BIOSAFETY IN BIOTECHNOLOGY

Jean-Marc Collard, Didier Breyer, Suzy Renckens, Myriam Sneyers, Ellen Van Haver, Bernadette Van Vaerenbergh, and **William Moens**
Institute of Public Health, Service of Biosafety and Biotechnology, Brussels, Belgium

Keywords: biotechnology, biological safety, hazard, risk assessment, risk management, regulation, containment, environment, GMO, vaccines, gene therapy, transgenic plant, novel food/feed, biodiversity, public acceptance, consumer, LMO.

Contents

Summary

Summarising the various aspects of biological safety in biotechnology is a matter of difficult choice among many interesting priorities.

Among biotechnological applications, the design of transgenic plants, as crops, food sources or medicinal factories, the development of new tools for a curative medicine at the cellular and genetic levels, the eradication of long persisting animal or human diseases using life recombinant vaccines, or more modestly but equally important the production of high quality and cheap medicinal proteins such as non-allergenic human insulin are trends feeding the believes and the fears of both the investors and the public.

Modern biotechnology is just learning to express itself in an open market of science technologies, multi-sectorial applications and recently, internet-wired consumer interactions. In such a context, the best ideas and derived products might encounter perception blockages illustrating that an open market also means an open place for perception diversity.

Resulting from a historical wedding with the traditional agro-food and pharmaceutical sectors, the discrete world of modern biology and genetics has still to stabilise ways of communication and behavioural and ethical practices in the real world. In such interactive and real-time evolving situation, any summary should appear, at the best, as a flashed picture.

Biosafety is an emerging discipline built from traditional risk assessment and risk management rationale originating from chemistry, toxicology, microbiology, epidemiology, ecology, human and veterinary medicines, agronomy and all related basic or engineering sciences. It is composed of a spectrum of ways of thinking from the pure scientific analytical way to the most global conceptual way merging regulatory science, ethical issues, economics, and sociology.

Biosafety is basically a case by case methodology exploiting pertinent safety criteria embedded in the history of sciences and of human practices. Risk assessment is and must be science-based only. However risk-assessment is evaluating multi-factorial situations and necessarily only leads to a set of certainties but also of uncertainties. Risk management leads to a binary decision: should an activity or a product be authorised or not, given a certainties/uncertainties ratio. Risk communication motivates the final decision and is a complex mixture of local and transboundary education, information and public interaction, dialectics, democratic respect, and transparency.

These three aspects of biosafety are complementary and mutually beneficial if properly managed.

To illustrate such a concept and its complexity, the present article gathers examples of the biosafety management of present biotechnological key developments.

As it might be understood further, biosafety meets the challenge to be at the boundary of hard and soft sciences, the place where, in many societies, skill requires wisdom, on top of expertise.

1. Introduction

Biotechnology, broadly defined, includes any technique or process that uses living organisms, or parts of such organisms, to create or improve products, to modify plants, animals, or microbes for specific uses. Consequently, its scope ranges from the traditional biotechnology originating from the ancient times to the so-called modern biotechnology in which the technology of recombinant DNA (often called genetic engineering) has become a central part [see also - Biotechnology].

As the productivity of any living cell used in biotechnological processes mainly depends on its genetic background, genomics has been an area of fundamental and commercial interests in biotechnology. Methods such as mutagenesis, microbial or cell fusion, plant and animal breeding have been widely used to improve productivity. These methods are further exposed in the different parts of this book.

The development of new techniques of genetic modification in the early 1970s initiated a wide discussion on safety of biotechnological products. The so-called "recombinant-DNA" debate originated from the scientific community itself which suggested that certain types of experiments should be deferred until their potential risks could be assessed. In 1975, scientists gathered in Asilomar to debate about the potential risks issued from the technology of recombinant DNA. One year after, preliminary guidelines were issued by the National Institutes of Health (NIH). According to the first recommendations, recombinant organisms had to be handled under containment measures that far exceeded those for the safe handling of non-recombinant pathogenic organisms. After few years of safe practical use, and a better scientific understanding of the risks posed by recombinant-DNA organisms, nowadays called genetically modified organisms (GMO's), experience-based guidelines were redrafted in 1979.

The paradigm of the mid-1980s was that recombinant DNA techniques are an extension of conventional genetic procedures and that potential risks inherently associated with recombinant organisms are not qualitatively different from and intrinsically more hazardous than those posed by "natural" organisms. Experience has supported such a scheme except in very few cases.

