

The safe handling and distribution of microorganisms under the law

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There is extensive legislation concerning the safe handling and distribution of microorganisms at the national, regional and international levels. However, it is evident that many microbiologists are still unaware of several aspects of these regulations if the condition of samples received by culture collection deposit and identification services is to be explained. Although this article presents the position in the UK in particular, and also that in Germany, much of what is described applies internationally now and is likely to do so increasingly in the future.

Microorganisms of hazard groups 2, 3 and 4 are hazardous substances under the UK Control of Substances Hazardous to Health (COSHH) legislation, they fall under the EU Biological Agents Directive 93/88/EEC. They are also considered dangerous goods as defined by the International Air Transport Association (IATA) Dangerous Goods Regulations where requirements for their packaging for transport are defined. Further, there are restrictions on distribution imposed by National Postal Authorities where an increasing number of countries prohibit receipt of Infectious, Perishable Biological Substances (IPBS) and, in some cases, Non-infectious Perishable Biological Substances (NIPBS). The Universal Postal Union (UPU, 1998) publishes such information. There are many other aspects of handling and distribution of microorganisms that raise questions. How many shippers of organisms provide health and safety information with, or more appropriately before, despatch of a sample containing known microorganisms and how many are aware of the training requirements before shipping of dangerous goods? The World Federation for Culture Collections (WFCC) Committee on Postal, Quarantine and Safety (<http://wcdm.nig.ac.jp/wfcc/index.html>) attempts to keep abreast of the forever changing regulations and inform their membership through newsletters and reports of new and changing rules (Smith, 1996). This paper extends the dissemination of such information with the aim to improve the awareness of microbiologists and of their obligations.

Introduction

Microorganisms are shipped by various means, by mail, courier or by hand, from one laboratory to another within countries and often across borders or continents. They are sent for identification, reference, research or for production purposes from colleague to colleague, from and to culture collections in a variety of packages. Thought must be given to the regulations that control these matters which cover information provision, packaging, postal and shipping, quarantine and safety. Not only does the legislation exist but from time to time it is changed or added to (Smith, 1996). Over the last few years there have been a number of extra requirements placed upon shippers. The EC Directive 93/88/EEC on Biological Agents and 90/679/EEC setting mandatory control measures for laboratories require that risk assessments are carried out on all microorganisms worked with and held in laboratories. This requires the assignment of each strain to a hazard group including a positive inclusion into hazard group 1 following a thorough risk assessment. Copies of EC Directives are available from the Office for Official Publications of the European Communities, L-2985 Luxembourg. The risk assessment should include an assessment of all hazards involved, not just infection, but also all others amongst which are, the production of toxic metabolites and the ability to cause allergic reactions. Organisms that produce volatile toxins or aerosols of spores or cells present a greater risk. It is the responsibility of the microbiologist to provide such assessment data to a recipient of a culture to ensure its safe handling and containment.

The importance of a laboratory's health and safety procedures stretch beyond the laboratory to all those who may come in contact with substances and products from that laboratory. A microorganism in transit will put carriers, postal staff, freight operators and recipients at risk, some organisms being relatively hazard free whilst others are quite dangerous. It is essential that safety regulations, such as

COSHH, and shipping regulations are followed to ensure safe transit. The more stringent shipping regulations have evolved because of increasing careless and negligent handling. If sound packaging, correct labelling and information were used then we might see a relaxation in the prohibition of the use of mail systems. There are several other pieces of legislation that restricts the distribution of microorganisms of which a microbiologist must be aware. This article will draw attention to these and give information sources to help microbiologists remain within the law.

Health and Safety

Whether it is compliance with the law, or duties of a caring employer, the basic requirements in order to establish a safe workplace are:

- Adequate assessment of risks
- Provision of adequate control measures
- Provision of health and safety information
- Provision of appropriate training
- Establishment of record systems to allow safety audits to be carried out
- Implementation of good working procedures

Good working practice requires assurance that correct procedures are actually being followed and this requires a sound and accountable safety policy.

The UK Management of Health and Safety at Work (MHSW) Regulations 1992 (Anon, 1992) are all-encompassing and general in nature but overlap and lead into many specific pieces of legislation. The Control of Substances Hazardous to Health (COSHH) regulations require that every employer makes a suitable and sufficient assessment of the risks to health and safety to which any person whether employed by them or not may be exposed to through their work (Anon, 1996a). These assessments must be reviewed regularly, additionally when changes in procedures or regulations demand, and must be recorded when the employer has more than five employees. The distribution of microorganisms to others outside the workplace extends these duties to protect others.

