THE CENTRAL ROLE OF NANOTECHNOLOGY IN TARGETED DELIVERY OF BIOLOGICAL AGENTS: IMPLICATIONS FOR BIOSECURITY

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EXECUTIVE SUMMARY

The concern that advances in science and technology might lead to the creation of novel biological warfare agents is compounded by the fact that new and improved ways of delivering such agents are already at hand and are likely to be developed further at a rapid pace. An indication of this rapid development can be found in the reports of experimental and clinical applications of advances in these areas, which are available in the open scientific literature.

The scientific research discussed in this paper, is focused on therapeutic applications, where the methods of delivery do not exactly mimic dissemination of biological agents in a weapons deployment scenario. However, an assessment of the feasibility of using these advances for targeted delivery of biological agents for terrorism or biological warfare can still provide insights into how advances in science and technology continue to make the development of novel weapon systems increasingly feasible. The two fields of work that have progressed most significantly and appear to be most relevant for both drug delivery and biological warfare are aerosol and vector-directed technologies, which are the focus of this study.

Significantly, great strides are being made as a result of investments in drug development and delivery as part of cancer treatment, as well as gene and immuno-therapy. Nanotechnology has played a fundamental role in many developments in these areas. For example, aerosol delivery of bioactive compounds has been greatly improved through the development of defined nanoparticles and new methods for making such substances absorbable through nasal and respiratory routes as well as the blood-brain barrier. In addition, improvements in targeting and gene transfer efficacy of viral and non-viral vectors, along with progress in protective packaging of these agents in nanoparticles that can also direct them to specific tissues, have made them much more feasible delivery systems. Clinical trials with humans have shown that several of the vectors already developed and armed to deliver specific payloads in cancer and gene therapy have proven to be successful in principle and in some cases in effect. Furthermore, the delivery of viral and non-viral vectors over the aerosol route is increasingly being explored so that this is rapidly becoming a feasible option. For example, in some 20 clinical trials that have been carried out, use of gene-transfer agents including adenoviruses and adeno-associated viruses as vectors have demonstrated proof of principle for gene transfer through the airway.

The most sophisticated of these advances in science and technology are certainly not easy to put into practice, but require extensive expertise (both scientific and tacit knowledge), well-equipped laboratories and substantial funds. Thus, state-supported actors are more likely than terrorists to have such means, although the possibility of hostile use by terrorists can by no means ruled out. While the potential for misuse is certainly given, it is difficult to assess the likelihood or consequences of misuse. Nevertheless, there still remains a need for pro-active approaches to this issue area, to reduce the risk of misperceptions between states, as well as the risk of misuse by other actors.

An appropriate response should involve the development and dissemination of criteria to help identify research of potential concern, communicate concerns to relevant communities, and where necessary to amend or expand upon existing governance systems.

To this end, this paper makes two key recommendations:

1. Education of the scientific community about relevant aspects of dual-use biosecurity

   This is the basis of a dual-use biosecurity oversight policy. Such education involves not only making scientists aware of the illegality of biological weapons, but also developing a clearer understanding of their legal, professional and ethical responsibilities under the aegis of the Biological and Toxin Weapons Convention. Only when those carrying out the work are fully aware of dual-use biosecurity issues will measures such as codes of conduct and risk management procedures be effective. In order to make sure that scientists are fully aware of security issues, it is essential to establish dual-use biosecurity education in the life sciences and related fields at the university level.

2. The development of risk management guidelines to direct the responsible scientist in taking the necessary steps to achieve mitigation of risks that are of particular dual-use concern

   This would be best pursued on the multilateral level within the context of the Biological and Toxin Weapons Convention (BTWC). Such work would ideally be carried out by an open-ended experts working group, which would be tasked with developing ‘best practice’ criteria, for consideration by the BTWC body as a whole. The ultimate aim of such work should be to help foster the development of systems of assessment and oversight at the national level in accordance with national implementation and compliance assurance agendas of the BTWC. Such systems would help foster the emergence of appropriate, transparent, legitimate and legally grounded systems of governance in this area.

1. INTRODUCTION

The successful application of bioactive materials for either therapeutic or hostile purposes depends to a great extent on how effectively they are delivered to their target. Effective delivery of a biological agent is also said to be the most difficult and crucial step in producing a biological weapon, and the one in which a terrorist would be most likely to fail.[1]

The classical biological agents are infectious microorganisms, as well as toxins produced by microorganisms and other living beings. However, recent advances in areas of the life sciences such as genomics and systems biology have revealed new potential targets for biological attack and thus extended the biological agent spectrum to include bioregulators. Bioregualors are small biochemical molecules that are produced by the body itself and that regulate the functions of vital physiological systems; these include such molecules as hormones, neurotransmitters and cytokines.[2] As long as these substances are produced in normal, physiological amounts, vital systems function normally. If bioregulators are present in abnormal amounts, the effects on the functions of vital systems can be detrimental.

Advances in science and technology relevant to biosecurity, including those relevant to the development of new and improved methods of delivering biological agents, were dealt with extensively in the 2006 Lemon-Relman report of the US National Academies.[3]. The report identified several potential methods of delivering biological agents, however this paper focuses primarily on aerosol and vector-directed technologies, which are areas that have progressed significantly in recent years and appear to be most relevant.

The aerosol route has always been the classical way of delivering biological agents over wide areas.[4] Indeed, many infectious diseases begin at the mucosal surfaces of the body and most biological agents can be effectively delivered by this route, as long as the substances can be absorbed and taken up through the tissues. With the methods available today, biological agents can be constructed so that they can be directed to specific tissues and cells in the body. Interests in

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[1] Kuhn, J. H. 2007. Defining the terrorist risk. *Bulletin of the Atomic Scientist* Roundtable "Is the availability of genetic information dangerous?" Available at: http://thebulletin.org/availability-genetic-information-dangerous/defining-terrorist-risk. [accessed 31.10.2014]

[2] Kelle, A., Nixdorff, K. and Dando, M. 2006. *Controlling biochemical weapons. Adapting multilateral arms control for the 21st century*. Basingstoke: New York.

[3] National Research Council. 2006. Globalization, biosecurity, and the future of the life sciences. National Academies Press: Washington, D. C. Available at http://www.nas.edu/. [accessed 31.10.2014]

[4] s. GlobalSecurity. Biological warfare agent delivery. Available at http://www.globalsecurity.org/wmd/intro/bio_delivery.htm [accessed 31.10.2014]; U.S. Department of Defense. 1998. The militarily critical technologies list. Part II: Weapons of mass destruction technologies. Available at http://www.fas.org/irp/threat/mctl98-2/mctl98-2.pdf. [accessed 31.10.2014]

delivering therapeutic drugs to fight cancer and other serious illnesses have led to the development of novel viral and non-viral vectors to act as ferries for bioactive substances.[5]

In the past ten years great strides have been made in the development of new and improved targeted delivery methods. In all this work, nanotechnology has played a central role, and improving delivery of biological agents is the most relevant aspect of nanotechnology for our discussion. Nanotechnology encompasses a very diverse range of technological approaches involving the development of new materials on the nanoscale.[6] Most relevant for consideration of targeted delivery technologies are nanoparticles ranging in size between 1 nanometer (a billionth of a meter, or around 10 times the size of an atom) and 100 nanometers (the size of large molecules).[7] The absorption (uptake) of bioactive substances can be greatly facilitated by packaging them into nanoparticles, as smaller particles can pass through tissues and into cells of the body more easily than larger ones. Furthermore, nanoparticles can be engineered to contain substances that improve absorption, control their release in the body, or direct them to specific cells or tissues for uptake. This will be described in more detail in connection with different delivery techniques.

