

# **Decontamination of Warfare Agents**

## Enzymatic Methods for the Removal of B/C Weapons

*Edited by*

*André Richardt and Marc-Michael Blum*



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## Foreword

The present book is intended to give a comprehensive overview of the history and state-of-the-art of a novel experimental technique: the enzymatic decontamination of biological and chemical warfare (C/BW) agents. By this, it not only intends to point out the actual relevance and need of modern technologies, but also tries to contribute efforts to a better international coordination of promising research and development work in this field. Decontamination in general defines a technical process used to reduce or ideally remove or destroy contaminants, and moreover, to prevent the spread of contaminants from persons and equipment. Contamination with C/BW agents can occur without warning, as a result of attacks and collateral damage during military operations, as well as by deliberate release of highly toxic materials and infectious agents, or industrial accidents and natural outbreaks of diseases. Historically, military communities have the highest requirement for powerful decontamination technologies, not at least presumably because they are potentially confronted with a higher abundance and severity of C/BW agent attacks in comparison with possible terrorist or criminal scenarios with localized release of minor quantities.

The main reason to search for new technologies is that the current decontamination procedures for C/BW agents depend on higher temperature treatment and harsh chemical reactive components e. g. strong oxidants and chemical disinfectants. These techniques and compounds are often not suitable for different materials, cause logistic problems and are themselves hazardous, corrosive and environmentally critical. In many Armed Forces, aggressive and toxic substances like hypochlorite and formaldehyde are still the pivotal reactive components for C/BW-decontamination. The common goal of the various decontamination measures is to rapidly destroy known CW agents as well as to disinfect bacteria, fungi and protozoa, inactivate viruses and detoxify toxins.

For all decontamination procedures of clothing, material and vehicles, the military user would rather prefer a modern technique that is equally effective for CW and BW agents. However, this approach is technically very ambitious. The decontamination of BW agents including bacterial spores, which are known to be highly resistant to temperature and disinfectants, represents a particular challenge with respect to optimizing the proportions of efficiency and logistic burden, as well as

its environmental impact. On the other hand, with the search for novel reactive components, biotechnology-based decontaminants became increasingly important with respect to these requirements. As will be outlined in this book, biotechnological approaches based on biochemical catalysis using enzymes on a technical scale may be competent alternatives or at least additives for existing protection measures against C/BW agents. They allow decontamination reactions under ambient (mild) conditions and follow a highly effective catalytic, rather than a reagent-consuming, stoichiometric principle. The data, as presented here, outline a good prognosis for such reagents. However, there is also strong evidence that more research and development efforts in this field—ideally on the basis of internationally coordinated cooperations—are indispensable to efficiently exploit this valuable biotechnology potential for future decontamination techniques, be it for military or civil defense.

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## Preface

Since the end of the Cold War, the threat scenarios arising from the potential use of chemical and biological (CB) warfare agents have changed fundamentally. During the Cold War, both blocks stockpiled tens of thousands of tons of chemical warfare agents and pursued biological warfare programmes. In case of a military conflict between NATO and the Warsaw Pact Central, Europe was the most likely battlefield and large-area attacks with CB agents were among the most realistic scenarios. Decontamination systems were designed to be able to return contaminated equipment back into combat service as quickly as possible. Environmental concerns and potential long-term damage to equipment due to the use of aggressive and harmful chemicals were not an issue. Human casualties and fatalities were likely and although military planning tried to minimize these casualties, large-scale attacks would have resulted in substantial numbers of affected personnel. As the civilian population had only rudimentary protection against these agents, the numbers of affected persons would be even higher. After the end of the Cold War, the scenario shifted towards the use of CB agents, both finally banned by international treaty regimes, in smaller conflicts where terrorists, religious fanatics or other insurgents could use these weapons in asymmetric scenarios to cause fear, panic and uncertainty, especially affecting the civilian population. In addition to this, the last twenty years saw revolutionary advancements in the life sciences and the phenomenon of globalization went along with an enormous growth of worldwide traffic of both goods and people. Both developments could add to a growing threat, making the use of CB agents even more likely.

When monitoring public discussion, it is often striking that the fear of being faced with new dangerous substances is based on unrealistic assumptions and insufficient information. Media, scientists and other actors sometimes abuse the veil of mystery that has rested on the field of chemical and biological warfare agents since their first use to raise attention and to justify the requests for money. But even a realistic and unemotional assessment of the current threats reveals that the use of CB agents, especially against civilian population, is possible and becomes even more likely as we see a growing technical sophistication among insurgents and terrorist movements. Also, several countries remain outside the treaty regimes

banning CB agents. Several of them are thought to pursue active weapon programs and raise proliferation concerns.