The effect of COSHH on Culture Storage and Supply

The Control of Substances Hazardous to Health (COSHH) regulations (Simpson & Simpson, 1991) aim to stimulate and enforce an improvement in health and safety in the workplace. All principles embodied in the COSHH regulations are contained in the UK Health and Safety at Work Act 1974. COSHH formalises, enforces, and in some instances, extends certain sections of this Act. COSHH requires a suitable and sufficient risk assessment for all work that is liable to expose an employee to any substance that may be hazardous to health. This UK legislation has equivalents in other countries but, in common with all health and safety legislation, is not so comprehensive and leaves much open to interpretation.

It can be much simpler dealing with a known chemical than with a named microorganism. The full metabolic and biochemical potential of a microorganism is rarely known and therefore assessing the risk when the hazard is not clearly defined becomes difficult. This is where the COSHH regulations are realistic, leaving room for interpretation. The regulations incorporate terms 'as far as reasonably practicable', 'adequate control', and 'suitable measures' which enable the employer to set relevant safe procedures that are workable. Microorganisms present different levels and kinds of hazard, leaving an enormous, but necessary, task for microbiologists. A risk assessment for example, must take into account the production of potentially hazardous toxins. In the final analysis a safe laboratory is the result of applying good techniques, a hallmark of technical excellence. Containment level 2 is easily achievable and should be standard practice in all microbiological laboratories. Good aseptic techniques applied by well-trained personnel will ensure pure cultures and will minimise contact with the microorganism. However, the unexpected, the accident, must also be taken into account when assessing the risk involved. The employment of good laboratory practice, good housekeeping, workplace and equipment maintenance and ensuring that staff have the relevant information and training, will minimise the risk of accidents (Smith & Onions, 1994). The establishment of emergency procedures to reduce potential harm is an additional and sensible approach.

Classification of Microorganisms on the Basis of Hazard

Various classification systems exist which include World Health Organisation (WHO); United States Public Health Service (USPHS); Advisory Group on Dangerous Pathogens (ACDP); European Federation of Biotechnology (EFB) and European Community (EC). In Europe, the EC Directive (93/88/EEC) on Biological Agents sets a common base line which has been strengthened and expanded in many of the individual member states. In the UK the definition and minimum handling procedures of pathogenic organisms are set by the ACDP who list four hazard groups 1-4 with corresponding containment levels. The Advisory Committee on Genetic Manipulation (ACGM) in the UK prescribe separate but similar regulations for those organisms that have been genetically modified. Similarly other European countries have advisory committees, in Germany it is the Zentrale Kommission für die Biologische Sicherheit (ZKBS), Robert Koch-Institute, Berlin. The Trade Corporation Association of the Chemical Industry (BG Chemie) advises on how individual Genetically Engineered Microorganisms (GEMs) should be classified. The assessment of risk in handling GEM or GMOs is more difficult as the hazards of the donor and recipient have to be taken into account as well as those of the resulting GEM.

Risk assessment

Microorganisms are normally classified on their potential to cause disease, their human pathogenicity, into four groups (Anon, 1995).

Group 1 A biological agent that is most unlikely to cause human disease.

Group 2 A biological agent that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread in the community. Laboratory exposure rarely produces infection and effective prophylaxis or treatment is available.

Group 3 A biological agent that may cause severe human disease and present a serious hazard to laboratory workers. It may present a risk of spread in the community but there is usually effective prophylaxis or treatment.

Group 4 A biological agent that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread in the community and there is usually no effective prophylaxis or treatment.

The containment level numbers correlate with the risk group in which the organism falls (i.e. organisms in Risk Group 1 require Containment Level 1 and so forth, see Table 1 below).

The species of bacteria and fungi falling into hazard groups 2 and 3 are defined (Anon, 1995, 1996a). All bacteria from the Approved List of Bacterial Names (Skerman *et al.* 1980) have been assigned to an appropriate hazard group in Germany (Anon, 1997a & 1998b). The sister publication on the fungi has not assigned the species to hazard group 1 (Anon, 1995, 1996a). There has been an attempt to categorise medically important fungi to relevant hazard groups by de Hoog (1996). To meet the UK and European legislation all microbiologists will have to make a risk assessment on the organisms with which they work or hold in collections. In the case of fungi it is recognised that many organisms infect following traumatic inoculation through the skin, or infect the compromised patient but do not infect healthy individuals. Most fungi from clinical specimens require Containment Level 2 (Anon, 1995) unless a higher degree of containment is specified (see Table 1). In the UK, Genetically Modified Organisms (GMO's) also require Containment Level 2 for handling and all potential work with such organisms must first be referred to the place of work's Biological Safety Officer and the place of work's Biological Safety Committee. Again legislation can be different in other countries, for example, in Germany some manipulated organisms can be handled at Containment Level 1.

