings took place in 1992-93 and the group submitted a positive report in 1993. A special Conference of States Parties examined the report in late 1994, and then mandated the work of an Ad Hoc Group (AHG), which met in Geneva from 1995 until 2001. Though the mandate of the AHG was much broader than just the strengthening of the Convention through improved verification measures, it crucially was:

"...to consider appropriate measures, including possible verification measures, and draft proposals to strengthen the Convention, to be included, as appropriate, in a legally binding instrument..."

So this marked a clear change in approach. States Parties agreed that there should be an attempt to agree verification measures to be included in a legally binding instrument (i.e. compulsory, not voluntary) as part of the process of strengthening the BWC.

The initial stage of the Ad Hoc Group's work was devoted to building on the studies of VEREX in order to identify the elements required in a legally binding Protocol to the BWC. This initial stage of work lasted from 1995 through to mid1997. It was only in the July-August session of 1997 that the group made a transition in its work to the consideration of a rolling text of the Protocol. The Fourth Review Conference of the Convention itself, in 1996, had called for an intensification of the work of the AHG and with the transition to a rolling text, more detailed provisions could be included in a systematic manner. According to Ambassador Tibor Tóth, the chairman of the AHG, a third stage of negotiations...
began in January 1999 with "the move to a final framework for the Protocol and the detailed negotiation on key elements". Numerous statements made to the AHG by ministers from States Parties at the March 2000 session, which coincided with the 25th anniversary of the Convention, stressed that the text was at an advanced stage and that an agreement could be reached prior to the Fifth Review Conference of the BWC in late 2001.

Ambassador Tóth subsequently produced what is called the chairman’s compromise text, which is an impressively long and complex document, containing 30 Articles and numerous Annexes which extend to over 200 printed pages, and it was possible to see how the remaining contentious issues might be successfully resolved to produce a strong Protocol. However, it was clear at the same time that numerous points of serious disagreement remained to be resolved. For example, there were concerns that some states wished to use the Protocol definitions to limit the General Purpose Criterion of the Convention. There were also strong divisions between the developing and developed world over how export controls and assistance and co-operation in biotechnology should be handled in the future. Finally, there were differing views concerning crucial elements of the central compliance (verification) measures of the Protocol.

The European Union has consistently held the view that the compliance measures should consist of the mandatory declaration of the most relevant facilities and activities and a system of visits to ensure the accuracy of these declarations, backed up by the possibility of challenge investigations in the event of well-founded concerns. Furthermore, a modest organisation to ensure that the undertakings in the Protocol are carried out was also required. In effect, this would provide the same overall compliance architecture as is being successfully implemented in the Chemical Weapons Convention (CWC), but with a smaller organisation and costs. Comparison of these proposals with the requirements of the Ad Hoc Group's mandate suggests that such a BWC Protocol would be just as adequate as the CWC in achieving the desired ends.

A difficult problem arose from the position taken by the pharmaceutical industry in the United States. Its trade association (PhRMA) says that it supports the strengthening of the Convention but it has consistently opposed a key element of the European Union's position. It has stated often that it is opposed to the kinds of visits necessary to adequately check the mandatory declarations (earlier called routine visits). It is clear that this influenced the position taken by other trade associations and, more particularly, the United States government. If this position of industry were to be accepted, then declarations would essentially be equivalent to the previously agreed - and demonstrably ineffective - Confidence Building Measures.

At the last meeting of the Ad Hoc Group in July-August 2001 and after over six years of negotiations, an agreement on the chairman’s compromise text was supposed to be reached. However, the US government decidedly rejected not only the Protocol text but also the whole process of further negotiations over the Protocol. As a result, no agreement could be reached, the meeting ended in disarray, and the future of negotiations was placed in an uncertain position. One reason Ambassador Mahley gave for rejecting the Protocol was that its provisions were too weak to test compliance. This appears to be in accord with the long-held belief of the US government that effective verification of the BWC is not possible (see discussion above). Another reason offered was that the verification measures contained in the Protocol would pose a risk to the protection of sensitive confidential national security as well as commercial proprietary information. At the same time, it has been pointed out that the Protocol contains more far-reaching measures to protect confidential information and is less intrusive than the CWC.

Potentially embarrassing for the US government in the light of its position taken in rejecting the Protocol was the revelation of some secret activities in the area of biological defense research that were being carried out or were being planned for the future. On the one hand, a factory was built that was capable of producing biological agents in large amounts (by the pound). Although only harmless simulants were actually produced, the operation was supposed to show that a clandestine BW production facility could be built from commercially available materials and equipment. In addition, the US Department of Defense has plans to repeat some experiments carried out by Russian scientists, which are potentially dangerous and could be viewed as contributing to arms proliferation. Briefly, the Russian investigators changed the
anthrax bacillus by genetic engineering so that the immunity provided by the normal vaccine was no longer effective against this manipulated strain. US defense researchers want to develop a vaccine that would be effective against such a manipulated strain. They have apparently tried unsuccessfully to obtain the strain from the Russian researchers who produced it, so they plan to repeat the experiments themselves. According to the provisions and prohibitions formulated in Article I of the BWC, these activities would be allowed, because they would be carried out with the intention of building a defense against potential biological weapons. Nevertheless, because of the nature of the activities, they can be interpreted as being dangerously close to the line of illegality.