New comprehensive concepts to counter the CB threat are needed. The changed scenarios call for a unified strategy that covers the military side as well as civil defense. In these concepts, decontamination plays a crucial role flanked by detection and protection technologies. Modern decontamination should be fast, safe, environmentally benign and useable in civilian environments. New and highly advanced decontamination technologies have been under investigation for many years and some of them are on the brink of being introduced into service in the very near future. Biotechnology-based methods using enzymes are among these technologies and are the main topic of this volume.

Our intention to write this book with the help of many specialists in the field was driven by the perception that scientists should not only know the basics of their field, but also be aware of the requirements for effective decontaminants and of general concepts if they plan to introduce new technologies in this security-relevant field. Therefore, this book is intended to serve as a bridge between basic science, on the one hand, and applied engineering and product development on the other. Biotechnology-based decontaminants could be used to fill capability gaps where other highly corrosive and harmful decontaminants fail. However, new technologies rarely replace old ones right away. In an interim phase, new and old technologies will coexist and acknowledging that public procurement decisions are often made for a decade or more, this phase might turn out to be quite long. We also intend to add to the public debate by trying to demystify chemical and biological agents and the possible threat posed by them. Discussion should be based on scientific knowledge, which all the authors of this volume try to put forward in a way that should enable the reader to take part in the debate with the necessary background to either make valuable contributions themselves, or to be able to assess and judge arguments put forward by others.

We would like to thank our friends, colleagues, co-authors as well as the editorial staff at Wiley-VCH for their support, ideas and remarks. Special thanks go to our families for their patience during the endeavour of this book.

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# 1

## The History of Biological Warfare

*Friedrich Frischknecht*

### 1.1

#### Introduction

Poisons have been used for assassinations for as long as humans can remember. It is unclear when they were first used intentionally for the purpose of warfare. However, during the last century, several tens of thousands of people were killed when disease agents or toxins were used in warfare, mainly during Japanese attacks on China in World War II. Two international treaties were established, first in 1925 and then in 1972, to prohibit the use of biological weapons in war, but both failed to inhibit research and large-scale productions of biological and toxin weapons in several countries. Since the letters containing anthrax that were sent in the wake of the September 11, 2001 terrorist attacks on the United States, it has been feared that pathogens could again be used in large scale for either terrorist or warfare purposes and billions of dollars have been poured into research of questionable scientific merit. To put the potential of such future threats into perspective, I recount some historical examples of biological warfare and terrorism.

### 1.2

#### Pre-Twentieth Century Examples

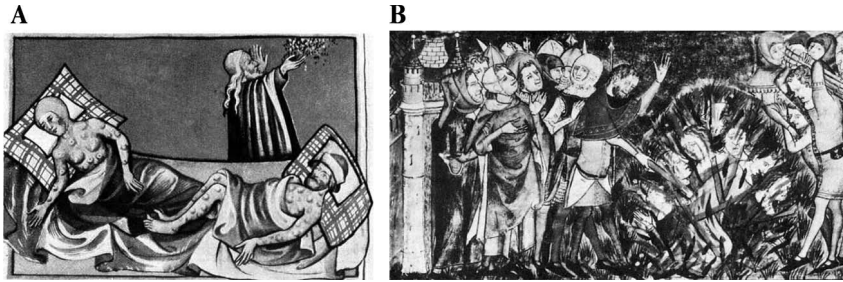
When humans first brought war upon each other, more soldiers were incapacitated from diseases than from the hand of their human enemies. This observation might have lead to the early deployment of poisoning substances during war (see Table 1.1). To our current knowledge, poisons were administered to enemy water supplies as early as the sixth century BC. Animal cadavers substituted for poisons during the Greek and Roman eras and Emperor Barbarossa used human corpses to the same end, although it is likely that a simple spoiling of the water supply, rather than the spreading of disease, was intended [1–3]. Hannibal suggested a more active approach when advising the Bithynians to catapult jars filled with snakes towards enemy ships in 184 BC [4]. The panic created, rather than