The COSHH regulations work well and can be easily applied in establishments with designed laboratories but may not work so well in the industrial environment where very large volumes and more hazardous techniques may be used. Total containment is rarely applicable.

Assessment of microorganisms

Microorganisms are more difficult to name, less predictable and more difficult to enumerate or measure than chemicals. Virulence and toxicity may vary from strain to strain within a species. In addition to the risk of infection other hazards exist, such as toxin production or allergenicity.

To meet COSHH requirements a step by step evaluation of a laboratory procedure or an industrial process must be carried out. This is necessary as different organisms present different hazards and different size inocula can be required to cause a problem. The assessment must cover the procedure from the original inoculum or seed culture to the final product or the point where the organism is killed and disposed of. It is not adequate to say that the microorganism is of ACDP hazard group 2 or less and therefore work can be carried out on the laboratory bench apart from those procedures that may create aerosols. Some individuals may respond differently to exposure, being more sensitive than others are. It is therefore critical that the full potential of organisms is taken into account and this is related to the effect they may have on the particular individual carrying out the work.

Microbial toxins - Mycotoxins

One of the better known hazards associated with fungi is the ability to produce toxic secondary metabolites. The presence of these in culture media adds to the hazard status of the growing organisms. The toxins produced may be carcinogenic, nephrotoxic, hepatotoxic, haemorrhagic, oestrogenic or cause inflammatory effects. The most commonly known is aflatoxin which is considered to be carcinogenic, hepatotoxic and potentially mutagenic and is produced by strains of *Aspergillus flavus* and *A. parasiticus*. Table 2 lists some mycotoxins that may be present in growth media and present additional problems in both use and disposal.

Mycotoxicoses are poisonings caused by the ingestion of food contaminated (and sometimes rendered carcinogenic) by toxin producing microfungi. Toxins are also produced by many other fungi, for example, citreoviridin, citrinin, islanditoxin and patulin by species of *Penicillium*, ochratoxin by *Aspergillus* and trichothecenes and zearalenone by species of *Fusarium*, and various other compounds including cochliodinol by *Chaetomium*. It should always be remembered that many fungi have not been studied chemically and because mycotoxins are not reported for a species does not mean it does not produce them. The handling of materials contaminated by these toxins can lead to their ingestion and subsequent poisoning. Inhalation of mycotoxins can also be dangerous. Toxins from *Aspergillus* and *Fusarium* species have caused problems in patients when inhaled. The death of two factory workers from liver disease was associated with the inhalation of dust containing aflatoxin.

Microbial toxins – Bacterial toxins

As infection patterns caused by bacterial pathogens are so different and depend on the bacterial pathogen and the individual host, every infection is an extremely individual process.

Diseases caused by bacteria may be grouped as follows (Anon, 1998b):

Local infections.

Manifestation of the pathogen in a localised tissue.

Examples: *Staphylococcus aureus*, *Neisseria gonorrhoeae*.

Local infections with production of a potent toxin.

Low invasiveness as above, but general diffusion of the toxin via the lymphatic and blood stream.

Examples: *Clostridium tetani*, *Corynebacterium diphtheriae*.

Acute generalised infections.

Usually highly invasive distribution of the pathogens after infection leading to possible septic-toxic shock. Sometimes tissue specific (organotrop) manifestation, examples: plague, typhoid, brucellosis, some types of tuberculosis.

