



Some Bioterrorism Issues of Quantitative Biosafety

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Abstract

With the increased recognition of a bioterrorism threat, the realm of biosafety is substantially expanding and merging with biosecurity. Two issues pertinent to bioterrorism and biosecurity, or biosafety in its new broader sense, are discussed in this article. The first concerns airborne exposure limits (AEL) for biological threat agents (including biowarfare (BW) agents) and a possible approach to determine these limits in the absence of precise data on infectious doses for most of these agents. The second issue concerns the sensitivity limits for the real-time biosensors that should detect BW agents and the significance of these limits for biosafety and biosecurity. To what levels of infection would the different sensitivity limits correspond and how is sensitivity related to the ability to manage the consequence of an airborne threat? These issues were addressed recently in a model study on detection limits of real-time biosensors (Sabelnikov et al., 2005) but because of their significance for biosafety and biosecurity deserve a much wider scope of discussion.

Introduction

Like most of the sciences dealing with biological entities, biosafety is rapidly changing both conceptually and methodologically. Not long ago its main concern was primarily laboratory safety. And though it is still considered “an inexact science” by some (e.g., one of the editors of *Biological Safety*, 2000 [Fleming & Hunt, 2000]), research in biosafety and related topics is making it a more exact and defined science with quantitative and prediction power. Examples include enhancements and refinements to areas such as risk assessment and analysis, sampling methods to detect contaminants, and novel methods developed over the past decade for decontamination. At present, with the advent of a bioterrorism threat, the realm of biosafety is substantially expanding and merging with biosecurity, even though according to the thesaurus, they are synonyms.

Never in the past was there a more significant threat concerning the use of BW agents by nonstate actors such as terrorists as the one that exists in modern times. Emergency response strategies (guidelines) to deal with any kind of terrorist event should incorporate several impor-

tant factors such as immediate mitigation (medical, decontamination, etc.), organizational (sequestering the area, establishing a quarantine, evacuating the population, etc.), and postevent (clean up, decontamination, etc.) procedures. These should be based on scientifically proven exposure/health risk assessment for the materials involved (chemical, biological, or nuclear/radiological); effective detection, identification, and decontamination technologies; and accepted clean-up standards. In the case of a radiological terrorist event, when “dirty bomb” radiation could be detected and measured, the emergency response strategy has already been determined (NCRP, 2001). In the case of a terrorist event with the use of chemical warfare (CW) agents, the situation is not that clear because airborne exposure limits (AELs) obtained by extrapolation of toxicological data among animal species and from animals to humans have proven to be unreliable for many chemical agents (Johnson, 2003). For BW agents the situation is the worst: Lack of scientific data on infectious doses for most of them (Johnson, 2003; Raber et al., 2001) prevents the generation of their AELs. Furthermore, as recently concluded by the American Biosafety Association (ABSA), the attempts to develop quantitative values for human infectious dose are not currently feasible, and infectious dose values developed using past studies would not accurately characterize the relative hazard of pathogenic organisms in humans (Johnson, 2003).

Does this mean that AEL for bioterrorist agents cannot be generated at present and we have to wait years to fill the gap in our knowledge of infectious doses? The answer is “No.” An efficient, temporary solution to bridge this gap and to set provisional AELs for BW agents may be inferred from the recent model study of detection limits of real-time biosensors—present, future, or conceivable (Sabelnikov et al., 2005). Several other issues addressed in this article also may be relevant to biosafety and biosecurity. For instance, recent Department of Homeland Security (DHS) agent sensitivity requirements for future field devices capable of BW agent identification in aerosols indicated a rather wide range of airborne concentration from 100 to 100,000 organisms per liter in air (DHS/HSARPA, 2004). Are these concentrations too high or too low with regard to biosafety and biosecurity? To what degree of “risk of infection” would these sensor sensitivity limits correspond? Will real-time biosensors be able to detect provisional AEL for BW agents?

All these issues, because of their significance for biosecurity, or biosafety in its new, broader sense, deserve wider discussion among biosafety professionals.