**Table 1.1** Selection of possible events of biological warfare.

| <i>Year</i>                | <i>Event</i>  | <i>Disease agents and outcomes</i>   |
|----------------------------|---|--|
| <1000 BCE.<br>(Trojan War) | Legend of Scythian archers using poison arrows.                                 | Clostridium (?) causing gangrene and tetanus.                                    |
| <500 BCE.                  | Assyrians poisoned enemy wells.   | Rye ergot fungus causing hallucinations.   |
| 590 BCE.                   | Greeks poison water supply of Kirrha during the first Sacred War.               | Hellebore root causing diarrhea. Kirrha falls and the population is slaughtered. |
| 184 BCE.                   | Bithynians catapult jars filled with snakes towards enemy ships.                | Snakes causing panic. Sea battle is won.   |
| 1155                       | Emperor Barbarossa poisons water wells.   | Decomposing human bodies.  |
| 1346                       | Tartars catapult plague victims over the walls of Caffa.                        | Yersinia pestis (?) causing plague. City is abandoned.                           |
| 1495                       | Spanish sell wine mixed with blood from leprosy patients to enemy.              |  |
| 1763                       | British distribute blankets from smallpox patients to native Americans          | Variola virus causing smallpox. Epidemic develops.                               |
| 1797                       | Napoleon floods fields around Mantua to enhance malaria.                        |  |
| 1915–1918                  | Germans infect animals of Allies  | Anthrax and Glanders.  |
| 1932–1945                  | Japanese conduct large-scale human experiments and biological warfare in China. | Many different pathogens killing tens of thousands.                              |

Note that the understanding of disease causing agents does not predate the work by Pasteur and Koch and thus a clear distinction between chemical and biological warfare can often not be made prior to that time. Also cause and consequence as well as intention and outcome are not always clearly established for many events as discussed in the text.

poisonous bites, likely decided the battle, revealing human psychology as a second important dimension during biological attacks. Catapulting infected human bodies and excrement constituted a further step during the sieges of many towns, although it is not clear if these actions contributed much to the spread of disease. When the Tartars besieged the Crimean city of Caffa (now Feodosiya, Ukraine) in 1346, they catapulted victims of the bubonic plague into the city where the Black Death soon caused collapse. As the occupying Genovese fled and the victors moved on, both spread the disease that ended up killing some 50% of the European and Chinese population (Figure 1.1). This changed the course of human history forever [5, 6] (Figure 1.1). However, whether biological warfare was the beginning of this greatest of medieval disasters remains impossible to prove. The fleas that transmit the disease between humans leave dead bodies rather quickly, therefore calling into question if the corpses that flew into Caffa were flea infested [3, 7]. Rats moving in and out of the city walls might have spread the disease much more



**Figure 1.1** Late medieval illustrations of the Black Death. (A) Suffering plague victims as illustrated in a German bible from 1411. (B) The 1349 burning of Jews blamed for causing the pandemic as an example of the social repercussions unexplained infectious diseases caused throughout history.

efficiently, although it is not clear if they would have moved far enough. Furthermore, it is not clear if the attackers intended to spread disease or simply wanted to get rid of the stinking bodies of their dead comrades. And lastly, it is even disputed if *Yersinia pestis* was the cause of the Black Death at all [8]. These uncertainties illustrate one of the biggest problems in biological warfare history. How can one be sure an attack actually leads to the spread of infections, while they could just as well result from coincidental natural infection in an “unnaturally” large aggregation of—or new encounters between—humans.

With time, humans became more inventive and thought of more elaborate ways of distributing disease agents. In 1495, Spanish forces supplied their French adversaries with wine contaminated with the blood of leprosy patients during battles in Southern Italy [9]. In the seventeenth century, Polish troops tried to fire saliva from rabid dogs towards their enemies. The first pledge against the use of poisoned weapons was made between France and Germany in the 1675 Strasbourg Agreement. And while Russian troops might still have catapulted plague victims into the Baltic city of Reval during a war with Sweden in 1710, and the Tunisians tried plague infected clothing 75 years later, Napoleon went a step further. He attempted to use swamp fever during the siege of Mantua in 1797, by flooding the fields around the city, hoping to thus induce the spread of the disease now known as the mosquito-transmitted malaria. Twelve years later he looked on happily at British troops dying from swamp fever in the marches of Holland, where they set up camp [10].