The third activity that was revealed is even more controversial and more difficult to justify as biodefense. The Central Intelligence Agency (CIA) constructed a model of a small Soviet bomb filled with a biological agent (once again, in this case a harmless simulant) with the aim of testing the dispersion properties of the agent contained in the bomb. It was argued that these tests were made in order to build a proper defense against such a weapon. However, some critics of these activities point to the possibility that the general purpose criterion of Article I of the BTWC does not apply here. Instead, the second paragraph of Article I explicitly forbids the development, production, storage, or acquisition of "weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict." In this respect, these experiments of the CIA were not in compliance with the rules and the spirit of the BTWC.  

The fact that these activities were kept secret together with the rejection of the Protocol on the grounds that it poses a risk to the protection of sensitive national security information raises the suspicion that the US government does not want its activities in the area of biodefense to be monitored. This position taken by the US definitely sends the wrong signal to other nations, encouraging them to act in like form. Following the Fifth Review Conference of the BTWC in November-December 2001, strengthening the Convention with measures to test compliance became even more uncertain. Once again, the USA played a major role. Two hours before the Conference was to come to an close, the US delegation proposed ending the mandate of the Ad Hoc Group, in order to crush any further attempts at negotiation over the Protocol and to prevent agreement on a Final Declaration of the Conference that would not be suitable to the US government. With that, there was a general agreement not to conclude the Conference but rather to adjourn it until November 2002, in order to achieve a "cooling off" period and to avoid total failure.  

The politics of the US government at the resumption of the Fifth Review Conference in November 2002 did not change. An agreement to resume negotiations over the Protocol or even verification measures was not possible. In the Final Document of the Fifth Review Conference, the States Parties agreed on a new procedure of work for the coming years until the Sixth Review Conference in 2006. It agreed "to hold three annual meetings of the States Parties of one week duration each year commencing in 2003 until the Sixth Review Conference, to be held not later than the end of 2006, to discuss, and promote common understanding and effective action on:

i. the adoption of necessary national measures to implement the prohibitions set forth in the Convention, including the enactment of penal legislation;

ii. national mechanisms to establish and maintain the security and oversight of pathogenic microorganisms and toxins;

iii. enhancing international capabilities for responding to, investigating and mitigating the effects of cases of alleged use of biological or toxin weapons or suspicious outbreaks of disease;

iv. strengthening and broadening national and international institutional efforts and existing mechanisms for the surveillance, detection, diagnosis and combating of infectious diseases affecting humans, animals, and plants;

v. the content, promulgation and adoption of codes of conduct for scientists."

The first two topics were discussed in 2003, while topics iii. and iv. were covered in 2004. In the eyes of many observers these discussions up to now have resulted in no substantive improvements or strengthening of the Convention. On the positive side, the States Parties have continued talks in a
multilateral arena, and some discussions have been useful. For example, more time than usual has been devoted to discussions over implementation of the Convention, which is a crucial topic. Still, little has been forthcoming in the way of promoting effective action.

A great deal of hope is being placed on the last topic, that of the formulation and adoption of codes of conduct for scientists, which will be the topic of the Meeting of Experts and the Meeting of the States Parties in 2005.

2. The Technology Available for BW Verification

After the end of World War II, the USA began several programs that were designed to strengthen defenses against biological weapons. Above all, the development of the capacity to detect BW on the battlefield was a high priority. The design of automatic, remote sensing systems similar to the types used to detect chemical weapons was intended. The names of some of the programs that are in development include the Biological Integrated Detection System (BIDS), Long Range Biological Stand-off Detection System (LR-BSDS), Short-Range Biological Stand-off Detection System (SR-BSDS), Joint Biological Remote Early Warning System (JBREWS) and the Joint Biological Point Detection System (JBPDS).39

In addition to the remote sensing systems, other methods of detection based on sample-taking are being developed. All of these systems are not so highly developed that they can detect biological agents automatically and differentiate among them. Some systems can detect and differentiate up to 4-7 different agents, and improvements can be expected. Some of the innovations include attempts to integrate antibody or DNA detection methods into these systems, in order to achieve a better differentiation of the agents. Although definite progress has been made in this area, the methods are not yet suitable for battlefield use. The following represents an analysis of further new developments in biotechnology that may be useful for verification. Some parts have been excepted from a recent study.40

2.1. Antibodies as Diagnostic Reagents

Normally, when an animal is immunized with an antigen (usually a protein or a polysaccharide molecule that is foreign to the animal), it responds by producing antibodies to that antigen. These antibodies are directed against a small region of the molecule, for example four to six amino acids in the protein or four to six sugar molecules in the polysaccharide. These regions are called antigenic determinants or epitopes, and the respective antibodies bind with these epitopes in a very specific manner. Since, for example, proteins contain a characteristic (usually large) number of amino acids, the immune system produces antibodies directed against several different regions or epitopes of that protein molecule when responding to the antigenic stimulus. Each of these specific antibodies is produced by a single antibody-producing cell, which multiplies to a clone of cells during the response, producing identical antibody molecules termed monoclonal antibodies. Normal immune responses are composed of a mixture of clones of antibody-producing cells, and are therefore polyclonal in nature. Accordingly, polyclonal antibodies are a mixture of antibodies with varied specificity, secreted into the serum of that individual.

