EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

On a generic approach to the safety assessment of micro-organisms used in feed/food and feed/food production

A working paper open for comment

Comments are invited from interested parties. Please send your comments before 30th June 2003 to the following e-mail address:

sanco-sc2-secretariat@cec.eu.int

This document for public consultation has been produced by a Working Group consisting of members of the Scientific Committee on Animal Nutrition, Scientific Committee on Food and the Scientific Committee on Plants of the European Commission.

Background

1. A wide variety of bacteria and microfungi are used to produce fermented foods in Europe and in other parts of the world. The bacterial genera involved include *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Micrococcus, Streptococcus*, *Staphylococcus*, *Propionibacterium* and *Acetobacter*. In addition to the well recognised use of *Saccharomyces* in the production of food and beverages, other fungal genera such as *Kluyveromyces, Pichia, Kloeckera, Candida*, *Penicillium*, *Aspergillus* and *Mucor* are used in the production of a variety of foods. Consequently, products of microbial action (alcoholic drinks, fermented milks, butter, cheeses, leavened and sourdough bread, pickled vegetables and fruit, cured meats, chocolate, tea and coffee) and/or the organisms themselves are part of an everyday diet. Some of these foods are manufactured using defined starter cultures, but many, even in industrialised processes, are produced either by spontaneous fermentation or by back-slopping.

2. With the exception of those micro-organisms not previously used to a significant degree in the preparation of a human food within the Community (captured by the Novel Foods Regulation[1]), micro-organisms for food use are not subject to Community regulation. Implicit in this absence of any formal requirement for a safety assessment is the recognition that there has been a long history of presumed safe use.

3. This is in marked contrast to micro-organisms entering the food chain in association with animal feeds or as plant protection products, both of which are comprehensively regulated in Europe[2,3]. Although many of the organisms used in animal feed or as a source of processing aids are the same or closely similar to those used in human food production, there is currently no mechanism for extrapolating from the experience of the food industry. This is partly because there is no recognised means for a micro-organism *formally* to be considered as safe for human food applications.

4. This has already led to situations where the same or closely related strains used freely in human foods have been the subject of stringent safety assessments when seeking Community approval as a feed additive. Conversely, legitimate concerns, such as the presence of antibiotic resistance factors, which determined the need for a safety assessment for microbial feed additives, are not addressed when the same organism has only a traditional food use.

Scope and purpose

5. The purpose of this document is to explore the possibility of introducing a system, similar in concept and purpose to the GRAS (Generally Recognised As Safe) definition used in the USA, which could be applied to micro-organisms and eventually their products and to invite comments on its practicality. It is evident that such a scheme should not compromise on safety but should ideally improve, extend, clarify and make more consistent the approval procedures for micro-organisms and, where possible, allow a more generic approach to be taken in place of a full case-by-case assessment.

6. Such an approach should not seek to reproduce the GRAS system but should take account of the different social and regulatory climate present in Europe. This is necessary since issues of importance to Europe would not necessary influence a GRAS listing. An example of this in the context of micro-organisms would be the presence of acquired antibiotic resistance factors, considered highly undesirable in Europe but currently of lesser issue in the USA.

---

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (E.C. O.J. n° L 43 of 14/2/1997, p. 1)

[2] Council Directive 93/113/EC of 14 December 1993 amending Council Directive 70/524/EEC concerning additives in feedingstuffs (E.C. O.J. n° L 334 of 31/12/1993, p. 17)

[3] Council Directive 2001/36/EC of 16 May 2001 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market (E.C. O.J. n° L 164 of 20/06/2001, p. 1)

7. Consequently it is proposed that any "generic listing" of a micro-organism should be **qualified**, allowing the general safety of the organism/group of organisms to be concluded provided that certain specific criteria are met. For example, for many of the live organisms currently used in the manufacture of, or added to, dairy products, this may simply be a requirement to demonstrate the absence of acquired antibiotic resistance factors.

Qualified presumption of safety

8. It is suggested that these requirements could be met by a system which would allow a:

   Qualified presumption of safety (QPS), presumption being defined as "an assumption based on reasonable evidence" and qualified to allow certain restrictions to apply.

