# Biotechnology and Biochemical Weapons

MARK WHEELIS[1]

*Dr. Mark Wheelis is Senior Lecturer in Microbiology at the University of California (UC), Davis, where he has been teaching in the field since 1970 and is also Director of the Program in Nature and Culture. He has authored numerous pieces on the history of biological warfare and the control of biological weapons, including two chapters on biological warfare in* ***Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*** *(Oxford University, 1999). Dr. Wheelis has also developed a website devoted to the threat of agricultural biowarfare and bioterrorism for the Federation of American Scientists.*

Biomedical sciences and the pharmaceutical industry are in the midst of a revolution in the science and technology of drug discovery that will significantly complicate the control of chemical and biological weapons (CBW). The 1993 Chemical Weapons Convention (CWC)[2] and the 1972 Biological and Toxin Weapons Convention (BWC)[3] prohibit the development and possession of these weapons, and the 1925 Geneva Protocol prohibits their use.[4] All three treaties are thus threatened by these technological developments. Scientists in fields that are contributing to this revolution must understand these implications of their work. Likewise, arms control experts must recognize that there is a profound revolution underway in biology and that the technical landscape of chemical and biological arms control is rapidly changing.[5] This article seeks to bridge the gap between science and arms control, in order to raise awareness in both fields of the potential ramifications that this scientific and technological revolution may have on CBW proliferation.

New drugs have traditionally been discovered by screening naturally occurring compounds for biological activity in bacterial or viral cultures, tissue cultures, or live animals. Once a compound with biological activity was discovered, it would be chemically modified in various ways in the hopes that one of the variants would have increased activity. Sometimes the spectrum of effectiveness seen with the variants would suggest the critically important chemical features of the molecule (e.g., the ß–lactam ring of the penicillins and cephalosporins), allowing a semi-rational approach to further modification.

For scientists seeking to develop new drugs, the principal bottleneck used to be discovering the initial compounds for screening; however, significant technological advances have now alleviated this problem, and further significant advances are on the horizon. Currently, new compounds are generated in large numbers by combinatorial methods and assayed for potential activity by ultra-high-throughput screening techniques. In the future, genomic and proteomic methods (described in more detail below) will encourage increasing use of computer modeling techniques to identify new drugs. These same scientific developments will also rapidly deepen our understanding of physiological processes in both healthy and diseased states. This understanding will provide the necessary knowledge base for identifying new drug targets and for predicting the

consequences of interfering with their normal functioning.

While the drivers of this revolution are to a large extent methodological, the result is a shift in the underlying strategy of drug discovery. Rather than first identifying compounds with biological activity and then determining their mode of action, the new approaches generally rely on identifying likely targets first, then finding compounds that can bind to them and affect their functioning. Drug targets are usually proteins (which are responsible for most of the activities of living organisms) that have binding sites on their surfaces that normally bind specifically to particular compounds (called *ligands*). Drugs (and many toxins) generally bind in place of the natural ligands and alter the ability of proteins to perform their normal function. Increasingly, the strategy is to identify particular proteins that, because of their function in the body, are likely drug targets, and then to use the techniques described here to find artificial ligands that bind to them. Thus the process is becoming less empirical and more rational, a trend that will accelerate as our physiological understanding deepens. These trends have significant implications for chemical and biological weapons control, because they are driving a rapid increase in the identification and development of new potential CBW agents. The pace of this technological revolution threatens to outstrip current biological and chemical arms control treaties, and it opens up new possibilities for states and terrorist groups seeking to develop biological and chemical weapons.

This article will review the principal technologies involved in this revolution in the drug discovery process, and point out their relevance to the discovery of new chemical/biological weapons agents.These technologies include: combinatorial chemistry, genomics, microarrays, proteomics, toxicogenomics, and database mining. The relevance of these developments to CBW control under the CWC and the BWC are then discussed, with particular attention to the destabilizing effect of non-lethal weapons development. It concludes with an evaluation of what is needed to prevent a renewed biochemical weapons threat.