The virulence of strains of pathogenic bacterial species is determined by their invasiveness, production of aggressins and toxigenicity. Most bacterial toxins are capable of damaging or killing normal host cells and are effective upon infection. In contrast, most mycotoxins are effective without invasion or infection. The role played by bacterial invasiveness in damaging the host varies widely: sometimes infection can be extremely localised (e.g. *Corynebacterium diphtheriae*), the toxin diffuses and reaches almost all tissues. Alternatively, pathogens may invade and need to multiply to large numbers in order to generate enough toxin to cause damage to the host (e.g. *Bacillus anthracis*). Two classes of bacterial toxins have been designated which can be distinguished by their chemical nature. The first are protein-like exotoxins (examples are diphtheria, tetanus, botulinum toxins and enterotoxins) and the second are endotoxins which are molecular complexes containing protein, lipid and polysaccharide components. Generally, endotoxins are relatively non-specific, are derived from the outer layers of cell walls of Gram-negative bacteria and released after bacterial lysis. Cells of nearly all Gram-negative pathogenic bacteria are intrinsically toxic. The best known endotoxins exhibiting pyrogenicity and toxicity are those of the enteric bacteria of the genera *Escherichia*, *Salmonella* and *Shigella*. Endotoxins are also inflammatory agents increasing capillary permeability.

Aggressins are enzyme-like substances e.g. proteases, collagenases, lipases, phospholipases or neuraminidases which usually support the invasion of a pathogen by damaging host tissue.

A complete list of all known bacterial toxins cannot be given here, some examples are given in Table 3 and further toxin producers can be found in Annexe III, Community Classification of the EU Directive 90/679/EEC. In addition to those bacteria that produce toxins during infection there are also those that are non-infectious toxin producers. The most important group of the latter is the Cyanobacteria of which there are *ca.* 2000 species and are currently considered as hazard group 1. Further information can be found on bacterial toxins in Collier *et al.* 1998.

Quarantine regulations

National

Clients in the UK who wish to obtain cultures of non-indigenous plant pathogens must first obtain a Ministry of Agriculture Fisheries and Food (MAFF) license. Under the terms of such a licence the shipper is required to see a copy of the Ministry permit before such strains can be supplied. Such licences are available in England and Wales from Ministry of Agriculture, Fisheries and Food, Room 340, Foss House, Kings Pool, 1-2 Peace Holme Green, York YO1 2PX and in Scotland from Plant Health Section, Agricultural Science Agency, East Craigs, Edinburgh EH12 8NJ.

Non-indigenous tree pathogens can only be supplied if the customer holds a current permit issued by The Forestry Commission: Forestry Commission Headquarters, 231 Corstorphine Road, Edinburgh EH12 7AP.

All shipments to Canada and the USA for plant pathogens must be accompanied by import mailing labels, without which entry of cultures to these countries is refused. Applications for these labels, stating the names of the organisms and the purpose for which they are required, should be made for Canada to the Chief of the Plant Protection Division, Agriculture Canada Science Division, Science Service Building, Ottawa, Ontario, Canada K1AS 0C5 and for the USA to USDA Agricultural Research Service, Plant Protection & Quarantine, Room 764, 6505 Belcrest Road, Hyattsville, Maryland 20782, USA.

The specified Animal pathogens order 1998 makes it an offence to possess or spread a listed animal pathogen (e.g. *Brucella*) within Great Britain without a licence. It is supplemented by the importation of Animal Pathogens Order 1980 which makes it an offence to import any animal pathogen, or potential or actual carrier, of an animal pathogen from a non-EC country, except under license. Both the supplier and recipient must hold the appropriate licences and undergo regular inspections from MAFF. Requests for strains must be refused where the requestor is unable to produce a copy of the appropriate licence. Such licences can be obtained in the UK from MAFF, AHDC Branch C, Tolworth (Toby Jug), Hook Rise, South Tolworth, Surbiton, Surrey KT6 7NF.

Regional

Information on the transport of plant pathogens throughout Europe can be obtained from the European and Mediterranean Plant Protection Organisation (EPPO), 1 rue le Nôtre, 75016 Paris, France.

Postal Regulations and Safety

Countries have their own regulations governing the packaging and transport of biological material in their domestic mail. International Postal Regulations regarding the postage of human and animal pathogens are very strict on account of the safety hazard they present. There are several organisations that set regulations controlling the international transfer of such material. These include the International Air Transport Association (IATA), International Civil Aviation Organisation (ICAO), United Nations Committee of Experts on the Transport of Dangerous Goods, the Universal Postal Union (UPU) and the World Health Organisation (WHO). It is common place to send microorganisms by post, as this is more convenient and less expensive than airfreight. However, many countries prohibit the movement of biological substances through their postal services. The International Bureau of the UPU in Berne publishes all import and export restrictions for biological materials by national postal services. This information can also be found in the countries table published in the DSMZ *Shipping of infectious, non-infectious and genetically modified biological materials. International Regulations* brochure (Anon, 1998).