9. QPS would provide a qualified generic approval system that would harmonise the safety assessment of micro-organisms throughout the food chain. This could be done without either compromising the standards set for micro-organisms used in animal feedingstuffs or requiring all organisms used in food production with a long history of use to be subjected to a full and unnecessary safety review. Thereafter it would aid the consistency of assessment and make better use of assessment resources without compromising safety.

10. A case-by-case safety assessment then could be limited to only those aspects that are relevant for the organism in question (*e.g.* the presence of acquired antibiotic resistance determinants in a lactic acid bacterium or known virulence factors in a species known to contain pathogenic strains).

11. However, to have any value a QPS scheme must be seen as assuring safety both by food/feed manufacturers and consumers. Similarly, QPS must show clear advantages over a full case-by-case approach allowing Notification to substitute for a repeat assessment when another use or production method is found for an organism already granted QPS status.

General considerations in a QPS scheme

12. Whatever the use and identity of the organism(s) there are a series of general conditions that would have to be met before QPS status could be established (see Figure 1). The starting point must be identity at whatever taxonomic level for which QPS status is sought. This could be at the genus level, but more likely would be for a named species, a recognised subgroup of a species or for a single well recognised/characterised strain (e.g. *E. coli* K12, a strain selected for its lack of pathogenic potential).

13. Thus a pre-requisite for QPS would be identity, unambiguously established at the taxonomic level claimed. The appropriate biochemical and molecular biological methods must exist to enable this to be done. The importance of taxonomy in the

risk assessment of micro-organisms is recognised internationally and is the subject of a guidance document currently being produced by the OECD Working Group on Harmonization of Regulatory Oversight in Biotechnology.

14. Many industrial strains of micro-organisms will be a product of a selection/mutagenesis programme designed to improve their phenotype for a particular purpose. In the majority of cases cryptic mutation or selection for the same use (*e.g.* increased phage resistance or over-production of an enzyme) will not affect taxonomic status. Use of recombinant technology for strain improvement is the subject of separate existing legislation[4,5].

15. If the taxonomic unit cannot be related *via* the existing and any historic nomenclature to a body of knowledge, then QPS status is not applicable. This is most likely to occur when an isolate identified to the genus level cannot be assigned to an existing species/sub-species or when the species is a newly recognised taxonomic unit.

16. The second test that would have to be applied is the question of *familiarity* and, in particular, the degree of familiarity.

17. Familiarity in this context is taken to include practical experience of use of the organism(s) including its history of use for particular purposes and any body of literature on the biology of the taxonomic unit. Judgement as to whether the organism(s) can be considered familiar should be based on a weight of evidence approach. This must be sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and predictable.

18. For organisms not commonly used in food production or without a long history of use, this implies a need for experimental data on the genetics of the taxonomic unit and the growth and biochemical characteristics of the component strain(s) under a variety of relevant environmental conditions. This should provide sufficient material for the third test applied – that of pathogenic potential (human or animal).

19. Many micro-organisms can, under extreme conditions (*e.g.* in the severely immuno-compromised), be found associated with diseased tissue. The occasional clinical report of a micro-organism or group of micro-organisms being isolated from clinical specimens should not necessarily result in the taxonomic unit being treated as potential pathogens.

20. If a taxonomic group is commonly responsible for pathological conditions, then QPS does not apply. However, if pathogenicity is limited to selected strains and if the mechanism underlying the pathology is understood and testable, then the taxonomic unit might still be eligible for QPS status; the qualifications attached

---

[4] Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms (E.C. O.J. n° L 330 of 5/12/1998, p. 13)

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/20/EEC - Commission Declaration (E.C. O.J. n° L 106 of 17/4/1990, p. 1)

being used to exclude the pathogenic strains. This is important in relation to, for example, *Bacillus* species and their toxigenic potential or to the virulent/avirulent forms of enterococci.

