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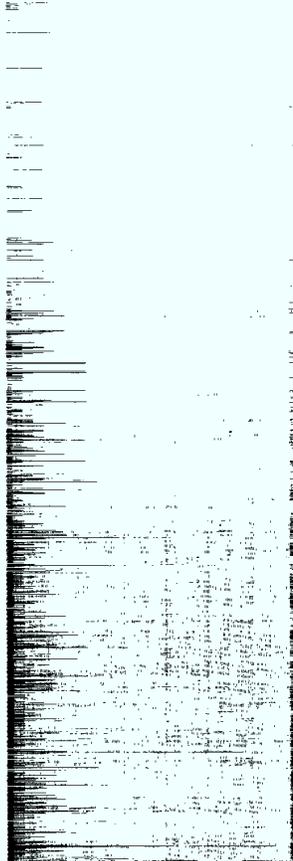
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Toxins: The Emerging Threat

BY

Murray G. Hamilton
Therapy Group
Medical Countermeasures Section



APRIL 1996

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Abstract

Toxins, which are non-replicating chemical agents of biological origin, are relatively anonymous chemical warfare agents even though some are among the most exquisitely lethal substances known. This report categorizes toxins, also known as mid spectrum agents, especially with respect to similarities and differences to classical chemical and biological agents. Areas of potential legitimate and non-legitimate use are discussed and examples of some recently identified toxins are provided. Defensive research initiatives and the impact of emerging technologies in biology, chemistry and computer science are explored. Finally, recommendations for the efficient use of limited Canadian defence research and development resources are presented.

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Résumé

Les toxines, qui sont des agents chimiques d'origine biologique ne se reproduisant pas, sont des agents de guerre chimique relativement peu connus même si certaines d'elles sont parmi les substances les plus terriblement meurtrières connues. Le présent rapport classe les toxines, qu'on peut qualifier d'agents de milieu de spectre, surtout en fonction des ressemblances et des différences avec les agents chimique et biologique conventionnels. On y aborde la question des utilisations légitimes et non légitimes possibles et on donne des exemples de certaines toxines récemment identifiées. Les programmes de recherche en défense et l'impact des nouvelles technologie en biologie, en chimie et en informatique y sont couverts. Enfin, on présente des recommandations concernant l'utilisation efficace des ressources limitées dont le Canada dispose pour dont le Canada dispose pour la recherche et la développement en défense.

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MONTREAL, Canada (Canadian Press)- Officials today are at a loss to explain the attack yesterday that left 8 dead and hundreds hospitalized after an apparent exposure to a biological warfare agent in the crowded Place de Congres in downtown Montreal.

"Nobody has come forward to claim responsibility", said a government spokesman. "The toxin was apparently disseminated through the ventilation system of the Place de Congres. a vast underground concourse in the heart of downtown Montreal. The casualties are suffering from what appears to be massive cardiovascular problems, but physicians are at a loss for an explanation because the causative organism has not yet been identified.

Cardiovascular problems include high blood pressure, heart attacks, strokes, aneurysms and other types of unexplained bleeding. Lack of treatment leads to death from a variety of causes related to disruption of the heart and vascular system. Treatment is symptomatic, consisting of reducing blood pressure and eliminating bleeding and cardiac arrhythmias if present. However, until the bacteria or virus responsible is identified, no specific vaccine or antibiotic

can be given.

Witnesses said the victims were situated near a ventilation shaft in this large underground concourse. The attack occurred at approximately 12:30 pm EST during the busy lunch hour period. The Place de Congres was particularly congested because many of the some 20,000 delegates attending the United Nations World Congress on Population Planning at the nearby Palais de Congres conference centre were having lunch in the concourse. Some victims said they noticed a faint smoke or fog about 15 to 30 minutes before they began to feel sick, but others do not remember anything noteworthy.

"We are expecting more deaths", said a hospital source. "We were lucky initially that the dose used was so low because it gave us time to get so many people to proper care. But, we do not have the facilities to provide round-the-clock intensive care for 600 to 1000 people at the same time, especially when a large number of them require specialized intensive care. It's tragic, but without a specific vaccine, all we can do is stabilize them and hope they come out of it themselves."