2.1.1. Hybridoma Technology

Hybridoma technology involves the production of monoclonal antibodies in cells known as hybridomas. Monoclonal antibodies have a single specificity, being derived from a single clone and directed against one single antigenic determinant with which they can bind. They are thus exquisitely specific reagents that can be used to detect the presence of a particular antigen, thus identifying a particular microorganism in an unequivocal manner. Antibody-producing cells do not divide in culture and die after a few days, never living long enough in vitro to produce significant quantities of their monoclonal antibodies. Malignant cells, on the other hand, are immortal and can be kept in culture continuously, where they divide and multiply. Malignant antibody-producing cells can be isolated from individuals afflicted with the cancerous disease myeloma, but these cells secrete monoclonal antibodies of unknown specificity (the antigen epitope against which they are directed is unknown). In 1975, Georges Köhler and Cesar Milstein devised a method for generating monoclonal antibodies in vitro41 that has revolutionized all areas of biological research. In this case, a normal, mortal, antibody-producing cell of defined specificity
(obtained from an animal immunized with a particular antigen) is fused with a malignant antibody-producing cell that has, however, lost the capacity to produce antibody itself. The result is a hybrid cell (hybridoma) that is immortal and produces monoclonal antibody of a defined specificity. The hybridoma can be placed in cell culture, where it continues to divide and produce relatively large quantities of a monoclonal antibody. Usually, the antibody-producing cell is obtained from a mouse, but other small animals such as rats and hamsters have also been used routinely. Monoclonal antibodies have been invaluable for research, diagnostic, and therapeutic purposes.

2.1.2. Enzyme-linked Immunosorbent Assays (ELISA)

Reference has already been made to the exquisite specificity of monoclonal antibodies in binding to antigens. They therefore represent a powerful potential means of identifying specific epitopes on antigen molecules, such as unique surface structures on microorganisms or unique antigenic determinants on toxin molecules. Their usefulness in supporting verification measures in a compliance regime depends to a large extent on how they are coupled to detection systems, which in turn determines their sensitivity and facility in identifying agents. Solid-state enzyme immunoassays (EIA) based on enzyme-linked immunosorbent assays (ELISA), have in the past few years been very useful tools in diagnostic laboratories for detecting microorganisms and cell products. Naturally, it is essential to obtain antibodies with high affinity binding capabilities, and this is the first limitation often encountered; experimentally, not all hybridomas produce high affinity antibodies. Some amount of skill is needed to set up the sandwich ELISA for new antigens, especially because of the requirement for two antibodies recognizing non-overlapping epitopes on the antigen. However, once the system functions well the test is extremely simple to perform, even for less-skilled personnel, and it is relatively sensitive, having a level of detection capability in the picogram (\(10^{-12}\) grams) range. Most microorganisms and toxins can be identified by this method.

While the ELISA can be carried out and results measured in a mobile laboratory, it does take one or two hours to perform, and the sensitivity may not be adequate for some situations. Modifications of the system are therefore being explored. One reported example is a semiautomated EIA test system using paramagnetic beads to capture the antibodies. The assay can be performed in suspension, which increases its sensitivity. The first and second antibodies are added to the antigen and allowed to react. The beads, which are coated with a substance that can bind antibodies non-specifically, are subsequently added to the suspension to capture the antibodies. The beads with the captured antibodies can be immobilized magnetically to facilitate washing. The second antibody of the two employed in the system is linked to a substance that produces a chemiluminescent reaction when activated, which can be measured in a special instrument. With this method, femtogram (\(10^{-15}\) grams) sensitivity levels were achieved for several antigens tested. Also, the testing time was reduced to 20-40 minutes.

The application of ELISA methods in the form of high-throughput microarrays is a development that is especially promising for the identification of biological agents. The principle is illustrated in Figure 1. Using a bifunctional N-hydroxysuccinimid ester, specific antibodies are bound to a micro glass plate (a chip) in a designated order. Antigens that bind to these antibodies can be detected with monoclonal antibodies (specific for those antigens) that have been labeled (tagged) with biotin. Biotin binds to a streptavidin molecule that has been coupled to an enzyme (in this case alkaline phosphatase). After addition of a fluorescent substrate of the alkaline phosphatase, the enzyme cleaves a phosphate group from the substrate, allowing the substrate to become fluorescent, which can be visualized under ultraviolet light. Fluorescence would then indicate that an antibody-antigen reaction had occurred, that is, that the antigen was present in the sample under investigation.