All of this work holds the promise of improving health and health security in general, and it is essential that advances in these areas continue. At the same time however, it cannot be ignored that some of this work points to lines of research which can be misused for hostile purposes and thus carries a biosecurity risk. Although work on targeted delivery of bioactive materials published in the open, scientific literature is focused on therapeutic applications where the methods of delivery do not exactly mimic dissemination of biological agents in a weapons deployment scenario, an assessment of the feasibility of using these advances for targeted delivery of biological agents for terrorism or biological warfare can still be valuable in reflecting upon how science and technology could facilitate the development of new biological weapon systems. In particular, it reveals how misperceptions might emerge among states in relation to certain areas of research, as well as the potential for some lines of research to be misused by non-state actors in the development of weapons.

2. Aerosol Delivery

Aerosols are particles in the form of a liquid or a powder that are suspended in air and can be inhaled. Many infectious microorganisms can enter the body through the mucous membranes lining the nasal and respiratory tracts as well as the intestinal tract. The size of the droplets determine to a

[5] Nixdorff, K. 2010. Advances in targeted delivery and the future of bioweapons. *Bulletin of the Atomic Scientists*, 66 (1), 24–33.
[6] "Nano" describes a dimension dealing with a billionth of some unit of measurement.
[7] Suri, S.S., Fenniri, H. and Singh, B. 2007. Nano-technology-based drug delivery systems. *Journal of Occupational Medicine and Technology* 2: 16-21.

great extent where they will be deposited in the airway after inhalation. Particles up to 5 micrometers in diameter can reach deep lung areas (alveoli or air sacs); larger particles will be deposited in more anterior parts of the respiratory tract.[8]

A pertinent example[9] of the efficacy of aerosol dissemination of biological agents involved the use of the bacterium *Bacillus thuringiensis* (which produces an insect toxin) to control insurgent populations of the European gypsy moth, which was posing a threat to the lumber industry in and around the Victoria region of British Columbia. This study demonstrates just how effective the aerosol route can be for delivering microorganisms successfully over a large area, even without using sophisticated technology. The report describes the results of aircraft spray application of the biological insecticide Foray 48B (a solution of *B. thuringiensis* endospores) over approximately 30,000 acres including residential and rural areas in the Victoria, B.C. region. The undertaking resulted in greater than 99 % mortality of the gypsy moth population in that area. A surprising finding was that enough small (2-7 micrometers) droplets were formed that could penetrate houses and contaminate the nasal passages of residents inside their homes, even though the equipment used was designed to generate droplets of 110-130 micrometers in diameter. While exposure to *B. thuringiensis* should have no detrimental effects on humans, this tells us something about how effective a similar type of operation using highly infectious microorganisms could be.

2.1. Aerosol Delivery of Vaccines

A further use of aerosol delivery of biological agents concerns vaccination with microorganisms via the aerosol route, which has been practiced for quite some time and is known to be effective. Vaccination via aerosol delivery has several advantages over the traditional methods of subcutaneous (beneath the skin) or intramuscular injection using needles and syringes. The drawbacks to these conventional methods include limited acceptance to the use of needles, transmission of diseases through needle pricks and the need for trained healthcare workers.[10] Field trials in Mexico established the effectiveness of mass immunization of children with the measles vaccine virus via the aerosol route.[11] The children were exposed to the aerosol output of a classic jet nebulizer driven by an air compressor for a 30-second period via a paper conical mask held over the mouth and nose. Subsequent tests showed that this type of vaccination compared favourably with that of conventional methods of administration and used a third of the dose normally required. In

[8] Scheuch, G., Kohlhaeufl, M.J., Brand, P. and Siekmeier, R. 2006. Clinical perspectives on pulmonary systemic and macromolecular delivery. *Advanced Drug Delivery Reviews* 58: 996-1008.
[9] Levin, D.B. and Valadares de Amorim, G. 2003. Potential for aerosol dissemination of biological weapons: lessons from biological control of insects. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice and Science* 1: 37-42.
[10] Tonnis, W.F., Kersten, G.F., Frijlink, H.W., Hinrichs, W.L.J., de Boer, A.H. and Amorij, J.-P. 2012. Pulmonary vaccine delivery: A realistic approach? *Journal of Aerosol Medicine and Pulmonary Delivery* 25(5): 249-260.
[11] Cutts, F.T., Clements, C.J. and Bennett, J.V.. 1997. Alternative routes of measles immunization: a review. *Biologicals* 25: 323-328.

the former Soviet Union, thousands of people were successfully vaccinated with aerosols of live, attenuated strains of anthrax, plague, tularemia and smallpox agents using tent-exposure systems.[12] Although the aerosols were produced in an enclosed environment (direct via a mask or in a tent), these practical experiments demonstrated that vaccination against a wide array of biological weapons-relevant microorganisms could be achieved by inhaling aerosol clouds containing the agents.

Aerosol delivery of vaccines has the added advantage that direct delivery to the mucous membranes of nasal and lung surfaces is achieved, where many infections begin. Among the various mucosal sites, nasal delivery is especially attractive for vaccine administration because this is a site of relatively high tissue permeability, low activity of destructive enzymes and the presence of a considerable number of immune response cells.[13] In order to avoid the risk of reversion to the active state of attenuated microorganisms used as vaccines as well as to reduce possible side effects that some whole cell vaccines cause, the use of sub-unit vaccines consisting of only the immunogenic protein components of microorganisms instead of whole cells has gained interest in recent years. Particularly, packaging these components for aerosol delivery into nanoparticles offers improved absorption and uptake. In addition, a more effective antigen recognition and response by immune cells in the mucosa would be achieved.[14] At the same time, these techniques could be misused to deliver biological agents that have the potential to exert detrimental effects on the immune system. This is elaborated in the following section 2.2.

2.2. Aerosol Delivery of Biochemical Agents and Therapeutics

Systems biology is rapidly proving knowledge about the regulation of vital physiological processes through bioregulators such as hormones, peptides, neurotransmitters and cytokines, along with the targets with which they interact. This work is being carried out with the aim of modulating disease processes in the direction of better health. There is, however, increasing concern that such knowledge can be used to pinpoint ways of disrupting the normal functions of vital physiological processes such as respiration, heartbeat and immunity, or affecting mood and cognizance negatively. The potential of aerosols for delivery of drugs is a current area of particular interest, and here nanotechnology plays a central role in improving aerosol delivery of bioactive substances:

[12] Laube, B. 2005. The expanding role of aerosols in systemic drug delivery. *Respiratory Care* 50: 1161-1176; Roth, Y., J.S. Chapnik and P. Cole. 2003. Feasibility of aerosol vaccination in humans. *Annals of Otology, Rhinology and Laryngology* 112: 264-270.
[13] Csaba, N., Garcia-Fuentes, M. and Alonso, M.J. 2009. Nanoparticles for nasal vaccination. *Advanced Drug Delivery Reviews* 61: 140-157.
[14] Ibid.

> *"A major challenge in nanomedicine is to engineer nanostructures that can efficiently encapsulate drugs at high concentration, cross the cell membrane, and controllably release the cargo at the target site over a prescribed period of time".*[15]

Delivery of therapeutics via the aerosol route is attractive for a number of reasons. The surface area of the lung is between 80 and 140 square meters. Also, the alveolar (air sac) epithelium (cell lining) in most pulmonary regions is only about 0.1-0.2 micrometers thick, and the distance between epithelial surface and the blood is much less than it is in the bronchial system, which should facilitate drug uptake.[16] There are, however, a number of absorption barriers in the human lung including the mucus layer, the alveolar lining fluid layer, and competing uptake pathways such as particle engulfment by macrophages (white blood cells that can take up and destroy foreign particles).