21. For some groups of organisms, such as those used as plant protection products, a consideration of impact on the wider environment may be appropriate. However, this would exclude organisms considered either to be of healthy gut origin and regularly introduced into the wider environment or to be of soil/water origin. In both cases the organisms are naturally occurring and therefore free of any need for an environmental impact assessment.

22. The final general question relates to end use; and has, in essence, three possible outcomes:

    - *A live organism is a component of a final product intended to enter the food chain directly (it is consumed);*

    - *A live organism is a component of a final product but is not intended to enter the food chain although it may enter it adventitiously (*e.g. *a plant protection product);*

    - *The organism(s) is used only as a production strain with the final preparation containing fermentation product(s) intended to be free of live organisms.*

23. The end use will influence the nature and degree of familiarity needed to determine whether the taxonomic unit is suitable for QPS status. It will also influence the qualifications imposed. It is envisaged that for products of fermentation there would be separate considerations for QPS status for the production strain and the product itself. Thus QPS status for a production strain would allow a presumption of safety to be applied to the production system but not to the product.

Qualifications

24. It is envisaged that virtually all organisms considered suitable for QPS status would have qualifications attached. Possible exceptions to this generalisation are some fungal genera/species, such as *Saccharomyces* spp. and some *Kluyveromyces* strains.

25. Although each consideration for QPS status would have to be on a case-by-case basis and so some qualifications may be unique to a particular organism and its application, there are a number of qualifications likely to be more widely applied, particularly to bacteria. For example:

    - Live bacteria entering the food chain *via* animal feed, or live and dead bacteria directly consumed by humans should be free of any acquired resistance to antibiotics of importance in clinical and veterinary medicine. The presence of antibiotic resistance determinants, however, would not exclude their safe use for production purposes provided that only the fermentation product(s) are retained in the final product.

- Similarly organisms entering the food chain or used for production purposes should not be capable of producing antibiotics with structural similarities to those of importance in human and veterinary medicine likely to encourage development of resistance.

- Bacteria from taxonomic groups known to contain some strains capable of toxin production (*e.g. Bacillus subtilis*), should be demonstrated free of any toxigenic potential.

26. Where qualifications applied to strains within a taxonomic unit granted QPS status require demonstration of a lack of a particular potential (*e.g* toxin production, production of virulence factors), where possible, evidence should be sought at a genotypic rather than a phenotypic level.

Establishing QPS

27. If QPS were to be introduced, initially at least it would be as part of an assessment process and as such it would carry no legal status. Since an assessment process has to be consistent in its application, no exclusive advantage could accrue to a Notifier.

28. Consequently, QPS would have to be established by those responsible for risk assessment rather than resulting from the cumulative applications of Notifiers. This might initially centre on the more commonly encountered genera, in particular those used for food application to which some form of regulation might be usefully introduced (lactic acid bacteria, bifidobacteria, *Bacillus* spp.). Thereafter, additions may be at the request of and with the help of Notifiers.

29. A mechanism should also exist for Notifiers to add to the data required to establish QPS where the weight of evidence would otherwise be considered insufficient. The advantage of the QPS status for the Notifier may be the ability to change production conditions (media etc) with only a requirement for notification rather than generating a need for an additional safety assessment.