The UK Post Office leaflet on "Infectious and non-infectious perishable biological substances in the overseas post" is available from The Post Office, Corporate Headquarters, 30 St James Square, London SW1 4PY. Tel: +44 171 490 2888; Fax: +44 181 681 9387 and provides the relevant information. Some countries will not accept human pathogens through the post for carriage overseas and this now includes the UK. A list, which changes from time to time, of these countries can also be obtained from the Post Office (also see Anon, 1998; Smith, 1996).

It is probably not uncommon for cultures to be transported on the person. This is a practice that should be resisted. Such an act contravenes public transport regulations and where aircraft are concerned cultures are considered dangerous goods under the IATA regulations with the possibility of heavy penalties imposed on those caught. Carriage on the person also circumvents all the controls described herein, which are designed to promote safety.

Packaging

IATA Dangerous Goods Regulations (DGR) require that packaging used for the transport of hazard group 2, 3 or 4 must meet defined standards, IATA packing instruction 602 (class 6.2) (IATA, 1998). The DSMZ has collected all relevant guidelines for the shipping of microorganisms and updated it on a regular basis (Anon, 1998) this will also be available shortly on the DSMZ web-site (<http://www.gbf.de/dsmz/shipping/shipping.htm>). Packaging must meet EN 829 triple containment requirements for hazard group 1 organisms (Anon, 1996b). However, microorganisms that qualify as dangerous goods (class 6.2), must be sent in UN certified combination packages. These packages must be sent by airfreight or courier if the postal services of the countries through which it passes do not allow the organisms in their postal systems. IATA (1998) Sections 2.4.1, 2.4.2 and 2.4.2.1, the carriage of dangerous goods in the mail is forbidden by UPU except as permitted in sections 2.4.2.1 which states:

Infectious substances, provided a "Shipper's Declaration" accompanies the consignment, and Carbon dioxide, solid (dry ice) when used as a refrigerant for infectious substances.

There are additional costs above the freight charges and package costs, if the carrier does not have its own fleet the package and documentation will require checking at the airport DGR Centre for which a fee is charged. There are currently very few private carriers that transport dangerous goods internationally. These private carriers do provide assistance in completing the Shipper's Declaration forms. The shipper is exclusively responsible for the shipment, its correct packaging, documentation, marking and labelling. Ready to use, re-usable packaging can be obtained from Air Sea Containers Ltd. at www.air-sea.co.uk and SAF-T-Pak Inc. at www.saftpak.com and both can provide useful information for the shipper of dangerous goods.

Regulations governing distribution of cultures

The IATA Dangerous Goods Regulations require that shippers of microorganisms of hazard groups 2, 3 or 4 must be trained by IATA certified and approved instructors. They also require shippers declaration forms, which should accompany the package in duplicate, and specified labels are used for organisms in transit by air (IATA, 1998). There are several other regulations that impose export restrictions on the distribution of microorganisms. These include control of distribution of agents that could be used in biological warfare, EU Council Regulation 3381/94/EEC on the control of export of dual-use goods (Official J. L 367, p1) and more generally countries are currently implementing Access Regulations to Genetic Resources under the Convention on Biological Diversity. It is critical that microbiologists are aware of and follow such legislation.

Some cultures represent a health hazard, and for post and packaging purposes these are placed into four classes by the UPU as follows:

Class 1. Agents of no recognised hazard under ordinary conditions of handling. Unrestricted distribution for bona fide teaching, research industry, etc.

Class 2. Agents of ordinary potential hazard. Distribution is restricted to professional investigators (includes *Trichophyton rubrum*).

Class 3. Pathogens involving special hazard. Distribution is restricted to professional investigators.

Class 4. Agents of potential danger to the public health, animal health or of hazard to laboratory personnel requiring special facilities for their containment. Distribution by permit, this includes *Fusarium moniliforme*.

For further details see *Packaging and Shipping of Biological Materials at ATCC* (Alexander & Brandon, 1986) and *Shipping of infectious, non-infectious and genetically modified biological materials, International Regulations* DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (1998), IATA Dangerous Goods Regulations (IATA, 1998).