## THE CBW IMPLICTIONS OF THE PHARMACOLOGICAL REVOLUTION

### Combinatorial Chemistry and Ligand Identification

The increasingly widespread use of combinatorial chemistry is one technology driving the pharmacological revolution. Combinatorial chemistry refers to techniques that produce complex sets ("libraries") of related compounds.[6] Typically it involves multiple rounds of reaction between a base compound and other compounds that can react with it, which may in turn provide additional reactive sites. If the process is sequential, batteries of computer controlled microreactors perform each synthesis by adding appropriate reactants and catalysts, and the products then provide starting material for the next round of synthesis. The result of a number of rounds of robotic synthesis and separation is a library of hundreds to thousands of separate, related compounds. Each can then be tested for biological activity against a target—purified protein molecules, tissue cultures, microbial cells, etc. The screening techniques are conducted robotically, allowing extremely high throughput rates.[7]

If the reactions are simultaneous, the result is a mixture of all products, typically thousands to tens of thousands of different compounds. Ligand binding to a target protein can be detected by affinity selection methods: the library is incubated with the target protein, which is then separated from unbound small molecules by micro-scale molecular sieving.[8] Bound ligands are then separated from the protein and identified.

Currently, a single industrial research facility can screen several hundred thousand new compounds per day against several dozen different proteins. In aggregate, the pharmaceutical industry is screening several million new potential ligands per year, and the results are stored in proprietary databases. In the course of toxicity testing of ligands identified in this way, about 50,000 compounds are identified each year that are highly toxic.[9] For the pharmaceutical company, such toxic compounds have little potential as drugs and further development is halted. However, any one of these is a potential lethal chemical weapon (CW) agent.

### Genomics and Target Identification

With the complete sequence of the human genome nearly in hand, and with many hundreds of different single-nucleotide polymorphisms (individual sequence variations) identified, a new set of drug development techniques is becoming available to scientists.[10] Genomic sequences allow the identification of many new possible targets for drugs. For instance, many currently effective drugs target either ion channels or membrane receptor proteins. Many new proteins of these types are being identified in genomic sequences, since they have homology to already identi-

fied proteins. Others possess features that are easily recognized in deoxyribonucleic acid (DNA) sequences (e.g., transmembrane domains, ATP- or GTP-binding domains, etc). Once a new target has been identified, the gene can be cloned and the protein produced in quantity for study and for use in screening combinatorial libraries. Thus, as genomic sequences are annotated (assigned a function), the number of potential targets for pharmaceutical development will skyrocket. So too will the potential targets for novel CW agents.

### Microarrays and the Measurement of Gene Expression

How genes are expressed into ribonucleic acid (RNA) sequences, and then (usually) proteins, can be important information. The conditions under which genes are expressed at high levels can give hints to their function (important because many genes identified in genomic sequences have unknown functions). Furthermore, comparison of the levels of expression can give an indication of possible therapeutic targets. For instance, genes expressed at high levels in cancer cells but not in normal tissue would be potential targets for anticancer drugs; and microbial genes that are turned on during infection of a host would be potential targets for antimicrobial drugs.

Such differential gene expression is now readily measured using DNA microarrays—glass slides or silicon chips on which thousands of DNA sequences are imprinted. Each spot on the microarray contains millions of identical single-stranded DNA molecules, whose sequence matches that of one of the genes of the organism being tested. A single slide can have tens of thousands of spots, representing each gene of the organism.

These microarrays are exposed to fluorescently-labeled RNA (or a DNA copy of the RNA) from an organism, and then the amount that hybridizes with each gene is measured by determining the amount of fluorescence from each spot. With this method, the cellular levels of expression under a range of conditions can be readily measured, aiding an understanding of the cellular function and importance of each gene, and pointing to the most likely targets of new drug (or weapons agent) design.

### Proteomics and Rational Agent Design

Proteomics is the study of the full complement of proteins of the cell.[11] Unlike the genome, the proteome is intrinsically dynamic: the cellular complement of proteins changes throughout the cell cycle in every cell, is different in different tissues, and can alter in response to environmental changes. Some of these changes can be measured by DNA microarrays, but some of them are the consequence of modification of proteins after synthesis and can only be studied at the protein level.