The American NIH guidelines constituted the reference for the development of rules for laboratory work using genetic engineering techniques and were at the basis of specific worldwide rules or national laws in many countries.

The first worldwide development inspired from these guidelines was the publication in 1986 of the OECD (Organisation for Economic Co-operation and Development) report on « Recombinant-DNA Safety Considerations» (also known as the "blue book"). It sets out the first international safety guidelines for the use of recombinant-DNA organisms in industry, agriculture and the environment.

From 1986, general biosafety regulations applicable to biotechnological products and activities appeared in several countries as well as at multi-national levels such as in the European Union. From our experience acquired these last 15 years, the remainder of this paper will describe the general principles of biosafety and document them in the cases of four relevant biotechnological areas:

- Contained use
- Deliberate release of transgenic plants
- Food and feed as or derived from transgenic crops
- Medicinal products

2. General Principles of Risk Assessment

The safety of any biotechnological application, like the safety of any human activity, is achieved by carrying out two sequential steps:

- Assessing the risks. Risk assessment is defined as an estimation of risks in terms of likelihood of occurrence of hazards and severity of their consequences (damages).

- Minimising the level of risks, where indicated by the results of the risk assessment, either by applying adequate management strategies, or by deciding not to carry out a given activity if the risks are unacceptable.

When applying these general principles to biotechnology, the risk assessment should take into account the following points:

- the characteristics of the organisms involved, including any newly introduced traits;
- the intended use(s) of the organisms (contained by physical, chemical and/or biological barriers versus released into the environment);
- the characteristics of the area where the biotechnological process, activity or release will take place; and the interactions between these.

The risk assessment is performed to protect the human health and the environment from any adverse effect. It is based on the principle of familiarity; i.e. knowledge of, and experience with the organisms used and their historical exploitations. Familiarity does not necessarily imply that the organism is safe. On the other hand, lack of familiarity with a novel organism used in a particular new manner does not necessarily mean that the process is hazardous. In that case, risk managers have to cope with uncertainties.

2.1. Classification of Natural Organisms on the Basis of Hazard

For natural organisms, hazard identification always relates to the pathogenicity of the organism and to the potential for epidemics. It is important to recall that the great majority of micro-organisms are harmless and many are beneficial. About 90 percent of micro-organisms used in biotechnology are harmless, either as wild types or mutant derivatives thereof. Nevertheless, pathogenic micro-organisms receive much attention because they represent a threat for the human health, the agriculture or the environment [see also - Environmental Biotechnology].

Several attempts have been made to classify human, animal and plant pathogens according to the risks they present to the laboratory staff first, and next to the collectivity and the environment should they escape from the biotechnological process or from the laboratory. A worldwide agreement exists on the four-group classification system (Table 1) for human pathogens (bacteria, fungi, viruses and parasites) ranking from those that pose no or negligible hazard (class /group 1) to those responsible for very serious diseases (class/group 4). Examination of the different classifications of biological agents performed by various national committees of experts shows a uniform result. However some disagreements still exist between and even within individual states to allocate specific agents to one hazard or risk group. One of the problems in allocation of risk group arises obviously from the geographic and climatic distribution of the micro-organisms, their reservoir and vectors, especially when animal or plant pathogens are concerned.

Risk Group I (low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.

Risk Group II (moderate individual risk, limited community risk). A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.
Risk Group III (high individual risk, low community risk). A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual to another.
Risk Group IV (high individual and community risk). A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

Table 1. World Health Organization classification of infective microorganisms by risk groups [WHO 1983 and 1993].

2.2. Assessing Risks of Genetically Modified Organisms

There has been long and sometimes controversial debates about the risk potentials and the classification of organisms modified by recombinant-DNA techniques. The discussions lead in many countries to the elaboration and implementation of regulations specifically dealing with GMO's [see also - Biotechnology in the Environment: Potential effects on biodiversity]. It is now accepted that the assessment of the risks of GMO's and their uses should be based on the full set of their characteristics rather than on how they were obtained.

An assessment of the risks to human health and the environment associated with the use of a GMO is based of the following key parameters, when applicable:

(i) the novel organism, taken into account

- the recipient/parental or host organism;
- the donor organism;
- the vector used;
- the insert or the introduced trait;
- any empirical data on the novel organism:

(ii) the intended use (contained or release), including the scale and any management procedures;

(iii) the potential receiving environment.

