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COMMISSION DECISION

of 28 February 2005

establishing guidance notes supplementing part B of Annex II to Council Directive 90/219/EEC on the contained use of genetically modified micro-organisms

(notified under document number C(2005) 413)

(Text with EEA relevance)

(2005/174/EC)

(OJ L 59, 5.3.2005, p. 20)

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**COMMISSION DECISION****of 28 February 2005****establishing guidance notes supplementing part B of Annex II to Council Directive 90/219/EEC on the contained use of genetically modified micro-organisms***(notified under document number C(2005) 413)***(Text with EEA relevance)**

(2005/174/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms ⁽¹⁾, and in particular the introductory paragraph of part B of Annex II thereto,After consulting the European Food Safety Authority ⁽²⁾,

Whereas:

- (1) The criteria listed in part B of Annex II to Directive 90/219/EEC must be met in order to establish the safety of a genetically modified micro-organism (GMM) for human health and the environment and its suitability for inclusion in part C of Annex II to that Directive.
- (2) The application of those criteria should be facilitated through the provision of guidance notes for Member States, as an aid to ensuring that the national competent authorities carry out their preliminary assessment in an appropriate manner and provide appropriate information to users as to the content of dossiers to be submitted.
- (3) The measures provided for in this Decision are in accordance with the opinion of the Committee established under Article 21 of Directive 90/219/EEC,

HAS ADOPTED THIS DECISION:

Article 1

The guidance notes set out in the Annex to this Decision shall be used to supplement part B of Annex II to Directive 90/219/EEC.

Article 2

This Decision is addressed to the Member States.

⁽¹⁾ OJ L 117, 8.5.1990, p. 1. Directive as last amended by Regulation (EC) No 1882/2003 of the European Parliament and of the Council (OJ L 284, 31.10.2003, p. 1).

⁽²⁾ The EFSA Journal (2003) 18, pp. 1 to 15.

Guidance notes supplementing part B of Annex II to Directive 90/219/EEC

INTRODUCTION

Types of GMMs are only judged to be suitable for inclusion in Annex II, part C when both the general and specific criteria set out in Annex II, part B are met.

All GMMs included in Annex IIC will be published in the Official Journal along with appropriate identifying characteristics or reference sources of the GMM. When considering whether a type of GMM is suitable for inclusion in Annex II, part C all components and where relevant the process used to construct the GMM should be considered. It should be noted that whilst consideration of all aspects must be undertaken it is only the properties of the GMM which will be judged against the criteria in Annex II, part B. If all the components of the GMM were individually considered and determined to be safe it is likely that the GMM will meet the safety criteria. This should not, however, be assumed and must be thoroughly examined.

If GMMs are produced as intermediate organisms in the production of a final GMM, these intermediates should also be judged against the criteria in Annex II, part B for each type to be exempt and thus de facto allow exemption of the whole contained use to be acceptable. Member States should ensure that the following guidelines are used, by users, to facilitate compliance with these criteria in producing appropriate dossiers establishing the safety, for human health and the environment, of types of GMMs to be included in part C to Annex II and by the national competent authorities to assess compliance.

Dossiers should include detailed and substantiated evidence to enable Member States to judge whether statements concerning the safety of GMMs in terms of the criteria are justified. A precautionary approach should be adopted where scientific uncertainty exists and only where there is convincing evidence to demonstrate that the criteria have been met will GMMs be considered for exemption.

The national competent authority receiving a dossier for this purpose should, following a positive appraisal as to compliance with the criteria, forward it to the Commission, which in turn should consult the committee, formed according to Article 21 of the Directive, as to inclusion of the GMM in question in its Annex II, part C. Definitions of the terms used are set out in Appendix 1.