While lipophilic (having an affinity for fat) substances are readily absorbable over the nasal mucosa, more polar (hydrophilic, having an affinity for water) compounds such as peptides and proteins or DNA are taken up relatively poorly, so that methods aimed at improving their permeability properties have been developed. In this context, packaging drugs into nanoparticles that are coated with cationic (positively charged) substances such as chitosan (a polysaccharide derived from shellfish chitin), polymeric nanocarriers such as poly lactic acid or poly lactic-co-glycolic acid, or a combination of these substances, greatly improves uptake. In addition, encapsulation of the particles with poly ethylene glycol or polyoxyethelene derivatives can increase their stability. Further improvements have been achieved by cross-linking chitosan with tripolyphosphate in order to increase the release time of encapsulated peptides and proteins or enhance gene expression of DNA-based vaccines[17], and competing uptake of the particles by phagocytosis can be reduced by packaging substances into porous particles.[18] Shoyele and Slowey[19] have offered a list of some 15 proteins/peptides that could feasibly be delivered via the lungs in order to treat various illnesses. These include the interferons, several interleukins, erythropoietin, calcitonin, insulin, amylin and growth hormone.

As in vaccine administration (see above), the nasal route has emerged as being particularly advantageous for the delivery of drugs. This route also has the added potential of providing direct access of drugs to the brain, and many peptides and proteins (among others: Orexin-A, insulin,

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[15] Liu, J., Stace-Naughton, A., Jiang, X. and Brinker, C.J. 2009. Porous nanoparticle supported lipid bilayers (protocells) as delivery vehicles. *Journal of the American Chemical Society* 131:1354-1355.
[16] Scheuch et al., 2006, op. cit.
[17] Csaba, Garcia-Fuentes and Alonso 2009, op. cit.
[18] Scheuch et al., 2006, op. cit.
[19] Shoyele, S.A. and Slowey, A. 2006. Prospects of formulating proteins/peptides as aerosols for pulmonary drug delivery. *International Journal of Pharmaceutics* 314: 1-8.

leptin, erythropoietin) can be detected in the central nervous system after intranasal delivery. [20] Orexins (or hypocretins) are neuropeptides that stabilise the waking condition, and it has been suggested that

> *"Clearly, if it were possible to interfere with the function of the orexins then there would be available to those with malign intent an impressive means of incapacitation".*[21]

Uptake after nasal delivery is achieved by various methods: absorption into olfactory blood vessels and entry into the general circulation; absorption into olfactory lymphatic vessels draining to the deep cervical lymph nodes of the neck; and extracellular diffusion or convection in compartments associated with olfactory nerve bundles and entry into the cranial compartment.[22]

Again, packaging bioactive materials into nanoparticles that have been constructed with absorption-enhancing substances such as chitosan can enhance uptake via the nasal route. Such constructs may also be used to enable bioagents present in the circulation to cross the blood-brain barrier. Normally, the brain is protected from the potentially harmful effects of most substances or cells in the circulation by the extremely tight junctions between the endothelial cells lining blood capillaries. This barrier has been called a double-edged sword:

> *On the one hand, this cellular interface helps to maintain a constant, optimal environment for neuronal function through a combination of barriers and selective transport systems that regulate the passage of wanted and unwanted molecules. But on the other hand, it presents a formidable challenge to medicine because it stops most drugs from passing from the bloodstream to the brain.*[23]

The mechanism of absorption enhancement by chitosan and other polycation (positively charged polymers) substances appears to be a combination of adhesion (sticking to tissues) and a transient opening of the junctions in epithelial cell layers lining the mucosal surface of the nasal compartment and respiratory tract.[24] This is also relevant for delivery of drugs across the blood-brain barrier, as the cancer drug doxorubicin was able to cross the intact blood-brain barrier when attached to nanoparticles coated with polysorbate, another absorption enhancer.[25]

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[20] Lochhead, J.J. and Thorne, R.C. 2012. Intranasal delivery of biologics to the central nervous system. *Advanced Drug Delivery Reviews* 64_614-628.

[21] Dando, M. 2011. Advances in neuroscience and the Biologixcal and Toxin Weapons Convention. Biotechnology Research International 2011: 9 pages, http://www.hindawi.com/journals/btri/2011/973851/. [accessed 31.10.2014]

[22] Lochhead and Thorne, 2012, op. cit.

[23] Betsholtz, C. 2014. Double function at the blood-brain barrier. *Nature* 509: 432-433.

[24] Sadeghi, A.M.M., Dorkoosh, F.A., Avadi, M.R., Weinhold, M., Bayat, A., Delie, F., Gurny, R., Larijani, B., Rafiee-Tehrani, M. and Junginger, H.E. 2008. Permeation enhancer effect of chitosan and chitosan derivatives: Comparison of formulations as soluble polymers and nanoparticulate systems on insulin absorption in Caco-2 cells. *European Journal of Pharmaceutics and Biopharmaceutics* 70: 270–278.

[25] Suri, Fenniri, and Singh, 2007, op. cit.

Nanotechnology can be applied in aerosol delivery of drugs to fight pulmonary infectious diseases such as pneumonia, tuberculosis or fungal infections. For example, nanoscale delivery systems can enhance the absorption of drugs and uptake through the epithelial layer of the mucosa, target the drugs to specific cells/tissues/organs and release them in a controlled manner in response to a specific stimulus.[26] The drugs can be protected from degradation and their release in the body controlled by coating the nanoparticles with substances such as poly(lactide-co-glycolic acid) (PLGA) or polyethylene glycol (PEG). Indeed, changing the degree of surface PEGlycolation, of either bare drug or encapsulating particles can influence the rate of particle degradation and can be manipulated to favor longer release; similar effects can be achieved with liposomes (vesicles of fatty molecules that can enclose bioactive substances).[27]

Several examples of clinical applications and experimental studies have shown that aerosol delivery of drugs and other bioactive biochemicals is feasible not only in principle but also in effect. Inhaled insulin delivery has been explored for over a decade, and some formulations for inhalation administration have been marketed and been found effective in the past but have since fallen from the market. Recently, a powdered formulation of the drug, Afrezza ("Technosphere insulin"), has been approved by the U.S. Food and Drug Administration and will be developed and manufactured by MannKind in partnership with Sanofi for the treatment of either Type I or Type II diabetes.[28]

Another example of successful aerosol delivery of a drug concerns the neuropeptide oxytocin, which was reported to increase trusting behaviour in humans given a single dose by nasal spray (Kosfeld et al. 2005).[29] It has even been marketed by Vero Labs as a liquid spray to be used like perfume, "formulated to emphasize its key role in human bonding to improve confidence, enhance relationships and strengthen bonds". [30] A growing number of studies have investigated the effects of oxytocin after nasal administration on human behaviour, cognition and brain activation, particularly in connection with specific disorders such as anxiety autism and schizophrenia.[31] Churchland and Winkielman have pointed out the difficulties in assigning a specific role to oxytocin in the complex realm of social cognition and that "*it may turn out that the best clinical use of intranasal OXT is primarily as an effective (and perhaps non-addictive) anti-anxiety [drug]*".[32]

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[26] Andrade, F., Rafael, D., Videira, M., Ferreira, D., Sosnik, A. and Sarmento, B. 2013. Nanotechnology and pulmonary delivery to overcome resistance in infectious diseases. *Advanced Drug Delivery Reviews* 65: 1816-1827.

