CHAPTER 4

CLASSIFICATION OF BIOLOGICAL AGENTS

ACCORDING TO RISK

4.1 GENERAL

Judgements of the inherent risks of a pathogen are made on the basis of such factors as the severity of the disease it causes, the routes of infection, its virulence and infectivity. This judgement should take into account the existence of effective therapies (e.g. antibiotic resistance), immunization, the presence (or absence) of vectors, quantity of agent and whether the agent is indigenous to Canada as well as possible effects on other species, including plants and animals. Emerging pathogens and novel agents, because of their unknown characteristics, may require specialized practices and procedures for handling.

With these factors as the prime consideration, biological agents are classified according to risk groups which are analogous to the levels of containment described in section 4.6. These classifications presume ordinary circumstances in the research laboratory, or growth in small volumes for diagnostic and experimental purposes.

The classifications of biological agents primarily reflect the judgements made on their inherent risks. Section 4.5 lists the general criteria. The agents listed in Section 4.6 were chosen because they seem to be frequently used in Canada. Agents not listed should be classified on the basis of similarity to those listed; risk groups for others will be identified on request to the Office of Laboratory Security, Laboratory Centre for Disease Control.

4.2 RECOMBINANT DNA AND GENETIC MANIPULATION

Genetic methods such as natural selection, cross breeding, conjugation and transformation have been used for many years to change biological species and organisms. These methods have recently been supplemented by newer and much more efficient ones, of which the best known are the techniques of recombinant DNA. This technology allows scientists to transfer genes between unrelated organisms and species, and has spawned the recent surge in biotechnology.

The initial fear of possible risks arising from organisms altered by this technology led Canada, the United States and Great Britain, among other countries, to develop stringent biosafety guidelines. Experience rapidly showed that the initial fears were not justified. By 1980, many of the containment requirements of 1975-1977 had been removed.
Guidance in how to assess potential risks in recombinant DNA research can only be very general; each case needs individual assessment. It is not realistic to try to define in advance all of the possible genetically engineered organisms which might be created or used in the laboratory. The vast majority of this research involves only the remotest possibility of creating a hazard because the source of the DNA being transferred, the vector and the host are all innocuous. However, some genetic manipulation does raise significant possibility of risk. In general, if none of the components of the genetic manipulation presents any known hazard, and none can be reasonably foreseen in their combination, then no biohazard restrictions are needed. If one of the components of the reaction is hazardous, then, in general, discussion of the containment level required should start at the level appropriate to the known hazard. Its containment level might be increased or decreased according to such considerations as: the particular gene being transferred; the expression of the gene in the recombinant organism; the biological containment offered by the host vector system; the envisaged interactions between the gene being transferred and the host vector system; and other such factors. In any research with genes coding for hazardous products, host vector systems of limited ability to survive outside the laboratory (i.e. offering biological containment) should be used; their use will reduce the level of containment required.

### 4.3 USE OF MAMMALIAN CELLS IN CULTURE

The biological hazards of mammalian cells arise from the possibility that they might contain or transmit infectious agents. It is prudent to consider all cell lines to be potentially infectious. Cells known or suspected to contain such agents, or primary cultures from animals and humans known or reasonably suspected to be infected, should be in the risk group for the suspected agent. Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all samples of human tissues and fluids, all primate tissue, all cell lines new to the laboratory (until proven to be free of adventitious agents), all virus-containing primate cell lines, and all mycoplasma-containing cell lines should be handled at containment Level 2.

### 4.4 RISK LEVELS ASSOCIATED WITH THE USE OF LABORATORY ANIMALS

The use of experimental animals and insects poses special problems. Animals can harbour infectious organisms which are acquired naturally. These infections can give rise to a chronic carrier state, or the agent might persist in a latent non-infective form which can be reactivated periodically or as a result of certain stimuli. If the possibility that such an agent may be excreted by an animal during the course of an experiment cannot be excluded, all those animals should be kept at a containment level appropriate to the risk.

Animals may also be deliberately inoculated with microorganisms in each of the four risk groups or with viable materials (i.e. transformed cells) suspected of containing these organisms. Under these circumstances, the animal should be kept at the containment level appropriate to the risk of the organism, recognizing that, in some cases, in vivo work may increase that risk.
In all situations, it is the responsibility of the scientist and the host institution in consultation with the Government and the Animal Care authorities, to determine the risk levels inherent in the proposed activity.