1 Introduction

The fictional "news" story printed above underscores the confusion and uncertainty concerning the conception of the word "toxin". Although the story may appear on the surface to be internally consistent, the underlined words and phrases point out gaps in the understanding of the differences between biological and chemical warfare agents. If the same story, for example,

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had talked about the use of sarin, as happened in Japan, virtually everybody in the world would have understood that a "chemical warfare agent" had been used. Furthermore, considerable expertise, both civilian and military, could be consulted and used to optimise the medical treatment, definitively identify the agent, predict the spread and persistence and estimate the sophistication of the perpetrators (in terms of making the agent, storing and disseminating it, quantities used, *etc.*) Similarly, an attack using an organism such as *Yersinia pestis* (plague) or *Francisella tularensis* (rabbit fever) would also be recognized for what it was: the use of a "biological warfare agent". Again, civilian and military physicians and researchers could bring a large armamentarium of knowledge and experience in handling "diseases" to bear on the problem.

Toxins on the other hand, occupy a much less well-defined area and recognition among most people. Saying "chemical warfare agent" to someone and images of dead Kurds lying in the street and in their homes or the huge blisters on Iranian soldiers caused by sulphur mustard contact would probably come to mind. Biological warfare would probably conjure images of dying from "the black plague" or acute respiratory anthrax. Toxin warfare or toxin warfare agent, however, would probably just generate a question: What? There would not be any images or any examples that came immediately to mind. (Some, although, may associate toxins with environmental concerns (for example, dioxins, PCBs, Agent Orange)).

Why is there this anonymity to "toxin warfare agent" when toxins, like botulinum toxin and the toxin described above, constitute some of the most exquisitely lethal poisons known? First, unlike both chemical and biological warfare agents, toxins have not been weaponized by very many countries. There are reports that Iraq had botulinum toxin weaponized prior to the Gulf War (Taylor, 1996) and the United States has some toxin stockpiles that were manufactured essentially as "proof of concept" only. Secondly, toxin chemical weapons are sometimes referred to as "mid-spectrum agents". This designation was intended to imply that toxins bridge the gap between biological agents and chemical agents, in terms of toxicity (although toxicity estimates for biological agents are not an appropriate measurement), applicability and cost/casualty. Unfortunately, the term has been perceived by some as suggesting that toxins are "less toxic" biological agents. Thirdly, defence R&D was quite reasonably directed at the real and accepted threat of the known chemical and biological capabilities of the Eastern bloc, and therefore there was little active research with toxins. In fact, after the early 1970s when then president Nixon unilaterally cancelled military research with toxins in the U.S., almost no defence R&D with toxins was conducted among the western countries. Similarly, academic research with toxins was not very robust, until just recently, primarily because of supply: toxins had to be labouriously extracted from fermentation tanks or homogenates of organisms, venom or plants and therefore were not generally available. Furthermore, even if one could obtain sufficient toxin to do research, it was very expensive.

For the foregoing reasons, toxin research, both defence oriented and academic was relatively dormant during the 1970's and early 1980's. But the quiescent status of the research community does not explain the confusion that exists when the terms toxin or toxin weapons are

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used. Some think of toxins as being biological agents, some as being chemical agents and some as being a mixture of the two. By the end of this report, the reader should have a clear understanding of differences between chemical and biological warfare agents and where toxins or mid-spectrum agents fit into this classification.

2. Threat Definition

Let us begin with a definition of what exactly is a toxin and then it will be easier to discuss the nature of the threat, the effects on militarily significant performance and what defence research and development (R&D) must do to provide a rational response and preparedness for toxin use.

DEFINITION

"Toxins are non-replicating chemical agents of biological origin."

This definition places toxins in a category of their own, distinct from both the classical chemical and the biological warfare agents and also perhaps, highlights why confusion exists concerning the term. Toxins are chemicals (since they are "non-replicating"), but they are often confused with biological agents ("of biological origin"). They therefore share properties of both groups of warfare agent. However, in contrast to the biological agents, toxins are neither infectious nor contagious. And they are not treatable with antibiotics or chemotherapeutic drugs. Unlike chemical agents, they are not the products of a chemist's imagination or synthetic skills (although that distinction may be changing; see Paragraph 9.2.2 *ff.*) nor are they by-products of some other process (like *e.g.* organofluorines or dioxins). The differences among these three categories of warfare agents are summarized in Table 1. This table makes it clear that toxins occupy a discrete classification with properties of both chemical and biological warfare agents (CWAs and BWAs). In concert with chemical agents, toxins do not reproduce and therefore the duration and area of contamination are more predictable. Like biological agents, toxins are (mostly) effective only after inhalation of appropriately sized particles and have a variable delay in onset of action that is substantially longer than the seconds to minutes effected by CWAs such as nerve agents or cyanide.

