National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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RIVM Letter report 205084002/2012
M.R. Klein

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Colophon

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Abstract

Classification of biological agents

This report contains an inventory of the issues of classification of biological agents, which may cause disease in humans.

The conclusion is that the European list with classifications is dated and needs to be updated and expanded. The current list contains conceptual and textual omissions. There is a lack of information on animal pathogens, opportunistic pathogens, genetically modified organisms, attenuated strains, toxins and biological agents with high risk for human health in particular situations (e.g. pregnancy). This is seen as an important drawback of the current European list.

In the Netherlands the classification of biological agents is embedded in the national Worker's protection legislation. As such the employers are responsible for classification of biological agents that are not on the European list, when specific work is carried out with these biological agents. However, the definitions, instructions and procedures for determining the classifications are equivocal.

The recommendation is to remove the appendices with classifications from the EC Directive, in order to be able to maintain the list on a more frequent basis. There are a number of online and interactive tools available to set up a centralized database with all the relevant information at our disposal. We suggest a central curator of such a resource, for instance the European Centre for Disease Prevention and Control (ECDC) and/or the European Agency for Safety and Health at Work (EU-OSHA).

Keywords:
Classification, Biological agent, Occupational Health and Safety, Biosafety

Rapport in het kort

Classificatie van biologische agentia

Deze rapportage omvat een inventarisatie van de problematiek rond de classificatie van biologische agentia, die ziekte bij de mens kunnen veroorzaken.

De constatering is dat de Europese lijst met classificaties verouderd is en geactualiseerd en uitgebreid zou moeten worden. De lijst bevat conceptuele en redactionele omissies. Het ontbreken van informatie over ondermeer dierpathogenen, opportunistische pathogenen, genetisch gemodificeerde micro-organismen, verzwakte stammen, toxines en biologische agentia met een verhoogd risico in bepaalde situaties (bijv. zwangerschap), wordt gezien als een belangrijke tekortkoming van de Europese lijst.

In Nederland zijn vanwege de inbedding in de Arbowetgeving de werkgevers verantwoordelijk voor de classificaties van biologische agentia die niet op de lijst staan, indien er gericht gewerkt wordt met deze agentia. Echter, de gebruikte definities, instructies en voorschriften voor het opstellen van classificaties zijn niet altijd even éénduidig.

Een aanbeveling is om de bijlagen met classificaties, los te koppelen van de Richtlijn, zodat het mogelijk wordt om deze op een meer frequente basis te onderhouden. Er zijn voldoende online en interactieve mogelijkheden om een centrale database op te zetten met informatie over biologische agentia, inclusief de actuele classificaties. Het beheren van een dergelijke informatiebron zou bijvoorbeeld neergelegd kunnen worden bij een organisatie als het European Centre for Disease Prevention and Control (ECDC) en/of het Europese Agentschap voor Veiligheid en Gezondheid op het Werk (EU-OSHA), in Bilbao (Spanje).

Trefwoorden:
Classificatie, Biologisch agens, Arbowet en -regelgeving, Bioveiligheid

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Summary

This report contains an inventory of the issues encountered with the classification of biological agents. The findings can serve as the basis of and can facilitate a multidisciplinary project in which the European Community classification of biological agents is subjected to a comprehensive assessment, updated and harmonised.

The Dutch Working conditions legislation stipulates that Annex III of EC Directive 2000/54/EC is the only authoritative list of classifications of biological agents in the Netherlands. These classifications are based on the level of pathogenicity in healthy workers (*i.e. virulence / pathogenicity*), contagiousness, or risk of spreading to the community (*i.e. transmissibility*) and the availability, if any, of effective prophylaxis or therapy (*i.e. treatment*). This approach identifies four risk groups with four corresponding (bio)containment levels, representing protective measures pursuant to the Occupational Hygiene Strategy (*Arbeidshygiëne Strategie*).

The current European list of classifications of biological agents has not been updated in the past decade. The conclusion is that in addition to conceptual and textual omissions the nomenclature is dated and that the entire list fails to reflect the latest scientific knowledge. Emerging biological agents are not included on the list and have no formal EU classification.

Although the EC Directive has a clearly defined ‘scope’, the lack of information on animal pathogens, opportunistic pathogens, genetically modified organisms, attenuated strains, toxins and biological agents posing a high risk to human health in particular situations (e.g. pregnancy) is seen as an important drawback of the current European list.

The European list can only be reviewed or amended as part of a formal procedure. Pending an EU classification, Member States draw up their own classifications of biological agents that cause or may cause human disease. As the EC Directive is embedded in Dutch Worker's protection legislation, employers are ultimately held accountable for the provisional classification of biological agents when work specifically involves the use of unclassified pathogenic biological agents.

However, the definitions used for the classifications are not entirely unequivocal, and the impact of the various contributing factors is uncertain. Virulence more so than transmissibility and availability of treatment appears to be the primary factor in the classification of risks. Furthermore, there are no definitive instructions or guidelines for the classification of biological agents. In this regard also, no effective provisions for exceptions or derogations from regulations are provided for in either the EC Directive or the Dutch Worker’s protection legislation.

List of abbreviations

AB	Working Conditions Decree *('Arbobesluit')*
AI	Working Conditions Fact Sheet
ARBO	Working Conditions
BA	Biological Agents
BAH	Biosafety-Occupational Hygiene principle
BGGO	Genetically Modified Organisms Agency
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSL	Biosafety Level (containment level)
CDC	Centres for Disease Control and Prevention
CIb	Centre for Infectious Disease Control Netherlands
COGEM	Committee on Genetic Modification
GMO	Genetically Modified Organism(s)
KIZA	Infectious Disease and Occupational Health Knowledge System
LCI	National Coordination Centre for Outbreak Management
MSDS	Material Safety Data Sheet
NVMM	Netherlands Society for Medical Microbiology
NVvM	Netherlands Society for Microbiology
NVP	Netherlands Society for Parasitology
OMT	Outbreak Management Team
PHAC	Public Health Agency Canada
RI&E	Hazard identification and risk assessment
RIEBA	Hazard identification and risk assessment for biological agents
VMT	Safe microbiological techniques
WHO	World Health Organisation
Wm	Environmental Management Act

Disclaimer

The views expressed in this report, about the problems with the current European list with classification of biological agents and possible ways to address this, should be considered as the author's opinion.

This report is a translation of the corresponding report in Dutch (RIVM 205084001-2012), which is leading.

1 Introduction and scope

This project was started in anticipation of a potential request from the EU to review the current European list of classifications of biological agents.[1] However, this report should be seen as an inventory of the issues of classification of biological agents and can serve as the basis of and a facilitator for a multidisciplinary project in which the classification of biological agents is subjected to a comprehensive assessment, updated and harmonised.

A sounding board meeting to further refine the definition of the project was held on 27 May 2010. The sounding board group comprised:

- Prof. A. van Belkum (Erasmus Medical Centre),
- Prof. J Galama (University Medical Centre St. Radboud Nijmegen (UMCN),
- Dr T. Boekhout (CBS-KNAW Fungal Diversity Centre),
- Dr C. van der Vlugt-Bergmans (BGGO),
- Dr P. Odinot (Chair of the Biological Safety Officers Platform (BVF Platform)),
- Dr J. Maas (Netherlands Centre for Occupational Diseases (NCvB));

Plus a delegation from the RIVM:

- Dr H. Vennema (virologist, Laboratory for Infectious Diseases and Perinatal Screening (LIS)/CIb, on behalf of Prof. M Koopmans),
- Dr T. Kortbeek (physician-microbiologist, parasitologist, LIS/CIb),
- Dr D. Notermans (physician-microbiologist, bacteriologist, LIS/CIb)
- Dr M. Klein (Team Leader High Containment, immunologist, HiC/LIS/CIb), chair, projectleader and author.

It became clear during the sounding board meeting that updating the European list of classifications would require such a significant amount of time and such a multidisciplinary approach that it would exceed the term and scope of this project. Moreover, it would be best if the classifications were carried out and assessed by the relevant professional groups/associations of microbiologists/ medical microbiologists, as they have by far the best depth of knowledge in this field. For the same reasons, no partial lists will be presented as part of this project for related issues, such as annotations on pregnancy and biological agents, sensitivity to disinfectants or the risks of certain biological agents in the context of bioterrorism,[2] etc. Although certainly relevant to risk assessment as a whole and to the taking of appropriate measures, these aspects fall outside the project's scope.

This report addresses the following questions: 1) What is the legislative framework for the classification of biological agents? (see Section 2); 2) What are the problems with the current European list of classifications? (see Sections 3-5); and 3) What are the guidelines and procedures for determining classifications? (see Sections 6-8).

[1] DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 83/391/EEC).
[2] Council Regulation (EC) No. 428/2009 of 5 May 2009 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items (see in Articles 1C351 up to and including 1C354).

2 Legislative framework

2.1 Legislation

EC Directives establish the larger framework for the national legislation of EU Member States. Although EC Directives have no direct implications for Dutch companies, institutions and citizens, they do apply because they are usually implemented and embedded in national legislation. In the Netherlands, guidelines and provisions relating to occupational health and safety are embedded in the national worker's protection legislation. Mandatory provisions are laid down in the Working Conditions Act (*Arbeidsomstandighedenwet*), the Working Conditions Decree (*Arbobesluit* (AB)) and the Working Conditions Regulations (*Arboregeling*), while non-mandatory and other more specific information is included in, for instance, the Working Conditions Fact Sheets (*Arbo-Informatiebladen* (AI)).

The classification of biological agents is governed by EC Directive 2000/54/EC, which contains instructions regarding the protection of workers against the risks of exposure to biological agents at work. In the Netherlands, this EC Directive is included as Section 9 of Chapter 4 in the Working Conditions Decree ("Biological agents"; AB Articles 4.84 up to and including 4.102). That part of the Decree contains the obligations for working safely with biological agents that are stated below (see also Table 1 for an overview of the relevant articles).[3]

Working Conditions Fact Sheet 9 (AI-9) on "Biological Agents" (5th edition, 2010) and various online sources, including the occupational health and safety site Arbozone[4] and the website of the Infectious Diseases and Work Knowledge System (KIZA)[5], offer more detailed expert practical information about biological agents and current legislation with commentary and explanatory notes on their practical application. In addition, 'adjoining' legislation is essential for working with biological agents, including for instance the Environmental Management Act (Wm), the Environmental Permitting (General Provisions) Act (Wabo), transport legislation,[6] the Public Health Act (Wpg)[7] and the Disaster and Crisis (Provision of Information) Decree (Biro).

2.2 Definition of biological agents

The following definitions are used in the Dutch Working Conditions Decree (AB article 4.84(2); originating from the EC Directive 2000/54/EC, Art. 2):

'Biological agents shall mean micro-organisms, including those which have been genetically modified, cell cultures and human endoparasites, which may be able to provoke any infection, allergy or toxicity.' 'Micro-organism shall mean a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material.' 'Cell culture' shall mean the in-vitro growth of cells derived from multicellular organisms.'