**Figure 1:** Schematic representation of an indirect ELISA format for the detection of antigens in antibody arrays. Specific antibodies are bound to a glass plate in a designated order using a bifunctional N-hydroxysuccinimid reagent. Antigens that can bind these antibodies can be detected with biotin-labeled monoclonal antibodies specific for those antigens. The antigen-antibody reaction is visualized by the addition of streptavidin coupled to an enzyme that can change a substrate to a fluorescent molecule. **Source:** Mendoza et al. (1999), modified.

The schematic representation of a glass chip of this kind can be seen in Figure 2. In this case, a microarray plate consisting of 96 wells has been used. Each of the wells consists of an area containing 5 x
6 antibody arrays. With this system, 96 different samples can be tested for the presence of antigens that can bind the antibodies in the arrays.

**Figure 2:** Antibody arrays for use with microtiter plates with 96 wells. Each well contains an antibody array consisting of 5 x 6 different antibody specificities. In this system, 96 different samples can be tested for the presence of antigens that are able to bind the antibodies in the array. Source: Mendoza et al. (1999).46

Developments in antibody-detection systems are advancing at a rapid pace, with accuracy, ease of performance, and automation in mind. There should be no problems involved in applying these methods in a practicable manner to situations calling for verification of compliance to the BWC.

### 2.2. Nucleic Acid Hybridization Techniques

In general, the composition of the genetic material that a microorganism carries determines its characteristics. However, the activity of a particular gene can be turned on and off according to regulation, so that some traits may not always be expressed under certain conditions, even though the genes are present and functional. While traits are of great practical importance in handling microorganisms, it is their nucleic acid base sequence that most accurately identifies the organisms and classifies them into phyla, groups, genera, species and strains. In the past decade, there has been a virtual revolution in the area of science describing the evolutionary relatedness of living beings, using nucleic acid base sequence analysis as a tool.47 In general, highly conserved nucleic acids such as ribosomal RNAs have been most useful for such analyses, although the genes encoding particular proteins have also yielded valuable information. Nucleotide base sequence analysis can also provide information regarding traits of microorganisms, such as the possession of genes determining virulence factors, for example, toxins. This would be of particular interest in the case of microorganisms that are potential biological weapons.

To identify microorganisms or trait-determining genes on the basis of nucleic acid base sequence, it is not necessary to actually sequence DNA or RNA. Instead, the technique of nucleic acid hybridization has proved to be very practical. Hybridization reactions take advantage of the ability of heat to break the bonds holding the nucleotide bases on opposing DNA strands together. If a labeled „probe“, consisting of a synthetic oligonucleotide or a DNA fragment, is added and the mixture allowed to cool, a single strand of the probe will anneal or bind (hybridize) to one of the nucleic acid strands, if it contains the proper complementary base sequence. The molecule thus produced is a hybrid of the DNA target strand and the probe. Hybridization is accordingly a measure of the nucleotide sequence match between target and probe nucleic acids. The label (reporter group) on the probe allows the visualization of successful hybridization.

A schematic representation of direct and indirect detection methods for hybridization of a probe with its target can be seen in Figure 3.48 In this procedure, the target DNA is heated to separate the two strands. When these are allowed to cool in the presence of a probe that has a nucleotide sequence complementary to one of the DNA strands, that probe will bind or hybridize with the target DNA. By using probes defining specific DNA sequence regions, the target DNA can be identified. Reporter groups for visualizing the hybridization reaction are attached to the probe. These are very often radioactive isotopes such as [32P] that have been incorporated into the nucleic acid, or enzymes that produce either a color or a chemiluminescent reaction when the appropriate substrate is added. Hybridization tests using nucleic acid probes are being applied extensively by many laboratories today for the detection of specific microorganisms.

**Figure 3:** Schematic representation of labeling and detection methods for nucleic acid probes. The nucleic acid probe hybridizes (binds) with homologous base sequences on the target nucleic acid. The label (reporter group) allows visualization of successful hybridization. In (A), the label is bound directly to the probe. In (B), a modifying group has been attached to the probe. The label is placed on a molecule that can bind specifically to the modifying group on the probe. Indirect methods are in some cases more sensitive than direct methods. Source: Reprinted from Kluwer Academic Publishers book *Molecular Methods for Microbial Identification* © 1993, pages 64-92, Chapter 3, Identification and typing by nucleic acid hybridization techniques, K.J. Towner and A. Cockayne, figures 3.1 and 3.2, with kind permission from Kluwer Academic Publishers.
A requirement for identification by hybridization procedures is the possession of specific probes for the genes that are to be detected. Specific probes for many microorganisms and viral genes are available, and others can be made by applying various gene cloning procedures. Even if nucleotide base sequences are not known, gene probes and hybridization analyses can still be usefully applied by employing methods designed for scanning nucleic acids by amplification with arbitrary oligonucleotide primers.49

A particularly promising application of hybridization procedures for the detection and identification of microorganisms is the use of high-density DNA microarrays.50 In this case, DNA sequences (oligonucleotides) specific for the DNA of different microorganisms are used. These oligonucleotides are immobilized on micro glass plates or chips. For the immobilization of the oligonucleotides, microscope slides that have been coated with poly-L-lysine are employed. Alternatively, other substances such as different silicium compounds, can be used for coating the slides. The oligonucleotides are "printed" on the slides in a designated pattern, and more than 250,000 different oligonucleotides pro square centimeter can be placed in the array.