Are there characteristics of (potential) toxin weapons that make them more desirable than either of the other types of classical agent? They are relatively expensive to produce and the reactants, or manufacturing expertise, is not readily available. However, some of the toxins (*eg* botulinum toxin, sarafatoxin) are among the most toxic substances known (in some cases up to 1,000,000 times more toxic than nerve agents; see Table 2) and because of this, although expensive initially and requiring at least a moderate technology base, the "cost per casualty" can be actually quite moderate. Further, unlike chemical weapons for which sensitive and specific

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Table 1.

Comparison of Some Characteristics of Chemical and Biological Warfare Agents

Characteristic	Nerve Agent (eg. Soman, Sarin)	Toxin (eg. Tetrodotoxin, Botulinum toxin)	Biological (eg. Anthrax)
Cheaply Manufactured	YES	NO (POTENTIALLY USING GENETIC ENGINEERING, FERMENTATION, PEPTIDE SYNTHESIS, ETC.)	YES
Reactants readily available	YES	NO	YES
Stable: A-Storage B-In weapons C-Dissemination	YES YES YES	YES ?? SOME	YES SOME PROBLEMATIC
Safe to users (eg binaries)	YES	SOME	PROBABLE (VACCINATION)
Persistent	YES	YES	YES (SOME FOR DECADES)
Volatile	YES	NO	NO
Detectable	YES	NO OR NOT EASILY	SOME
Therapy	YES	SUPPORTIVE	ANTIBIOTICS CHEMOTHERAPEUTIC DRUGS
Pretreatment or Prophylaxis	YES	SOME (Vaccine)	SOME (Vaccine)
Sub-microgram Lethality	NO	YES	NOT APPLICABLE
Time to Effect	PREDICTABLE; SECONDS TO MINUTES	Predictable; minutes to hours	Unpredictable; hours to days
Exposure Risk	Dermal, oral, inhalation	Inhalation, oral	Inhalation, oral

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detectors are available, there are not yet similar detectors for toxins; and unlike biological agents where the time to onset can only be estimated, toxins are much more predictable in their delay. Other onset variables such as safety to users (which is similar to that of binary nerve agents), stability in the atmosphere (which is better than BWAs), treatment, which is usually supportive, not specific, and therefore resource intensive, underscore the potential for a significant place for a toxin type chemical weapon in the arsenal of an aggressor nation. Further, the extreme potency (therefore requiring small quantities), predictable time to onset, lack of specific detectors and treatment, safety to user (i.e. no or low dermal/oral exposure risk) and defined duration of hazard certainly recommend their use for covert military operation or by terrorist groups in addition to the potential for full scale military use.

3 Toxin Categorization

Since toxins can indeed be a threat in the hands of at least some groups, then which toxins might be encountered or "militarized" by an aggressor nation or group? Table 2 lists a few of the many hundreds of toxins that that are known and which have toxicity data associated with them. The new discipline of toxinology is one of the most active research areas in both academic and applied (pharmaceutical) pharmacology. There are a variety of reasons for this, but perhaps the most germane is that toxins have evolved in animals, plants and microorganisms over many thousands of years to have specific and unique effects. Often toxins target specific "receptors" or enzymes; the same receptors and enzymes that are disrupted, out of control or non-functional in some disease states. Toxins, therefore, can be used as probes for diseased systems, as models for the synthesis of new drugs or, in their native form, as drugs or remedies themselves. (Some examples of toxins that are in current use by the medical community include botulinum toxin [blepharospasm], endothelin derivatives [hypertension], curare [surgical muscle relaxant], cisplatin derivatives [cancer chemotherapy]). Consequently, scores or hundreds of new toxins are being "discovered" each year and a table such as Table 2 is inadequate to describe all the toxins in the medical/research/agricultural domain. The rows in this table offer examples of this diversity in structure and origin for a sample of toxins. A similar table for CWAs would have rows for organophosphates, cyanide, sulfur mustard, choking agents and perhaps arsenicals. The much vaster array of (potential) toxin weapons is one reason for the anxiety caused by considering toxins as weapons. However, the table provides some useful ways that toxins may be categorized which are helpful in evaluating the potential as a mass casualty/covert or terrorist weapon.