[3] Sections 1-8 of Chapter 4 of the Working Conditions Decree do not apply to biological agents.
[4] http://gevaarlijkestoffen.arbozone.nl/artikelen/biologische-agentia
[5] http://www.kiza.nl
[6] http://www.bvfplatform.nl/item.html&objID=2569
[7] http://www.rivm.nl/cib/themas/wetgeving

Table 1. Guidelines and instructions in the Working Conditions Decree (*Arbobesluit* (AB)) for working safely with biological agents.[8]

Chapter 4	**Hazardous substances and biological agents**
Section 9	**BIOLOGICAL AGENTS**
§1 Definitions and scope	
Article 4.84	- Biological agents, cell cultures and micro-organisms
§2 Hazard identification, risk assessment and classifications	
Article 4.85	- Further RI&E guidelines
Article 4.86	- Consequences of the classifications
§3 Measures in response to exposure	
Article 4.87	- Avoid exposure; replace
Article 4.87a	- Avoid or minimise exposure
Article 4.87b	- Measures to prevent or minimise exposure to Legionella bacteria in the deployment and maintenance of air humidification and water systems
Article 4.88	- Safety awareness
Article 4.89	- Hygiene protection measures
Article 4.90	- Registration
§4 Occupational health study	
Article 4.91	- Study and vaccinations
§5 Works Council	
Article 4.92	- Information for accidents or incidents
Article 4.93	- Other information
§6 Supervision	
Article 4.94	- Notification
Article 4.95	- Accidents or incidents
Article 4.96	- Transfer of data/information
§7 Special provisions with work other than laboratory diagnostics in the health care and veterinary sectors	
Article 4.97	- Health care and veterinary care
Article 4.98	- Protective measures
§8 Special measures for laboratories, spaces with laboratory animals and industrial processes	
Article 4.99	- Containment levels for laboratories and spaces with laboratory animals
Article 4.100	- Containment levels for industrial processes
Article 4.101	- Containment levels for biological agents that are not listed in Annex III
§9 Special provisions for the provision of information and training	
Article 4.102	- Provision of information and training
Section 10	**SPECIAL SECTORS AND SPECIAL CATEGORIES OF WORKERS**
§2 Youth	
Article 4.105	- Prohibited from working with hazardous substances and biological agents
§3 Pregnant and lactating workers	
Article 4.109	- Prohibited from working with certain biological agents

[8] See http://www.wetten.nl

2.3 Classification criteria for biological agents

In the Dutch Working Conditions Decree biological agents are divided into four risk groups (AB Article 4.84(3). No classifications of biological agents are included in the Decree. These can be found in Annex III of EC Directive 2000/54/EC ("Community classification - biological agents known to infect humans"). In the Decree there is a specific reference to this list, making the latter the only formal list of classifications in the Netherlands (AB Article 4.84(4)).

The European list of classifications is available both online via the *Arbozone* occupational health and safety website and the Infectious Diseases and Work Knowledge System (KIZA) website, as well as in print in Annex 4 of Working Conditions Fact Sheet 9 (AI-9)). The list of classifications was also included in previous editions of the so-called 'grey booklet'[9] published by the Netherlands Society for Microbiology (NVvM). The current version only includes references to foreign websites for further information.[10]

The following criteria are used to classify biological agents into risk groups:

Table 2. Classification of biological agents in four risk groups:

Classification	**Ability to cause disease in humans** *(virulence)*	**Hazard to workers**	**Spread to the community** *(transmission)*	**Effective prophylaxis or** *(treatment)*
Category 1	Unlikely	No hazard	Not applicable	Not applicable
Category 2	Likely disease	Potential hazard	Unlikely	Available
Category 3	Likely severe disease	Serious hazard	Likely	Available
Category 4	Severe disease	Serious hazard	High risk	Not available
Similar definitions are also used by other countries and international organizations:				
WHO	Laboratory Biosafety Manual, 3rd Edition (2004)[11]			
PHAC	Laboratory Biosafety Guidelines, 3rd Edition (2004)[12]			
CDC	Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (2009)[13]			
Note. An extensive overview of the criteria for risk classification of biological agents is presented on the website of the American Biological Safety Association – ABSA.[14]				

2.4 Scenarios and consequences for classification into categories

The Dutch Working Conditions Decree distinguishes between three situations involving exposure to biological agents (AB Article 4.86):

[9] A. van Belkum, T. Boekhout, et al. (2009). Working safely with micro-organisms, parasites and cells in laboratories and other working areas. Theory and Practice. (*Veilig werken met micro-organismen, parasieten en cellen in laboratoria en andere werkruimten. Theorie en Praktijk*). 3rd edition, ISBN 978-90-8559-549-6.
[10] http://www.nvvm-online.nl/modules/nvvm_classification
[11] http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
[12] http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/
[13] http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
[14] http://www.absa.org/riskgroups/index.html

1. Situations which entail possible exposure to non-pathogenic biological agents (risk category 1). The requirement in these situations is to be as careful, orderly and tidy as possible and to take all necessary measures in relation to hygiene. These situations include among others, working in the food industry or in the hotel and catering sector.

2. Situations that do not specifically involve the use of biological agents, but nonetheless entail real risks of exposure to category-2, -3 or -4 pathogenic biological agents. The obligations laid down in AB Articles 4.87(a), 4.87(b), 4.89, 4.91, 4.93, 4.95, 4.97, 4.98, 4.99(2) and 4.102 apply. These situations include, for instance, healthcare, veterinary and agricultural workers, as well as refuse disposal/waste management workers (see also the 'Indicative List' of examples in Annex I of the EC Directive).

3. In situations that specifically involve the use of category-2, -3 or -4 pathogenic biological agents, the obligations laid down in AB Articles 4.87 up to and including 4.102 apply. These situations include, for instance, work involving pathogenic biological agents in microbiological laboratories in the biotech industry, pharmaceutical industry, hospitals and research institutes.

2.5 Risk management

In accordance with Article 5 of the Dutch Working Conditions Act (*Arbowet*), a hazard identification and risk assessment (RI&E) must always be performed when a worker risks exposure to biological agents. An RI&E for biological agents (RIEBA) is useful in assessing the nature, degree and duration of exposure in order to determine any risk to the worker (AB Article 4.85). Containment measures proportionate to the hazard must be implemented to rule out or minimise the risk. There is a direct relationship between the level of risk and the measures required. Greater risks require more extensive measures (AB Article 4.86). The body of measures associated with a certain level of risk is referred to as a (bio)containment level.

Preventive and protective measures for risks related to exposure to biological agents in accordance with the Occupational Hygiene Strategy for Work with Biological Agents, which is also referred to as the Biosafety-Occupational Hygiene (*Bio-ArbeidsHygiënisch* (BAH)) principle.[15]

If the nature of the work permits, the biological agent should be replaced with another that is not or is less hazardous to worker's health and safety (AB Article 4.87). In short, the first task is to address the issue at the source. If replacement is not an option, measures must be taken to prevent exposure – starting with technical measures (physical containment measures).

Depending on the outcome of the RIEBA, Containment Levels 2, 3 or 4 respectively (also known as Biosafety Levels 2 (BSL2), 3 (BSL3) and 4 (BSL4)),[16] (AB Article 4.99) should be applied as a minimum to work specifically with category-2, -3 or -4 biological agents in laboratories, and spaces containing test animals that have been deliberately infected with category-2, -3 or -4 biological agents or are (possible) carriers of one of these categories of

[15] http://www.kiza.nl/content/bio-arbeidshygiënisch-bah-principe
[16] In the Netherlands, for work with genetically modified organisms (GMOs), similar but differently named containment levels apply (namely ML-I, ML-II, ML-III, ML-IV).

biological agents. In this respect, classification of pathogenic agents can also be seen as a formal tool to determine which precautionary measures are at least required to ensure the worker's health and safety.

Annex V of EC Directive 2000/54/EC contains instructions concerning containment levels and the corresponding physical containment measures for microbiological laboratories, which are also included as provisions in the Working Conditions Decree. Annex VI of the EC Directive outlines containment for industrial processes.

The next layer of protection is ensured via organizational, procedural and administrative control measures addressing issues such as personal hygiene, safe microbiological techniques (VMT), safety procedures, emergency procedures, worker vaccinations, training and the provision of information. Finally, in addition to collective or general protection measures, further individual measures can be employed by using personal protection equipment (PPE).

Article 18(2) of the EC Directive stipulates that, pending a formal Community classification, Member States shall establish their own classifications of biological agents that are not listed and that are or may be a hazard to human health based on the definitions in the same Directive. Since the EC Directive is implemented in the Dutch Worker's Protection legislation, it means that in the Netherlands this is the primary responsibility of the employers. If specific work is performed involving pathogenic biological agents outside the formal classification framework, the employer will need to provide a provisional classification based on the definitions presented above in addition to completing the compulsory risk identification and risk assessment for hazardous biological agents (RIEBA).

3 Editorial comments

3.1 Omissions in the list of classifications

The previous section clearly showed that the primary role of the classification of biological agents is to determine which precautions are required to safeguard health and safety when working with the biological agents in question and that Annex III of EC Directive is the only authoritative, formal list of classifications of biological agents in the Netherlands.

While it should be mentioned that the current European list of classifications is largely sufficient for use in everyday practice, it still contains omissions, language errors, typing errors and outdated nomenclature. These are the issues which at the very least should be addressed the next time the list is reviewed and amended.

Assuming the European list is applicable in all EU Member States, it is notable that the Dutch translation of the classifications of a number of biological agents differs (compared to other versions)[17] from what it probably should be. This may be attributed to editorial errors:

* *Coccidioides immitis* – according to the Dutch version – is a category-3 biological agent, while other translations of the EC Directive state place it in category 2.

* *Edwardsiella tarda* and *Morganella morganii* – according to the Dutch version – are category-3 biological agents, while some of the other translations of the EC Directive say category 2. In practice, category 2 is assumed in the Netherlands as well (see AI-9 and www.kiza.nl).

* *Coxiella burnetii* – according to the Dutch version – is a category-2 biological agent, while some of the other translations of the EC Directive place this agent in category 3. In practice, category 3 is also assumed in the Netherlands.[18]

Similar errors and omissions occur in the other translations of the European list, including:

* *Campylobacter fetus* has not been classified in the Italian version.
* *Madurella mycetomatis* is missing in the French and Italian versions.
* *Taenia solium* – according to the Italian version – is a category-2 biological agent, while some of the other translations say category 3. On the other hand: *Toxocara canis* – according to the Italian version – is a category-3 biological agent, while some of the other translations say category 2.
* *Yersinia enterocolitica* is missing in the Spanish version.

Other shortcomings of the current list are described in the next sections based on the *Introductory Notes* of Annex III of Directive 2000/54/EC.