Probability of Detection and Probability of Infection

Highly sophisticated and powerful biosensors, in addition to their use as constant surveillance devices and as mandatory equipment for the first responders, may also become working tools for laboratory biosafety personnel.

The evaluation of how the probability of detection corresponds to the probability of infection by an agent on the same time scale was achieved by a generated mathematical model. The model was based on the probabilistic nature of the events of detection of aerosolized microbes (including BW agents) and the events of infection, if they are pathogenic. The model included three generated equations for detection, one equation for the probability of infection by inhalation, and several general and specific assumptions. In particular, it was assumed that:

1. There is a space that contains aerosol particles with microbes. Microbes within aerosol particles and the latter within the space volume (both are *discrete* entities) are distributed according to Poisson distributions with parameters equal to their mean concentrations.
2. Aerosols contain both viable and nonviable (inactivated) forms of microbes. Only viable organisms are infectious; however, both forms can be identified.
3. Every *viable* pathogenic organism inhaled by the body (as aerosol) can initiate the infection process with a probability of *p* independently of other organisms.

Probability of Infection

The equation for the probability of infection by inhalation (equation 3 below) was derived based on the above assumptions from the earlier equations of Peto (1953), Mayorov et al. (1989), and Chermashentsev et al. (1993). In short, the probability that none of the *viable* organisms, *N*, infects the cell is $(1 - p)^N$ can be approximated by $\exp(-pN)$. Further, according to the second assumption, $N = D_i D_v$, where D_i is a total number of inhaled viable and nonviable microorganisms, and D_v is the fraction of viable organisms. So, the probability of infection, **P**, may be written as:

$$P = 1 - \exp(-pD_i D_v) \quad (1)$$

By definition, **P** is equal to 0.5 when a dose becomes equal to **ID50** value (the infectious dose, or the number of microbes capable of producing the disease in 50% of those exposed). From that *p* can be easily found as:

$$p = \ln 2 / \text{ID50} \quad (2)$$

Substitution of *p* in equation (1) with its value from (2) yields the final equation for **P** that includes several key parameters, such as D_i , D_v , and **ID50**:

$$P = 1 - \exp(-D_i D_v \ln 2 / \text{ID50}) \quad (3)$$

Recently, a conceptual modeling approach was successfully used for the theoretical estimation of the risks of infection with smallpox virus and *Francisella tularensis* (Jones et al., 2005; Nicas et al., 2004). Though for most BW agents the infectious doses have not yet been precisely established (Johnson, 2003; Raber et al., 2001), there are reasons to believe that some agents, such as smallpox (Henderson et al., 1999; Nicas et al., 2004), tularemia (Dennis et al., 2001; Jones et al., 2005), and Q-fever (Fournier et al., 1998; Johnson, 2003), have a very low ID50 index (in single units). For others, like Marburg virus (Chermashentsev et al., 1993) and plague (Iglesby et al., 2000), it seems to be higher—approximately 100 units and approximately 1,000 units for anthrax (Iglesby et al., 2002; Meselson, 1994).

Taking these values into account, it seemed reasonable to group BW agents by their currently inferred ID50 values into three categories, with low, intermediate, and high ID50 values. It was suggested that such grouping might be an efficient, temporary solution for the purpose of the modeling (Sabelnikov et al., 2005). Apparently, it might also be efficient in setting “provisional” safety limits for BW agents. Three levels were assigned for inhaled ID50 indices and used for simulations: low (5 organisms), intermediate (100 organisms), and high (1,000 organisms).

Our first objective here is to consider what this approach and grouping provides with regard to provisional airborne exposure limits (AEL).

Similar to PEL (permissible exposure limits) for hazardous chemicals (including chemical warfare agents), several AEL may be considered for BW and other infectious agents depending on the *time of exposure* via the inhalation route. Further, AEL as a “permissible airborne concentration” of an agent would determine a certain, *critical risk of infection* by inhalation in a certain period of time (exposition). So, in order to find “safe,” permissible, concentration limits, we first have to agree about what are the critical (“acceptable”) risks of infection after a certain time of exposure for every BWA agent, and then find permissible AEL corresponding to those risks.