For the investigation of a sample, either RNA or DNA is isolated from the sample and labeled with fluorescent compounds (e.g. Cy3- and Cy5-dUTP). These labeled nucleic acids are then pipetted onto the oligonucleotide arrays, and the presence of a particular microorganism in the sample can be seen by a positive hybridization reaction with one of the oligonucleotide spots in the array (fluorescence of the particular spot).

2.3. The Polymerase Chain Reaction

One main problem in using molecular biology procedures is having enough of a specific DNA molecule or gene sequence in hand to work with. Cultivating organisms, extracting DNA, identification of specific gene sequences and amplification (reproduction) of the material by cloning can be time-consuming undertakings. The polymerase chain reaction (PCR) was developed in the mid 1980’s and has since revolutionized molecular biology and genetics. It is a method for the rapid amplification of DNA in vitro (in a test tube). It can multiply DNA molecules by up to a billionfold in the test tube in the span of a few hours, yielding sufficient amounts for identification, cloning, sequencing, or for use in mutation studies.

For the replication of DNA, the enzyme DNA polymerase synthesizes a new strand of DNA, using a single strand of the old DNA as a template. In addition, the polymerase requires a short piece of double-stranded DNA as a primer, to begin the synthesis. By using primers with specified nucleotide sequences, the starting point and the stopping point of the DNA synthesis can be precisely determined. Thus, one major advantage of the PCR is that a specified region of the DNA used as a template will be amplified, dictated by the nucleotide sequence of the primers that define the boundaries of the region. The PCR method requires that at least a portion of the nucleotide sequence of the gene to be amplified be known in order to make the specific primers. The primers with the desired nucleotide sequence can be readily synthesized, and many biotechnology firms offer production of these at affordable prices for research. In the PCR reaction (Figure 4), a cycle of synthesis includes melting the DNA by raising the reaction temperature to about 94°C to form single DNA strands, cooling the reaction mixture to about 72°C to allow annealing (binding) of the single strands with the primers to create the short pieces of double-stranded DNA needed by the polymerase, and extention of the primers by the polymerase, using the DNA single strands as a template. After extention, the mixture is heated again to separate the strands and a new cycle begins. The exact temperatures used for melting and annealing, the time intervals optimal for each step, and a variety of other parameters must be standardized for each system. A final PCR product is obtained, which represents many copies of the DNA region specified by the two primers, which has a characteristic nucleotide chain length. To detect and identify this product, the different molecules in the reaction mixture are separated according to length by electrophoresis in an agarose gel, and visualized with a special dye.

Figure 4: The polymerase chain reaction (PCR).

(a) Starting material is double-stranded DNA that acts as a template. (b) The strands are separated by heating and subsequently cooled to allow annealing with primers. The primers bind on the ends of the section to be replicated. (c) The polymerase
synthesizes new DNA strands complementary to the template (primer extension). (d) The cycle is repeated. Drawing by Mark Hotz.

The PCR method has certainly had a major impact on basic research, allowing a totally new approach to the study of molecular genetics. Reagents for investigations can be made readily and with greater precision than before, and knowledge about the regulation of gene function can be gained at a faster pace. In many cases it can replace time-consuming cloning procedures. In regard to biological weapons control, the PCR has to be viewed as a dual-purpose technique with respect to the BTWC and its verification; because manipulations are generally facilitated by the PCR, it could serve an aggressor in a negative way. On the other hand, the PCR method can have a definite positive impact on verification, specifically in the area of identification of microorganisms in the environment. Recent investigations using the PCR have been centered on determining the diversity of microorganisms in a particular habitat, even when these organisms are not able to be cultured. The PCR can be combined with new, rapid nucleotide sequencing methods. This represents a relatively rapid procedure for investigating possible changes in nucleotide sequence of genes, which may provide information about mutations or genetic manipulations.

The successful application of hybridization analyses depends upon how well all parameters and reaction conditions in the PCR and in hybridization techniques have been standardized for a particular system in question, such as a signature sequence probe or a specific toxin gene probe in reaction with their corresponding target DNAs. While it is conceivable that some analyses (such as detection of virulence genes) using systems that have been properly standardized may be carried out in appropriately equipped mobile laboratories on-site, more often than not investigations will require extensive analyses that can best be performed in better equipped off-site laboratories.

The combined equipment needed for the different phases of analysis, such as DNA extraction, amplification with PCR, electrophoresis, hybridization, and hybridization detection represents a rather bulky array for a mobile on-site laboratory. However, more compact versions of instruments in use today can surely be expected in the future, because clinical laboratories and the pharmaceutical industry, which represent the primary markets for desk-top, automated equipment for nucleic acid analyses, are very interested in such developments. The identification of biological agents by molecular biological procedures such as PCR are being used more and more frequently in clinical laboratories.