[17] http://eur-lex.europa.eu/ (Dutch translation was compared to the versions in English, German, French, Spanish and Italian).
[18] www.kiza.nl/content/gevaarsklasse-bacterien

3.2 Outdated nomenclature

"The nomenclature of classified agents used to establish this list reflects and is in conformity with the latest international agreements of the taxonomy and nomenclature of agents at the time the list was prepared" [Introductory note 5].

As a result of increased understanding and developments in science, the nomenclature and taxonomy used for a number of biological agents are no longer generally accepted.[19] This applies in particular to a number of viruses included on the European list:

* Hepatitis D virus (Delta) is not classified in the Hepadnaviridae family.
* Hepatitis E virus is not assigned to a virus family. It was previously classified in the Caliciviridae family.
* Papovaviridae has been split into Papillomaviridae and Polyomaviridae.
* Toroviridae is now classified in the Coronaviridae family as Torovirus.
* Equine morbillivirus is now called Hendra virus.
* A more systematic naming system is now more commonly used for the Herpesviridae family:

HHV-1, -2	Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2)
HHV-3	Herpesvirus varicella-zoster (VZV)
HHV-4	Epstein-Barr virus (EBV)
HHV-5	Cytomegalovirus (CMV)
HHV-6, -7	Human B-lymphotropic virus (HBLV); Roseolovirus
HHV-8	Kaposi's sarcoma-associated herpesvirus (KSHV)

As is the case in the other translations, Annex III includes several virus names that have been translated into Dutch (e.g. 'Apenpokkenvirus'). As a result, specific information is used that is not common knowledge either from a scientific or international perspective. In this particular example, 'Monkeypox virus' is more commonly used. The use of translations could also lead to confusion, and should be avoided.

The nomenclature for several bacteria has also changed. Examples:

* *Rickettsia tsutsugamushi* is now *Orientia tsutsugamushi*.
* *Chlamydia psittaci* is now *Chlamydophila psittaci*; the other non-avian strains are *Chlamydophila abortus, C. caviae* and *C. felis*.
* *Anaplasma* is separated from *Ehrlichia* spp.; the most commonly known example being the human pathogen *A. phagocytophilum*.
* In the naming system for the *Salmonella* species, the serotype (serovar) is written with a capital letter and not in italics after the family name. For example: *Salmonella* Typhimurium (instead of *Salmonella typhimurium*) and *Salmonella* Paratyphi A, B and C (instead of *Salmonella paratyphi* A, B, and C).

In addition, the old names of some bacteria are still included between brackets. These names have now fallen out of use or are incorrect such that they should probably not be used anymore (or perhaps it would be better to give the old names separately to avoid confusion).

[19] See www.ictvdb.org

4 Latest scientific and technical knowledge

In addition to the errors in the European list discussed in the previous section, there is the issue of whether the current list is exhaustive and whether it properly reflects the latest scientific and technical knowledge. In this respect, the following introductory comment in Annex III of the EC Directive is relevant: *"The list of classified biological agents reflects the state of knowledge at the time that it was devised"* [Introductory note 6].

Although last adopted in the year 2000, the substance of the current European list largely dates back to a previous version from 1990, namely EC Directive 90/679/EEC. We can therefore confidently state that the current European list no longer reflects the present state of scientific and technical knowledge and developments as it has not been updated in the past decade.

4.1 Knowledge and expertise

Since the European list was last adopted, spectacular successes have been achieved in the research field of synthetic genomics. For instance, the J. Craig Venter Institute was the first to jump-start the synthetic genome of *Mycoplasma mycoides* (a bacteria present in cows and goats).[20] Another example, the Influenza virus, which caused the 1918 Spanish flu pandemic, has also previously been reconstructed.[21] Although the first example does not appear on the European list as it is not a human pathogen and 'only' an indirect reference is made to the second example (it is covered by the heading 'Influenza viruses type A'), it is clear that advances in *in vitro* technology and synthesis methods are enabling us to create new biological agents that not only do not appear on the European list, but also in some cases are unlikely to occur in the natural world. The question is how to deal with such cases in terms of classification and risk management, because synthetic genomes – and (taking a broader perspective) genetically modified organisms as well – were not taken into account when the European list of classifications was drafted.

4.2 Recent outbreaks of new infectious diseases

The SARS Coronavirus – which caused a major outbreak in 2003 – is the best example of a new wild-type biological agent that is not included on the current European list. Similarly, the New Influenza A(H1N1) virus, which caused a global pandemic in 2009, is not on the list either.[22] The literature study by Woolhouse and Gowtage-Sequeria (2005)[23] revealed that more than 1,400 human pathogens had already been identified, of which 177 were classified as 'emerging of re-emerging' biological agents. We can confidently say that new pathogens (by definition) do not appear on the current European list. Ways to address this issue are discussed later.

[20] Gibson et al., Creation of a bacterial cell controlled by a chemically synthesized genome. Science. 2010, 329:52-6.
[21] Tumpey et al., Characterization of the reconstructed 1918 Spanish Influenza pandemic virus. Science. 2005, 310:77-80.
[22] Strictly speaking, both viruses are covered by their respective family names: 'Coronaviridae' and 'Influenza viruses A'. According to the European list, both would fall into category 2, but it is widely known that a higher category was used during and after the outbreaks.
[23] Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and re-emerging pathogens. Emerg. Infect. Dis. 2005, 11(12):1842-7.

4.3 How exhaustive is the list?

In determining how exhaustive the current European list of classifications of biological agents is, we can ask ourselves how it compares to other sources of information. It is notable that lists from, for instance, Belgium,[24] Germany[25] or the United States[26] include many biological agents as human pathogens that are not included as such on the European list.

The European list includes 375 biological agents: 151 bacteria, 129 viruses, 69 parasites and 26 fungi and yeasts. However, the actual number of biological agents covered is larger (but how much larger is not known) because in some cases a generic name is used, instead of specific species names. For instance, the partial lists of bacteria, parasites, fungi and yeasts sometimes include the note 'spp' for certain biological agents: ‘spp’ refers to species that are known to be pathogenic in humans. Not all known biological agents are therefore named individually on the European list.

In addition, the list of viruses does not specifically name each pathogenic virus. Certain viruses are only covered indirectly as they are assumed under headings such as:

* 'Other LCM-Lassa complex viruses',
* 'Other Tacaribe complex viruses',
* 'Other hantaviruses',
* 'Other bunyaviridae known to be pathogenic',
* 'Other Caliciviridae',
* 'Other flaviviruses known to be pathogenic',
* 'Other known alfaviruses', and
* 'Hepatitis viruses not yet identified'.

With the exception of the last item, all of these viruses are classified in category 2. There is some disagreement in this regard at the international level, however, with some of these viruses classified in category 3:

* Other bunyaviridae known to be pathogenic:
  - Akabane virus (AKAV)
  - Douglas virus (DOUV)
  - Enseada virus (ENSV)
  - Great Saltee virus (GRSV)
  - Germiston virus (GERV)
  - Issyk-Kul virus (ISKV)
  - Ngari virus (NRIV)
  - Oropouche virus (OROV)

* Other hantaviruses:
  - Orán virus (ORNV)
  - Pergamino virus (PRGV)
  - Saaremaa virus (SAAV)
  - Topografov virus (TOPV)

* Other flaviviruses known to be pathogenic:
  - Deer Tick virus (DRTV)
  - Koutango virus (KOUV)
  - Murray Valley encephalitis virus (MVEV)
  - Negishi virus (NEGV)

Of the viruses that are identified individually on the European list, there are examples of differences in classification compared to the more recent US-CDC

[24] http://www.biosafety.be/RA/Class/ClassBEL.html
[25] http://www.dsmz.de/microorganisms/?menu_id=2
[26] BMBL, 5th Edition, Section VIII-F: Arboviruses and Related Zoonotic Viruses, Table 6. Alphabetic Listing of 597 Arboviruses and Haemorrhagic Fever Viruses.

list. These differences concern a number of vector-borne pathogens in particular, including certain tick-borne Flaviviruses. Notably, this US-CDC list includes seven category-4 pathogenic viruses that are classified as category 3 on the European list, namely:

- Absettarov (ABSV)
- Hanzalova (HANV)
- Hypr (HYPRV)
- Kyasanur Forest (KFDV)
- Kumlinge (KUMV)
- Omsk (OHFV)
- Russian spring-summer encephalitis (Tick-borne encephalitis; RSSEV)

The opposite situation also occurs, i.e. the risk category of a certain virus can be lower on the US-CDC list than on the European list. Examples include:

- Dengue virus (DENV)
- Mayaro virus (MAYV)
- Ndumu virus (NDUV)

Despite the largely uniform category definitions used at international level (see Table 2), there are still significant differences between the lists of classifications of biological agents both in terms of quantity (the number of biological agents) and quality (risk assessments based on the definitions). This could be associated with improvements in our understanding of the virulence and pathogenicity of some biological agents, which may have been incorporated into more recently drafted lists. The reasons can also be more trivial in nature, however, such as nuances in the perception of risk or the precise way in which definitions are applied (weighting of the individual factors). Classifications and the reasons for those classifications based on today's knowledge is a subject for further investigation *(falls outside of the scope of this report)*.

We note that the procedure for adopting and amending the formal European list with classifications (see later in this report) leaves little opportunity to respond promptly to new developments in science and in the world, including natural outbreaks of infectious diseases or bioterrorism.

5 Scope of the classifications

5.1 Scope of the EC Directive

The current European list of classifications has a clearly defined scope. However, as this is the only formal “tool” to assess the risks of biological agents, it unintentionally gives rise to a grey area for which no instructions or procedures have been laid down. We suggest that this is one of the major shortcomings of the current European list. In any event, it currently poses practical problems as regards adopting new classifications and assessing the risks posed by biological agents in a broader sense.

"In line with the scope of the Directive, only agents which are known to infect humans are to be included in the classified list. Where appropriate, indicators are given of the toxic and allergic potential of these agents. Animal and plant pathogens which are known not to affect man are excluded. In drawing up this list of classified biological agents consideration has not been given to genetically modified micro-organisms" [Introductory note 1].

5.2 Zoonoses

Animal pathogens can pose additional risks to human health, both in specific and non-specific work, because some of these biological agents can also be transmitted to humans and cause disease. Given the apparent increase in the number of zoonoses over time, it is vital to monitor and gain an understanding of this situation.[27] The literature survey by Woolhouse (2005) clearly showed that the majority (58%) of emerging pathogens is of animal origin. When a biological agent can cause disease in humans, it should be included on the list irrespective of its origin. The question is whether this has always happened in practice.

A review of more recent sources of information reveals that the current European list does fail to include a number of zoonotic animal pathogens, such as *Anaplasma phagocytophila* and *Capnocytophaga canimorsus*. These and other biological agents currently missing from the list should therefore be added to the next revised version of the list of classifications.

Finally, there would be advantages to linking all available information on animal pathogens and zoonoses in some way with the European list of classifications of biological agents. This would ensure a clear overview for work involving animal pathogens and whether that work poses additional risks to humans and the environment. The RIVM has posted extensive information on zoonoses on its website.[28] There are also EC Directives,[29] as well as dedicated zoonoses research networks,[30] all of which offer useful information to supplement the current European list of classifications.