Much of proteomics is currently concerned with identifying cellular proteins using two-dimensional gels and mass spectrometry, matching them to their genes in genomic sequences, and determining their interactions with other proteins.[12] These efforts will complement genomics in helping to understand pathological states and to identify promising targets for new drug design.

Protein microarrays are under rapid development; a nearly complete microarray of the yeast proteome was recently produced.[13] Comparable human proteome chips are on the horizon, as well as ones for a variety of other organisms of interest. Protein microarrays, combined with combinatorial chemistry, will dramatically broaden the search for new ligand-target combinations with therapeutic (or weapons) applications. They also allow the identification of protein-protein interactions, a critical part of cellular communication systems, and another possible set of drug/weapon targets.

Furthermore, rapid progress is being made in predicting protein three-dimensional structure from genomic sequences.[14] It is now possible to predict the structure of simple proteins with fairly high accuracy, as well as that of more complex proteins when they are homologous to proteins whose structure has been determined experimentally. In the near future it should be possible for most protein structures to be predicted with a high degree of accuracy from their genomic sequences alone. Knowing the structure of the active site allows rational design of ligands with a shape and charge distribution that is precisely complementary to it. This computer modeling approach to drug design promises to complement, and probably eventually supplant, traditional wet chemistry methods of ligand identification (although of course any design has to then be validated by traditional experimental approaches). The same techniques would allow rational design of new weapon agents.

### Toxicogenomics, Database Mining, and the Prediction of Toxicity

Most drug candidates are eliminated in clinical trials due to toxicity problems. Since this constitutes a significant cost to the pharmaceutical companies, there is intense interest in predictive algorithms for toxicity, so that toxic

compounds can be eliminated before they enter clinical trials. Of course, exactly the same approach would be useful if the goal were to develop more toxic compounds.

Two approaches have shown significant promise. First, toxicogenomics employs proteomic and microarray techniques to analyze the response of cells to known toxins.[15] If the changes in patterns of gene expression or in the proteome induced by a novel compound are similar to the response to known toxins, the likelihood is that the new compound will prove to be toxic. This allows probable toxins to be screened out at an earlier stage; however, it also allows early identification of potential new biochemical warfare agents.

Second, the analysis (using sophisticated neural network approaches) of databases of drugs and nondrugs allows the selection of a range of descriptors that together can predict whether a compound is likely to be drug-like (pharmacologically active, with low toxicity), or non-drug-like (not pharmacologically active or toxic).[16] Similar algorithms could possibly predict compounds with a variety of other desirable traits for novel biochemical weapons agents, in addition to high toxicity.

## THE RATE OF PROGRESS IS VERY HIGH AND ACCELERATING

An immense amount of time and money are being invested into these biomedical fields, and the rate of discovery is very rapid. Furthermore, this is a field in which fundamentally new methodologies are one of the principal drivers. Since new methods open up entire new categories of questions, they act to stimulate the rate of progress significantly.

The intellectual base of the methodologies is supported by an immensely sophisticated and rapidly growing micro-scale instrumentation and computational base. The computer-controlled reaction vessels, ultrahigh throughput screens, robotic microarray printers and readers, time-of-flight mass spectrometers, high speed sequencers, and other devices have been critical to the development of the field. So, too, has the exponential growth of computer speed and memory, as well as the sophistication of software, since all of these laboratory technologies depend on computers for the collection and analysis of data. Indeed, bioinformatics is probably now the rate-limiting technology, as the flood of genomic and proteomic data is overwhelming the capacity to integrate and understand it.

The intellectual momentum of this science is immense and clearly unstoppable. Thus a very large number of new, highly toxic compounds with precisely understood and controllable physiological effects will soon be discovered. Many of these will enter production as drugs or as research reagents. The range of known potential CW agents will thus broaden by a very large factor in a very short period of time, and most of them will be synthesized from precursors that are not currently regulated under the CWC.