2.4. Genetic profiles of microorganisms using molecular typing with nucleotide sequence information

Nucleotide sequencing and genome analyses are concerned with the determination of the nucleotide base sequence of the genomic (chromosomal) DNA of organisms. The complete sequencing of the genomes of some 100 prokaryotic microorganisms and many viruses has been achieved, and many others are currently being sequenced. Recently, considerable progress has been made in the area of high-throughput automated DNA sequencing in connection with many genome sequencing projects that will ensure an even more rapid pace of data gathering in the future. These methods are being intensively applied to the sequencing of the genomes of pathogenic microorganisms, with the aim of discovering and identifying new virulence determinants. It is hoped that targets for the development of diagnostic and chemotherapeutic reagents as well as vaccines can be defined in the course of these investigations. Naturally genomic sequencing has dual use relevance for the BWC.

At the same time, these activities can play a very positive role for verification of compliance to the BWC. The need for effective methods of identifying microorganisms with increased virulence or transmissibility as well as antibiotic-resistant strains has prompted a novel approach to molecular typing primarily designed for global epidemiology. This approach is called multilocus sequence typing (MLST), which involves using the polymerase chain reaction (PCR) to amplify DNA fragments of a limited set (for example seven) of designated genes of a particular bacterium and then sequencing the PCR products either manually or by using an automated sequencer. For each gene, deviating sequences in different isolates of the bacterium are designated as alleles of that gene and the alleles of the seven loci provide an
allelic profile, which unambiguously defines the sequence type of each isolate. The accumulation of nucleotide changes (mutations) in what is known as conserved genes is relatively slow, and the allelic profile based on such slowly evolving genes is stable enough over time for the method to be well suited for global epidemiology. Genes that change more rapidly may be useful for short-term, local epidemiology to determine, for example, if different isolates from a localized outbreak of disease are the same or different strains.

The technique has been successful in identifying antibiotic resistant clones of *Streptococcus pneumoniae* isolated from an outbreak in Taiwan, and in tracing the origin of these clones. In these studies, some isolates were identified as members of a multiply-antibiotic-resistant clone originating from Spain, while others had a far east origin. Further successful applications have been made, such as in the case of *Neisseria meningitidis* strains, as well as with many other microorganisms.

Especially pertinent to BWC compliance, a similar approach was recently used to study genetic relationships within *Bacillus anthracis*. Even though this bacterium is one of the most genetically homogeneous pathogens known, the authors of the study were able to determine genomic regions containing enough variability to allow discrimination among different *Bacillus anthracis* isolates. The sequences used for profiling were those found in DNA areas known as variable number tandem repeat (VNTR) sequences, whose function is essentially unknown.

These studies have been extended in a comparison of whole-genome sequences that identify further markers that can be used to distinguish among *Bacillus anthracis* strains. Particularly useful markers in addition to VNTRs were single nucleotide polymorphisms (SNPs) and inserted or deleted sequences (indels). For example, the investigators observed two SNPs and two indels that differed between *Bacillus anthracis* isolated from the letter attack in Florida and the Ames strain from Porton Down, which lacks both virulence plasmids. In another example, the authors found that two other *Bacillus anthracis* strains, each of which carried one of the two virulence plasmids lacking in the Porton Down stain, differed from the Florida strain by 38 SNPs, three indels and eight VNTRs. The researchers hypothesize that polymorphisms can appear after relatively few generations of the bacteria. Their work shows in any case that genome-based analyses can indeed be useful in determining the origin of *B. anthracis* strains.

The applicability to particular microorganisms of relevance that have not yet been examined in this context will of course have to be rigorously tested. In this regard, intensive research involving the participation of many scientists working with pathogenic organisms all over the world is called for, to contribute by determining allelic profiles of isolates in their locations and submitting the sequences to open genome databases. The method has proved its usefulness in several cases and has tremendous potential with regard to cooperative measures in the area of disease surveillance and tracking of pathogenic organisms. Genome-based analysis of microbial pathogens will certainly provide a powerful new tool for investigation of infectious disease outbreaks. As such it could contribute decidedly to promoting transparency and building confidence in a BWC compliance regime, which is a strong criterion for preventive arms control.

2.5. Beyond genomics

We are right in the middle of what has been termed the pharmacological revolution, in which combinatorial chemistry, genomics and proteomics all play essential roles in drug-discovery. Combinatorial chemistry refers to the methods used to create complex sets or repertoires of compounds, whose reactivities with other molecules can be tested. One example of this is phage display, in which a set of recombinant bacteriophage clones are made to display a peptide component, whose structure may be varied from clone to clone. These displayed peptides can then be tested with various other molecules for their reactivities in systems similar to ELISA.