1. GENERAL CRITERIA

1.1. *Strain verification/authentication*

The identity of the strain should be established and authenticated and the vector/insert should be well characterised for its structure and function as it occurs in the final GMM. A detailed strain history (including the history of genetic modifications) provides useful information for safety evaluation. The taxonomic relationship to closely related, known, harmful micro-organisms should be understood, as this can give information about possible harmful characteristics not normally expressed but which as a result of the genetic modification may be expressed. For eukaryotic cell and tissue culture systems these should be verified for their identity in accordance with international classifications (ATCC or others).

Relevant literature should be searched for history, safety records, taxonomic detail, phenotypic and genetic markers, e.g. Bergeys Manual of Determinative Bacteriology, scientific papers and journals, information from commercial companies supplying the DNA. Useful information can also be obtained from culture collections and culture collection organisations such as the World Federation of Culture Collections (WFCC), who publish the World Directory of Collections of Cultures of Micro-organisms, and the European Culture Collections Organisation (ECCO). Major European culture collections which maintain broad groups of micro-organisms should also be taken into account. In the case of a novel isolate or a strain that has not been extensively studied, any issues still unanswered should be addressed by the tests carried out to confirm identity of the GMM. This could well arise where the GMM strain differs appreciably from its parent strain(s), for instance if it is derived from cell fusion or is the result of multiple genetic modifications.

Where tests are necessary to confirm the identity of the strain, these tests can include morphology, staining, electron microscopy, serology, nutritional profiles based on utilisation and/or degradation, iso-enzyme analysis, protein and fatty acid profiles, % G + C, DNA/RNA fingerprints, amplification of taxon specific DNA/RNA sequences, gene probing, hybri-

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disation with rRNA specific DNA probes and DNA/RNA sequencing. The results of such tests should be documented.

For the identification of the genes in the GMM, the optimum situation is where the complete nucleotide sequence of the vector and insert are known. The function of each genetic unit can then be accounted for. The vector and insert should be limited in size, where possible, to the genetic sequences required to perform the intended function. This decreases the probability of introduction and expression of cryptic functions, or the acquisition of unwanted traits.

1.2. *Documented and established safety*

Documented evidence of safe use of the GMM should be provided. This may include results from tests previously performed, data from a literature search or established record of the safety of the organism. It should be noted that a history of safe use does not necessarily establish safety, especially when the GMM has been used under highly controlled conditions for reasons of safety.

Documented evidence of established safety of the recipient or parental strain will be a key element of support in deciding whether a GMM meets this criterion. However, the GMM may have significant changes compared with the parent(s) which could affect safety and these must be investigated. In particular, care should be taken if the genetic modification was designed to remove a harmful or pathogenic trait from the recipient or parental strain. In such cases clear, documentary evidence of the successful removal of harmful or potentially harmful traits should be provided to prove safety. If data is not available for the particular recipient or parental strain, it may be possible to use data collected for the species. This data, supported by a literature survey and taxonomic investigation of the strain variation within the species, may provide evidence of the safety of the recipient or parental strain concerned.

If information to prove safety is not available, then appropriate tests would have to be carried out to establish the safety of the GMM.

1.3. *Genetic stability*

The genetic modification should not increase the stability of the GMM over the unmodified micro-organism in the environment if it could lead to harm.

Where any instability in the genetic modification could adversely affect safety, evidence of stability must be provided. This is especially so in cases where a disabling mutation has been introduced into the GMM to attenuate harmful properties.

2. SPECIFIC CRITERIA

2.1. *Non-pathogenic*

The GMM should not be capable of causing disease or harm to healthy humans, plants or animals under any normal conditions or as the result of a reasonably foreseeable incident such as a needlestick injury, accidental ingestion, aerosol exposure, and escape leading to environmental exposure. Where there is an increased likelihood that immunocompromised individuals are exposed to the GMM, for example, where the GMM is to be used in a clinical setting, the possible effects of such exposure should be taken into account when judging the overall safety of that GMM.

The literature searches and background information gathered for the general criteria should provide much of the information required here. Historical data on handling and safety of the species and closely related strains should be investigated. Lists of human, animal and plant pathogens should also be searched.