[27] http://www.rivm.nl/bibliotheek/rapporten/330214002.pdf (Emerging zoonoses: Early warning and surveillance in the Netherlands).
[28] http://www.rivm.nl/ziekdoordier/zoon_op_rij
[29] Council Directive 97/22/EC of 22 April 1997 amending Directive 92/117/EEC concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications.
[30] http://www.medvetnet.org; Med-Vet-Net is the European Network of Excellence; it works to prevent and control zoonoses and food-borne diseases.

5.3 Genetically modified micro-organisms

While the definition of biological agents does allow for the inclusion of genetically modified micro-organisms (GMOs), it is remarkable that "in drawing up this list of classified biological agents, consideration has not been given to genetically modified micro-organisms". This is a potential conceptual omission in the EC Directive.

In practice, laboratory experiments and research ("specific work") involving GMOs are based on the classification of wild-type biological agents. An expanded risk analysis determines whether to move to a higher (or lower) category, based on host, donor and vector sequences and other technical manipulations conducted in the microbiological laboratory. This process ensures that adequate containment measures are taken to ensure the health and safety of workers and the environment.

The Dutch GMO Agency (BGGO) maintains two lists: 1) a list of biological agents that are harmless to humans, plants and animals and 2) a list of pathogenic micro-organisms and their category of pathogenicity, as applied in the risk analyses for their contained use (*ingeperkt gebruik* (IG)).[31]

There are a few differences between the BGGO and European classifications of wild-type biological agents. For instance, *Trypanosoma brucei gambiense* – according to the European list – is a category-2 biological agent, whereas category 3 is used as the starting point for genetic modification. In contrast, Hepatitis C virus and *Plasmodium falciparum* – according to the BGGO list – are category-2 biological agents, while they are category 3 on the formal European list. Of course, strictly speaking, this last situation is prohibited.

In the past, the Dutch Committee on Genetic Modification (COGEM) has published classification recommendations for a number of biological agents (wild-type and modified).[32] This was done primarily in response to requests for contained use in particular when the European list lacked the particular classifications. This information too, should be added to the next revised version of the European list of classifications of biological agents.

Another question is the extent to which genetic manipulation can be tolerated before a biological agent is created that clearly differs from wild-type biological agents in terms of virulence / pathogenicity. A key tool in GMO legislation in this regard is the compulsory risk assessment to be conducted when an application is made for the relevant permit. Strictly speaking, however, every single mutation – irrespective of its impact on virulence – could motivate departures from the formal classifications. This could and does give rise to various lists of classifications. Generally this issue does not appear to be a major problem. However, we would like to make a case for for having a single authoritative source of classifications of biological agents (genetically modified or wild-type; pathogenic or not).

[31] http://bggo.rivm.nl/paginas/doc-ig.htm; Appendix A of the GMO Regulations (*Regeling GGO*) of June 1998 and the Guidelines of the Committee on Genetic Modification (*Commissie Genetische Modificatie* (COGEM)) accompanying the GMO Regulations.
[32] http://www.cogem.net/zoekenxNL.aspx?Pageid=1&loc=4&sStr=classificatie

5.4 Opportunistic pathogens

"The list of classified agents is based on the effect of those agents on healthy workers. No specific account is taken of particular effects on those whose susceptibility may be affected for one or other reason such as pre-existing disease, medication, compromised immunity, pregnancy or breast feeding" [Introductory note 2].

Not all biological agents cause disease in healthy humans. There are also biological agents that only pose a health risk when the host is temporarily or chronically more susceptible to the agent. The EC Directive clearly states that the list only includes biological agents if they pose a risk to healthy workers. Accordingly, the list does not include opportunistic pathogens. This approach may introduce unintended risks to working in a healthy and safe manner with biological agents.

Obviously, the compulsory hazard identification and risk assessment (RI&E) must take into account the heightened risk faced by some workers. Any extra measures or precautions should then be implemented.

It is not always easy to distinguish between biological agents that are always pathogenic and those that are only pathogenic if the host is more susceptible to the agent due to environmental factors or other (personal) circumstances. The current European list includes several biological agents, such as *Mycobacterium fortuitum*, of which we know now that they are opportunistic pathogens and therefore do not belong on the list. It is possible that this classification principle was not applied consistently when the list was drawn up and this should be revisited.

AIDS research has taught us that as a result of the immune system becoming increasingly compromised, patients are becoming more susceptible to opportunistic infections over the course of a progressive HIV-1 infection. It seems that there is a relationship between susceptibility to certain opportunistic infections and the CD4 T cell count. At relatively high CD4 count there is an increased susceptibility to some conditions (e.g. tuberculosis, Kaposi's sarcoma and oral candidiasis), while at extremely low CD4 count results there is increased susceptibility to others (e.g. a CMV infection). Thus, some opportunistic infections can therefore occur in relatively healthy people.

Although organisational measures (e.g. medical check-ups, vaccinations, training and supervision) are often taken, they are of course not an absolute guarantee that the worker will remain healthy. When affected by illness, stress, use of medicines, pregnancy or other factors, normally healthy workers can be more susceptible (temporarily or otherwise) to certain opportunistic pathogens that would normally not pose a risk to the health of these workers. Consequently, it may well be better to include on the European list as many biological agents as possible that are known for their ability to cause infectious diseases in humans irrespective of the status of the host's immune system. This information could also be added to the list as an additional annotation.

In this regard, the website of the Netherlands Association for Parasitology (*Nederlandse Vereniging voor Parasitologie* (NVP))[33] represents an excellent example. The site includes a summary of all known human parasites. There are definitely a few dozen parasites that are not included on the European list either individually by name or under a family name. Many of the parasites identified can infect humans asymptomatically, but only some will cause illness in people when their immune system is compromised to some extent.

5.5 Pregnancy

The Working Conditions Decree (AB) establishes a number of rules and regulations for pregnant women, who may be more susceptible to certain biological agents. There is an increased risk to the foetus in any event if the mother is exposed to pathogenic biological agents as part of her job. This is why several biological agents, including Toxoplasma and Rubellavirus, are the subject of specific “occupational bans” as they are a potential threat to pregnant women (AB, Article 4(109)).

Although the compulsory RI&E must also address the possibility of pregnant workers, there has been no thorough examination conducted of all the biological agents on the list to determine whether they could pose a heightened risk during and after pregnancy. The RIVM has set up a dedicated website on which it has posted valuable information on this subject. There are also several publications worth mentioning that also contain relevant information. There is an argument for adding this type of information to the list as an extra annotation.

5.6 Non-pathogenic biological agents

The scope of the European list is expressly limited to biological agents that can cause infectious diseases in humans, see also Introductory note 1, and the following: *"Biological agents which have not been classified for inclusion in groups 2 to 4 of the list are not implicitly classified in group 1. For agents where more than one species is known to be pathogenic to man, the list will include those species which are known to be the most frequently responsible for diseases, together with a more general reference to the fact that other species of the same genus may affect health. When a whole genus is mentioned in the classified list of biological agents, it is implicit that the species and strains known to be non-pathogenic are excluded".* [Introductory note 3].

"Member States are to ensure that all viruses which have already been isolated in humans and which have not been assessed and allocated in this Annex are classified in group 2 as a minimum, except where Member States have proof that they are unlikely to cause disease in humans" [Introductory note 7].

This is a clear case of the well-known phrase 'the absence of evidence is not necessarily evidence of absence'. Caution should be exercised if it has not been definitively determined that a certain biological agent cannot cause disease in humans. The possibility that the agent is a pathogen must definitely be taken into account when there are indirect indications of the existence of a related

[33] http://www.parasitologie.nl/index.php?id=12; Netherlands Society for Parasitology Parasite Fact Sheets: Human (*NVP Parasieten factsheets: Humaan*).

type or another species that is clearly pathogenic. In such a case, the agent should be classified in category 2 at least.

Although it is stated that non-pathogenic biological agents are implicitly excluded from classification, this is not strictly speaking true, because these agents are given the lowest classification according to the definition (i.e. risk group 1); this is a matter of semantics. It would be better to create a list or a database including all known biological agents, indicating which are pathogenic or non-pathogenic in humans (stating otherwise that this is information is not known). This would avoid any misunderstandings.

A good example is the aforementioned Annex I of the Dutch GMO Regulations (*Regeling GGO*): "Hosts (prokaryotes, yeasts and fungi) which are suitable for the production of GMOs and belong to group I".[34] The Netherlands Genetically Modified Organisms Agency (BGGO) maintains this overview of biological agents known to be non-pathogenic in humans, animals and plants. Work involving these biological agents can be performed without any risk to the health and safety of the workers. Of course, as stated earlier in this Report, the usual hygiene measures will continue to apply.

5.7 Attenuated strains

"Where a strain is attenuated or has lost known virulence genes, then the containment required by the classification of its parent strain need not necessarily apply, subject to assessment appropriate for risk in the workplace. This is the case, for example, when such a strain is to be used as a product or part of a product for prophylactic or therapeutic purposes" [Introductory note 4].

It is suggested here that the classification of the attenuated strain need not differ from that of its pathogenic main strain. Strict application of the classification definitions would usually allow a lower classification. It is indeed noticeable that the list does not state live attenuated vaccines separately. Only Bacillus Calmette-Guérin (BCG) is named separately. An attenuated strain of the human pathogen *Mycobacterium bovis*, BCG is used to vaccinate against tuberculosis. Sometimes live attenuated vaccines are not the product of natural selection, but rather genetic manipulation. Even then, it would still be a challenge to classify such a vaccine strain in the framework of the current list. The US-CDC list, however, specifically names several vaccines, including a few category-3 and category-4 viruses that have been attenuated to the extent that Biosafety Level 2 (BSL2) will suffice to achieve a safe and healthy workplace (see Table 3).

5.8 Toxin producing bacteria

Although several bacteria on the list are known to be capable of producing toxins, this information is not stated in each case (see Table 4). These toxin-producing strains – the toxins in particular – are often hazardous to humans. Notably, the strict definition of biological agents does not include toxins as they are not micro-organisms, although they cause toxicity (AB; Article 4.84(2)). It is unclear why some bacteria are classified as category 2 and

[34] http://bggo.rivm.nl/paginas/doc-reg.htm; the list is part of Article 3 of the GMO Regulations (*Regeling GGO*). Appendix I is based on the list kept by the Institute of Medical Microbiology, Immunology and Parasitology (IMMIP) in Bonn (Germany).

others as category 3. In general, there are effective vaccines against the toxins and antibiotics against the bacteria involved.