Taking into account that $D_i = t W_h C$ (where *C* is the concentration of an agent in the air, *t* is the time of exposure, and W_h is the adult inhalation rate), and making a simple rearrangement of equation 3, we obtain:

$$C = - \ln (1 - P) \text{ID50} / (D_v t W_h \ln 2) \quad (4)$$

where all the symbols are the same as in equation (2 and 3). (Note that *C* is inversely dependent on D_v .)

In Table 1 airborne concentrations corresponding to different risks of infection after 1 hour exposure are presented for all three groups of BW agents. Throughout all the simulations that were performed with equation 4, 11 liters/min was used as the adult inhalation rate, W_h (Allan & Richardson, 1998).

Table 1

Airborne concentrations (microbes/m³) corresponding to risks of infection by different ID₅₀ groups of BW agents after 1 hour exposure.

Risk of Infection with BW agents (after 1hour)	Corresponding Airborne concentration of a BWA (units/cubic meter)			
	Bacillus anthracis (ID ₅₀ =1000)		Marburg virus (ID ₅₀ =100), Dv= 0.1	smallpox virus (ID ₅₀ =5), Dv = 0.1
	Dv= 0.5	Dv = 0.1		
0.000001	0.004358	0.021789	0.002179	0.000109
0.00001	0.043579	0.217894	0.021789	0.001089
0.0001	0.435808	2.179041	0.217904	0.010895
0.001	4.360045	21.80022	2.180022	0.109001
0.01	43.798	218.99	21.899	1.09495
0.1	459.1468	2295.734	229.5734	11.47867

Let us consider what risks of infection, if any, might be considered critical. Taking into account the lack of epidemiological potential of *B. anthracis*, even the value of about 1×10^{-4} for the risk of infection (column 1) might initially appear to be an acceptable candidate for a safety limit for this BW agent. That would correspond to AEL of 0.44 organisms/m³ air (with 50% viability, Dv=0.5, column 2), and 2.18 organisms/m³ air (with 10% viability, Dv=0.1).

The situation involving agents with epidemiological potential, such as smallpox, is, however, less clear. Zero risk might be a necessity in this case because according to the model and other recent theoretical estimations (Nicas et al., 2004), even tiny airborne concentrations of the agent seem to have unacceptable risks of infection. For instance, concentrations as small as 11 virions/1000m³ still pose an infection risk of the order of 1×10^{-4} (Table 1). The final assessment in setting the critical risk level of infection and critical air concentrations for every particular BW agent should belong to all interested stakeholders, such as safety and occupational health professionals, medical doctors, emergency response authorities, regulatory agencies, public organizations, and the general public via regulatory review practices.

The results presented in Table 1 show that even very small airborne concentrations of BW agents, and especially highly infectious agents such as smallpox, are still able to provide significant risks of infection for adult humans. It is conceivable that such small airborne concentrations of BW agents might originate from a terrorist act when very small amounts of a BW agent are used, especially if the attack is conducted indoors. Tiny concentrations of a BW agent may also arise from the reaerosolation of BW agent remnants left after incomplete decontamination of the sites of terrorist attacks. The likelihood

of such reaerosolation, especially for spore-forming agents, was suggested in the notorious case of *B. anthracis* accidental release from the warfare facility in Sverdlovsk in 1979 (Alibek, 1999) and demonstrated recently during the decontamination of affected indoor areas after the anthrax attacks of 2001 in the United States (Altman, 2001).

It is important to know whether these small concentrations of BW agents can be detected by real-time biosensors. This question was among the original goals of our earlier model study (Sabelnikov et al., 2005). The bio-safety implications are discussed below.