Accidental spreading of diseases like smallpox, influenza, measles and tuberculosis took the lives of most indigenous people of the Americas, making the Spanish conquest possible [6, 11, 12]. Later, in North America, the French and English used smallpox-drenched clothing that they distributed among Indians [2, 13]. However, similar to the situation in Caffa, it is not clear if the following smallpox epidemic was caused by this measure, or rather because of contacts from trading activities between Indians and colonists [7, 14]. During the civil war in the United States, the Confederate doctor Luke Blackburn attempted to infect federal troops

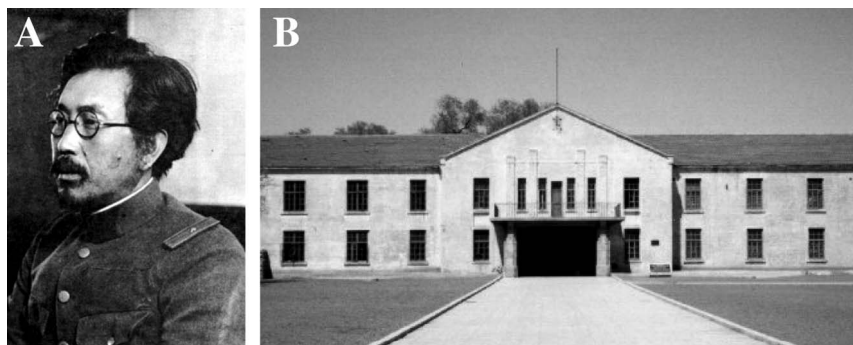
using clothing carried by smallpox and yellow fever patients. However, yellow fever was soon discovered to be solely transmitted by mosquitoes, leaving claims that soldiers died from such an attack rather unbelievable. On the other hand, Union troops were forbidden by an army order to use poison in any manner.

### 1.3

#### From World War I to World War II

With the foundation of microbiology by Pasteur and Koch, bio-warfare could take new dimensions as weapons could be designed on a rational basis. Recognizing this, two international declarations (1874 in Brussels and 1899 in The Hague) tried to prohibit the use of poisoned arms [15]. Nevertheless, as with chemical weapons, the Germans pioneered the use of biological weapons during World War I, albeit on a miniscule scale. Covert operations were using both anthrax and glanders to infect animals directly or to contaminate animal feed in Romania, France, Mesopotamia, Argentina and the United States [7, 13]. Fearing each other and relying on misinformed intelligence reports, a number of European countries had biological warfare programmes established before the onset of World War II.

In North America, Sir Frederick Banting, Nobel Prize-winning discoverer of insulin, started what could be called the first private biological weapon research center [16]. The United States joined their British allies, who, like the French, feared a German biological attack [16]. However, unlike the Nazis, some Japanese were truly enthusiastic about the potential of biological weapons [16–20]. The radical nationalist Shiro Ishii started his research in 1930 and became the first head of Japan's bio-weapon program during World War II (Figure 1.2). At its peak, the program employed over 5000 people, killing as many as 600 prisoners a year in human experiments in the largest of its many centers. At least 25 different



**Figure 1.2** Japanese biological warfare during World War II.  
(A) Shiro Ishii in 1932, photographed by Masao Takezawa.  
(B) Reconstructed building of Unit 731 at Harbin as photographed in 2002 by Markus Källander. Both images are public domain pictures from Wikipedia.



disease-causing agents were cruelly tested on prisoners and unsuspecting civilians. Water wells in Chinese villages were poisoned to study cholera and typhus outbreaks. Plague-infested fleas were dropped by plane over Chinese cities or distributed by saboteurs in rice fields and along roads, causing epidemics in areas where the plague was unknown, some persisting for years after the war ended. It is estimated that several tens of thousands of people died as a consequence of offensive biological research, including soldiers on both sides of Soviet-Japanese battles when suicide squads and artillery shells were employed to spread disease in 1939 [18, 19].