In Europe non-pathogenic biological materials of risk group 1 are transported by road packed according to EN 829 requirements. Transport by road is regulated by the Accord Européen relatif au transport international des marchandises dangereuses par routes (ADR). This clearly separates class 6.2 into two subclasses, A: highly infectious material (hazard groups 3 and 4) and B: other infectious material. These two groups A and B, have different packaging requirements. Therefore the UN specification containers for class 6.2 materials must be used for both subclasses. The EU have made an attempt to co-ordinate Member State laws on transport of dangerous goods by road with the 'ADR-Directive' EC Council Directive 94/55/EC of 21 November 1994 on the approximation of the laws of the Member states on the transport of dangerous goods by road (EC, 1994, 1996).

The basis for all regulations governing the safe transport of goods for all carriers are laid down in the Orange Book, Recommendations on the Transport of Dangerous Goods (Anon, 1997d).

Some service culture collections such as the National Collection of Type Cultures (NCTC) and DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen maintain registers of persons authorised by their employer to request hazardous pathogens. This measure is designed to protect the customer by ensuring orders are authorised by a responsible person who will ensure that the hazardous microorganisms are handled by appropriate staff under suitable conditions of containment. Requests for such organisms are accepted only when countersigned by one of the authorised signatories.

Control of Distribution of Dangerous Organisms

There is considerable concern over the transfer of selected infectious agents capable of causing substantial harm to human health. There is potential for such organisms to be passed to parties not equipped to handle them or to persons who may make illegitimate use of them. Of special concern are pathogens and toxins causing anthrax, botulism, brucellosis, plague, Q fever, tularemia and all agents classified for work at Biosafety Level 4 (hazard group 4). The 'Australia Group' of countries has strict controls for movement outside their group but has lower restrictions within. The UK National Culture Collections are implementing a system involving the registration of customers to ensure *bona fide* supply. The USA have rules that include a comprehensive list of infectious agents, registration of facilities that handle them, requirements for transfer, verification and disposal. These rules carry criminal and civil penalties. In the UK all facilities handling hazard group 2, 3 or 4 must be registered and strict control of hazard group 3 and 4 organisms is in place. The UK Department of Trade and Industry (DTI) require that certain infectious agents are exported to members of the 'Australia Group' under an Open General Export Licence (OGEL) which is granted only to organisations registered with the DTI. Exports of these agents outside the 'Australia Group' require an Individual Export Licence (IEL) and only individuals nominated by their senior management and who are registered with the DTI may submit an application for an IEL. Failure to comply with these requirements is a criminal offence. Persons being supplied with these infectious agents should not avoid these regulations by providing subcultures to third parties.

In Germany, permission to import, distribute, store and handle microorganisms allocated to risk group 2 and higher (pathogenic or "hazardous" biological material able to multiply) are subject to restrictions laid down in the Federal German Infectious Diseases Act of December 1979 with its amendments in on microorganisms pathogenic to humans. A laboratory must be registered with the local health authority. Furthermore, the scientific leader of the responsible institution whether industry, hospital, university etc. or the head of the laboratory must have a personal permit issued by the local health authority. It is not sufficient for an institution to have registered laboratories, additionally there must be at least one authorised qualified person registered. If the person leaves the institution, a new authorised person must be registered. However, the person does not lose authorisation (personal authorisation is transferrable to another institution). Handling microorganisms which are exclusively pathogenic to animals (in Germany), is subject to restrictions according to the Federal Infectious Diseases of Animals Enactment. The position is similar to that with human pathogens, the institution has to have registered laboratories and at least one authorised person. However it is the district authority that is responsible for granting permission in this case. The district authority is also responsible for permits to laboratories working on genetically manipulated microorganisms. A similar registration is necessary for handling Genetically Engineered Microorganisms (GEMs) allocated to safety level 1, the laboratory must be registered, there must be a deputy biological safety officer (authorised person as above) and a project leader who is responsible for the genetic engineering project. Additionally each S2 project must be registered separately with the district authority.

Convention on Biological Diversity

The Convention on Biological Diversity requires that microbiologists seek prior informed consent from the country in which they wish to collect organisms. They will be required to agree terms on which benefits will be shared should they accrue from the use of the organisms. The benefit sharing may include monetary elements but may also include information, technology transfer and training. The supply of every organism will now be under agreed terms and the future will no doubt require material transfer agreements between supplier and recipient to ensure benefit sharing with, at least, the country of origin. Many culture collections have operated benefit sharing since they began giving organisms in exchange for deposits and re-supplying the depositor with the strain if they require a replacement. The huge rewards that may accompany the discovery of a new drug remain a pipe dream as the hit rate is often reported at being less than 1 chance in 250 000. However, access legislation and the hope for substantial financial returns from isolated strains are restricting the free deposit in service culture collections and their free movement for research and development. An EU DG XII project, Microorganisms Sustainable Use and Access Regulation International Code of Conduct (MOSAICC) is working toward standard material transfer agreements to facilitate access to genetic resources whilst

adhering to the spirit of the CBD and National and International law governing the distribution of microorganisms (Davison et al. 1998).