## THE PROBLEM OF NON-LETHAL AGENTS UNDER THE CWC

The CWC allows states to possess chemical agents and delivery systems designed for riot control and other law enforcement purposes. Non-lethal chemical agents are otherwise illegal: the Convention defines a CW agent as "any chemical, which through its chemical action on life processes can cause death, temporary incapacitation or permanent harm to humans or animals."

Furthermore, the CWC explicitly prohibits the use of riot control agents "as a method of warfare." However, at least one State Party (the United States) has interpreted this wording as limiting the prohibition to interstate armed conflict.[17] This reading leaves open a wide variety of military operations in which such agents could be legally used, including counterterrorism, peacekeeping, monitoring, and the like. Given the potential tactical utility of non-lethal chemical agents in such "military operations other than war," their development, and the development of munitions to deliver them, is being actively pursued. Unless the States Parties to the CWC can reach consensus that the prohibition of riot control agent use covers a much wider range of hostile actions than merely international military conflict, there is certain to be widespread development of this capability.

New "riot control" agents are likely to be of a variety of different kinds.[18] Neuropharmacology is one of the areas in which rapid expansion of knowledge can be confidently predicted. The toll of mental illness, and the growing promise of chemical treatment, makes it certain that a wide range of new psychoactive chemicals will be discovered, as well as chemicals that affect transmission across neuro-muscular and neuro-endocrine synapses. It is likely that in the near future a range of agents will be developed that affect perception, sensation, cognition, emotion, mood, volition, bodily control, or alertness. Given the great potential for such agents to be abused, it would be prudent to delay arming the militaries of the world with

them until their long-term implications have been carefully analyzed.

In fact, a categorical distinction between lethal and non-lethal chemical agents is not strictly possible, since "non-lethal" agents may be lethal at high concentration or for specific individuals. More seriously, synergy between two different non-lethal agents may make their combination highly lethal. The molecular techniques I have discussed will soon allow rational strategies to discover such synergistic pairs. Thus the development of multiple non-lethal agents may provide a lethal CW capability, in violation of the intent of the Convention.

Furthermore, allowing states to develop stockpiles of incapacitating chemical agents and munitions for their delivery in combat situations would defeat one of the fundamental purposes of the Convention: to prevent states from entering wars with a stockpile of CW whose use is proscribed, but which might nevertheless be considered under the doctrine of military necessity.

Finally, a legal development program of new riot control agents would provide a nearly impenetrable cover for a covert development program for new lethal agents, thus reducing the capacity of the international community and the Organization of the Prohibition of Chemical Weapons (OPCW) to detect violations of the CWC. For all of these reasons, continued development of non-lethal CW threatens the stability of the regime.

## RELEVANCE OF THE BWC

A better case can be made that the BWC prohibits non-lethal biochemical weapons, although it, too, possesses weaknesses. It prohibits the development, production, and stockpiling of biological weapons (BW) agents and delivery devices, as the CWC does for CW, but it lacks the CWC's verification provisions. Furthermore, the scope of its terms "microbial or other biological agents, or toxins whatever their origin or method of production" is ambiguous. However, there appears to be a consensus that "other biological agents" includes all of the biochemical products of the living body that in abnormal doses can be used as toxins, including bioregulators, neurotransmitters, and hormones.[19] Since the final document of the Second Review Conference affirmed that the Convention applied to analogues of toxins as well as to their native form, it would seem that the BWC would apply to all of the biochemical compounds whose discovery I discuss here.[20] Since their activity is a function of their ability to bind specifically to an active site on a protein, they are by definition analogues of the natural ligands and thus covered by the BWC. As toxic chemicals, they are also covered by the CWC. The BWC and the CWC thus overlap quite substantially, and the term "biochemical" weapon agents can be used to describe toxic chemicals in this overlap category.

The BWC prohibits the possession of devices designed to employ biological agents "for hostile purposes or in armed combat." It thus contains a more expansive prohibition than the CWC—hostile purpose is clearly a broader category than armed conflict, which is, in turn, broader than war. Furthermore, there are no exclusions in the BWC for riot control or for other law enforcement purposes. For these reasons, it would appear that the agents outlined here would be categorically prohibited by the BWC.