Probability of Detection of BW Agents with Real-time Biosensors

In order to simultaneously estimate the probability of infection and the probability of detection of BW agents, the time of sampling (by a sensor) and the time of inhalation of infectious agent by an individual were set as equal. That allowed us to exclude from all calculations, if needed, time and concentration factors. The model yielded quantitative results upon the input of several incoming parameters such as an infectious dose of a microbe, parameters of a model sensor, etc. A model biosensor was defined as a single device that included an aerosol sampler and a device for identification by any known or conceived method. A network of biosensors was defined as a set of several single biosensors that operate in a similar way and deal with the same amount of agent. The three model biosensors used for the simulation employ different and widely used techniques (for a recent review, see Graham & Sabelnikov, 2004) to include polymerase chain reaction (PCR), antibody/antigen binding, and mass spectroscopy (MS). Neither the particular deployment of sensors within the network nor the spatial and

temporal distribution of agent aerosols due to wind, ventilation, humidity, temperature, etc. was considered by the model.

According to one of the assumptions of the model, the probability of agent identification in the individual sample was set equal to the probability of finding the agent in that sample in quantities not less than the threshold value, I , called the *sensitivity* of the biosensor. Further, it was assumed that the number of organisms in the sample volume V_s follows a Poisson distribution with a parameter, λ , equal to the mean concentration (# per volume) of viable and nonviable organisms in the sample volume. So, the probability of identification, P_{is} , of the agent in one sample with the volume V_s was expressed as:

$$P_{is} = 1 - \sum_{k=0,1-I} F(k), \quad (5)$$

where $F(k) = (\lambda V_s)^k e^{-\lambda V_s} / k!$ is the probability of finding exactly k organisms in the sample volume V_s , $\lambda = K_c D_i W_i / (W_h V_c)$ —mean concentration of organisms, both viable and nonviable in collective, V_c , and individual, V_s , samples; a sampler intakes the air with a flow rate, W_s , and concentrates aerosolized particles with an efficiency, K_c , into a liquid collective sample of volume V_c .

Since a sensor may detect BW agents in several parallel samples, n , the probability of agent identification with one device, P_{id} , is equal to the probability of identification at least in one of n and was expressed as:

$$P_{id} = 1 - (1 - P_{is})^n \quad (6)$$

By analogy, the probability of agent identification with the net of m similar devices, P_{im} , was expressed as:

$$P_{im} = 1 - (1 - P_{id})^m \quad (7).$$

To simultaneously estimate the probability of identification and infection for various BW agents, several hundred simulations were performed for all three types of model sensors (see above) with variable parameters that encompassed metric features used in modern, commercially available biosensors, in laboratory devices, and only theoretically achievable. It turned out that an overwhelming amount of simulation data yielded extremely low values of probability of detection for doses less than 500 microbes, so only the simulation results obtained with the best existing or conceivable metric characteristics for a particular model sensor were presented in the paper (Sabelnikov et al., 2005). Evidently, those metric parameters provided the most beneficial conditions for detection, rarely achievable in real life with equipment currently used in the field or in laboratories. However, as was shown by the results of the simulations, none of the model sensors analyzed could identify the quantities of agents corresponding to inhalation doses equal to or less than $D_i = 5$ microbes.

Using the model we can compare how efficient the best model sensors are in detecting the infection risks presented in Table 1. Listed are some metrics of the model sensors that were used in the simulations. A model, PCR-based sensor with the *best combined* metrics