Table 3. Live attenuated viral vaccines

Virus	Vaccine strain	Category CDC list[35]	Category European list
Chikungunya virus	181/25	2	3
Junin virus	Candid #1	2	4
Rift Valley fever virus	MP-12	2	3
Venezuelan equine encephalomyelitis virus	TC83 & V3526	2	3
Yellow fever virus	17-D	2	3
Japanese B encephalitis virus	14-14-2	2	3

Table 4. Toxin-producing bacteria

Bacterium	Toxin	Classification
Bordetella pertussis	Yes; not on the list	2
Clostridium tetani	Yes	2
Clostridium botulinum	Yes	2
Clostridium perfringens	Yes; not on the list	2
Clostridium difficile	Yes; not on the list	2
Corynebacterium diphtheriae	Yes	2
Pseudomonas aeruginosa	Yes; not on the list	2
Staphylococcus aureus	Yes; not on the list	2
Streptococcus pneumoniae	Yes; not on the list	2
Streptococcus pyogenes	Yes; not on the list	2
Vibrio cholerae	Yes; not on the list	2
Bacillus anthracis	Yes; not on the list	3
Escherichia coli (e.g. O157:H7)	Yes	3
Shigella dysenteriae	Yes	3

5.9 Retroviruses

Of the Retroviridae, only HIV, SIV and HTLV are on the list. There are a great number of retroviruses, however, although the majority are probably not human pathogens. It is common knowledge that retroviruses can be transmitted

[35] BMBL, 5th Edition, Section VIII-F: Arboviruses and Related Zoonotic Viruses, Table 5. Vaccine Strains of BSL-3 and BSL-4 Viruses that May Be Handled as BSL-2.

between different hosts/species[36], and sometimes with very serious (e.g. fatal) consequences, with HIV-1 infection / AIDS) as a infamous example.

However, it is not inconceivable that people aquire asymptomatic infections with retroviruses as a result from working with infected laboratory animals, particularly involving non-human primates.[37] Co-infections with other retrovirsues, or other triggers, may reactivate endogenous viruses and result in disease. We note that the current European list pays insufficient attention to interactions between viruses and the potential associated risks, let alone the criteria or guidelines how to address this issue.

[36] J Denner 2007. Transspecies transmissions of retroviruses: new cases. Virology. 2007; 369:229-33.
[37] DG Baker. 1998. Natural Pathogens of Laboratory Mice, Rats, and Rabbits and Their Effects on Research. Clin. Microbiol. Rev. 11(2): 231-266.

6 Creation of new classifications

6.1 Definitions and weighing of criteria

"Pending Community classification Member States shall classify biological agents that are or may be a hazard to human health on the basis of the definition in the second paragraph of Article 2, points 2 to 4 (groups 2 to 4)."
(2000/54/EC, Article 18(2)).

When work specifically involves the use of biological agents, the compulsory hazard identification and risk assessment for biological agents (RIEBA) must always be performed. If no formal classification of the biological agent in question exist, Member States (which in the Dutch situation should be interpreted as 'employers') have the duty of establishing a provisional classification based on the definitions provided in Article 2. Once a classification has been established, the corresponding precautions follow. The greater the health risk, the more comprehensive the measures to ensure health and safety of the workers and the environment.

The EC Directive's classification, however, focuses exclusively on human pathogens and therefore not on non-pathogenic biological agents, animal or plant pathogens, GMOs or opportunistic pathogens. As stated earlier, this approach comes with limitations and unintended risks. Moreover, the classification system demands strict adherence to formal definitions and poses a number of challenges (see below).

For instance, the definitions in the EC Directive are not unequivocal. The individual factors and their impact (weight) in the classification are not entirely clear and are often difficult to quantify in practice. In addition, the exceptions granted are not always specifically named. There are also more situations conceivable in practice than are strictly speaking taken into account in the current system of classification definitions.

6.2 Introductory notes

The Introductory notes of Annex III of the EC Directive further outline the scope of the Community classifications. In theory, therefore, these notes could help to determine provisional classifications.

6.3 The current list as an example

Reviewing the current list of classifications could also help to understand how the definitions should be applied. However, the EC Directive's lack of any substantiation or explanation of the classifications is a significant drawback in thi srespect. Therefore one can only guess the reasons behind certain classifications.

6.4 Virulence is decisive in classification

The classification definitions take into account the following factors (Table 2):

1) An estimate of the biological agent's potential to cause disease in healthy workers *(virulence / pathogenicity)*;

2) An estimate of the biological agent's contagiousness (i.e. risk of spreading to the community) *(transmissibility)*;

3) Information as to whether any effective prophylaxis or therapy is available *(treatment).*

A general principle seems to be for virulence to be given greater weight in the classification definitions compared to transmissibility. For instance, the extremely virulent Ebola virus is actually not that infectious in terms of the risk it poses to the entire population. As regards transmissibility, the Ebola virus would be classified as either category 2 or category 3. The fact that it is currently listed as a category-4 biological agent is because of its virulence profile and the continued lack of effective prophylaxis or therapy.

The seasonal flu viruses have been classified as category 2, given the relatively mild clinical picture they generally produce. They should, however, be classified as category 3 given their high degree of transmissibility. The fact that Influenza viruses are listed as category 2 is based on their virulence profile and not – or to a lesser extent – on their transmissibility.

6.5 Limited risk of infection

"Certain biological agents classified in group 3, which are indicated in the appended list by two asterisks (**), may present a limited risk of infection for workers because they are not normally infectious by the airborne route. Member States shall assess the containment measures to be applied to such agents, taking account of the nature of specific activities in question and of the quantity of the agent involved, with a view to determining whether, in particular circumstances, some of these measures may be dispensed with" [Introductory note 8].

Some category-3 biological agents only pose a limited risk of infection to workers, because they are normally not transmitted by air (e.g. *Salmonella* Typhi and HIV-1) or because there is no transmission vector in the laboratory (e.g. Plasmodium falciparum). The European list includes 36 such biological agents, including 8 bacteria, 21 viruses and 7 parasites, but no fungi or yeasts. Member States (which in the Dutch situation should be interpreted as 'employers') are free to determine whether departures from the required containment measures are permissible, taking into account the nature of the work being performed, the quantity of biological agent involved and other risks. In this case, however, the classification itself is not adjusted.

6.6 Parasites

"The requirements as to containment consequent on the classification of parasites apply only to stages in the life cycle of the parasite in which it is liable to be infectious to humans at the workplace" [Introductory note 9].

As in the previous section, there is also a similar situation for a number of parasites. It should be noted, however, that the containment measures derived from the classification only pertain to the various stages of the parasite's life cycle that pose a risk of infection for workers.

The previous two examples therefore demonstrate that departures from individual containment measures are permissible when the worker's risk of infection is lower. However, the formal classifications themselves must not be changed. This again seems to suggest that virulence weighs more heavily in the classification definitions than the other factors (i.e. transmissibility and treatment).

6.7 Effective treatment

Although it should be the case according to the definitions, the availability of effective treatment and also resistance to antimicrobial agents are hardly taken into consideration at all in the final classification of biological agents.

In 1980, the WHO declared the global eradication of the Smallpox virus. The Smallpox vaccine played a major role in this achievement. If we apply the definitions, the Smallpox virus, which is fatal in approximately 30% of cases, would be classified as category 3 or category 4. As an effective vaccine is available, it should be classified as category 3. Now that the Smallpox virus has been eradicated and Smallpox vaccines are no longer administered, however, the virus has been classified as category 4. The same thing is expected to happen with the Poliovirus, which is currently listed as category 2, but will probably be changed to category 3 after it is eradicated. The current definitions make no provision for this type of biological agent in such exceptional circumstances.

According to the EC Directive's classification definitions, the availability of effective new prophylaxis or therapy should lead automatically to reclassification. However, this is not expected to happen quickly for high-risk pathogens. For instance, vaccines against several category-4 Viral Haemorrhagic Fevers (VHFs) are within reach (see also Table 4). Of course, vaccination does nothing to change the intrinsic virulence of these biological agents. Given the fatal consequences in a large number of cases of infections involving high-risk biological agents, it is assumed that the highest level of containment will continue to be enforced.

6.8 Resistance

The EC Directive's classification definitions also state that changes in resistance to antimicrobial agents should lead to a classification review. Strictly speaking, this would involve multi-drug, ‘extensively’ or totally resistant biological agents (e.g. MDR-TB and XDR-TB) being placed in a higher category. It is the current view, however, that the development of resistance does not by definition change the virulence of biological agents. Quite the opposite situation usually occurs, as the development of resistance to antibiotics generally comes at the expense of the biological agent's *‘fitness’* and virulence. Accordingly, resistance 'only' impacts on the outcome of infection and the possibility of treatment, not on the severity of the disease.

The conclusion is therefore that biological agents should be classified according to the clinical picture they can produce (i.e. virulence). The remaining criteria (transmissibility and treatment), circumstances and actions taken determine which additional containment measures are required.

6.9 Link to containment measures

Although the classification model is basically meant as a tool to help determine which containment measures are required, its application is not without challenges.

The 'automatic' correspondence of formal classification in categories 1 to 4 to containment levels BSL 1 to 4 seems a relatively simple model: the greater the risk, the more comprehensive the measures. Our conclusion, however, is that this model can only be used in practice if the link between risks and measures only serves as a starting point for the final package of containment measures and does not necessarily need to be applied in this way.

For instance, if we consider the definitions for category 2 and category 3, we can see that the primary difference is the degree of virulence and the risk of transmission among the population. However, the main differences between BSL2 and BSL3 laboratory facilities lie in the implementation of physical or technical containment measures (see Annex V of the EC Directive 2000/54; also Annex IV of EC Directive 2009/41). These measures are implemented without exception to prevent the spread of biological agents to the outside world (e.g. air extraction using HEPA filters). In short, the measures are designed to protect the people and the environment outside of the laboratory.

As the more organisational measures (e.g. safety procedures and techniques, vaccinations, training and the use of PPE) can be implemented at every level, no real distinction is made. There is consequently an argument in favour of microbiological laboratories always operating at BSL3 given the work performed with pathogenic biological agents that can be transmitted by air and in favour of that work being performed in biological safety cabinets ('flowkast').

6.10 Unknown risk of infection

If the biological agent under assessment cannot be clearly classified in one of the groups as defined in the Dutch Working Conditions Decree (AB Article 2(2)), it must be included among the alternatives in the highest-risk group (AB Article 18(3)).

It is difficult to imagine how this instruction can be followed during outbreaks involving high-risk pathogens. This instruction seems just as unachievable when the behaviour and properties of a biological agent (new or otherwise) are insufficiently known, because the highest-level containment measures (i.e. BLS4) will have to be implemented. That is not possible during a large-scale outbreak due to the limited availability of laboratory facilities offering such a high-level of containment.

This situation occurred in 2009 during the New Influenza virus A(H1N1) pandemic. The virulence of the virus was initially unclear. Initial reports were very unsettling and reminded people of previous flu pandemics, including the 1918-1919 Spanish flu pandemic which was caused by an A(H1N1) strain. BSL3

working conditions were at first maintained, though later BSL2 conditions for laboratory work (primarily diagnostic procedures) were permitted.