#### 1.4

#### Secret Projects and Cold War Allegations

After the war, the Americans granted freedom to all researchers in exchange for information on their human experiments [17–20]. In this way, mass murderers and war criminals became respected citizens, including founders of pharmaceutical companies. Masaji Kitano, the successor of Ishii, published post-war papers on human experiments using “monkey” instead of “human” when referring to the experiments in wartime China [18]. A cover-up was almost successful when, 45 years after the war, numerous bones were found during construction work near Ishii’s old laboratory in Tokyo [17]. In 1947, to profit from the secret Japanese knowledge, United States President Truman withdrew the Geneva Protocol from Senate, which still had not ratified it. Soon after, the US military conducted open-air tests releasing both pathogenic and non-pathogenic microbes on test animals, human volunteers and unsuspecting civilians [2, 16, 21]. *Bacillus globigii* and *Serratia marcescens*, two harmless bacteria, were released from naval vessels on the Virginia coast and off San Francisco, infecting around 800 000 people in San Francisco alone. Furthermore, bacterial aerosols were released at bus stations and airports [16]. The most infamous test was the 1966 contamination of the New York metro system with *Bacillus globigii* (a so-called anthrax simulant) to study the spread of a pathogen in a big city [16, 22]. However, with the opposition to the Vietnam War and his own wish for a Noble Peace Prize growing, US President Nixon decided to abandon offensive biological weapon research and signed the Biological Weapon and Toxin Convention (BWTC) in 1972. While most stocks were destroyed the same year, some quantities of toxins were kept aside by the CIA for several years [22].

Allegations that other countries produce biological weapons have served as excuses to develop these weapons and have been exploited as welcome means for propaganda. During the Korean War the Chinese, North Koreans and Soviets accused the US-led troops of deploying biological weapons of various kinds. Although it is now largely seen as a propaganda move, the secret deal between the US and Japanese bio-weapon researchers did not help diffuse the allegations [23]. In reverse, the United States accused their Vietnamese enemy of dropping fungal toxins onto the American Hmong allies in Laos. After some confusion it

turned out that the yellow rain associated with the reported variety of syndromes was bee feces [24].

Closer to home, Cuba complained frequently about American biological warfare, and in 1997 was the first country to officially file a complaint under article 5 of the BWTC, accusing the US of dissipating a plant pathogen [15]. Although this allegation is probably not substantial, the US did invest in biological warfare against Fidel Castro and Frederik Lumumba of Congo [22]. However, not everything ridiculed by politicians or scientists turned out to be fiction. The outbreak of anthrax in the city of Sverdlovsk (now Ekaterinburg, Russia) in 1979 was seen by many in the West as a breach of the BWTC. The Soviets naturally refuted such accusations.

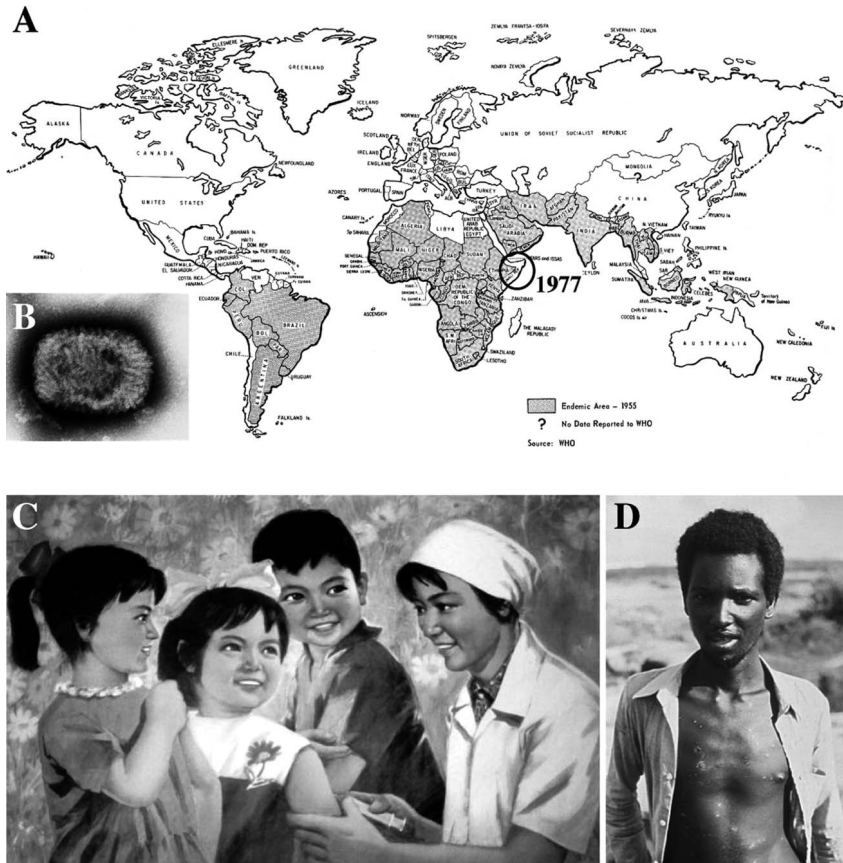
After signing the BWTC, the Soviet Union created a gigantic project employing at its height over 50 000 people directly working on the development and production of biological weapons [25]. While stockpiling large amounts of anthrax and smallpox, also for the use on missiles, an unknown number of people lost their lives. Most famously, when a filter was not replaced between shifts. The resulting outbreak of inhalation anthrax in Sverdlovsk killed at least 66 civilians [25, 26]. In another accident within a high security lab, a virologist injected himself with marburg virus [25]. After his death, his colleagues re-isolated a more virulent virus from his body and continued to work on this particular strain! Similarly, there are speculations about the association of a bio-weapon research center located on a small island in the Aral Sea with an outbreak of smallpox in the city of Aralsk, as well as the occasional deaths of fisherman and researchers [22, 27]. With the fall of the Soviet Union it is now generally feared that not all stocks of dangerous pathogens including smallpox (Figure 1.3) have been destroyed, and may have been taken abroad by scientists now working in other countries [22, 25, 28]. Furthermore, it is not clear to what extend Russia is continuing with secret programs.