of some currently available commercial devices (Idaho Tech. Inc., 2004; Smiths Detection, 2004; BDS/Invitrogen, 2004; Cepheid, 2004; Sceptor Industries, 2004; etc.) would be able to analyze 16 identical samples of an agent ($n=16$) of volume, $V_s = 12.5 \mu\text{l/sample}$, with the sensitivity, I , of 15 organisms. It would be attached to a sampler with $W_s = 1000 \text{ l/min}$ and $K_c = 0.8$. An antibody/antigen-based model sensor (such as that used by, for example, Laricchia-Robbio & Revoltella [2004], Naimushin et al. [2002], and Uttenthaler et al. [2001]) would combine the best available metrics: $W_s=1000$; $K_c=0.8$; $V_c=10$; $V_s=1$; $n=4$, and $I=25$. The operational concept of MS-based model biosensor (such as that used by Doroshenko et al. [2002], Madonna et al. [2003], and Warscheid & Fenselau [2003]) employs the following metrics: Aliquots of 0.001 ml (V_s) would be withdrawn from 10 ml of concentrated collected sample (V_c) and applied onto a target plate. They would be evaporated, ionized with a laser beam, and analyzed. Accordingly, n is set as 1, and I as 1. All other parameter values are the same as for the other sensors (see above).

Detection of BW Agents with Real-time Model Biosensors

Table 2 shows the simulation results on detection of different doses of a BW agent with ID50 index of 1000 (*Bacillus anthracis*, etc) by the model PCR-, Antibody/antigen-, and MS-based sensors with the metrics described above.

Amazingly, both the model PCR-based and MS-based sensors failed to detect microbes of *B. anthracis*-level of infectivity ($ID_{50}=1000$) in doses 100 times higher than those providing suggested “safe”/accepted risk limits (0.0001). In fact, that dose could not be detected by any model sensor tested (Sabelnikov et al., 2005). A similar or even worse situation with regard to detection is observed with the more infectious agents ($ID_{50}=100$), such as Marburg virus, *Y. pestis*, etc. (Table 3), or smallpox virus (Table 4). In fact, those small, undetected doses can still provide rather high risks of infection even for the high level ID_{50} organisms, such as *B. anthracis*. In the case of aerosols containing highly viable microbes (with $D_v > 0.1$) of intermediate and high levels of infectivity/virulence (intermediate and low ID_{50} level organisms) and especially of those with high contagious potential, such as smallpox, lack of detection would have catastrophic epidemiological consequences.

As predicted by the model for PCR-, antibody-, and MS-based sensors, the reliable identification of small BW agent doses equal to or less than single microbes could be theoretically achieved either by increasing the identifier sensitivity or enhancing concentration of the agent in the sample. However, in reality it seems unlikely because the increase in sensitivity will undoubtedly result in raising

Table 2

Detection of different doses of a BW agent with ID₅₀ index of 1000 by the model PCR-, Antibody/antigen-, and MS-based sensors with the best metric characteristics.

Risk of Infection	Dose of inhaled BW agent (ID ₅₀ = 1000)	Probability of detection, P _{id} of BW agents by:		
		PCR-based model biosensor	Antibody/antigen-based sensor	MS-based model biosensor
0.000001	0.002886	0	0	2.1E-05
0.00001	0.02886	0	0	0.00021
0.0001	0.288615	0	1.39E-13	0.002097
0.001	2.887447	0	0.615705	0.020781
0.01	29.0053	0	1	0.190184
0.1	304.0708	1	1	0.890455

Table 3

Detection of different doses of a BW agent with ID₅₀ index of 100 by the model PCR-, Antibody/antigen-, and MS-based sensors with the best metric characteristics.

Risk of Infection	Dose of inhaled BW agent (ID ₅₀ = 100)	Probability of detection, P _{id} of BW agent by:		
		PCR-based model biosensor	Antibody/antigen-based sensor	MS-based model biosensor
0.000001	0.001443	0.00	0.00	0.00
0.00001	0.01443	0.00	0.00	0.00
0.0001	0.144307	0.00	0.00	0.00
0.001	1.443723	0.00	0.00	0.01
0.01	14.50265	0.00	1.00	0.10
0.1	152.0354	1.00	1.00	0.67

false positives, especially for highly “loaded” environmental samples. It is likely that the same factors will affect the increase of the agent concentration in the sample.