Chiefly, the choice of these criteria means that the risk groups/classes system is equally valid for both genetically modified organisms and for "natural" ones taking into account genetic and ecological mechanisms occurring in the environment such as gene flow, invasion, persistence and dissemination potential, fitness and impact on the biodiversity.

In the early 90's, the general perception of risk and familiarity was very different for transgenic plants, animals and micro-organisms. While genetically-modified micro-organisms were mainly concerned by research, enzymes production and pharmaceutical applications, transgenic animals were not perceived as a biosafety issue.

On the contrary, the rise of molecular botany in the early 80's and the start of official field tests of transgenic tobacco's in 1986 in Belgium, UK and USA at a very small scale were perceived as the prelude to a giant developmental phase and the short coming source of commercial transgenic crop varieties. Consequently, in the early 90's, the lack of experience with transgenic plant development and commercialisation did justify to take precautions at the highest levels.

Development of transgenic plants or veterinarian vaccines were consequently allowed on a case-by-case and through stepwise procedures of authorisation. The regulations on both sides of the Atlantics imposed to the operators to work gradually from a highly contained and controlled situation to more open and less controlled one (see section 4). Additionally, field monitoring was either advised or imposed by regulatory authorities and justified as a way to objectivate knowledge and experience.

However, the lack of experience itself has made the monitoring parameters questionable themselves. Therefore also, national or international authorities did support basic research on specific biosafety topics, the BAP and BRIDGE Biosafety programs of the European Commission being quoted here as an example.

In practice, every transgenic plant that has been released in the environment so far should have been classified, and were officially classified as such in certain countries, as belonging to the class 1 of biological risks

In 2000, the perception of risk has evolved a lot since transgenic crops have started to be commercialized in many countries of the world since 1996. Presently, the concept of "release" itself does encompass the development, the large scale production and the placing of GM-based products on the market including the multiple uses of GM-based products from the field down to the waste chains.

Both intentional and accidental releases are now considered and do include processing, distribution and recycling pathways. Moreover, long term impact of the different uses, the delayed and/or indirect risks will have to be assessed in the next future provided scientific criteria of assessment and the financial means of assessment become available.

More recently, transgenic plants being a source of food and feed and being genetically traceable by nucleic acids-based technologies, traceability of transgenic plants as a product or a by-product on the market is more and more perceived by the consumers as safety and public acceptance issues.

Monitoring or surveillance are now *de facto* coupled to concepts of quality management and certification applied to all agro-food developmental, industrial and commercial practices.

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Biographical Sketches

Jean-Marc COLLARD, born in 1961, obtained his PhD degree from the University of Liège (Belgium) in 1989 where he conducted researches on the genetic and biochemical bases of cadmium resistance in unicellular algae. His post-doctoral fellowship done at Nuclear Research Centre in Mol on the bacterial resistance to heavy metals allowed him to acquire experience in molecular biology and biotechnology. He then worked for two years on bacterial gene transfer at the Flemish Institute for Technological Research, on a European programme on the Fate of genetically engineered micro-organisms and genetically engineered sequences in some environmental hot spots. Since 1993 he has been working for the Service of Biosafety and Biotechnology (the secretariat of the Belgian Biosafety Council) at the Institute of Public Health whose primary duties involve scientific assessments in the field of contained use and deliberate release of GMO's. He is a member of the steering committee of the European network of inspectors for Directive 90/219/CEE. He also teaches Biosafety at the University of Liege and conducts a research programme on the spread of antibiotic resistance genes in the environment.

Didier BREYER received his Ph.D. in Biology from the University of Liege (Belgium) in 1989 and conducted research activities for 6 years in the field of molecular biology applied to micro-organisms. Since 1995 he has been working in the Service of Biosafety and Biotechnology (the secretariat of the Belgian Biosafety Council) whose primary duties involve the scientific and technical assessments for the Belgian competent authorities of any activities using GMO's and pathogens, including genetic and ecological aspects related to biodiversity. Since 1996, he has been closely involved in the negotiation and the implementation of the Cartagena Protocol on Biosafety. He has been designated as national Focal Point for this international agreement. He is also representing Belgium in various international bodies acting in the field of biosafety: OECD (Working Group on the Harmonisation of Regulatory Oversight in Biotechnology), UNEP, CEN.

Suzy RENCKENS graduated as Engineer in Biotechnology at the Free University of Brussels (VUB). In 1994 she obtained a PhD in Applied Biological Sciences at the same university, carrying out fundamental research in the area of plant molecular biology, more specifically on gene silencing and transposable elements in plants.