3.1 Toxicities.

The columns in this table offer some basic ideas on how toxins could be classified. (For comparison, the classical CWA soman is categorized in the same way in the last row.) One obvious category is toxicity (or in the case of non-lethal incapacitants, the effective dose (50%) or ED₅₀). Toxins span the entire range of toxicities from relatively non-toxic (domoic acid) to

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TABLE 2:
PROTOTYPICAL TOXIN AGENTS

TOXIN/CHEMICAL	SOURCE	CATEGORY	MECHANISM OF ACTION	TARGET SYSTEM	TOXICITY LD ₅₀ (µg/kg, ip) in MICE
CONOTOXIN	VENOM OF <i>CONUS GEOGRAPHUS</i>	POLYPEPTIDE	Ca ²⁺ CHANNEL BLOCKER	NERVE CELLS	5
NEUROPEPTIDE Y	MAMMALIAN BRAIN	BIOREGULATOR	Ca ²⁺ CHANNELS (?)	NERVE CELLS	?
ENDOTHELIN	HUMAN ENDOTHELIAL CELLS SNAKE VENOM#	BIOREGULATOR/ TOXIN	Ca ²⁺ CHANNEL ACTIVATOR	SMOOTH/VASCULAR MUSCLE	< 3
DOMOIC ACID	ALGAL (CONCENTRATED BY SHELLFISH)	CHEMICAL TOXIN	Ca ²⁺ CHANNEL BLOCKER	NERVE CELLS	10-50 (mg/kg)
TETRODOTOXIN	FISH GONADS	CHEMICAL TOXIN	Na ⁺ CHANNEL BLOCKER	NERVE AXONS	8
SAXITOXIN	DINOFLAGELLATES	CHEMICAL TOXIN	Na ⁺ CHANNEL BLOCKER	NERVE AXONS	3
MAITOTOXIN	DINOFLAGELLATE (CIGUATERA POISONING)	POLYPEPTIDE	Ca ²⁺ AGONIST	SMOOTH/VASCULAR MUSCLE NERVE CELLS	0.13
BOTULINUS TOXIN	<i>Clostridium botulinum</i>	PROTEIN	INHIBITION OF (Ca ²⁺ CHANNEL DEPENDENT?) ACETYLCHOLINE RELEASE	ACETYLCHOLINE NERVES	< 5 x 10 ⁻⁵
PALYTOXIN	<i>Palythoa</i> sp. (SOFT CORAL)	POLYETHER	K ⁺ CHANNEL BLOCKER	CARDIAC/VASCULAR MUSCLE NERVE	0.15
BATRACHATOXIN	FROG SKIN SECRETIONS	CHEMICAL TOXIN	Na ⁺ CHANNEL ACTIVATOR	NERVE CELLS	.1
SOMAN	ORGANIC SYNTHESIS	ORANOPHOSPHORUS	ACETYLCHOLINESTERASE INHIBITION	NEURO/NEUROMUSCULAR TRANSMISSION	250 (subcutaneously)

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equitoxic with classical nerve agents (tetrodotoxin) to 10 to 1,000,000 times more toxic than nerve agents (botulinum toxin, maitotoxin). Based on toxicity alone, toxins offer a far greater assortment of agents than the classical nerve/lung/blister chemical agents. It is easy to identify, for example, at least 100 toxins having, say, 10 times the toxicity of VX, the most toxic (weaponized) nerve agent. However, considerations other than toxicity may play a major role in assessing whether, or not, a toxin is a threat.

3.2 Sources.

For example, palytoxin is exquisitely potent: the $LD_{50} < 0.15 \mu\text{g}/\text{kg}$ in mice. But, as shown in the CATEGORY column, its polyether structure is so complex that chemical synthesis is not feasible and the extraction on a militarily significant scale from the producing organism (*Palythoa* sp.; SOURCE column) is not a viable option yet. Similar availability problems have been encountered with saxitoxin, where chemical synthesis has proved too complicated and extraction from the native organism too inefficient. In fact production of relevant concentrations of toxins has been, heretofore, the major determinant of the military significance of toxin weapons. It still represents one of the more important hurdles to the incorporation of toxins into the sphere of weapons of mass destruction. Recent advances, however, in areas as diverse as computer modelling, biotechnology and protein chemistry, along with those that can reasonably be expected in the near future, may have reduced the critical nature of this shortcoming.