Proteomics is the large-scale study of proteins, normally by using biochemical methods for protein preparation and identification. For example, one and two-dimensional gel electrophoresis systems can be used to separate complex mixtures of proteins, which can be identified with the help of antibodies. Other techniques such as affinity chromatography or high pressure liquid chromatography can also be used
to separate and isolate proteins. The most significant breakthrough in proteomics has been the matrix-assisted laser description ionization time of flight mass spectrometry (MALDI-TOF-MS), in which pulsed energy from a laser is transferred to the molecules to be analyzed with the help of a matrix.\textsuperscript{66} The molecules are ionized and released into the gas phase of the mass spectrometer, which results in a time-of-flight distribution of molecules in a mixture. These can then be identified by their characteristic peaks in the mass spectrum. MALDI-TOF has also been used for rapid identification of microorganisms.\textsuperscript{67}

Furthermore, peptide sequencing can be achieved by a two-step procedure employing mass spectrometry.\textsuperscript{68}

One of the stated goals of genomics and proteomics lies in drug discovery, which is developing at a rapid pace. Within the drug discovery campaign, it can be expected that many of the substances produced will fall into the category of bioregulators (compounds that are chemical in nature and regulate the operation of physiological systems). Bioregulators will be gaining more and more significance for biochemical arms control as time progresses.\textsuperscript{69, 70}

There is clear evidence of a shift in focus from the agents themselves to the targets of interacting physiological systems which they can affect.\textsuperscript{71} In the light of this shift, dealing with advances in the life sciences becomes enormously more complex. The BWC, which has no treaty organization and does not contain adequate measures for verifying compliance, is running into the danger of being completely overwhelmed by scientific and technological developments in the future in the sense that the states parties to the convention will not be able to cope with the complexity of science and technology advances.

Renewed interest in so-called „non-lethal“ chemical weapons (which include bioregulators) threatens to undermine the current CBW control regimes and calls into question their future robustness.\textsuperscript{72} For one, the US military shows a strong interest in developing this kind of capability.\textsuperscript{73, 74, 75} The BWC prohibits any agent categorically “for hostile purposes or in armed conflict”. The CWC prohibits all chemical agents for non-peaceful purposes, but the convention contains an undefined exception, permitting the use of such agents for purposes of “law enforcement”, in which case this is difficult to define. From a scientific and technical point of view the major problem with „non-lethal“ weapons lies in the fact that they are not non-lethal, as the Moscow theater hostage crisis in 2002 has clearly demonstrated.\textsuperscript{76} The fentanyl derivative used by the Russian security forces represents just the tip of the iceberg. Although it can be claimed that the Russian fentanyl use falls under the CWC law enforcement provision, a thorough discussion of the matter in the interest of clarification at the First Review Conference was prevented by a few powerful states.\textsuperscript{77} This does not speak well for the capability of the CWC to deal with changes that might affect the sustainability of the prohibitory norm against chemical weapons.\textsuperscript{78}

3. The problem of dual use biotechnology

The problem of biochemical incapacitants reaches new proportions when viewed from the arena of interacting biological systems. It is being recognized more and more that the immune system interacts intricately and extensively with the nervous and the endocrine systems. 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. 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.\textsuperscript{79}

To illustrate how the one system can affect the other, with possible detrimental effects for both, the interaction of soluble bioregulators of the immune system (cytokines) and the neuroendocrine system (hormones and neurotransmitters) within the HPA axis will be taken as an example. The proinflammatory cytokines IL-1\(\beta\), TNF\(\alpha\) and IL-6 are produced by cells of the immune system after contact with microorganisms or their products.\textsuperscript{80} The cytokines gain entry into the circulation from sites of the immune response in tissues and organs. Normally, these cytokines are of sufficiently large size that would prevent them from passing the blood-brain barrier. However, a window in the barrier can be found in a particular area of the hypothalamus (a part of the brain), which allows the entry of the cytokines into this region.\textsuperscript{81, 82} They subsequently bind to receptors on cells in the hypothalamus and trigger reactions
collectively known as sickness behaviour, which is characterized by fever, drowsiness, lethargy and loss of appetite. With this reaction, the brain signals the body that a fight against infection is underway.

Another effect the proinflammatory cytokines have on the hypothalamus is to induce the production of corticotropin-releasing factor (CRF), which in turn causes the pituitary to produce adenocorticotropic hormone (ACTH). This hormone enters the circulation and acts on the adrenal cortex to induce the production of glucocorticoids, which have a profound effect in suppressing immune responses, as outlined in the previous section. However, CRF also has an effect on the central nervous system. In this regard, overproduction of the hormone has been implicated with neurotoxicity and neurodegeneration in animal studies. For example, in an animal model of acute ischemia (stroke), it was shown that CRF antagonists could protect against the loss of neurons which occurs as a result of a stroke. In addition, CRF has been associated with major depression, anorexia nervosa and Alzheimer’s disease. Normally, these interactions within the HPA axis work as a check and balance system to keep reactions from getting out of hand. However, it is easy to see that a selective overproduction of proinflammatory cytokines could tip the balance to potentiate effects on both the immune and the neuroendocrine systems, leading to debilitating sickness behaviour, significant immune suppression and even damage to neurons.