[27] Rubin, B.K. and Williams, R.W. 2014. Emerging aerosol drug delivery strategies: from bench to clinic. *Advanced Drug Delivery Reviews* 75: 141-148.

[28] Kling, J. 2014. Sanofi to propel inhalable insulin Afrezza into market´. *Nature Biotechnology* 32(9): 581-582.

[29] Kosfeld, M., M. Heinrichs, P.J. Zak, U. Fischbacher and E. Fehr. 2005. Oxytocin increases trust in humans. *Nature* 435: 673-676.

[30] Vero Labs. http://www.verolabs.com. [accessed 31.10.2014]

[31] Weissman, O., Zagoory-Sharon, O. and Feldman, R. 2012. Intranasal administration of oxytocin is reflected in human saliva. *Psychoneuroendocrinology* 37: 1582-1586.

[32] Churchland, P.S. and Winkielman, P. 2012. Modulating social behavior with oxytocin: How does it work? What does

Other clinical studies on the aerosol administration of drugs have reported further progress in the using this route for the treatment of *Pseudomonas* infections in cystic fibrosis patients.[33] There has also been a great deal of interest in using the gene silencing RNA interference (RNAi) system therapeutically. This can be a potent, effective and practical method of interfering with or silencing the expression of unwanted gene activities. Effectors of this method of gene silencing are, among others, short, (21-26 nucleotides) interfering RNA molecules (siRNA). These are recognized by a so-called silencing complex (RISC) which mediates the degradation of gene transcripts (specific messenger RNA expressed by that gene). This essentially ablates, turns off or "knocks-down" the activity of that specific gene since no product can be synthesised from that degraded gene transcript. [34] A multitude of new formulations are being investigated that package siRNA into nanoparticles suitable for uptake by cells, and some studies have used the nasal route for delivery.[35] Recently, a double-blind, placebo-controlled clinical trial of a siRNA-based therapeutic directed against respiratory syncytial virus and delivered by nasal administration showed that this method had therapeutic activity. It represents the first proof-of-concept efficacy test of the therapeutic effect of RNAi in humans.[36]

The most prominent example of the feasibility of the aerosol delivery of drugs is the incident in which Russian military special forces tried to rescue hostages held at the Moscow Dubrovka Theatre Center by introducing an unidentified "gas" (supposed to have incapacitating effects) into the theatre ventilation system. Of the 800 hostages held in the theatre, 127 died and more than 650 of the survivors required hospitalization (Wax, Becker and Curry 2003). Many of the patients had classic signs of opioid (narcotic) intoxication, and the Russian Health Minister announced several days later that a derivative of the opioid fentanyl had been used.

2.3. ADVANCES IN AEROSOL DELIVERY DEVICES AND TECHNIQUES

Aerosol delivery of biological agents for experimental and therapeutic clinical applications is increasingly becoming the preferred and most relevant route, but also the least straightforward.[37] Due to the advantages of using the aerosol route for the delivery of biologically active substances (as outlined above in sections 2.1. and 2.2.) a great deal of research into the development of aerosol delivery devices for therapeutic purposes has been carried out in the last two decades. A huge

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it mean? *Hormones and Behavior* 61: 392-399.

[33] Geller, D.E., Flume, P.A., Staab, D., Fischer, R., Loutit, J.S. and Conrad, D.J. 2011. Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*. *American Journal of Respiratory and Critical Care Medicine* 183: 1510-1516.

[34] Sandy, P., Ventura, A. and Jacks, T. 2005. Mammalian RNAi: a practical guide. *BioTechniques* 39: 215-224.

[35] Merkel, O.M, Rubenstein, I. and Kissel, T. 2014. siRNA Delivery to the lung:What's new?. *Advanced Drug Delivery Reviews* 75: 112-128.

[36] Reviewed in DeVincenzo, J.P. 2012. The promise, pitfalls and progress of RNA-interference-based antiviral therapy for respiratory viruses. *Antiviral Therapy* 17: 213-225.

[37] Merkel, Rubenstein and Kissel, 2014, op. cit.

increase in activity occurred in the 1990s after the Montreal Protocol banned chloroflurocarbons as propellants. This resulted in the development of hydrofluorocarbon-driven metered dose inhalers (MDIs) as well as dry powder inhalers (DPIs) for aerosol drug delivery (see Figure 1, Figure 2). In the most recent phase, engineered porous powders of low density have been developed that can achieve increased lung deposition of 40-60 % at present, compared with 5-20 % in the past. Dry powder inhalers (DPIs) can deliver higher doses and are therefore particularly useful for one-dose delivery programmes. While MDIs can deliver doses in the range of 1 mg or less, DPIs can deliver loads of 50 mg of powder per inhalation.[38]

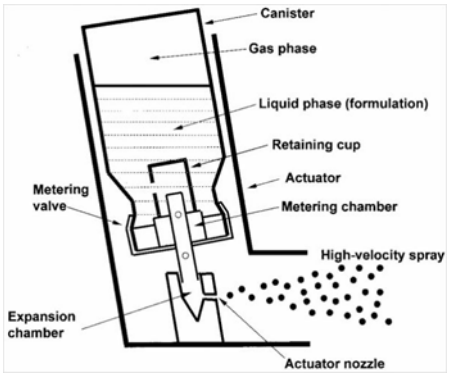

FIGURE 1 SCHEMATIC DIAGRAM OF A METERED DOSE INHALER

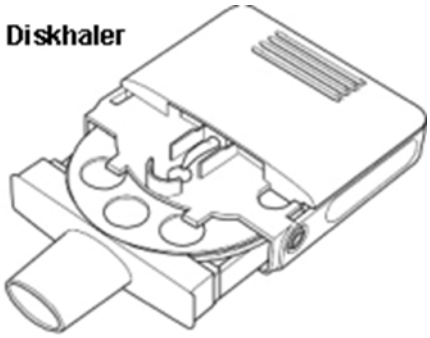

FIGURE 2 SCHEMATIC DIAGRAM OF A DRY POWDER INHALER (DISK TYPE)

Nebulizers or atomizers are also used to create aerosols (see Figure 3). These are devices that pump air or oxygen through a liquid to produce a mist, which is inhaled through a face mask. A classic jet-type nebulizer driven by an air compressor was used in the successful measles virus aerosol vaccination programme in Mexico discussed above. Newer devices use a vibrating mesh or ultrasound systems for generating aerosols of fine particle fractions of precisely controlled size with minimal shear forces.[39] They can be applied to delivering a wide variety of drug formulations including highly viscous fluids, proteins, peptides, surfactants and DNA for gene therapy purposes. Indeed, advanced aerosol technologies "*continue to expand therapeutic options*".[40]

[38] Leach, C.L. 2007. Inhalation aspects of therapeutic aerosols. *Toxicologic Pathology* 35: 23-26.

[39] Daniels, T., Mills, N. and Whitaker, P. 2013. Nebuliser systems for drug delivery in cystic fibrosis (Review) *The Cochrane Library* Issue 4, http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD007639.pub2/pdf.[accessed 31.10.2014]

[40] Waldrep, J.C. and Dhand, R. (2008) Advanced nebulizer designs employing vibrating mesh/aperture plate technologies for aerosol generation. *Current Drug Delivery*, vol. 5, pp. 114-119.