This can be attributed to the fact that the flu virus was included under the heading 'Influenza virus type A', which is classified in the European list as category 2. It would have otherwise been formally required to work under BSL4 conditions. The same applies to the outbreak of SARS Coronavirus in 2003, when the diagnostic laboratory work was performed under BSL3 conditions.

Finally, therefore, there is an argument in favour of the establishment of a single authoritative national or European list or database, addressing both work with wild-type biological agents and GMOs. This system would compile information on both human pathogens and harmless microbes, including opportunistic pathogens, as well as the well known animal and plant pathogens that are a potential risk to human health.

In addition, given its current less-than-optimal performance, there is a need for greater nuance between classification (i.e. risk assessment) and (bio)containment / biosafety level (i.e. containment measures). More attention should be focused on the risks and gearing measures accordingly. For instance, measures should be implemented when working with pathogens that are transmitted by air in order to prevent or reduce this risk. Other more appropriate measures should be implemented for agents that have other means of transmission. All laboratories should have a basic set of equipment and facilities, as well as supplementary equipment and facilities according to the risks encountered, the biological agents used and the procedures performed. The (bio)containment / biosafety levels are therefore the minimum requirements.

7 Risk management

Risk management consists of a hazard identification and risk assessment (RI&E) and effective measures to control risks. As stated previously, the classifications serve as a tool to determine quickly the health risk associated with working with biological agents and the corresponding control measures. In practice there is the need for easy and readily accessible information about (emerging) biological agents. Moreover, there is a growing need for effective standardised and customised measures which are rooted in evidence-based research (i.e. scientific/semi-scientific) research.

7.1 Fact sheets

In an ideal world, a centrally managed file would be maintained on every biological agent, containing all relevant information and including as much quantitative data as possible, structured according to a standard system and used to arrive at an objective and evidence-based substantiation of the risks involved. This, however, is not always the case in practice.

The website of RIVM's National Coordination Centre for Outbreak Management (*Landelijke Coördinatie Infectieziektebestrijding* (LCI)) includes directives, contingency plans and other relevant information for a large number of infectious diseases and biological agents.[38] Elsewhere online, there are various examples of standardised safety fact sheets. These are excellent sources on which to base a sound risk assessment. Other examples include the website of the Netherlands Society for Parasitology (*Nederlandse Vereniging voor Parasitologie* (NVP))[39] which offers fact sheets for both human parasites (pathogenic and non-pathogenic) and animal parasites. In addition, the Material Safety Data Sheets (MSDS) for Infectious Substances of the Public Health Agency of Canada (PHAC)[40] demonstrate wonderfully how relevant information can be compiled and presented.

These fact sheets provide information on biological agents, the associated health risks and medical aspects (including diagnosis and treatment), infectivity and transmissibility (including vectors and zoonoses), risks specific to the laboratory environment, high-risk work tasks and sensitivity to antimicrobial agents and biocides (disinfectants). Combined with an inventory of the tasks posing a risk when working with biological agents and other issues, including the quantity of agent involved and the duration and frequency of possible exposure to a biological agent, it is possible to make a sound estimate of the risks associated with working with biological agents.

7.2 Risk management

After the hazards have been identified, the available data must be processed and a risk assessment conducted to select the most effective containment measures. This process is generally not quantitative, but more based on intuition

[38] http://www.rivm.nl/cib/infectieziekten-A-Z/infectieziekten
[39] http://www.parasitologie.nl/index.php?id=12
[40] http://www.phac-aspc.gc.ca/msds-ftss

and subjective opinion. This can clearly be improved by implementing a more structured approach.

This raises another important point. First, pursuant to legislation on biological agents, a step-by-step plan was developed in the year 2000 for identifying and assessing risks pertaining to biological agents (RIEBA) in the healthcare sector – hospitals in particular.[41] This was the result of an initiative by the Safety Professionals in Healthy Care (*Veiligheidskundigen in de Gezondheidszorg* (VIG)) group of the Dutch Association of Safety Professionals (*Nederlandse Vereniging voor Veiligheidskunde* (NVVK)) and the liaison group Dutch Occupational Hygiene Society (*Nederlandse Vereniging voor Arbeidshygiëne* (NVvA)). More useful information can also be found in other Working Conditions Fact Sheets (*Arbo-Informatiebladen*) about risk assessment which may also apply to working with biological agents.[42]

Sandia National Laboratories recently developed a more standardised model for mapping out relative risks using a systematic and reproducible method.[43] This model produces more objective and semi-quantitative estimates of the risks associated with exposure to biological agents. The role played by the different risk factors, the scoring and their respective relative weight was developed in part using the expertise of biosafety professionals from around the world. Our conclusion is too that classification is only really worthwhile if developed using a multidisciplinary approach and if it can be verified independently.

7.3 Diagnostics

An integral aspect of the risk management of biological agents is the capacity to conduct laboratory diagnostics. This is relevant for regular control of infectious diseases and treatment of patients, but also during outbreaks. It is also vital for those working with biological agents. In case of laboratory incidents involving exposure (e.g. needle injuries) or what are referred to as 'silent infections' where the worker only reports that he or she is ill after some time has passed (apparently without any link to a specific incident), it is essential to determine whether the transmission or infection was actually related to laboratory work involving biological agents.

In 2007, the RIVM published an overview of microbiological laboratories in the Netherlands with the facilities to conduct laboratory diagnostics for certain biological agents. This Memorandum on Pathogens (*Pathogenennota*) sets out who is doing what and where as regards laboratory diagnostics for infectious diseases.[44] Despite its focus on regular laboratory diagnostics as they relate to fighting infectious diseases, it remains a valuable source in managing the associated risks when specifically working with biological agents.

[41] See AI-9 (Biological Agents), Section 6: Hazard identification and risk assessment (RI&E). Section 6.4: Expert opinion on health risks (*Deskundig oordeel over de gezondheidsrisico's*). See also Report by Tauw bv. on 'Biological agents in the cleaning sector and commercial linen hire, laundry and dry cleaning sector' (*Biologische agentia binnen de schoonmaak- en reinigingsbranche en de linnenverhuur- wasserij- en textielreinigersbranche*), 13 June 2003.

[42] See AI-45 (Risk management: task risk analysis, personal risk analysis, work permit regime) (*Risicobeheersing: taakrisicoanalyse, persoonlijke risicoanalyse, werkvergunningsysteem*); http://sdukennisportaal.rijksweb.nl/arbozone/11416/risicobeheersing-taakrisicoanalyse-persoonlijke-risicoanalyse-werkvergunningsysteem.

[43] http://www.sandia.gov/ram/BIORAM.htm

[44] Katchaki J, Kortbeek LM, Notermans DW (2008); An inventory of the laboratory diagnostics of micro-organisms that can impact public health (*Een inventarisatie van laboratoriumdiagnostiek van volksgezondheids-relevante micro-organismen*). Update 2007. RIVM report no. 230071001.

8 Classification procedures

As stated previously, Annex III of the EC Directive is the only authoritative list of classifications in the Netherlands. If work is performed specifically involving pathogenic biological agents that fall outside of the formal classification framework, Member States (i.e. employers in the Netherlands) bear responsibility for establishing a provisional classification.

8.1 Expert opinion and the latest scientific and technical knowledge

The Committee on Genetic Modification (COGEM) recently started several projects to more systematically map out the classifications of bacteria, yeasts and fungi, viruses and parasites. The available international lists of classifications are compared to the European list, as well as other lists already used by COGEM and the GMO Agency (BGGO). During the writing of this report, reports on classifications of fungi, bacteria and parasites were published on the COGEM website. With these projects completed (only a revised list with virsus is currently lacking), the Netherlands has a high-quality database of classifications reflecting the current state of scientific and technical knowledge, as well as an expert consensus on the classifications of biological agents.[45]

8.2 Classification during an outbreak

An unusual situation arises when an outbreak of a new infectious disease occurs and the biological agent involved has not been given a Community classification. A provisional classification must be drafted by the employer to facilitate the implementation of effective protective measures for workers who specifically or indirectly use the agent in question.

If there is a threat of an epidemic, the RIVM's Centre for Infectious Disease Control (CIb) convenes and chairs a multidisciplinary group of experts known as the Outbreak Management Team (OMT). The group's task is to offer the Minister of Health, Welfare and Sport (VWS) expert advice on ways to fight the infectious disease. The CIb advises the Minister indirectly via the Administrative Consultative Committee (BAO), which is convened on a case-by-case basis in line with the OMT. The OMT could include the classification of the biological agent as a recurring item on its agenda during an outbreak because the relevant professionals are brought together in the OMT, including the Chair of the Working Conditions Management Team (*Arbo Management Team*). A Biosafety Officer (*Bioveiligheidsfunctionaris* (BVF)) could be invited, when required, to work out and propose classifications in cooperation with the microbiologists/clinical microbiologists. These classifications should be seen as expert opinion, but do not represent formal classifications like the ones on the European list. However, they will help employers to conduct their own risk assessments for work involving a new biological agent. It would be an 'elegant' situation if the various professional groups of microbiologists/clinical

[45] Classification of 1) Fungi and Yeasts, 2) Bacteria, and 3) Parasites (respectively):
1) www.cogem.net/index.cfm/nl/publicaties/publicatie/classificatie-humaan-en-dierpathogene-fungi
2) ../publicaties/publicatie/classification-of-bacterial-pathogens
3) ../publicaties/publicatie/advies-classificatie-humaan-en-dierpathogene-parasieten
(Note, at this point is unknown whether the classifications of human pathogenic viruses will be revised).

microbiologists[46] were to support the OMT's recommendation in order to generate as much support as possible, but it is still up to each individual employer to decide whether or not to adopt the advice.

If there is a need to revise the classification after (or during) an outbreak, it then makes sense to consult the same group of professionals who also participated in the OMT during the previous discussions about classification.

We propose publishing the OMT's recommendations on the classification of emerging biological agents, for instance on the websites of the CIb and the NVvM and possibly to spread the information via the LabInf@ct message service.[47] The information will then reach especially the professional groups involved in fighting infectious diseases in the Netherlands. In addition, avenues should be sought to inform and support employers in other sectors, particularly in situations involving possible exposure resulting from work that indirectly involves biological agents (e.g. via the website of the RIVM Centrum Gezond Leven, Loketgezondleven.nl or via the Arbo-Inf@ct newsletter).[48]

In summary, employers are responsible for a provisional classification for biological agents that fall outside the formal European classification framework. A (national) database, containing all available information on biological agents, should be established in order to improve the quality of this process and to support employers. The relevant information should be structured in a clear manner in the form of standardised fact sheets. Risk analysis procedures should be more structured, involving an objective – preferably quantitative – method in which the different risk factors are given scores and weighted in relation to each other. Finally, the containment measures should be evidence-based and should be accompanied by a clear explanation of the reasons behind them and the intended objective.[49]

8.3 Procedure to change formal classifications

"Purely technical adjustments to the Annexes in the light of technical progress, changes in international regulations or specifications and new findings in the field of biological agents shall be adopted in accordance with the procedure laid down in Article 17 of Directive 89/391/EEC" (2000/54/EC, Article 19).