Safety information provided to the recipient of microorganisms

A safety data sheet must be despatched with an organism indicating which hazard group it belongs to and what containment and disposal procedures are necessary. In the UK, microorganisms are covered by the Control of Substances Hazardous to Health (COSHH) regulations (1988), HSW Act s.6(4)(c) and subject to the Approved Code of Practice for Biological Agents 1994 (Anon, 1994). Article 10 of the EU Directive 90/379/EEC regulates that manufacturers, importers, distributors and suppliers must provide safety data sheets in a prescribed format. A safety data sheet accompanying a microorganism must include:

The hazard group of the organism being despatched as defined by EU Directive 90/679/EEC Classification of Biological Agents and by the national variation of this legislation for example, in the UK, as defined in the Advisory Committee on Dangerous Pathogens (ACDP) *Categorisation of biological agents*, 4 edition, and the Approved Code of Practice (ACOP) for Biological Agents.

A definition of the hazards and assessment of the risks involved in handling the organism.

Requirements for the safe handling and disposal of the organism.

- Containment level
- Opening cultures and ampoules
- Transport
- Disposal
- Procedures in case of spillage

Summary

In the interests of the progress of science, microbiologists must be able to exchange their organisms upon which their hypotheses and results are based, but they must do this in a way that presents minimum risk to those who come into contact with the organism. They must not fall foul of the laws that control the shipping of microorganisms as this will inevitably result in ever more restrictive legislation that may make their exchange impossible. Health and Safety, packaging and shipping and controlled distribution legislation may be extensive and sometimes cumbersome but is there to protect us and must be followed.

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Table 1. Summary of laboratory containment levels for the UK (Anon 1995)

CONTAINMENT REQUIREMENT	CONTAINMENT LEVEL			
	1	2	3	4
Laboratory site: isolation	No	No	Partial	Yes
Laboratory: sealable for fumigation	No	No	Yes	Yes
Ventilation: inward airflow/negative pressure	Optional	Optional	Yes	Yes
Ventilation: through safety cabinet	No	Optional	Optional	No
mechanical: direct	No	No	Optional	No
mechanical: independent ducting	No	No	Optional	Yes
Airlock	No	No	Optional	Yes
Airlock: with shower	No	No	No	No
Wash hand basin	Optional	Yes	Yes	Yes
Effluent treatment	No	No	No	Yes
Autoclave site: on site	Yes	-	-	-
in suite	-	Yes	Yes	-
in lab: free standing	-	-	Optional	-
in lab: double ended	-	-	-	Yes
Microbiological safety cabinet/enclosure	No	Optional	Yes	Yes
Class of cabinet/enclosure*	-	Class I	Class I/III	Class I/III

- * Guidance on the use of Class II microbiological safety cabinets is given in the Advisory Committee on Dangerous Pathogens Report (Anon, 1995).

Table 2. Some common mycotoxins and examples of fungi producing them.