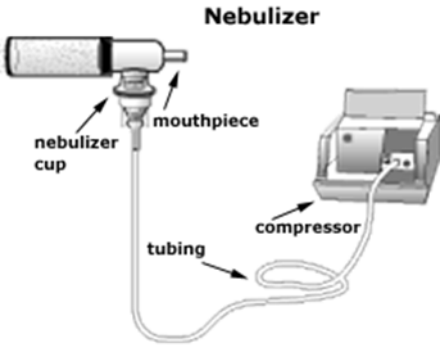

FIGURE 3 SCHEMATIC DIAGRAM OF A NEBULIZER

Currently, emerging inhalation therapies applying these delivery devices are being used for the treatment of various illnesses by delivering biologically active substances. These substances include antibiotics for treatment of respiratory bacterial infections, neuraminidase inhibitor for influenza, anti-pulmonary arterial hypertension agents, cyclosporine for transplant rejection, cytokines such as interferon beta (IFN-β) for treatment of asthmatic symptoms, chronic obstructive pulmonary disease (COPD) as well as infectious diseases and alpha 1 antitrypsin for treatment of cystic fibrosis.[41]

Experiments consisting of aerosol delivery of biological agents to animals have employed different methods depending on the animal type. In the case of mice, nose-only delivery systems (see Figure 4) are most frequently used[42], while for larger animals such as monkeys a Henderson head-only system is usually the choice.[43] In the Henderson system, a non-human primate is anesthetized and placed on a platform in a supine posture, with the head placed through an opening in a type of dental dam covering the round opening on the side of an aerosol chamber. Although these methods for clinical and experimental purposes are all designed to present the most concentrated agents directly to the subject and thus do not mimic aerosol delivery in a biological weapons type of scenario, data gained from these experiments can provide a calculated estimate of the feasibility of delivery of biological agents for terrorist or warfare purposes. For example, researchers experienced in aerosol delivery of ricin to non-primate humans using a Henderson head-only system have estimated that

> *"An aerosol cloud of micron-sized particles containing high concentrations of ricin toxin, **even in the event of significant atmospheric dilution**, could deliver a*

[41] Zhou, Q., Tang, P., Leung, S.H.Y., Chan, J.G.Y. and Chan, H.-K. 2014. Emerging inhalation aerosol devices and strategies: Where are we headed? *Advanced Drug Delivery Reviews* 75: 3–17.

[42] Mainelis, G., Seshadri, S., Garbuzenko, O.B., Han, T., Wang, Z. and Minko, T. 2013. Characterization and application of a nose-only exposure chamber for inhalation delivery of liposomal drugs and nucleic acids to mice. *Journal of Aerosol Medicine and Pulmonary Delivery* 26(6): 345-354.

[43] Roy, C.J., Song, K., Sivasubramani, Gardner, D.J. and Pincus, S.H. 2012. Animal models of ricin toxicosis. *Current Topics in Microbiology and Immunology* 357: 243–257; Nalca, A., Livingston, V.A., Garza, N.L., Zumbrun, E.E., Frick, O.M., Chapman, J.L. and Hartings, J.M. 2010. Experimental infection of cynomolgus macaques (*Macaca fascicularis*) with aerosolized monkeypox virus. *PLoS ONE* 5(9): e12880. doi:10.1371/journal.pone.0012880; Dabish, P. A., Kline, J., Lewis,C., Yeager, J. and Pitt, M.L.M. 2010. Characterization of a head-only aerosol exposure system for nonhuman primates. *Inhalation Toxicology* 22(3): 224-233.

potentially lethal dose to victims in a military battlefield or civilian terrorist scenario".[44] [emphasis added]

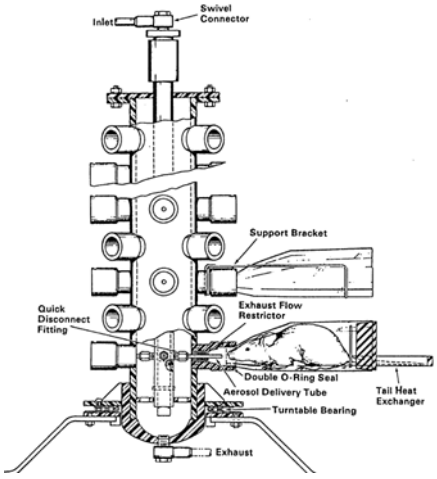

FIGURE 4 SCHEMATIC DIAGRAM OF A CANNON FLOW-PAST NOSE-ONLY AEROSOL EXPOSURE CHAMBER

3. VIRAL VECTOR TECHNOLOGY

Advances in molecular biology, immunology and tumor genetics have led to the design of novel viral vectors for legitimate use in vaccine therapy, cancer, drug and immunotherapy. In general, these viruses act as ferries or vehicles that carry and deliver foreign genes to the body. The strategy to this procedure is that infection with the modified virus would lead to the expression of the foreign genes in the cells of affected tissues. This would result in the synthesis of the selected active substance (the gene product), which can then exert its effect.

In the biomedical and clinical application context these are perfectly legitimate undertakings that can counter disease and promote health, but the dual-use implication here is that these same technologies could be used to arm viruses with a destructive or even deadly payload. The use of viral vectors may seem to be fairly straight-forward in principle, but there are several obstacles to success which have to be overcome and have been the subject of intensive research and trial, including directing the viruses to the right cells, improving gene transfer efficiency and gene expression. Nevertheless such progress has been made that it has prompted the prediction that

> *"...in spite of naysayers lacking vision,...there is now firm hope that gene therapy will soon do for medicine what aeroplanes did for transportation".*[45]

Clinical trials with humans have shown that several of the vectors already developed and armed to deliver specific payloads in cancer and gene therapy have proven to be successful in principle and

[44] Roy, Song, Sivasubramani, Gardner and Pincus, 2012, op.cit.
[45] Leboulch, P. 2013. Primed for take-off. *Nature* 500:280-282.

in some cases in effect. In clinical trials with metastatic melanoma patients, an engineered vaccinia virus armed to deliver an immunostimulatory substance to boost anti-tumor responses showed that the virus could in effect successfully deliver its package to selected tissues.[46] Vaccinia virus enhanced for tumor selectivity has also been armed with a pro-drug activation system which has been termed "suicide gene therapy".[47] In this case the virus delivers a gene encoding a non-toxic yeast enzyme that is converted to its highly toxic form when the gene is expressed in tumor cells.

3.1. ADENOVIRUSES

Adenoviruses (AV) and adeno-associated viruses (AAV) have also been used as gene vectors for therapy. Adenoviruses can cause mild respiratory and ocular infections, but most persons with an intact functioning immune system recover without any treatment. Adenoviruses have long been a popular viral vector due to their large host range as well as their ability to achieve efficient transgene expression (activation of the foreign gene that has been transferred by the virus) in both replicating and non-replicating cells, accommodate relatively large foreign genes, and code for proteins without integrating into the host cell genome, which can cause detrimental mutations. Adenoviruses have a fairly large host range, but the range of cell types that adenoviruses can infect has been broadened by engineering the virus to contain new surface proteins. This is what is known as changing the tropism of a virus, so that it can bind to and be taken up by cells that it does not normally infect.[48] Compared to other vector systems, such as lentiviruses and adeno-associated viruses, the clinical efficacy data has, in general terms, been disappointing. Apparently, this may be due to several reasons

> *"including longevity of transgene expression using conventional 'first-generation/E1-deleted' vectors and the prevalence of preexisting immunity to HAdV-5 [the most frequently used strain of AV] in the population"*[49].