Overall, the results of simulations performed with variable metric parameters of all three types of model sensors (Sabelnikov et al., 2005) indicated that small doses of aerosolized agents (less than 5 microbes) that are still able to provide significant risks of infection especially for highly infectious agents (e.g., for smallpox those risk are 1, 8, and 37 infected out of 1,000 exposed, depending on the viability of the virus preparation) would remain undetected by the present, most advanced, or even future, significantly refined real-time biosensors. Meanwhile, they are expected to help in selecting the proper emergency

response management (ERM) should a bioterrorist attack occur. The solution might be in using multicomponent networks of sensors (with more than 4 similar devices). However, it may be costly, at least at the present time.

Extending the Approach and the Results Beyond BW Agents

In contrast to the military strategy of BW agents’ application, the main desirable effect of a bioterrorist attack is to cause massive panic and to paralyze economic and social activities. To achieve this it is not absolutely necessary to use deadly BW agents. Perhaps, it might be suffi-

cient to cause the outbreak of diarrhea or some other readily detectable disturbance among a good portion of civil population. So, other infectious agents such as those, for instance, that cause food poisoning (*Bacillus cereus*, *Salmonella* strains, etc.) might also be employed by bioterrorists. Interestingly, as mentioned in the Russian guidelines on countermeasures against biological terrorism (CABT, 2003), "aerosol distribution of microbes might be considered universal, because even microbes with non-inhalation routes of infection may be distributed in this way." Nothing is known, however, about their possible inhalation infectious doses, though intuitively we may assume that their "acceptable" safety limits should be orders of magnitude higher than those of BW agents. If they are indeed retaining their infectious potential as aerosols, the approach discussed here may be used also for determination of their AEL.

Conclusion

Two additional points need to be made. First, it should be emphasized that real-time identification, no matter how rapid and sensitive, cannot prevent infection. In the best cases it can mitigate the consequences and help with a proper emergency management. Additionally, most of the time it should be expected that real-time identification, if it indeed takes place, will estimate at best only the lowest possible doses inhaled by the exposed individual, since this individual keeps on breathing during the identification time. Therefore, the fastest identification will be extremely beneficial. In this respect, MS-based sensors capable of identification within minutes might be the most promising.

Second, AELs undetected by the model real-time biosensors closely correspond to, or even extend, the high and very high levels of Index of Microbial Air contamination, IMA, introduced by Pasquarella et al. (2000) for environments at risk. They also correspond to, or extend, the highest classes of air contamination defined by several authoritative agencies such as NASA (1967), U.S. Federal Standards 209E (1992), European Union Good Manufacturing Practices (1997), and International Organization for Standardization, ISO/DIS (1998). The environments at high risk usually include aseptic and operating rooms at hospitals, microelectronics and pharmaceutical plants, and others. High risks of infection by small doses of BW agents, especially of those with high epidemiological potential, add a new "member" to the class of environments at very high risk.

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ERRATUM: Table 4 in *Applied Biosafety* (Volume 11, Number 2, 2006)

The article "Some Bioterrorism Issues of Quantitative Biosafety" by Alex Sabelnikov, Vladimir Zhukov, and Ruth Kempf (Center for Security Studies and Research, East Carolina University, Greenville, North Carolina) that appeared on pages 67-73 of *Applied Biosafety* (Volume 11, Number 2, 2006) should have had a fourth table included. Table 4 was inadvertently deleted from the published manuscript, and should be included in this article. We apologize for any inconvenience this may have caused.

Table 4

Detection of different doses of a BW agent with ID₅₀ index of 5 by the model PCR-, Antibody/antigen-, and MS-based sensors with the best metric characteristics.

Risk of Infection	Dose of inhaled BW agent (ID ₅₀ = 5)	Probability of detection, P _{id} of BW agents by:		
		PCR-based model biosensor	Antibody/antigen-based sensor	MS-based model biosensor
0.000001	7.22E-05	0.00	0.00	0.00
0.00001	0.000722	0.00	0.00	0.00
0.0001	0.007215	0.00	0.00	0.00
0.001	0.072186	0.00	0.00	0.00
0.01	0.725132	0.00	0.00	0.01
0.1	7.601769	0.00	1.00	0.05