The European list of Community classifications of biological agents can only be revised as part of a formal procedure in which the European Commission is assisted by a Committee of representatives from all Member States which is chaired by a Commission representative.

It makes sense to expect that changing, revising and expanding Annex III of the EC Directive would require a tremendous effort on the part of all Member States. Any revision of the classification definitions and other guidelines for drafting the list (see 'Scope of the EC Directive') will likewise be a time-consuming process. The dilemma posed by a list is that it is always a snapshot frozen in time. The list is by definition already outdated when it is adopted.

[46] NVvM, NVMM and NVP.
[47] As part of Inf@ct, the Labinf@ct information service targets clinical microbiologists, virologists working in a clinic and molecular biologists affiliated with a medical microbiology laboratory.
[48] http://toolkits.loketgezondleven.nl/infectieziekten
[49] Kimman TG, Smit E, Klein MR. Evidence-based biosafety: a review of the principles and effectiveness of microbiological containment measures. Clin Microbiol Rev. 2008; 21:403-25.

We recommend separating the Annexes from the main body of the EC Directive in order to make it possible to review and update the annexes on a more frequent basis. This would help to achieve the intention of the EC Directive, namely to ensure that the list of classifications always reflects the latest scientific and technical knowledge. There is sufficient information online and there are sufficient interactive tools available to establish a central database of information on biological agents, including the most recent classifications. The management of such a source of information could, for instance, be the responsibility of an organisation such as the European Centre of Disease Prevention and Control (ECDC)[50] and/or the European Agency for Safety and Health at Work (EU-OSHA) in Bilbao (Spain).[51]

[50] ECDC: http://www.ecdc.europa.eu
[51] EU-OSHA: http://osha.europa.eu/nl

Annex

European Directive 2000/54/EC ANNEX III, “Community Classification” with *Introductory notes* and classifications of bacteria, viruses, parasites and fungi.

ANNEX III

COMMUNITY CLASSIFICATION

Article 2, second paragraph, and Article 18

INTRODUCTORY NOTES

1. In line with the scope of the Directive, only agents which are known to infect humans are to be included in the classified list.

   Where appropriate, indicators are given of the toxic and allergic potential of these agents.

   Animal and plant pathogens which are known not to affect man are excluded.

   In drawing up this list of classified biological agents consideration has not been given to genetically modified micro-organisms.

2. The list of classified agents is based on the effect of those agents on healthy workers.

   No specific account is taken of particular effects on those whose susceptibility may be affected for one or other reason such as pre-existing disease, medication, compromised immunity, pregnancy or breast feeding.

   Additional risk to such workers should be considered as part of the risk assessment required by the Directive.

   In certain industrial processes, certain laboratory work or certain work with animals involving actual or potential exposure to biological agents of groups 3 or 4, any technical precautions taken must comply with Article 16 of the Directive.

3. Biological agents which have not been classified for inclusion in groups 2 to 4 of the list are not implicitly classified in group 1.

   For agents where more than one species is known to be pathogenic to man, the list will include those species which are known to be the most frequently responsible for diseases, together with a more general reference to the fact that other species of the same genus may affect health.

   When a whole genus is mentioned in the classified list of biological agents, it is implicit that the species and strains known to be non-pathogenic are excluded.

4. Where a strain is attenuated or has lost known virulence genes, then the containment required by the classification of its parent strain need not necessarily apply, subject to assessment appropriate for risk in the workplace.

   This is the case, for example, when such a strain is to be used as a product or part of a product for prophylactic or therapeutic purposes.

5. The nomenclature of classified agents used to establish this list reflects and is in conformity with the latest international agreements of the taxonomy and nomenclature of agents at the time the list was prepared.

6. The list of classified biological agents reflects the state of knowledge at the time that it was devised.

   It will be updated as soon as it no longer reflects the latest state of knowledge.

7. Member States are to ensure that all viruses which have already been isolated in humains and which have not been assessed and allocated in this Annex are classified in group 2 as a minimum, except where Member States have proof that they are unlikely to cause disease in humans.

8. Certain biological agents classified in group 3 which are indicated in the appended list by *two asterisks (**)*, may present a limited risk of infection for workers because they are not normally infectious by the airborne route.

   Member States shall assess the containment measures to be applied to such agents, taking account of the nature of specific activities in question and of the quantity of the agent involved, with a view to determining whether, in particular circumstances, some of these measures may be dispensed with.

9. The requirements as to containment consequent on the classification of parasites apply only to stages in the life cycle of the parasite in which it is liable to be infectious to humans at the workplace.

10. This list also gives a separate indication in cases where the biological agents are likely to cause allergic or toxic reactions, where an effective vaccine is available, or wher it is advisable to keep a list of exposed workers for more than 10 years.

These indications are shown by the following letters:

A: Possible allergic effects

D: List of workers exposed to this biological agent to be kept for more than 10 years after the end of last known exposure

T: Toxin production

V: Effective vaccine available

The application of preventive vaccination should take account of the code of practice given in Annex VII.

BACTERIA

and similar organisms

NB: For biological agents appearing on this list, 'spp.' refers to other species which are known pathogens in humans.

Biological agent	Classification	Notes
Actinobacillus actinomycetemcomitans	2	
Actinomadura madurae	2	
Actinomadura pelletieri	2	
Actinomyces gerencseriae	2	
Actinomyces israelii	2	
Actinomyces pyogenes	2	
Actinomyces spp.	2	
Arcanobacterium haemolyticum (Corynebacterium haenolyticum)	2	
Bacillus anthracis	3	
Bacteroides fragilis	2	
Bartonella bacillifonnis	2	
Bartonella quintana (Rochalimaea quintana)	2	
Bartonella (Rochalinea) spp.	2	
Bordetella bronchiseptica	2	
Bordetella parapertussis	2	
Bordetella pertussis	2	V
Borrelia burgdorferi	2	
Borrelia duttonii	2	
Borrelia recurrentis	2	
Borrelia spp.	2	
Brucella abortus	3	
Brucella canis	3	
Brucella melitensis	3	
Brucella suis	3	
Burkholderia mallei (Pseudomonas mallei)	3	
Burkholderia pseudomallei (Pseudomonas pseudomallei)	3	
Campylobacter fetus	2	
Campylobacter jejuni	2	
Campylobacter spp.	2	
Cardiobacterium hominis	2	
Chlamydia pneumoniae	2	
Chlamydia trachomatis	2	
Chlamydia psittaci (avian strains)	3	
Chlamydia psittaci (other strains)	2	
Clostridium botulinum	2	T
Clostridium perfringens	2	
Clostridium tetani	2	T, V
Clostridium spp.	2	
Corynebacterium diphtheriae	2	T, V
Corynebacterium minutissimum	2	
Corynebacterium pseudotuberculosis	2	
Corynebacterium spp.	2	
Coxiella burnetii	3	
Edwardsiella tarda	2	
Ehrlichia sennetsu (Rickettsia sennetsu)	2	
Ehrlichia spp.	2	
Eikenella corrodens	2	

Biological agent	Classification	Notes
Enterobacter aerogenes/cloacae	2	
Enterobacter spp.	2	
Enterococcus spp.	2	
Erysipelothrix rhusiopathiae	2	
Escherichia coli (with the exception of non-pathogenic strains)	2	
Escherichia coli, verocytotoxigenic strains (e.g. O157:H7 or O103)	3 (**)	
Flavobacterium meningosepticum	2	
Fluoribacter bozemanae (Legionella)	2	
Francisella tularensis (Type A)	3	
Francisella tularensis (Type B)	2	
Fusobacterium necrophorum	2	
Gardnerella vaginalis	2	
Haemophilus ducreyi	2	
Haemophilus influenzae	2	
Haemophilus spp.	2	
Helicobacter pylori	2	
Klebsiella oxytoca	2	
Klebsiella pneumoniae	2	
Klebsiella spp.	2	
Legionella pneumophila	2	
Legionella spp.	2	
Leptospira interrogans (all serovars)	2	
Listeria monocytogenes	2	
Listeria ivanovii	2	
Morganella morganii	2	
Mycobacterium africanum	3	V
Mycobacterium avium/intracellulare	2	
Mycobacterium bovis (except BCG strain)	3	V
Mycobacterium chelonae	2	
Mycobacterium fortuitum	2	
Mycobacterium kansasii	2	
Mycobacterium leprae	3	
Mycobacterium malmoense	2	
Mycobacterium marinum	2	
Mycobacterium microti	3 (**)	
Mycobacterium paratuberculosis	2	
Mycobacterium scrofulaceum	2	
Mycobacterium simiae	2	
Mycobacterium szulgai	2	
Mycobacterium tuberculosis	3	V
Mycobacterium ulcerans	3 (**)	
Mycobacterium xenopi	2	
Mycoplasma caviae	2	
Mycoplasma hominis	2	
Mycoplasma pneumoniae	2	
Neisseria gonorrhoeae	2	
Neisseria meningitidis	2	V
Nocardia asteroides	2	
Nocardia brasiliensis	2	
Nocardia farcinica	2	
Nocardia nova	2	

Biological agent	Classification	Notes
Nocardia otitidiscaviarum	2	
Pasteurella multocida	2	
Pasteurella spp.	2	
Peptostreptococcus anaerobius	2	
Plesiomonas shigelloides	2	
Porphyromonas spp.	2	
Prevotella spp.	2	
Proteus mirabilis	2	
Proteus penneri	2	
Proteus vulgaris	2	
Providencia alcalifaciens	2	
Providencia rettgeri	2	
Providencia spp.	2	
Pseudomonas aeruginosa	2	
Rhodococcus equi	2	
Rickettsia akari	3 (**)	
Rickettsia canada	3 (**)	
Rickettsia conorii	3	
Rickettsia montana	3 (**)	
Rickettsia typhi (Rickettsia mooseri)	3	
Rickettsia prowazekii	3	
Rickettsia rickettsii	3	
Rickettsia tsutsugamushi	3	
Rickettsia spp.	2	
Salmonella arizonae	2	
Salmonella enteritidis	2	
Salmonella typhimurium	2	
Salmonella paratyphi A, B, C	2	V
Salmonella typhi	3 (**)	V
Salmonella (other serovars)	2	
Serpulina spp.	2	
Shigella boydii	2	
Shigella dysenteriae (Type 1)	3 (**)	T
Shigella dysenteriae, other than Type 1	2	
Shigella flexneri	2	
Shigella sonnei	2	
Staphylococcus aureus	2	
Streptobacillus moniliformis	2	
Streptococcus pneumoniae	2	
Streptococcus pyogenes	2	
Streptococcus suis	2	
Streptococcus spp.	2	
Treponema carateum	2	
Treponema pallidum	2	
Treponema pertenue	2	
Treponema spp.	2	
Vibrio cholerae (including El Tor)	2	
Vibrio parahaemolyticus	2	
Vibrio spp.	2	
Yersinia enterocolitica	2	
Yersinia pestis	3	V
Yersinia pseudotuberculosis	2	
Yersinia spp.	2	

(**) See paragraph 8 of the introductory notes.