However, advanced adenoviral vectors have been developed that have achieved considerably longer transgene expression duration than first-generation adenoviral vectors.[50] One major disadvantage with the use of adenovirus vectors for therapy is the fact that they induce potent immune responses after delivery, which would limit their effectiveness, particularly upon re-administration. This

[46] Liu, T.C., Galanis, E. and Kirn, D. 2007. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nature Clinical Practice Oncology* 4: 101-117.
[47] Chalikonda, S., Kivlen, M.H., O'Malley, M.E., Dong, X.D.E., et al. 2008. Oncolytic virotherapy for ovarian carcinomatosis using a replication-selective vaccinia virus armed with a yeast cytosine deaminase gene. *Cancer Gene Therapy* 15: 115-125.
[48] Coughlan, L., Uusi-Kerttula, H., Ma, J., Degg, B.P., Parker, A.L. and Baker, A.H. 2014. Retargeting adenovirus serotype 48 fiber knob domain by peptide incorporation. *Human Gene Therapy* 25:385–394.
[49] Baker, A.H. 2014. Adenovirus-based vectors: maximizing opportunities and optimizing a rich diversity of vectors for gene-based therapy. *Human Gene Therapy* 25: 255-256.
[50] Brunetti-Pierri, N., Ng, T.,Iannitti, D., Cioffi, W. et al. 2013. Transgene expression up to 7 years in nonhuman primates following hepatic transduction with helper-dependent adenoviral vectors. *Human Gene Therapy*. 24:761–765.

problem has been difficult to solve. Nevertheless, adenoviral vectors are still the most commonly used viral vectors in clinical studies.[51]

3.2. Adeno-associated Viruses

Adeno-associated viruses (AAV) are small viruses of the parvovirus family that are found mixed with adenoviruses, as the name implies. This association with adenoviruses is practical, as AAV are defective and need a so-called helper virus (the AV) for replication (reproduction). Because AAV can infect cells but not replicate, they are considered safe vectors and indeed have never been found to be pathogenic. A major drawback for gene therapy is the development of an immune response against the viral vector, a problem they share with adenoviruses.[52] To deal with this problem many methods are presently being tried, including changing the serotype of the virus, introducing new capsid proteins, modulating the immune response or inducing tolerance. Alternatively, the virus has been administered to animals with an immature immune system, where long-term gene expression has been demonstrated.[53]

3.3. Lentiviruses

Much work has been invested in the development of lentivirus (the subfamily of retroviruses to which the AIDS virus belongs) vector delivery systems, as these viruses are very efficient in infecting even non-dividing cells and achieving stable expression of the transferred genes in those cells, and lentiviruses are only weakly immunogenic.[54] Although lentiviruses have a very narrow host range, this can be broadened or altered by pseudotyping, which involves exchanging the surface proteins of particular strains of viruses during packaging of the virus. Other innovative approaches include outfitting the virus with targeting ligands fused to virus surface proteins to infect specific cells as well as using tissue-specific gene regulating promoters to restrict gene expression to certain target cells and reduce the risk of gene-induced immune responses to new proteins expressed on the surface of targeted cells.[55]

The great promise of lentiviral vector development for clinical use is dampened by the fact that they are retroviruses that integrate randomly into the genome of the host, which could cause detrimental mutations as has been shown in past occurrence of vector-related leukaemia in a significant number

[51] Crystal, R.G. 2014. Adenovirus: the first effective *in vivo* gene delivery vector. *Human Gene Therapy* 25: 3-11.
[52] Carlon, M.S., Vidovic, D., Dooley, J., Mori da Cunha, M. et al. 2014. Immunological ignorance allows long-term gene expression after perinatal recombinant adeno-associated virus-mediated gene transfer to murine airways. *Human Gene Therapy* 25(6): 517-528.
[53] Ibid.
[54] Schambach, A. and Baum, C. 2008. Clinical application of lentiviral vectors – concepts and practice. *Current Gene Therapy* 8: 474-482.
[55] Frecha, C., Szecsi, J., Crosset, F.-L. and Verhoeyen, E. 2008. Strategies for targeting lentiviral vectors. *Current Gene Therapy* 8: 449-460.

of the children treated to correct severe combined immunodeficiency (SCID).[56] On the other hand, the property of retroviruses to integrate into the host genome has the advantage of potentially long-lived expression of the delivered gene due to its stable insertion, and third generation lentivirus vectors have been designed for improved safety as well as performance[57], although the safety factor would not be relevant for the use of lentivirus vectors as biological weapons.

There is a much interest in developing lentiviruses as vectors in combination with RNA interference (RNAi) as described above in section 2.2 to silence or block the expression of genes, thus preventing the synthesis of the gene products. Its use in combination with genetically engineered viral vectors, such as lentivirus, facilitates high efficiencies of small hairpin RNA (shRNA) delivery and/or integration into genomic DNA for stable shRNA expression.[58] Although *in vivo* delivery of shRNA to selected cell types has continued to be a major technical challenge, recent studies have shown selective delivery of a lentivirus-based shRNA to macrophages in the lungs of mice by intratracheal administration. The shRNA was specific for targeting a transcription factor that regulates the production of pro-inflammatory cytokines by macrophages. This treatment was successful in modulating the inflammatory response to a significant degree.[59]

RNAi has also been exploited for the sustained, efficient production of proteins in cell "factories".[60] One major problem with the production of recombinant proteins in heterologous cells is the degradation by proteolytic enzymes produced by that cell, which reduces the recombinant protein yield. In human embryonic kidney derived HEK293 cells that have been engineered to produce human recombinant erythropoietin, delivery by an engineered lentiviral vector of shRNA designed to block the synthesis of a proteolytic enzyme (that could destroy errythropoietin) in HEK293 cells resulted in an increased production of erythropoietin.

3.4. FEASIBILITY OF AEROSOL DELIVERY OF VIRAL VECTORS AS BIOLOGICAL WEAPONS

In most research and clinical studies viral vectors are administered by injection, in some cases using repeated application, which would not be practical for their delivery as biological weapons. However, some studies have indicated that administration over natural routes such as inhalation is feasible. For example, treatment of cystic fibrosis patients by inhalation of an adeno-associated

[56] Escors, D. and Breckpot, K. 2010. Lentiviral vectors in gene therapy: their current status and future potential. *Archivum Immunologiae et Therapiae Experimentalis* 58(2): 107–119. doi:10.1007/s00005-010-0063-4.
[57] Ibid; and Mátrai, J., Chuah, M.K.L. and VandenDriessche, T. 2010. Recent advances in lentiviral vector development and applications. *Molecular Therapy* 18(3): 477-490.
[58] Stovall, D.B., Wan, M., Zhang, Q., Dubey, P., Sui, G. 2012. DNA vector-based RNA interference to study gene function in cancer. *Journal of Visualized Experiments* (64): e4129 10.3791/4129, DOI : 10.3791/4129.
[59] Wilson, A.A., Kwok, L.W., Porter, E.L., Payne, J.G. et al. 2013. Lentiviral delivery of RNAi for *in vivo* lineage specific modulation of gene expression in mouse lung macrophages. *Molecular Therapy* 21(4): 825-833.
[60] Dhamne, H., Chande, A.G. and Mukhopadhyaya, R. 2014. Lentiviral vector platform for improved erythropoietin expression concomitant with shRNA mediated host cell elastase down regulation. *Plasmid* 71:1-7.

virus vector engineered with a gene to deliver the transmembrane conductance regulator, which is defective in cystic fibrosis, resulted in "encouraging trends in improvement in pulmonary function".[61] In some 20 clinical trials that have been carried out, use of gene-transfer agents including adenovirus and adeno-associated virus have demonstrated "proof of principle for gene transfer to the airway".[62]

It was further shown that lentiviral vectors pseudotyped with the glycoprotein from the Ebola Zaire EboZ filovirus outer surface for specific airway cell targeting could achieve gene transfer in the lungs of mice.[63] Although the mice were infected by direct instillation of a single dose of the vector, the potential for infection by inhalation was at least given by the investigation. In another study, a lentivirus vector carrying a foreign gene was administered to mice by inhalation in a nose-only exposure chamber.[64] The results showed that lentivirus-mediated delivery of the foreign gene via aerosol was effective to a significant degree.