Eukaryotic viral vectors, to be included in Annex IIC should not produce harmful effects on human health and the environment. Their origin should be known as well as the mechanism of their attenuation and the stability of the features concerned. Whenever practicable the presence of such features in the virus should be confirmed, before and after modification is carried out. Where such vectors are used, only deletion mutations should be employed. Constructs that use DNA or RNA vectors derived from viruses in cultured cells as hosts where no infective virus is involved or can be produced may also be appropriate.

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Non-virulent strains of acknowledged pathogenic species, such as live human and animal vaccines, could be considered as unlikely to cause disease and as such satisfy the criteria for Annex IIB provided that:

1. the non-virulent strain has an established record of safety with no adverse effects on human, animal or plant health (lit. survey); or
2. the strain is stably deficient in genetic material that determines virulence or has stable mutations known to sufficiently reduce virulence (pathogenicity tests, genetic investigation, gene probes, phage and plasmid detection, restriction enzyme mapping, sequencing, protein probes) and for which good evidence of safety exists. The risk of reversal of gene deletion or mutation by any incoming gene transfer event should be considered.

To obtain the information required, if not revealed by a literature and taxonomic survey, pathogenicity tests appropriate to the micro-organism in question should be carried out. These tests should be carried out on the GMM, although in some cases tests on the recipient or parental strain could be adequate. However where the GMM is considerably different to its parental organism(s) care should be taken to avoid false conclusions of non-pathogenicity.

Examples of recipient or parental strains of micro-organisms for the production of GMMs that could be considered suitable for inclusion in part C to Annex II include:

- adequately disabled derivatives of bacterial strains, e.g. *Escherichia coli* K12 and *Staphylococcus aureus* 83254 whose growth and survival depends on the addition of nutrients not available in humans or in the environment outside of culture media, e.g. di aminopimelic acid requirement, thymine auxotrophy,
- Eukaryotic cell and tissue culture systems (plant or animal, including mammalian) can be considered as adequately disabled hosts. The GMMs based on the cells should meet the other criteria listed here (e.g. no harmful adventitious agents and non-mobilisable vectors),
- strains of non-pathogenic, wild type hosts may have extremely specialised ecological niches for which accidental escape from control would have minimal environmental impact or have very widespread benign occurrence for which accidental escape from control would have minimal human, animal, or plant health consequences. Examples of such hosts include lactic acid bacteria, rhizobacteria, extreme thermophiles, antibiotic-producing bacteria or fungi. The above must be micro-organisms with an established record of well-developed genetics and molecular knowledge.

The vector and insert as they occur in the final GMM, should not contain genes expressing an active protein or transcript (e.g. virulence determinants, toxins, etc.) at a level and in a form which endow the GMM with a phenotype likely to cause disease to humans, animals and plants or to cause adverse effects in the environment.

Use of a vector/insert containing sequences which code for harmful traits in certain micro-organisms, but which do not endow the GMM with a phenotype likely to cause disease to humans, animals and plants or adverse effects in the environment, should be avoided. Care should also be taken that the inserted genetic material does not encode a pathogenicity determinant capable of substituting for a disabling mutation present in the parental organism.

The phenotype resulting from a vector may be dependent on the recipient or parental organism; what is true for one host should not be automatically assumed when the construct is transferred to a different host. For example, a disabled retrovirus vector in bacteria or most cell lines would be incapable of producing infective virus particles. However, the same vector in a packaging cell line would produce infectious virus particles and, depending on the nature of the disablement and insert sequences, may endow the GMM with a phenotype likely to cause disease.

2.1.1. Non-toxicogenic

The GMM should not produce unexpected toxins nor increased toxigenicity as a result of the genetic modification. Examples of microbial toxins are exotoxins, endotoxins and mycotoxins. Consideration of the recipient or parental strain would provide useful information on this point.