States Parties might argue that domestic riot control is necessary to preserve the public peace and thus legal under the BWC general purpose criteria of allowing "protective, prophylactic, or other peaceful purposes." However, an equally strong case could be made that even domestic riot control should be considered a hostile use, given the very general prohibition on hostile purposes beyond armed conflict, and that BW are not to be used even here. The BWC would, like the CWC, benefit from constructive Review Conference consideration of the boundary between permitted and prohibited activities.

## CONCLUSION

The emerging biotechnology of drug discovery promises great advances in medicine, biology, psychology, and a host of related sciences. However, the same tools that are revolutionizing drug discovery can be used to discover novel biochemical agents for the purpose of weaponization. Related developments in chemistry and chemical engineering have similar implications.[21]

Most of these novel agents will be synthesized from unlisted precursors and will be nearly invisible to the verification regime of the CWC, although their development, production, and stockpiling will be unambiguously prohibited. Containing proliferation will thus become significantly more difficult, especially in states with mature biotechnology and pharmaceutical industries. Given the rapid dissemination of industrial biotechnology, this will soon include a very large number of States Parties.

Effective responses from the Conference of States Parties and the OPCW will be difficult. Certainly a willingness to revise the "Schedules of Chemicals" regulated by the CWC as the need arises will be essential. Vigilance

will be necessary, especially during inspections of production facilities that produce discrete organic chemicals. States Parties with the capability may be able to use intelligence and national technical means to detect covert CW programs. This capability, coupled with a willingness to employ challenge inspections, could serve to some extent as a deterrent. In the end, however, the only effective long-term solution is a universal norm against such weapons, which can only be reached via sustained efforts for universality of both Conventions and transparency in chemical and biological defense programs.

Equally threatening is the interest of some States Parties in the development of non-lethal CW in the guise of riot control agents, and their assertion that such development is not prohibited as long as the agents are not intended for use in hostilities between states. This position opens the door to the widespread development, production, and stockpiling of non-lethal chemical agents and munitions designed for their use in military combat. This is clearly contrary to the intentions of the CWC.

If states want to avoid the widespread integration of non-lethal biochemical agents into military arsenals, with all the problems that this will bring, they will need to act decisively to affirm that one or both of the Conventions prohibits all military use of these agents (except perhaps for narrowly specified purposes, such as domestic riot control). Obviously, such an affirmation of the understanding of the meaning of the BWC or the CWC would require consensus; the States Parties that are now engaged in non-lethal weapons development would have to acquiesce in an affirmation that would force them to abandon their efforts.

Even if a consensus were to be reached, it would still be a challenging problem to distinguish the legal development of new riot control agents (if this is allowed under the BWC) from the prohibited development of new non-lethal biochemical weapons. Probably the best curb on the development of a military capability to wage chemical warfare with riot control agents would be to circumscribe legal munitions and delivery devices to those that are already in common use by police forces worldwide.

[1] Thanks to Dr. Lynn Klotz for helpful suggestions. An earlier version of this paper was delivered at the Pugwash Conferences on Science and World Affairs, Workshop on the Implementation of the Chemical and Biological Weapons Conventions, Oegstgeest, Netherlands, June 2001.

[2] The *Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction* opened for signature in Paris on January 13, 1993, and entered into force on April 29, 1997.

[3] The *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction* opened for signature in Washington DC, London, and Moscow on April 10, 1972, and entered into force on March 26, 1975.

[4] The *Protocol Prohibiting the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare* was signed in Geneva on June 17, 1925, and entered into force on February 8, 1928.

[5] Claire M. Fraser and Malcolm R. Dando, "Genomics and Future Biological Weapons: The Need for Preventive Action by the Biomedical Community," *Nature Genetics* 29 (2001), pp. 253-256; Mark Wheelis and Malcolm Dando, "New Technology and Future Developments in Biological Warfare," *Disarmament Forum* 4 (2000), pp. 43-50.