Since that time, more and more studies using aerosol administration of viral vectors have been reported. For example, the delivery of recombinant viral vector-based antigens for vaccine production is increasingly being applied. The delivery of recombinant vectors over the respiratory route is considered ideal for targeting vaccines encoding antigens from pathogens such as the causative agent of tuberculosis, respiratory syncytial virus, and influenza viruses. By targeting the immunogenic substances in a vector directly to the local, mucous membrane site, protective antibodies and cellular pathogen-specific immune responses can, in principle, be generated and remain at high levels at the portal of entry for these pathogens.[65] Furthermore, aerosol delivery of viral vectors has been used in cancer therapy experimental models. In this regard, the protein osteopontin has been involved in cancer metastases, and delivery of lentivirus-based shRNA targeting the osteopontin gene (to silence this gene) in mice with breast cancer via a nose-only inhalation system "*significantly decreased the expression level of osteopontin and altered the*

[61] Moss, R.B., Rodman, D., Spencer, L.T., Aitken, M.L. et al. 2004. Repeated adeno-associated virus serotype 2 aerosol-mediated cystic fibrosis transmembrane regulator gene transfer to the lungs of patients with cystic fibrosis. *Chest* 125: 509-521.
[62] Laube, B. 2005. The expanding role of aerosols in systemic drug delivery. *Respiratory Care* 50: 1161-1176.
[63] Medina, M.F., Kobinger, G.P., Rux, J., Gasmi, M., Looney, D.J., Bates, P. and Wilson, J.M.. 2003. Lentiviral vectors pseudotyped with minimal filovirus envelopes increased gene transfer in murine lung. *Molecular Therapy* 8: 777-789.
[64] Hwang, S.-K., Kwon, J.T. Park, S.-J., Chang, S.-H., Lee, E.-S., Chung, Y.-S., Beck, G.R. Jr., Lee K.H. and Piao, L. 2007. Lentivirus-mediated carboxyl-terminal modulator protein gene transfection via aerosol in lungs of K-*ras* null mice. *Gene Therapy* 14: 1721-1730.
[65] Roy, C.J., Ault, A., Sivasubramani, S.K., Gorres, J.P., Wei, C.J., Andersen, H., Gall, J.,Roederer, M. and Rao, S.S. 2011. Aerosolized adenovirus-vectored vaccine as an alternative vaccine delivery method. *Respiratory Research* 12:153 (21 November 2011), 7 pages, http://respiratory-research.com/content/12/1/153 [accessed 31.10.2014]; Song, K., Bolton, D.L., Wei, C.-J., Wilson, R.L. et al. 2010. Genetic immunization in the lung induces potent local and systemic immune responses. *Proceedings of the National Academy of Sciences USA* 107(51): 22213–22218.

expression of several important metastasis-related proteins".[66] This group of investigators reported later that nose-only aerosol delivery of lentivirus-mediated osteopontin to mice suppressed lung tumorigenesis.[67]

Many viruses are quite sensitive to environmental stress, reducing their ability to survive in the atmosphere, a property that would be disadvantageous for their dissemination as biological weapons via aerosols. However, an area of intensive investigation involves the development of methods for encapsulating or packaging sensitive substances for controlled drug delivery over the nasal and respiratory routes.[68] These studies could yield benefits for increasing the resistance of viruses and non-viral agents during aerosol dissemination.

Again, the methods used for experimental and clinical administration of viral vectors in aerosols (intratracheal application, nasal instillation, nose-only administration) do not exactly mimic the aerosol dissemination of biological agents as weapons, but they nevertheless demonstrate the principle of concept and provide data that could be used to extrapolate to a weapons type of delivery.

4. ARTIFICIAL VIRUSES *AS* VECTORS

One area of nanotechnology that is rapidly advancing and needs to be closely monitored is the creation of so-called "artificial viruses" for drug delivery as well as gene and cancer therapy.[69] These are polymer-based complexes of nanoparticle size containing DNA, and are being developed in an attempt to overcome the negative aspects of using viruses to deliver genes, such as safety and manufacturing problems, immunogenicity, limited targeting ability and limited transport capacity. Artificial viruses usually consist of DNA compacted into particles with polycationic substances such as polyethylenimine, oligoethylenimine coupled with short diacrylate linkages, polyaspartylhydrazide and chitosan.[70,71,72] Shielding molecules such as polyethylenegylcol to

[66] Yu, K.-N., Minai-Tehrani, A., Chang, S.-H., Hwang, S.-K. et al. 2010. Aerosol delivery of small hairpin osteopontin blocks pulmonary metastasis of breast cancer in mice. *PLoS ONE* 5(12): e15623. doi:10.1371/journal.pone.0015623.
[67] Minai-Tehrani, A., Chang, S.-H., Kwon, J.-T., Hwang, S.-K. et al. 2013. Aerosol delivery of lentivirus-mediated O-glycosylation mutant osteopontin suppresses lung tumorigenesis in K-rasLA1 mice. *Cellular Oncology* 36:15–26.
[68] Mahajan, H.S. and Gattani, S.G. 2009. Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: development and evaluation. *Chemical and Pharmaceutical Bulletin* 57(4): 388–92; van der Walle, C.F., Sharma, G. and Kumar, M.R. 2009. Current approaches to stabilising and analysing proteins during microencapsulation in PLGA. *Expert Opinion on Drug Delivery* 6(2): 177–86; Nayak, B., Panda, A., Ray, P. and Ray, A. 2009. Formulation, characterization, and evaluation of rotavirus encapsulated PLA and PLGA particles for oral vaccination. *Journal of Microencapsulation* 26(2): 154–65.
[69] Mastrobattista, E.; van der Aa, M.A.E.M., Hennink, W.E. and Crommelin, D.J.A. 2006. Artificial viruses: a nanotechnological approach to gene delivery. *Nature Reviews Drug Discovery* 5: 115-121.
[70] Douglas, K.L. 2008. Toward development of artificial viruses for gene therapy: a comparative evaluation of viral and non-viral transfection. *Biotechnology Progress* 24: 871-83.
[71] Russ, V., Elfberg, H., Thoma, C. Kloeckner, J. Ogris, M. and Wagner, E. 2008. Novel degradable oligoethylenimine acrylate ester-based pseudodendrimers for *in vitro* and *in vivo* gene transfer. *Gene Therapy* 15: 18-29.

protect the DNA cargo and particular surface structures that can target the vectors to specific tissues can be added to these basic nanoparticles. As a further example, stabilized nanoparticles of hexanediol diacrylate cross-linked oligoethylenimine have been developed as a non-viral polymeric vector designed to deliver siRNA.[73] However, the main problem with non-viral vectors is that they have not yet consistently demonstrated gene transfer efficiency comparable to that of viruses, which limits their practical use.[74] Nevertheless, a significant degree of effectiveness in gene delivery to airway cells in mice using a cationic non-viral vector administered through the nasal route has been demonstrated[75].