Mycotoxin	Organism
Aflatoxin	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Aflatrem	<i>Aspergillus flavus</i>
Altenuic acid	<i>Alternaria alternata</i>
Alternariol	<i>Alternaria alternata</i>
Austdiol	<i>Aspergillus ustus</i>
Austamide	<i>Aspergillus ustus</i>
Austocystin	<i>Aspergillus ustus</i>
Bentenolide	<i>Monographella nivalis</i>
Brevianamide	<i>Aspergillus ustus</i>
Citrinin	<i>Aspergillus carneus</i> , <i>A. terreus</i> , <i>Penicillium citrinum</i> , <i>P. hirsutum</i> , <i>P. verrucosum</i>
Citreoviridin	<i>Aspergillus terreus</i> , <i>Penicillium citreoviride</i>
Cochliodinol	<i>Chaetomium cochliodes</i>
Crotocin	<i>Acremonium crotocinigenum</i>
Cytochalasin E	<i>Aspergillus clavatus</i>
Cyclopiazonic acid	<i>Aspergillus versicolor</i>
Destruxin B	<i>Aspergillus ochraceus</i>
Fumagilin	<i>Aspergillus fumigatus</i>
Fusarin	<i>Fusarium moniliforme</i>
Gliotoxin	<i>Aspergillus fumigatus</i>
Islanditoxin	<i>Penicillium islandicum</i>
Malformin	<i>Aspergillus niger</i>
Maltoryzine	<i>Aspergillus</i> spp.
Moniliformin	<i>Fusarium moniliforme</i> , <i>F. oxysporum</i> , <i>F. equiseti</i>
Ochratoxin	<i>Aspergillus ochraceus</i> , <i>Penicillium viridictum</i>
Oxalic acid	<i>Aspergillus niger</i>
Patulin	<i>Aspergillus clavatus</i> , <i>Penicillium expansum</i> , <i>P. roquefortii</i> , <i>P. claviforme</i> , <i>P. griseofulvum</i>
Penicillic acid	<i>Aspergillus ochraceus</i>
Penitrem	<i>Penicillium crustosum</i>
Roridin	<i>Myrothecium roridum</i> , <i>M. verrucaria</i> , <i>Dendrodochium</i> spp., <i>Cylindrocarpon</i> spp., <i>Stachybotrys</i> spp.
Rubratoxin	<i>Penicillium rubrum</i>
Rubroskyrin	<i>Penicillium</i> spp.
Rubrosulphin	<i>Penicillium viridicatum</i>
Rugulosin	<i>Penicillium brunneum</i> , <i>P. kloeckeri</i> , <i>P. rugulosum</i>
Satratoxin	<i>Stachybotrys chartarum</i>
Slaframine	<i>Rhizoctonia leguminicola</i>
Sterigmatocystin	<i>Aspergillus flavus</i> , <i>A. nidulans</i> , <i>A. versicolor</i> , <i>Penicillium rugulosum</i>
Trichodermin	<i>Trichoderma viride</i>
Trichothecin	<i>Trichothecium roseum</i>
Trichothecenes	<i>Fusarium acuminatum</i> , <i>F. roseum</i> , <i>F. sporotrichioides</i>
T2 toxin	
deoxynivalenol	
(vomitoxin)	
nivalenol	
diacetoxyscirpenol	
fusarenone	
3-acetyldeoxynivalenol	
15-acetyldexonivalenol	
Tryptoquivalene	<i>Aspergillus clavatus</i>
Verrucaridin	<i>Myrothecium verrucaria</i> , <i>Dendrodochium</i> spp.
Verruculogen	<i>Aspergillus fumigatus</i>
Viopurpurin	<i>Trichophyton</i> spp., <i>Penicillium viridicatum</i>
Viomellein	<i>Aspergillus</i> spp., <i>Penicillium aurantiogriseum</i> , <i>P. crustosum</i> , <i>P. viridicatum</i>
Viriditoxin	<i>Aspergillus fumigatus</i>
Xanthocillin	<i>Eurotium chevalieri</i>
Zearalenone	<i>Fusarium culmorum</i> , <i>F. graminearum</i> , <i>F. oxysporum</i> , <i>F. roseum</i>

Data from the CABI Bioscience Genetic Resource Collection database and Smith & Moss (1985).

Table 3. Some bacterial exotoxins and examples of bacteria producing them (based on Stanier *et al.* 1987)

Toxin	Bacterium
Neurotoxins	
Botulinum toxins: A, B, C1, C2, D, E, F, G	<i>Clostridium botulinum</i>
Tetanospasmin	<i>Clostridium tetani</i>
Tetanolysin	
Cytotoxins	
α lecithinase	<i>Clostridium perfringens</i>
Necrotic factors	
Hemolysin	
Collagenase	
Diphtheria toxin	<i>Corynebacterium diphtheriae</i>
Streptolysin O	<i>Streptococcus pyogenes</i>
Streptolysin S	
Enterotoxins	
Enterotoxin	<i>Clostridium perfringens</i>
α toxin	<i>Staphylococcus aureus</i>
“Enterotoxin”	
Shiga toxin	<i>Shigella dysenteriae</i>
Cholera toxin	<i>Vibrio cholerae</i>
“Guinea pig toxin”	<i>Yersinia pestis</i>
Heat-labile enterotoxin (LT)	<i>Escherichia coli</i>
Heat-stable enterotoxin (ST)	<i>Escherichia coli</i>