## 1.5

### **Towards the Twenty-first Century: Madmen on the Run**

Apart from state-sponsored bio-warfare programmes, a number of single person or non-governmental group-driven attacks have occurred or were alleged [29]. Here just three examples. Just after World War II a peculiar bank robbery occurred in Tokyo. A man identified himself as an official from the Ministry of Health and Welfare and made every employee of the branch drink a potion with the effect that 12 out of 16 people died after several minutes. An alleged high level cover-up possibly led to the imprisonment of an innocent person who was sentenced to death, but never killed, fuelling speculations that former members of the Japanese bio-warfare program were somehow involved [17].

A large outbreak of salmonella in a small town along the Columbia River in Oregon in 1984 caused the gastroenteritis of 751 people [30] when a religious sect wanted to poison a whole community to influence an upcoming local election [22]. The bacteria were found in a number of salad bars in various restaurants in a



**Figure 1.3** Smallpox and its eradication. (A) Countries with endemic smallpox in the year 1955 are highlighted. The circle indicates the last case of naturally acquired smallpox in Somalia 22 years later. (B) Electron micrograph of a variola virus particle. (C) Pre-1979 Asian poster promoting youth

vaccination for Smallpox and Measles. (D) Ali Moaw Maalin at 23 years of age became the last person worldwide to contract smallpox (*Variola minor*) in Somalia in 1977. All images are from the Center of Disease Control Public Image Library and courtesy of WHO and CDC.

manner that could not be traced back to a naturally occurring outbreak and the sect was eventually identified as culprit due to the testimony of an insider [30]. The sect obtained the bacterial strain simply, from a commercial supplier, and produced it in a hospital setting in its own grounds. This clearly indicates how easy it might be for organized groups to collect a mix of reagents in order to put together a small bio-terrorism program. All it would seem to need are a few letters to “colleagues” at scientific institutions whose duty it is to send out published materials in order to share it with the rest of the community [31].

Another set of attacks occurred in 1995, when the Japanese Aum Shinrikyo cult in Tokyo killed 12 people and injured over 5000 by using the nerve gas sarin [32].

The investigations into the sect revealed that it had also tried to distribute anthrax within the city. However, this remained without success. The production of the spores was apparently possible, but the sect failed to produce the right kind of aerosol needed for successful dissemination [15, 33]. Sadly, the still unidentified perpetrator(s) of the 2001 anthrax attacks in the United States were more successful by sending contaminated letters that eventually killed 5 unsuspecting citizens. Together with copycat hoax attacks, the letters caused an estimated economic loss of several hundred million dollars [15, 22, 34].

## 1.6

### Future Threats—Science or Fiction?

Public interest in the potential danger of biological warfare and terrorism has been sparked by the events on and following September 11, 2001 as well as the depiction of fictitious biological attacks in books and movies [35, 36]. While some experts predict major outbreaks with devastating effects, others assume that, with reasonable precautions, such epidemics could be avoided [15, 37]. Leitenberg (2001) interprets the example of the Aum Shinrikyo attacks as evidence that terrorists might not find it straightforward to develop an effective biological weapon. In contrast, state-sponsored biological warfare programs and their successful concealment indicate that complacency might be the wrong strategy in dealing with this fuzzy potential threat. However, when deciding on how to distribute research money, we should always compare the speculative nature of potential future biological attacks with the grim reality of daily life for millions of people, who continue to suffer and die often from already preventable infections.

## 1.7

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