More recently the construction of so-called nanorobots[76] has been gaining attention. Nanorobots have endless applications in medicine. Because of their small size, they can interact directly with cells or even penetrate into them. They can be constructed from DNA or proteins that carry different types of active payloads, with ligands that can attach to specific cell surface structures to direct them to designated targets. They can be programmed by specific construction with "logic operations" to reconfigure in response to cell-surface cues to open up and deliver their payloads. To date only "dumb" nanorobots have been designed, that is, they cannot actively seek out the cells they have been constructed to attack but will reach these cells only by chance. Lenaghan et al.[77] propose an active nanorobot design for cancer therapy, discussing the challenges involved which include core design, propulsion, power, sensing and actuation, control, decision making and integration. There is great interest in developing these vectors further so that rapid advancement in this area can be expected, which could pose a huge potential for misuse in the near future, as these vectors could carry and deliver toxins or bioregulators that could cause the disruption of vital physiological or neurological processes.

5. CONCLUSIONS

Advances in science and technology over the years have enabled new and improved approaches to countering disease and promoting health in general. This progress in the life sciences is absolutely

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[72] Ogris, M., Kotha, A.K., Tietze, N., Wagner, N., Palumbo, F.S., Giammona, G. and Cavallaro, G. 2007. Novel biocompatible cationic copolymers based on polyaspartylhydrazide being potent as gene vector on tumor cells. *Pharmaceutical Research* 24: 2213-2222.

[73] Steele, T.W.J., Zhao, X., Tarcha, P. and Kissel, T. 2012. Factors influencing polycation/siRNA colloidal stability toward aerosol lung delivery. *European Journal of Pharmaceutics and Biopharmaceutics* 80: 14–24.

[74] Douglas, K.L. 2008, op. cit.

[75] Kim, T.W., Chung, H., Kwon, I.C., Shin, B.C. and Jeong, S.Y. 2005. Airway gene transfer using cationic emulsion as a mucosal gene carrier. *Journal of Gene Medicine* 7: 749-758.

[76] Douglas, S.M., Bachelet, I. and Church, G.M. 2012 A logic-gated nanorobot for targeted transport of molecular payloads. *Science* 335: 831-834;

Elbaz, J. and Willner, I. 2013. Nanorobots grab cellular control. *Nature Materials* 11:276-277;

Lenaghan, S.C., Wang, J., Xi, N., Fukuda, T., Tarn, T., Hamel, W.R. and Zhang, M. 2013. Grand challenges in bioengineered nanorobotics for cancer therapy. *IEEE Transactions on Biomedical Engineering* 60:667-673.

[77] Lenaghan, S.C., Wang, Y., Xi, N., Fukuda, T., Tarn, T., Hamel, W.R. and Zhang, M. 2013. Grand challenges in bioengineered nanorobotics for cancer therapy. *IEEE Transactions on Biomedical Engineering* 60(3): 667-673.

essential. At the same time the possibility of misuse of these developments for hostile purposes cannot be ignored. The misuse potential becomes more actual as the biological agent spectrum increases, due to the rapid accumulation of knowledge about new targets for the interaction of biological agents with vital physiological systems. These discoveries can point to ways in which physiological systems may be manipulated with the aim of improving health, but with the potential to be misused to disrupt vital functions. This is compounded by the equally rapid development and improvement of ways to deliver these agents to their targets. Two delivery methods that have progressed most significantly and appear to be most relevant are aerosol and vector-directed technologies. Nanotechnology plays a central role in almost all of these advances in ways to deliver bioactive substances more effectively.

In assessing the potential utility of using viral vectors to deliver bioactive substances, it must be stressed that the goals of using armed viruses for gene and cancer therapy are quite different from those of using armed viruses as weapons. For example, the stringent efficacy demands of therapeutic use might not be so crucial in the case of weapons delivery, and the concerns about the safety of highly efficient lentiviral or other viral vectors would presumably be of little concern for a determined aggressor bent on delivering a biological weapons agent to a chosen target.
The most sophisticated of these advances in science and technology are certainly not easy to put into practice, but require extensive expertise, well-equipped laboratories and substantial funds. While the application of these advances by non-state actors for hostile means can certainly not be ruled out, state-supported actors are more likely than terrorists to have such means. This places a particular responsibility on the States Parties to the Biological and Toxin Weapons Convention to ensure that illicit biological warfare programs using these technologies are not being developed.

The potential for misuse is certainly given, however, it is most difficult to assess just how actual the risk of misuse is. Trying to assess the risk of misuse of advances in individual technological fields would not be very productive. Indeed, combinations of these technological developments would be needed to design and produce a working biological weapon, so that it would be essential to assess the entire research programme to see just how these advances are being put into use. Risk is generally taken to be the product of the probability of occurrence of the event (production of an effective biological weapon) times the probable damage that would result. [78] Assessing the probable damage that would result is difficult enough when considering the risk potential in a biosecurity context, but determining the probability of occurrence is even more challenging, because assessing this component is heavily dependent upon making subjective judgements in many cases. This can

[78] For a discussion of risk assessment in the context of biosecurity see German Ethics Council. 2014. Chapter 3. Risk Assessment. In *Biosecurity – Freedom and Responsibility of Research*. Available at: http://www.ethikrat.org/publikationen/stellungnahmen/biosicherheit.

best be seen by looking at some relevant criteria for assessing the probability of occurrence, which might include

- Availability of the agent;
- Status of the technological development;
- Difficulty of application vs the de-skilling process;
- Availability of expertise: scientific and tacit knowledge;
- Properties of the facility;
- Availability of prophylactic and therapeutic measures.

The element of intent has to be judged in assessing the role of many of these aspects and a definitive decision is hard to make. In addition, the roles of the social and political context of the work that is being carried out and the international aspects involved have to be considered in this assessment.

With this in mind then, it is unlikely that a system of risk assessment in the context of biosecurity could ever guarantee the certainty of its result. This being said however, certainty can still be approached by considering all relevant factors involved. It is essential to carry out an assessment of the risk of misuse of advances in science and technology for hostile purposes in order to facilitate discussion of potential concerns among key stakeholders. With this in mind this report concludes with two key recommendations.

1. Education of the scientific community about relevant aspects of dual-use biosecurity

This is the basis of a dual-use biosecurity oversight policy. Such education involves not only making scientists aware of the illegality of biological weapons, but also developing a clearer understanding of their legal, professional and ethical responsibilities under the aegis of the Biological and Toxin Weapons Convention. Only when those carrying out the work are fully aware of dual-use biosecurity issues will such measures as codes of conduct and risk management procedures be effective. In order to make sure that scientists are fully aware of security issues, it is essential to establish dual-use biosecurity education in the life sciences and related fields at the university level.

2. The development of risk management guidelines to direct the responsible scientist in taking the necessary steps to achieve mitigation of risks that are of particular dual-use concern

This would be best pursued on the multilateral level within the context of the Biological and Toxin Weapons Convention (BTWC). Such work would ideally be carried out by an open-ended experts working group, which would be tasked with developing ‘best practice’ criteria, for consideration by the BTWC body as a whole. The ultimate aim of such work should be to help foster the

development of systems of assessment and oversight at the national level in accordance with national implementation and compliance assurance agendas of the BTWC. Such systems would help foster the emergence of appropriate, transparent, legitimate and legally grounded systems of governance in this area.