4.5 CRITERIA FOR CLASSIFICATION OF BIOLOGICAL AGENTS BY RISK GROUP

Risk Group 1 (low individual and community risk)

A biological agent that is unlikely to cause disease in healthy workers or animals.

Risk Group 2 (moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

Risk Group 4 (high individual risk, high community risk)

A pathogen that usually produces very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

4.6 CATEGORIES OF PATHOGENS

As a general precaution, the risk group for agents should be raised when manipulation may result in the production of infectious droplets and aerosols. Agents of similar pathogenic characteristics not included in these lists should be considered in the same risk category. It must also be understood that this is not a complete list - many agents are referred to in the literature by a variety of names and before assuming that an unlisted organism is classified in Risk Group 1, its characteristics, and pathogenicity must be verified in consultation with the Office of Laboratory Security, Health Canada.

4.6.1 RISK GROUP 1 AGENTS: REQUIRING CONTAINMENT LEVEL 1

Risk Group 1 (low individual and community risk)
This group includes those microorganisms, bacteria, fungi, viruses and parasites, which are unlikely to cause disease in healthy workers or animals.

4.6.2 RISK GROUP 2 AGENTS: REQUIRING CONTAINMENT

LEVEL 2

Risk Group 2 (moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease but under normal circumstances, is unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group 2 Bacteria, Chlamydia, Mycoplasma

Actinobacillus - all species
Actinomyces pyogenes (C. pyogenes)
Bacillus cereus
Bartonella bacilliformis, B. henselae, B. quintana, B. elizabethae
Bordetella pertussis, B. parapertussis and B. bronchiseptica
Borreliia recurrentis and B. burgdorferi
Campylobacter spp. (C. coli, C. fetus, C. jejuni)
Chlamydia pneumoniae, C. psittaci (non-avian strains), C. trachomatis,
Clostridium botulinum, Cl. chauvoei, Cl. difficile, Cl. haemolyticum,
Cl. histolyticum, Cl. novyi, Cl. perfringens, Cl. septicum,
Cl. sordellii, Cl. tetani
Corynebacterium diphtheriae, C. haemolyticum,
C. pseudotuberculosis, C. pyogenes (A. pyogenes)
Edwardsiella tarda
Erysipelothrix rhusiopathae (insidiosa)
Escherichia coli enterotoxigenic/invasive/hemorrhagic strains
Francisella tularensis Type B, (biovar palaeaectica), F. novocida
Fusobacterium necrophorum
Haemophilus influenzae, H. ducreyi
Helicobacter pylori
Legionella spp.
Leptospira interrogans - all serovars
Listeria monocytogenes
Mycobacteria - all species (except M. tuberculosis, and M. bovis (non-BCG strain), which are in Risk Group 3)
Mycoplasma pneumoniae, M. hominis Neisseria gonorrhoeae, N. meningitidis
Nocardia asteroides, N. brasiliensis
Pasteurella, all species (except P. multocida type B in Level 3)
Pseudomonas aeruginosa
Salmonella enterica (S. choleraesuis)
Salmonella enterica serovar arizonae (Arizona hinshawii)
Salmonella enterica ser. gallinarum-pullorum (S. gallinarum-pullorum)
Salmonella enterica ser. meleagridis (S. meleagridis)
Salmonella enterica ser. paratyphi B (S. paratyphi B) (Schottmulleri)
Salmonella enterica ser. typhi (S. typhi)
Salmonella enterica ser. typhimurium (S. typhimurium)
Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus spp. (Lancefield Groups A, B, C, D, G)
Treponema carateum, T. pallidum (including pertenue), T. vincentii
Ureaplasma urealyticum
Vibrio cholerae (incl. El Tor), V. parahaemolyticus, V. vulnificus
Yersinia enterocolitica, Y. pseudotuberculosis

Risk Group 2 Fungi

Cryptococcaeae
Candida albicans
Cryptococcus neoformans
Moniliaceae
Aspergillus flavus
Aspergillus fumigatus
Epidermophyton floccosum
Microsporum spp.
Sporothrix schenckii
Trichophyton spp.

Risk Group 2 Viruses

*Arthropod-borne viruses are identified with an asterisk. Only those viruses which may be associated with human or animal disease have been included in this list. Agents listed in this group may be present in blood, CSF, central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection.