This scenario is not at all far-fetched. The ability to attack biological systems through the use of biochemical incapacitants is intimately related to developments in targeting technology. This technology is being intensively developed for use in cancer and gene and drug therapy, and is still mainly in the experimental stage. Nevertheless, it is evident that cytokines as bioregulators can be delivered quite effectively by viruses engineered to carry cytokine genes, as was illustrated in the mousepox experiment with the cytokine interleukin 4. In addition, the US Army has apparently investigated the absorption of endogenous bioregulators through the aerosol route, to determine how effectively such substances can be taken up by inhalation. It has reported, for example, that the hormone insulin and the proinflammatory cytokine interleukin 1 were effective in aerosol form in basic pulmonary absorption studies. Indeed, it has been stated that the greatest potential for delivering drugs is through the pulmonary route by inhalation of particles of a particular size. There is a great deal of interest in developing drug delivery systems consisting of defined nanoparticles mixed with substances to enhance absorption.

A final point to be made here is that the interaction of these systems and the interdependence of the resulting reactions on this interaction raises the dual use dilemma to a new order of complexity. With the rapid advances in the accumulation of knowledge concerning the mechanisms of interaction of these systems that will surely occur, trying to deal with this information to exploit the benefits while minimizing the risks is going to become more and more of a Herculean task in the future.

4. Looking ahead

Rapid advances in life sciences research are essential for the fight against infectious diseases. However the same techniques used to improve health and protect against infections can be misused to produce new and more effective biological weapons. In this context, the dual use dilemma is absolute. Trying to exploit the benefits while minimizing the risks that these developments pose will be an enormous task in the future.

Biosecurity measures designed to counteract misuse of biotechnology for biological warfare and bioterrorist activities will invariably affect biomedical research developments and must therefore be carefully drafted so as not to impede this research. No blanket prohibitions should be placed on research activities of any kind that are carried out with peaceful intent. This is in agreement with the view expressed in the recent report of the National Research Council of the National Academies in the USA that even the results of research that present the most danger of being misused may still provide beneficial aspects essential for combatting infectious diseases.

Preventive arms control criteria emphasize the need for monitoring research, to provide possible early warning of potentially dangerous developments. An oversight program for reviewing research proposals involving work relevant to the BWC has been drafted in the Project on Controlling Dangerous Pathogens. In this proposal, the review process would be a mandatory, tiered procedure (at the local,
national or international level according to the potential danger) and it would be applied to government and industry as well as academia. If such a process could be implemented, this would definitely be a step forward. Transparency in science is just as essential. Results of legitimate research which can benefit progress in biomedicine should not be censored in any way.91

The new BWC process of yearly meetings of the States Parties preceded by an Experts Meeting to discuss ways of further controlling biological weapons could be beneficial. The issues that are being handled are, however, limited in scope and in international application. Nevertheless, the mechanisms being discussed would without a doubt be useful in strengthening the BWC, but as the meetings progress it is evident that there is a need for the States Parties to demonstrate that they are serious about living up to their obligations. In 2005 the subject of these meetings is the formulation and promulgation of a Code of Conduct for the Life Sciences. A Code of Conduct could represent an effective element in preventing the hostile use of biological agents, if it is designed to promote awareness of the complex dual use dilemma and at the same time pro-actively obligate the research scientist to engage in reflective activities such as risk assessments and consideration of alternative approaches during the research process.92 The scientist should therefore obligate himself to become informed and be aware of possible dual use aspects of biomedical and bioscience research, to carry out risk assessments at each stage of the research process as a reflective action and to consider alternative approaches as the risks demand.

Naturally, this code element can only be applied if the scientist engaging in biomedical and bioscience research is aware of the dual use problem and is well informed about ethical decision-making processes. Unfortunately, these subjects are not a part of the curriculum at many universities. Governments should therefore encourage universities to place such instruction into their biomedical and bioscience curricula as required courses. Special incentives should be offered to those universities that do so.

The registration or licensing of all facilities and their scientists working with pathogenic microorganisms (or genetic material and toxic products from these microorganisms) of biological weapons relevance would be another potentially beneficial biosecurity measure. Many States already issue licenses or permits to scientists allowing research in the areas of genetic engineering and work with pathogenic microorganisms. In this regard, the awarding of a license or permit should be contingent upon receiving instruction about the content of the Biological and Toxin Weapons Convention and the obligations of the scientist under this treaty, as well as instruction about ethical decision-making and risk assessment93 processes. It would take little effort to include such instruction in these licensing programs. Receiving a permit should further be contingent upon signing a code of conduct.
Figure 1.

Figure 2.
a) Double-stranded DNA

b) Strands are separated by heating; Oligonucleotide primers complementary to a sequence in each strand are added; Strands are cooled to allow annealing with primers

c) DNA polymerase synthesizes new DNA complementary to the template (primer extension)

d) The reaction is repeated for the next cycle


4 Ibid.


11 Nixdorff et al. (2003), op. cit.


27 Ibid.


32 *Ibid*


40 Nixdorff, K., M. Hotz, D. Schilling, and M. Dando (2003), *op cit*


45 *Ibid*

46 *Ibid*


Ibid.


Ibid.


Ibid.


See also the website of the Sunshine Project for documentation of the US non-lethal weapons programmes, at www.sunshine-project.org


Kelle, A. (2003): The CWC after its first review conference: is the glass half full or half empty? Disarmament Diplomacy, No. 71 (June/July), pp. 31-40.


Ibid.


Straub et al. (1998), op. cit.