VIRUSES (*)

Biological agent	Classification	Notes
Adenoviridae	2	
Arenaviridae		
LCM-Lassa-virus complex (old world arena viruses):		
Lassa virus	4	
Lymphocytic (strains)	3	
Lymphocytic choriomeningitis virus (other strains)	2	
Mopeia virus	2	
Other LCM-Lassa complex viruses	2	
Tacaribe-Virus-complex (new world arena viruses):		
Guanarito virus	4	
Junin virus	4	
Sabia virus	4	
Machupo virus	4	
Flexal virus	3	
Other Tacaribe complex viruses	2	
Astroviridae	2	
Bunyaviridae		
Belgrade (also known as Dobrava)	3	
Bhanja	2	
Bunyamwera virus	2	
Germiston	2	
Oropouche virus	3	
Sin Nombre (formerly Muerto Canyon)	3	
California encephalitis virus	2	
Hantaviruses:		
Hantaan (Korean haemorrhagic fever)	3	
Seoul virus	3	
Puumala virus	2	
Prospect Hill virus	2	
Other hantaviruses	2	
Nairoviruses:		
Crimean-Congo haemorrhagic fever	4	
Hazara virus	2	
Phleboviruses:		
Rift Valley fever	3	V
Sandfly fever	2	
Toscana virus	2	
Other *bunyaviridae* known to be pathogenic	2	
Caliciviridae		
Hepatitis E virus	3 (**)	
Norwalk virus	2	
Other *Caliciviridae*	2	
Coronaviridae	2	
Filoviridae		
Ebola virus	4	
Marburg virus	4	
Flaviviridae		
Australia encephalitis (Murray Valley encephalitis)	3	
Central European tick-borne encephalitis virus	3 (**)	V
Absettarov	3	
Hanzalova	3	
Hypr	3	
Kumlinge	3	
Dengue virus type 1-4	3	
Hepatitis C virus	3 (**)	D

Biological agent	Classification	Notes
Hepatitis G virus	3 (**)	D
Japanese B encephalitis	3	V
Kyasanur Forest	3	V
Louping ill	3 (**)	
Omsk (a)	3	V
Powassan	3	
Rocio	3	
Russian spring-summer encephalitis (TBE) (a)	3	V
St Louis encephalitis	3	
Wesselsbron virus	3 (**)	
West Nile fever virus	3	
Yellow fever	3	V
Other flaviviruses known to be pathogenic	2	
Hepadnaviridae		
Hepatitis B virus	3 (**)	V, D
Hepatitis D virus (Delta) (b)	3 (**)	V, D
Herpesviridae		
Cytomegalovirus	2	
Epstein-Barr virus	2	
Herpesvirus simiae (B virus)	3	
Herpes simplex virus types 1 and 2	2	
Herpesvirus varicella-zoster	2	
Human B-lymphotropic virus (HBLV-HHV6)	2	
Human herpes virus 7	2	
Human herpes virus 8	2	D
Orthomyxoviridae		
Influenza viruses types A, B and C	2	V (c)
Tick-borne *orthomyxoviridae*: Dhori and Thogoto	2	
Papovaviridae		
BK and JC viruses	2	D (d)
Human papillomaviruses	2	D (d)
Paramyxoviridae		
Measles virus	2	V
Mumps virus	2	V
Newcastle disease virus	2	
Parainfluenza viruses types 1 to 4	2	
Respiratory syncytial virus	2	
Parvoviridae		
Human parvovirus (B 19)	2	
Picomaviridae		
Acute haemorrhagic conjunctivitis virus (AHC)	2	
Coxsackie viruses	2	
Echo viruses	2	
Hepatitis A virus (human enterovirus type 72)	2	V
Polioviruses	2	V
Rhinoviruses	2	
Poxviridae		
Buffalopox virus (e)	2	
Cowpox virus	2	
Elephantpox virus (f)	2	
Milkers' node virus	2	
Molluscum contagiosum virus	2	
Monkeypox virus	3	V
Orf virus	2	
Rabbitpox virus (g)	2	
Vaccinia virus	2	
Variola (major and minor) virus	4	V

Biological agent	Classification	Notes
Whitepox virus ('*Variola* virus')	4	V
Yatapox virus (Tana & Yaba)	2	
Reoviridae		
Coltivirus	2	
Human rotaviruses	2	
Orbiviruses	2	
Reuviruses	2	
Retroviridae		
Human immunodeficiency viruses	3 (**)	D
Human T-cell lymphotropic viruses (HTLV), types 1 and 2	3 (**)	D
SIV (h)	3 (**)	
Rhabdoviridae		
Rabies virus	3 (**)	V
Vesicular stomatitis virus	2	
Togaviridae		
Alphaviruses		
Eastern equine encephalomyelitis	3	V
Bebaru virus	2	
Chikungunya virus	3 (**)	
Everglades virus	3 (**)	
Mayaro virus	3	
Mucambo virus	3 (**)	
Ndumu virus	3	
O'nyong-nyong virus	2	
Ross River virus	2	
Semliki Forest virus	2	
Sindbis virus	2	
Tonate virus	3 (**)	
Venezuelan equine encephalomyelitis	3	V
Western equine encephalomyelitis	3	V
Other known alphaviruses	2	
Rubivirus (rubella)	2	V
Toroviridae	2	
Unclassified viruses		
Equine morbillivirus	4	
Hepatitis viruses not yet identified	3 (**)	D
Unconventional agents associated with the transmissible spongiform encephalopathies (TSEs)		
Creutzfeldt-Jakob disease	3 (**)	D (d)
Variant Creutzfeldt-Jakob disease	3 (**)	D (d)
Bovine spongiform encephalopathy (BSE) and other related animal TSEs (i)	3 (**)	D (d)
Gerstmann-Sträussler-Scheinker syndrome	3 (**)	D (d)
Kuru	3 (**)	D (d)

(*) See paragraph 7 of the introductory notes.
(**) See paragraph 8 of the introductory notes.
(a) Tick-borne encephalitis.
(b) Hepatitis D virus is pathogenic in workers only in the presence of simultaneous or secondary infection caused by hepatitis B virus. Vaccination against hepatitis B virus will therefore protect workers who are not affected by hepatitis B virus against hepatitis D virus (Delta).
(c) Only for types A and B.
(d) Recommended for work involving direct contact with these agents.
(e) Two viruses are identified: one a buffalopox type and the other a variant of the Vaccinia virus.
(f) Variant of cowpox virus.
(g) Variant of Vaccinia.
(h) At present there is no evidence of disease in humans caused by the other retroviruses of simian origin. As a precaution containment level 3 is recommended for work with them.
(i) There is no evidence in humans of infections caused by the agents responsible for other animal TSEs. Nevertheless, the containment measures for agents categorised in risk group 3 (**) are recommended as a precaution for laboratory work, except for laboratory work relating to an identified agent of scrapie where containment level 2 is sufficient.

PARASITES

Biological agent	Classification	Notes
Acanthamoeba castellani	2	
Ancylostoma duodenale	2	
Angiostrongylus cantonensis	2	
Angiostrongylus costaricensis	2	
Ascaris lumbricoides	2	A
Ascaris suum	2	A
Babesia divergens	2	
Babesia microti	2	
Balantidium coli	2	
Brugia malayi	2	
Brugia pahangi	2	
Capillaria philippinensis	2	
Capillaria spp.	2	
Clonorchis sinensis	2	
Clonorchis viverrini	2	
Cryptosporidium parvum	2	
Cryptosporidium spp.	2	
Cyclospora cayetanensis	2	
Dipetalonema streptocerca	2	
Diphyllobothrium latum	2	
Dracunculus medinensis	2	
Echinococcus granulosus	3 (**)	
Echinococcus multilocularis	3 (**)	
Echinococcus vogeli	3 (**)	
Entamoeba histolytica	2	
Fasciola gigantica	2	
Fasciola hepatica	2	
Fasciolopsis buski	2	
Giardia lamblia (Giardia intestinalis)	2	
Hymenolepis diminuta	2	
Hymenolepis nana	2	
Leishmania brasiliensis	3 (**)	
Leishmania donovani	3 (**)	
Leishmania ethiopica	2	
Leishmania mexicana	2	
Leishmania peruviana	2	
Leishmania tropica	2	
Leishmania major	2	
Leishmania spp.	2	
Loa loa	2	
Mansonella ozzardi	2	
Mansonella perstans	2	
Naegleria fowleri	3	
Necator americanus	2	
Onchocerca volvulus	2	
Opisthorchis felineus	2	
Opisthorchis spp.	2	
Paragonimus westermani	2	

Biological agent	Classification	Notes
Plasmodium falciparum	3 (**)	
Plasmodium spp. (human and simian)	2	
Sarcocystis suihominis	2	
Schistosoma haematobium	2	
Schistosoma intercalatum	2	
Schistosoma japonicum	2	
Schistosoma mansoni	2	
Schistosoma mekongi	2	
Strongyloides stercoralis	2	
Strongyloides spp.	2	
Taenia saginata	2	
Taenia solium	3 (**)	
Toxocara canis	2	
Toxoplasma gondii	2	
Trichinella spiralis	2	
Trichuris trichiura	2	
Trypanosoma brucei brucei	2	
Trypanosoma brucei gambiense	2	
Trypanosoma brucei rhodesiense	3 (**)	
Trypanosoma cruzi	3	
Wuchereria bancrofti	2	

(**) See paragraph 8 of the introductory notes.

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Biological agent	Classification	Notes
Aspergillus fumigatus	2	A
Blastomyces dermatitidis (Ajellomyces dermatitidis)	3	
Candida albicans	2	A
Candida tropicalis	2	
Cladophialophora bantiana (formerly: *Xylohypha bantiana, Cladosporium bantianum* or *trichoides*)	3	
Coccidioides inunitis	3	A
Cryptococcus neoformans var. *neofonnans (Filobasidiella neofonnans* var. *neofonnans)*	2	A
Cryptococcus neoformans var. *gattii (Filobasidiella bacillispora)*	2	A
Emmonsia parva var. *parva*	2	
Emmonsia parva var. *crescens*	2	
Epidermophyton floccosum	2	A
Fonsecaea compacta	2	
Fonsecaea pedrosoi	2	
Histoplasma capsulatum var. *capsulatum (Ajellomyces capsulatus)*	3	
Histoplasma capsulatum duboisii	3	
Madurella grisea	2	
Madurella mycetomatis	2	
Microsporum spp.	2	A
Neotestudina rosatii	2	
Paracoccidioides brasiliensis	3	
Penicillium marneffei	2	A
Scedosporium apiospermum (Pseudallescheria boydii)	2	
Scedosporium prolificans (inflatum)	2	
Sporothrix schenckii	2	
Trichophyton rubrum	2	
Trichophyton spp.	2	

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