Adenoviridae
Adenoviruses, all serotypes
Arenaviridae
Lymphocytic choriomeningitis virus (laboratory-adapted strains)
Tacaribe virus complex: Tamiami, Tacaribe, Pichinde
Bunyaviridae*
Genus Bunyavirus
Bunyamwera and related viruses
California encephalitis group, including LaCrosse, Lumbo and snowshoe hare
Genus Phlebovirus
All species except Rift Valley fever virus (see Table 1)
Caliciviridae - all isolates (including Hepatitis E & Norwalk)
Coronaviridae
Human coronavirus, all strains
Transmissible gastroenteritis virus of swine
Hemagglutinating encephalomyelitis virus of swine
Mouse hepatitis virus
Bovine coronavirus
Feline infectious peritonitis virus
Avian infectious bronchitis virus
Canine, Rat and Rabbit coronaviruses
Flaviviridae*
Yellow fever virus (17D vaccine strain)
Dengue virus (serotypes 1,2,3,4)
Kunjin virus
Hepadnaviridae
Hepatitis B virus, includes Delta agent
Herpesviridae
Alphaherpesvirinae
Genus Simplexvirus: all isolates, including HHV 1 and HHV 2, except Herpes B virus which is in Risk Group 4
Genus Varicellovirus: all isolates, including varicella/zoster virus
(HHV 3) and pseudorabies virus (see Table 1)
Betaherpesvirinae
Genus Cytomegalovirus: all isolates including CMV (HHV 5)
Genus Muromegalovirus: all isolates
Gammaherpesvirinae
Genus Lymphocryptovirus: Epstein Barr Virus (HHV 4) and EB-like isolates
Genus Rhadinovirus: all isolates (except H. ateles and H. saimiri, see Risk Group 3)
Genus Thetalymphocryptovirus: all isolates
Unassigned Herpesviruses: includes HHV 6 (human α-lymphotrophic virus), HHV 7, HHV 8, etc.
Orthomyxoviridae
Genus Influenzavirus:
Influenza virus type A, all isolates
Influenza virus type B, all isolates
Influenza virus type C, all isolates
Papovaviridae
Genus Papillomavirus: all isolates
Genus Polyomavirus: all isolates
Paramyxoviridae
Genus Paramyxovirus: all isolates
Genus Pneumovirus: all isolates
Genus Morbilliviruses: all isolates (except Rinderpest—see Table 1)
Paroviridae
Genus Parovirus: all isolates
Picornaviridae
Genus Aphthovirus - See Table 1
Genus Cardiovirus - all isolates
Genus Enterovirus - all isolates (see Table 1 for restrictions)
Genus Hepatovirus - all isolates (Hepatitis A)
Genus Rhinovirus - all isolates
Poxviridae (see Table 1 for restrictions)
Chordopoxvirinae (poxviruses of vertebrates)
Genus Capripoxvirus
Genus Molluscipoxvirus
Genus Yatapoxvirus
Genus Avipoxvirus - all isolates
Genus Leporipoxvirus - all isolates
Genus Orthopoxvirinae - all isolates (except Variola and Monkeypox in Level 4)
Genus Parapoxvirus: all isolates
vGenus Suipoxvirus: Swinepox (see Table 1 for restrictions)
All other ungrouped poxviruses of vertebrates
Reoviridae
Genus Orbivirus - all isolates (see Table 1 for restrictions)
Genus Orthoreovirus, types 1, 2 and 3
Genus Rotavirus - all isolates
Retroviridae
Onyvirinae
Genus Oncornavirus C
Subgenus Oncornavirus C avian - all isolates
Subgenus Oncornavirus C mammalian - all isolates except HTLV-I, HTLV-II
Genus Oncornavirus B - all isolates
Lentivirinae - all isolates except HIV-I, HIV-II
Spumavirinae - all isolates
Rhabdoviridae
Genus Vesiculovirus (see Table 1 for restrictions) (All laboratory adapted strains)
Genus Lyssavirus: Rabies virus (Fixed Virus)
Togaviridae
Genus Alphavirus*
Semliki forest virus
Sindbis
O'Nyong-Nyong
Ross river virus
Venezuelan equine encephalitis (Strain TC-83 only, no animal inoculation, see Table 1)
Genus Rubivirus
Rubella virus
Genus Pestivirus
Hepatitis C virus
Bovine diarrhoea virus
Border disease virus
Genus Arterivirus
Equine arteritis virus
Unclassified viruses
Toroviridae
Other Hepatitis Viruses
Borna disease virus
Astro viruses
Chronic infectious neuropathic agents (CHINAs):
Scrapie, BSE (except Kuru, CJD, see Risk Group 3)