3.3 Mechanism of Action.

The vast array of known toxins renders discussion of individual toxin threats and peculiarities premature and probably impossible. Like CW agents, toxins act in many different ways on many different systems and processes in the body. Therefore, perhaps it is better to look at the other side of the coin: what would be the consequence if this particular system (process, nucleus, receptor) were affected by (whatever) toxin? Looked at from this angle, groups begin to emerge among the myriad of toxins, as shown in the MECHANISM column. For example, there are toxins that target critical pores in nerve membranes that allow the cell to communicate with, and adapt to, its external environment. These toxins are often found in the venom of carnivorous animals and are usually specific and very fast acting, like the Ca^{2+} channel blocker conotoxins found in the venom of fish eating cone snails. Other toxins, for example the exotoxins elaborated by anaerobic bacteria, like botulinum and tetanus toxin, act on specific parts of the apparatus in nerves that control the release of the chemical messenger from axons. These types of toxins are typically slower in onset than the channel blockers described above, but extremely potent. Yet other classes of toxins act on specific "receptors" and either overstimulate or block critical processes, like breathing (curare, α -bungarotoxin) or blood pressure (endothelin). These toxins are also rapid in onset. There are also others that inhibit certain biochemical processes or cause non-lethal performance degrading effects (hallucinations, ataxia, vertigo). So, grouping toxins according to their mechanism of action often puts seemingly unrelated compounds together in the same category, but allows a diverse and extremely large set to be visualized and studied more easily.

3.4 Structures.

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Toxins, as a class, constitute some extremely complex chemical structures as well as biological molecules like peptides, polypeptides and proteins. The diversity in structure is suggested in the CATEGORY column which identifies the type of compound represented by each toxin. It is obvious that toxins span a wide variety of chemical and biological molecular types which would make detection and subsequent identification very difficult. It will probably turn out that the best detector will be man and the most reliable identification will be based on analysis of signs and symptoms.

4 Hazard Assessment

All of the information presented above show that toxins would present considerable problems if they were actually used in the military sphere in terms of detection, identification and most importantly medical or therapeutic countermeasures. But are toxins a significant threat to the CF at present. Well, as an armamentarium of weapons right now, the answer would have to be no. But, as the experience in Iraq has shown, some toxins (botulinum toxin for example) have indeed been produced and weaponized by non first world countries. Additionally, recent experiences in Japan, the U.S. and Europe show that relatively open societies are vulnerable to terrorist attacks not just from explosives but also from CBW agents that could easily include toxins. Unlike CB agents, toxin research is not prohibited under terms of the CBW treaty and there are many valid and important toxin research programmes under way in all parts of the world. This valid interest in toxin research, by pharmaceutical and agricultural companies in particular, is spurring developments in parallel fields (modelling, targeting, gene transfer) that contribute to the feasibility of developing a toxin as a drug or a poison. Of course, if it is available for commercial use, it is available for other less admirable intentions. Given that the field of toxinology is vast and active then, the question is how can research and development target which toxins (or classes) to study.

4.1 Toxicity.

One seemingly obvious choice to eliminate certain toxins or specify which to study is toxicity. In fact some work along these lines has already suggested that toxins whose lethal dose is not at least 10 times less than that of VX be excluded from the list of active R&D consideration. The term toxicity should be interpreted with caution and certainly should include agents that are non-lethal but militarily incapacitating. Such toxins might include SEB (a nausea producing agent), batrachatoxin, an hallucinogen, mycotoxins which are both a contact and a systemic irritant and cholera toxin, a diarrhea producing agent. However, the potency of these toxins could still be a determining factor in judging whether significant threat was posed by non-lethal incapacitating toxins.

4.2 Technology.

One of the areas that will undoubtedly have a major impact on both the perception and the fact of a toxin threat is the rapidly advancing technology. This area incorporates not just so-

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called “biotechnology” with recombinant DNA, plasmid transfers, designer bacterial synthesis *etc.*, but also advances in mainstream technology like solid phase peptide synthesis and computer science. Toxins, as has been pointed out above, are generally quite complex molecules, whether peptides, proteins or some other organic structure. This molecular and structural complexity has contributed to their relative obscurity and the perceived lack of a menace. Technology has and is rapidly changing the ability of many countries to afford and build designer toxins:

- 4.2.1 Fermentation: This is still the same old “low tech” method that can be and is being) used to produce weaponizable quantities of easily obtained bacterial toxins like cholera, botulinum and SEB toxin. New relatively simple and commonplace gene splicing techniques can be used to force bacteria or yeast to grow toxins that are normally only produced in eukaryotes, that is in animal and plant cells. Although the toxin backbone can be easily produced, many toxins undergo significant post-translational modification that confers special properties (potency, specificity *etc.*) to the toxin and prokaryotic organisms (*i.e.* bacteria, yeast *etc.*) normally do not have the correct “machinery” to provide this step. Thus the toxins produced in this manner may lack several unique qualities of the native toxin but nevertheless still be a useful weapon.
- 4.2.2 Peptide Synthesis: Once more in the realm of magic than science, (poly)peptide synthesis is now an automated technique and custom synthesis of many complex polypeptides is increasingly affordable. Recently, a peptide with a length of more than 100 peptide bonds, was synthesized using solid phase synthetic techniques. Other techniques, like “combinatorial libraries”, an automated method of producing random analogues of peptides or nucleotides based on a parent structure, is commercially available and an increasing number of pharmaceutical companies are using this method to discover, synthesize and develop new drugs. So previously difficult to obtain peptide or protein toxins are rapidly becoming commonplace and easily affordable to obtain.
- 4.2.3 Delivery and Penetration: Many very potent and lethal toxins have evolved to reach the site of action by ingestion (botulinum or cholera toxin) or in the case of predation or self-defence, by injection (crotoxin and sarafatoxin from snakes). In order to be an effective weapon, toxins must be delivered in a form that can cause the desired effect. As mentioned earlier, with the exception of certain mycotoxins, toxins are not (normally) active per-cutaneously. The agent, therefore would have to gain access to the body through one of the other three routes: orally, mucous membranes of the eyes or through the lungs. Production of particles of optimal size for deep lung penetration is now routinely and economically achievable so that delivery of toxins in this manner, notwithstanding the limitations mentioned above, is not a problem. Exotic routes of entry, like oral and ocular, would not likely be considered for a toxin that is being used as a weapon of mass casualty. The conclusion to be reached here is that if a toxin exists that is stable in the atmosphere, the technology to deliver respirable

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particles easily, economically and reproducibly already exists. It is important to note here that the technology exists not only for large scale applications, for example many square kilometres, but also in miniaturized form that could find application in the "news" story that opened this article. Further, the technology, which is primarily agricultural, is neither restricted nor monitored.

Normally toxins were not intended to be inhaled. But given the ease of incorporation into respirable particles, can a toxin, once absorbed into the body, reach its target, for instance the central nervous system for neurotoxins? In many cases, tetanus toxin is one example, there exists an active transport for the toxin to its central site of action. But for others the barriers massed by the body, not the least of which is the lung/blood barrier, are quite formidable. Here again, there are instances whereby biotechnology and other technological advances have provided interesting potential and actual solutions to this problem. For example, American Cyanamid has submitted proposals to the U.S. Environmental Protection Agency to field test a viral/toxin combination that kills beet armyworm. A lethal scorpion toxin is inserted into a baculovirus, a type of virus that specifically infects larval stages of lepidoptera. The expression of the scorpion toxin after infection of the lepidopteran hosts, the beet armyworm, increases the toxicity and halves the onset delay by factors of 2 to 3. Interestingly, the specificity of this insect control is at two levels: the virus only infects lepidoptera and the scorpion toxin elaborated by the introduced gene only kills insects. It does not require a great leap of imagination to think of a "vector" that will infect susceptible organisms or people. Let's think, for example, of a new flu virus strain: "Hong Kong X³A". This virus could be delivered to a naive population, and express a toxin protein, say botulinum toxin, within a specified time period. Vaccine protection against the virus would be provided for the users, so safety would be essentially complete. The toxin produced after infection could be lethal, as with botulinum toxin, or debilitating. Such a scenario has not been accomplished in the field of CW agents, but if it is being done and commercialized in agriculture, certainly the technology is available, the methods proven and the training/education accessible. This begs the question: would a "harmless" viral or bacterial vector containing a toxin producing gene, but no toxin, contravene the CBW treaty? The speculation described above combines both biological (viral vector) and chemical (toxin) warfare agents, but in ways that have not been considered in treaty negotiations. In addition to the scenario described above, there are also the proven technologies, like encapsulation of toxins in liposomes or oligomeric liposomes with known biological dwell times. These encapsulation technologies are feasible, inexpensive, reproducible and generally available.

- 4.2.4 Computer Science: Without doubt, computer science is the field of research that is going to have the most significant impact on toxins and mid-spectrum agents as CBW threats. It is not a secret that the major drawback to new or exotic toxins as CW

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**Table 3.
Toxin Risk Assessment**

(in this Table, the lower the Total Number, the more dangerous the toxin)

Toxin	Production	Effect	Potency	Stability	Legality	Onset	TOTALS
Endothelin	4	7	1	3	1	1	17
Tetrodotoxin	9	5	3	5	2	4	28
SEB	2	2	4	3	8	6	25
Domoic Acid	3	8	10	7	6	10	44
Botulinum Toxin	1	7	1	2	1	3	15
Palytoxin	9	8	3	5	3	4	32

Production:

1=Low technology, low cost, widely available *e.g.* fermentation

10=Cutting edge, high cost, only available to specialized teams *eg.* solid phase synthesis of > 100 amino acid polypeptides, advanced genetic manipulation.