In June 1996 she left her post-doctoral research to join the Section of Biosafety and Biotechnology of the Institute of Public Health where she since is involved as biosafety expert with all notifications concerning the deliberate release and the placing on the market of genetically modified plants. She is the secretary of the Scientific Committee 'Transgenic plants' of the Biosafety Council, the Belgian advisory body on GMO's and is engaged as technical expert in meetings organised by the Belgian competent authorities and the European Commission on this topic.

Myriam SNEYERS, born in 1962, obtained her graduate of engineer in agronomy and her teaching diploma for higher secondary education at the University of Gembloux in 1985. She worked as research scientist in different area: she studied rotaviruses and pestiviruses at the University of Liege (1985-1988) and then the molecular endocrinology of bovine development at the University of Gembloux (1988-1994) where she received her PhD. She also gained experience in the pharmaceutical industry (SmithKline Beecham Biologicals) where she worked as research scientist on SIV and HIV (1988) and as quality control supervisor of vaccines (1994-1995). She expanded her formation by following MBA courses at the University of Louvain-La-Neuve (1992-1994). Since 1995, she is biosafety expert for the Service of Biosafety and Biotechnology at the Scientific Institute of Public Health. She is working within the framework of regulations on the contained use and deliberate release of genetically modified organisms; her specific biosafety domains of expertise are high containment levels, animal facilities, gene therapy, vaccines, growth factors, clinical trials, human and veterinary medicinal products. She is also an expert for gene therapy at the European Commission level.

Ellen VAN HAVER, born in 1975, graduated in 1998 as Bio-engineer (specialisation: food microbiology and food technology) at the University of Leuven, Belgium [1993-1998]. Afterwards she stayed at the University of Leuven from 1998 until 2000, working as a research assistant at the Laboratory of Food Technology of the Faculty of Agricultural and Applied Biological Sciences of the University of Leuven. Currently, she is involved in the administration of applications for the registration of genetically modified foods at the Section of Biosafety and Biotechnology of the Institute of Public Health of Brussels, Belgium.

Bernadette VAN VAERENBERGH, born in 1949, studied biology at the University of Leuven, Belgium (1968-1971) and biochemistry at the university of Ghent, Belgium (1971-1973).

She started working at the Laboratory of Experimental Cancerology, Academic Hospital, Ghent on the mechanisms of interaction between normal and cancer cells. (1973-1976).

Since 1976 she is working at the Institute of Public Health, first at the Department of Environment (1976-1983: study on radiotoxic effect of tritium in waste water from nuclear reactors, and 1983-1989: survey on air pollution by heavy metals), and from 1990 to 1995 at the Department of Microbiology, Section of Mycology, on molecular typing (PCR, RAPD) of fungal populations of medical interest.

She is now since 1995 working at the Section of Biosafety and Biotechnology as biosafety expert for the regional authorities on all matters related to the regional regulations of the contained use of genetically modified organisms

William MOENS, born in 1948, Zoologist of the Free University Brussels obtained a Ph.D. in Molecular Biology under supervision of Jean Brachet for the study of the role of cyclic nucleotides in the control of normal and cancerous proliferation. From 1978 to 1986, he studied the genetic rearrangements produced at the gene and chromosomal levels by genotoxic chemicals at the Institute of Public Health, he was a visiting scientist for 2 years in 1987 at the department of Molecular genetics of Weizman Institute of Science, Rehovot, Israel, where he contributed to a study of the regulation of gene expression along human foetal development of the three 6-phospho-fructokinase iso-enzymes encoded on different chromosomes.

Back to the Institute of Public Health, Brussels, he was mandated by the government in 1990 to implement the EU biosafety regulations of biotechnology in Belgium. A tenure position that led to the creation of the Biosafety Advisory Council and its permanent executive body, the Service of Biosafety and Biotechnology. Such a service gathers the experience of the risk assessment of transgenic plants, biotechnological research and production and clinical research with recombinant medicinal GMO's since 1986. He is currently involved as governmental expert for all biosafety matters at the EU and international levels.

In parallel, he developed a laboratory specialised in gene tracing using PCR-based technologies applied to biosystematics, molecular taxonomy of filamentous fungi and, recently, to the tracing of genetically-modified organisms in the food/feed chains and in environmental complex matrices. He is the chief-editor of the internationaly recognized "Belgian Biosafety Server" at http://biosafety.ihe.be. He admires the cathedral builders, Mozart and the structure of DNA.