It should be considered that where the recipient or parental strain was toxin free, attention must be paid to any possibility of the vector/insert introducing toxins or stimulating/de-repressing toxin production. The

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presence of toxin should be carefully considered although it does not necessarily exclude the GMM from being included in Annex IIC.

2.1.2. Non-allergenic

Whilst all micro-organisms are to some degree potentially allergenic, some species are noted allergens, these can be found in Council Directive 93/88/EEC ⁽¹⁾ and Commission Directive 95/30/EC ⁽²⁾ and amendments thereof. It should be considered whether the GMM belongs to this particular allergenic group. Allergenic components of micro-organisms can include cell walls, spores, naturally occurring metabolic products (e.g. proteolytic enzymes) and some antibiotics. If the vector and insert are expressed in the resulting GMM, the gene product must not possess biological activities which could lead to significant allergens. It should be noted that this criterion cannot be applied in absolute terms.

2.2. *No harmful adventitious agents*

The GMM should not harbour known adventitious agents such as mycoplasma, viruses, bacteria, fungi, other plant/animal cells, symbionts which can lead to harm. The use of a recipient or parental strain known to be free from harmful adventitious agents in the construction of the GMM is one method to avoid this. However, it must not be assumed that the GMM will be free of adventitious agents because the parent(s) were. New agents may have been introduced during the construction of the GMM.

Particular care should be taken when determining whether animal cell cultures contain potentially harmful adventitious agents such as lymphocytic chorio meningitis virus or mycoplasma such as *Mycoplasma pneumoniae*. Adventitious agents may be difficult to detect. Any limitations of screening efficiency should be taken into account.

2.3. *Transfer of genetic material*

The inserted genetic material in the GMM should not be transmissible or mobilisable if it could cause a harmful phenotype in a recipient micro-organism.

The vector and insert should not transfer any resistance markers to the GMM where resistance could compromise therapeutic treatment. Possession of such markers would not a priori exclude the inclusion of the GMM in Annex IIC but would place additional emphasis on the importance of non-mobilisation of such genes.

If the vector is a virus, cosmid or any type of virus-derived vector it should also be rendered non-lysogenic when used as a cloning vector (e.g. defective in the cI -lambda repressor). The insert should not be mobilisable, due to the presence of, for example, transferable provirus sequences or other functional transposing sequences.

Some vectors which are integrated into the host chromosome may also be considered non-mobilisable but should be investigated case by case particularly in consideration of mechanisms that may facilitate chromosome mobility (e.g. the presence of a chromosomal sex factor) or transposition to other replicons that may be present in the host.

2.4. *Safety for the environment in the event of an escape from containment*

Harm to the environment will normally only arise if a GMM can persist and possesses hazardous characteristics. When considering harm to the environment, account should be taken of the different environmental conditions that exist within Member States and where necessary, extreme case scenarios should be considered. Details of previous releases (deliberate or otherwise) and any associated impact on the environment should also be provided where available.

2.4.1. Organism survival

In deciding whether the GMM is likely to cause adverse effects on the environment or disease to plants and animals, consideration should be given to whether the biological characteristics of the GMM will enhance, leave unaltered or decrease the ability of the GMM to survive in the environment. If the GMMs are biologically disabled for survival in the environment these micro-organisms will not survive for any significant periods outside of the containment, and therefore the likelihood of interaction with the environment is reduced.

⁽¹⁾ OJ L 268, 29.10.1993, p. 71.

⁽²⁾ OJ L 155, 6.7.1995, p. 41.

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In considering possible adverse effects on the environment, the possible fate of GMMs that escape from containment into food webs should also be taken into account.

2.4.2. Dispersal

To be able to establish itself in the environment a GMM would have to survive dispersal to, and establish itself in, a suitable niche. Consideration should be given to the method of dispersal and the likelihood of survival during dispersal. Many micro-organisms survive, for example, when dispersed in aerosols and droplets and also via insects and worms.