Effect:

The number assigned here depends on whether the goal is to hinder the enemy or to kill. In this particular Table, overloading the logistic and medical support is considered to be a priority.

1=Severely incapacitating

10=Invariably lethal

Potency:

1= effective dose in the 10^{-9} g range

10=effective dose in the 10^{-3} g range

Stability:

This factor refers to stability in storage or weapons. This factor may be greatly affected by "packaging", for example in liposomes or particular solvents;

1=extremely stable in storage, no special conditions needed

10=labile or requires special conditions *eg* refrigeration, nitrogen atmosphere;

Legality:

Does the possession or research of quantities of this chemical contravene any treaties?

1=Completely legal, widely researched or used

10=No known research or medical use, specifically named in treaties;

Onset:

For this Table, immediate (*i.e.* battlefield relevant) onset rather than delayed (*e.g.* terrorist relevant) onset is considered desirable.

1=minutes to hours onset

10=multiple hours or days to onset

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agents is their availability: as a class toxins are generally structurally complex and diverse, not easily synthesized or extracted, and still require a relatively high level of technical competence and funding to produce. However, if the bulk of the research required to verify or develop "proof of concept" quantities of some particularly toxic or desirable or novel toxin could be carried out without actually ever producing the toxin or conducting laboratory experiments, the caveats mentioned above would disappear. Further, if the costs of conducting this "theoretical" research were exceptionally modest, the advantages of having and stockpiling toxin agents mentioned in Table 2, not the least of which are non-detectability and identification and compliance with the CBW treaty, become enormous.

This type of speculation is no longer fantasy. Faced with increasing costs of laboratory research, many pharmaceutical companies now routinely use what is called "structure based" drug design. With this approach, the target (for example a receptor or an enzyme) structure is known and likely stored along with thousands of others in the Protein Data Bank. A so-called "docking program" then screens a database of compounds and finds those that have good geometrical and chemical complementarity for the target. Computers solve these molecular jig saw puzzles and provide a list of about 100 compounds (from a starting list of >100,000) that are worthy of further investigation. Using computer graphics, several of these "hits" (rather than 100's) are then selected for classical laboratory investigation. This type of approach has led to several "new" drugs (anti-AIDS, anti-malarial) that are currently in clinical trials.

An alternative method actually designs new molecules to fit directly into the target site. With this approach completely novel "scaffolds" can be developed. For example, non-peptide based chemicals have been developed that mimic the action of a very potent bioregulator, angiotensin II, and which are now in clinical trials. Computer programs capable of designing *de novo* compounds for specific targets include GROW, GrowMol and Legend. It is important to remember that these programs, which actually design hitherto unknown or unsynthesized chemicals, are easily and commercially available and run on rather modest computer systems. Alternative software, including BUILDER, LUDI and FOUNDATION/SPLICE take preformed fragments and link them together, again with a specific target in mind. Nor are the targets difficult to obtain: several thousand are stored in the Protein Data Bank. Advances in this field are moving rapidly. Already libraries of peptides and peptoids are being planned from which "biased" databases can be built and expanded to ensure the maximum diversity and number of possible structures. Considering the availability of the databases and modelling software (through the Internet), the number of commercial sources of design and synthesis software packages and the power and low price of the computer platforms capable of using the software, it becomes apparent that a significant program of new threat (toxin or mid-spectrum) agent development could be undertaken with a very modest outlay of resources.

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(Something along the lines of “virtual” CW development.) Furthermore, the research, for the most part, could be done anywhere by a very small team with validation needed only in the final stages.

5 Discussion and Recommendations

What are the implications of the foregoing discussion for Canadian defence research and development (R&D)? First, it should be very obvious that even with unlimited resources, defence R&D in this country would not be able to investigate every potential toxin threat let alone those that may develop because of targeted synthesis and molecular modelling software. As mentioned above, certain defining categories, such as toxicity, can be used to reduce the potential number of emerging toxin/mid-spectrum threats, but even that leaves many hundreds of compounds to choose from. Table 3 suggests some ways to quantitate the potential risk and recommend which toxins should be of interest to the defence R&D community, but it is by no means exclusive. Other actions that should be undertaken are listed below.