[6] Jonathan Ellman, Barry Stoddard, and Jim Wells, "Combinatorial Thinking in Chemistry and Biology," *Proceedings of the National Academy of Sciences (USA)* 94 (1997), pp. 2779-2782.

[7] Ronald E. White, "High-Throughput Screening in Drug Metabolism and Pharmacokinetic Support of Drug Discovery," *Annual Review of Pharmacology and Toxicology* 40 (2000), pp. 133-157.

[8] Kollol Pal, "The Keys to Chemical Genomics," *Modern Drug Discovery* 3 (2000), pp. 47-55.

[9] Tamas Bartfaì, "Genomic Identification of Receptors and Their Polymorphisms, and Ligand Design," in Malcom Dando, Graham Pearson, and Bohumir Kriz, eds., *New Scientific and Technical Developments of Relevance to the Biological and Toxin Weapons Convention* (forthcoming).

[10] David J. Lockhart and Elizabeth A. Winzler, "Genomics, Gene Expression and DNA Arrays," *Nature* 405 (2000), 827-836.

[11] Akhilesh Pandey and Matthias Mann, "Proteomics to Study Genes and Genomes," *Nature* 405 (2000), 837-846; Rosamonde E. Banks et al., "Proteomics: New Perspectives, New Biomedical Opportunities," *Lancet* 356 (2000), pp. 1749-1756.

[12] John R. Yates, "Mass Spectrometry: from Genomics to Proteomics," *Trends in Genetics* 16 (2000), pp. 5-8.

[13] Heng Zhu et al., "Global Analysis of Protein Activities Using Proteome Chips," *Science* 293 (2001), pp. 2101-2105.

[14] David Baker and Andrej Sali, "Protein Structure Prediction and Structural Genomics," *Science* 294 (2001), pp. 93-96.

[15] Jeffery F. Waring and Roger G. Ulrich, "The Impact of Genomics-Based Technologies on Drug Safety Evaluation," *Annual Review of Pharmacology and Toxicology* 40 (2000), pp. 335-352.

[16] Ajay, W. Patrick Walters, and Mark A. Murko, "Can We Learn to Distinguish between 'Drug-Like' and 'Nondrug-Like' Molecules?" *Journal of Medicinal Chemistry* 41 (1998), pp. 3314-3324; Jens Sadowski and Hugo Kubinyi, "A Scoring Scheme for Discriminating between Drugs and Nondrugs," *Journal of Medicinal Chemistry* 41 (1998), pp. 3325-3329.

[17] Margaret-Anne Coppermoll and Xavier K. Maruyama, "Legal and Ethical Guiding Principles and Constraints Concerning Non-Lethal Weapons Technology and Employment," Presentation at the Non-Lethal Defense III Symposium in 1998, Defense Technical Information Center, <www.dtic.mil/ndia/NLD3/copp.pdf>. For details of U.S. non-lethal chemical agent development, see the Sunshine Project, "Non-Lethal Weapons Research in the US: Calmatives and Maloderants" and "Non-Lethal Weapons Research in the US: Genetically Engineered Anti-Material Weapons," <www.sunshine-project.org>.

[18] Malcolm Dando, *A New Form of Warfare: The Rise of Non-Lethal Weapons* (Washington DC: Brassey's, 1966).

[19] Personal communications from a number of diplomats and technical advisors to delegations to BWC Review Conferences and to the Ad Hoc Group, Geneva, Switzerland, 2000-2001. See, for instance, the comment by Sweden that hormones or transmitter substances might be developed as bioweapons in its background paper to the Second Review Conference, document BWC/CONF.II/4, 18 August 1986, p 3.

[20] Second Review Conference of the Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (1986), *Final Document*, BWC/CONF.II/13, United Nations, Geneva, Switzerland.

[21] George W. Parshall, "Scientific and Technical Developments and the CWC," in Jonathan B. Tucker, ed., *The Chemical Weapons Convention: Implementation Challenges and Solutions* (Washington DC: Monterey Institute of International Studies, 2001), pp 53-58.