2.4.3. Organism establishment in the environment

Establishment in a particular environment is dependent upon the nature of the environment into which the GMM escapes and its ability to survive transmission to the new environment. The potential for establishment in a suitable niche varies with the size of the viable population, the size of the niche and the frequency of suitable niches for the species. The probability will be different for each species. In addition resistance or sensitivity to biotic or abiotic stresses will have a great influence on the establishment of a GMM in the environment. The persistence of a GMM in the environment over a significant period is linked to its ability to survive and adapt to environmental conditions or to initiate a competitive growth rate. These factors may be influenced by the genetic modification and the site of integration. There are examples where the genetic modification would be unlikely to produce this effect, for example when:

- the gene product contributing to the formation of a secondary metabolite, formed at the end of growth, cannot promote growth initiation.

2.4.4. Transfer of genetic material

More information is becoming available on transfer of genetic material between micro-organisms. Even if the GMM has a very limited capacity to survive it will be important to decide on the potential for the introduced genetic material to persist in the environment or be transferred to other organisms and cause harm. Transfer of genetic material has been shown to occur, for example, under experimental conditions in soil (including rhizospheres), animal guts and water by either conjugation, transduction or transformation.

The chance of genetic material transfer from GMMs, with a low probability of growth and limited survivability is very low. If the GMM did not carry self-transmissible plasmids or transducing phages, active transfer is practically excluded. The risk would be very small if the vector/insert are not self-transmissible and are poorly mobilisable.



APPENDIX I

Definitions of terms used in this document

Adventitious agents — other micro-organisms, active or latent, existing alongside/inside the required micro-organism.

Antigen — any molecule which induces B cells to produce a specific antibody. A molecule which can be specifically recognised by the adaptive elements of the immune system, that is by B cells or T cells or both.

Allergen — an antigen which can sensitise individuals such that a hypersensitivity reaction is provoked in individuals on subsequent exposure to this allergen.

Allergy — immediate hypersensitivity reactions, occurs when an IgE response is directed against an innocuous antigen such as a non-pathogenic, non-viable bacteria cell. The resulting release of pharmacological mediators by IgE sensitised mast cells produces an acute inflammatory reaction with symptoms such as asthma, eczema, or rhinitis.

Conjugation — the active transfer of DNA from one host to another.

Cosmid — type of cloning vector comprising a plasmid in which the *cos* sequences of a lambda phage have been inserted.

Disease — any disturbance of structure or function in an immunocompetent human, animal or plant of such a degree as to produce detectable illness or disorder.

Expression — the process of producing RNA transcripts, proteins and polypeptides using the information contained in the genes, of the GMM. In this guidance expression is also a measure of the anticipated or known level of expression of the inserted genetic material.

Mobilisation — the passive transfer from one host to another.

Mobilisation defective — vectors defective in one or more transfer functions and which are unlikely to be mobilised by other elements which supply the missing functions.

Pathogenicity — the ability of the micro-organism to cause disease which can be by infection, toxicity or allergenicity. Pathogenicity is a taxonomically significant attribute and is the property of a species.

Plasmid — an extrachromosomal self-replicating piece of DNA, found in many micro-organisms, that generally confer some evolutionary advantage to the host cell.

Recipient or parental micro-organism — the micro-organism(s) to which the genetic modification occurred.

Rhizobacteria — bacteria which inhabit the rhizosphere, i.e. the soil adhering to plant roots, eventually entering the roots either intracellularly or intercellularly. Rhizobacteria are often used as microbial/seed inoculants in agriculture.

Transduction — the incorporation of bacterial DNA in bacteriophage particles and their transfer to recipient bacteria.

Transformation — the uptake of naked DNA by a cell.

Vector — a carrier DNA or RNA molecule, e.g. plasmid, bacteriophage into which a genetic material sequence can be inserted for introduction into a new host cell where it will be replicated *and* in some cases expressed.

Virulence — the capacity to cause harm. Individual strains of a micro-organism can vary widely in their ability to harm the host species.