5.1 The Generic Approach.

The R&D program in Canada, which is centred at the Defence Research Establishment Suffield, has chosen a so-called common or generic approach to the investigation of toxins. In this concept, critical physiological systems, for example specific channels in membranes, are studied with respect to function, including the effects of activators and blockers on the systems the channels control and ways to restore function or prevent dysfunction in the event of poisoning. The point of this type of research is to use the signs of poisoning of *a physiological system* to direct the therapeutic response. Subsequent attempts at detection/identification may also be assisted as classes of toxins can be excluded based on the pharmacology of known toxins to that system. In recent years, research in three important channels in nerve membranes that are very common targets of quite potent toxins has been undertaken in Canada, and in several other countries. Most of the active toxin research is aimed at defining the role of cation channels in excitable cells, like neurons, heart and muscle cells. Cooperation among various countries has expanded the knowledge base among all partners that allows a rational attempt at reasonable therapeutic measures, even if the identity of the specific toxin is not known.

5.2 Education.

The Force reduction plan applies to all areas of the Canadian Forces including the medical realm. It appears that more civilian medical personnel will increasingly be requested to provide essential services to DND, at least in emergencies. These health care workers, physicians, nurses and medics will require upgrading and training in fields relevant to the CF. This should include not only areas like tropical medicine but also CW, BW and mid-spectrum/toxin briefings. Of course, training for medical personnel within the CF must also continue. Emphasis should be on classes of agents and the drugs currently on the Canadian pharmacopoeia that may be useful in treatment until positive identification is made and a specific “antidote” is available. It is

important to remember that, increasingly, civilian populations are at risk whether through terrorist attacks or specifically targeted, and that CF medical personnel will be expected to provide, if not actual primary care, then at least advice.

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5.5 Collaboration.

The continued and rapid evolution of computer aided drug design should be a major interest of the R&D branch. The academic community and the pharmaceutical industry are

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devoting large amounts of resources to this new area of research. Along with the opportunity to tailor-make new drugs more quickly and cheaply, the opportunity to use the same and freely available technology exists for more nefarious motives. Collaborative programs with industry, academia and other countries, should be entertained to provide the R&D branch with the expertise and information required to evaluate whether this technology is easily adaptable to mid-spectrum agents. This type of collaborative program could also provide significant benefit to the CF in terms of other unresolved problems in CW, for example in designing or modelling therapeutic modalities for agents like phosgene, mustard and potentially other medical or health related questions unique to the CF.



MONTREAL, Canada (Canadian Press)-Police say that they still have no idea who planted the device that delivered a deadly fog during an international meeting taking place in this Canadian city two days ago. Twelve deaths have been reported following the surprise attack by terrorists during the busy lunch hour period at the Palais de Congres in this eastern Canadian city. "The deaths were caused by cardiovascular collapse, that is similar to a heart attack" says a hospital official. "We were very worried that many more casualties would succumb until we identified the toxic component of the fog and were able to administer a specific drug to counteract the substance's effects".

The toxic material was identified by a special team from the Department of National Defence. Based on the signs of poisoning, the DND experts were able to identify the toxin contained in the fog using sophisticated immunological methods. After the compound had been identified, the DND team was able to advise the use of a specific drug to counteract the toxin's

effects. "The poison used was a chemical derived from the venom of a snake" said a senior DND official. "It is related to a compound produced by our own bodies which is why it is so potent and specific.

"Our research had previously shown that moderate exposures to this particular class of poisons could be treated with a commonly available blood pressure medicine. More severe cases require a very specific antidote which is available only in limited quantities. Luckily, there were very few severely poisoned people who probably were sitting quite close to the ventilation shaft where the toxin cloud was released".

Police are continuing their investigation but wish to remind people that there is no danger of infection or spreading the disease. "This was not a virus or bacterial attack so it is not infectious" says the DND spokesman. "This was a chemical attack with a bioengineered, very potent toxin, but it behaves exactly like a poisonous chemical and will not cause any new or exotic disease"

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Toxins, which are non-replicating agents of biological origin, are relatively anonymous chemical agents even though many are among the most exquisitely lethal substances known. This paper categorizes toxins especially with respect to classical chemical and biological agents. Areas of potential use are discussed and examples of some identified toxins provided. Defensive research initiatives and the impact of emerging technologies in biology, chemistry and computer science are discussed.

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