Requirements of QPS

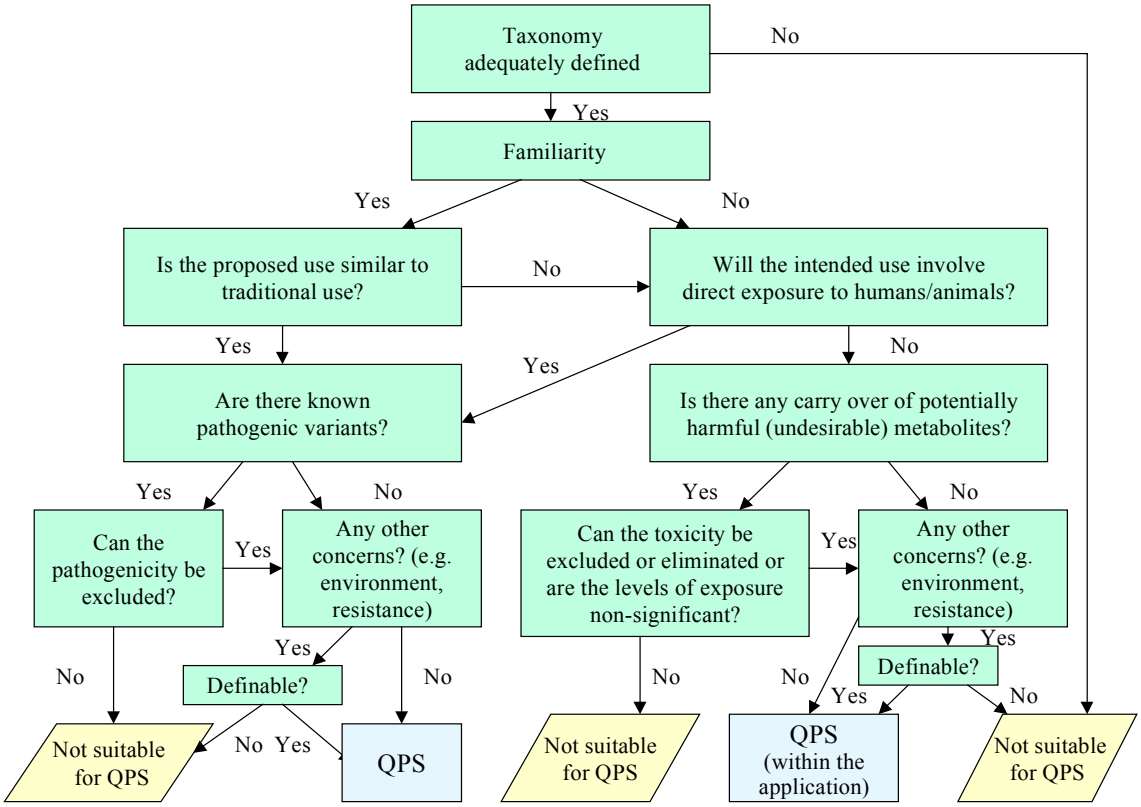
30. For a Notifier with a production strain falling within a taxonomic unit already granted QPS status the only requirement would be:

    - Registration of a production strain with accompanying evidence of its taxonomic status and that the strain meets all of the qualifications imposed for the particular taxonomic unit

    - Notification of any changes in use or to production conditions.

    Otherwise paragraph 28 might apply.

Figure 1. A general scheme for the assessment of suitability for QPS status of micro-organisms.



QPS as a practical exercise

31. The following are examples of how QPS might apply to micro-organisms widely used in the dairy food industry or to bacteria used primarily for bulk production by fermentation. These assessments are preliminary and designed only to illustrate how QPS might be applied. Any conclusions should not be considered definitive or binding.

Dairy lactobacilli

32. The genus *Lactobacillus* is a taxonomically heterogeneous group of organisms producing lactic acid, either as a sole fermentation product (homofermentative lactic acid bacteria -LAB) or together with acetic acid/ethanol and CO_2 (heterofermentative LAB). While certain species are obligatory homofermentative (*Lactobacillus acidophilus, L. helveticus, L. delbrückii*) or heterofermentative (*L. brevis*, *L. fermentum*, *L. buchneri*, *L. reuteri*), the majority metabolise hexose sugars homofermentatively and pentose sugars heterofermentatively. Although modern molecular biological classification methods have revealed clusters of species within the genus, these clusters do not correspond to the traditional grouping based on physiology, morphology and fermentation patterns. Nonetheless, species identification can in most cases be reliably done using few simple physiological and biochemical tests allowing connection to a long history of use and an extensive bibliography.

33. The lactobacilli have been traditionally used to produce a wide variety of fermented foods, including cheese, fermented milks, sourdough, cure meats and sausages etc. In dairy applications, particularly the obligate homofermentative species *L. delbrueckii* ssp. *bulgaricus* and *L. helveticus*, are well known as starters for yoghurt and Swiss cheese, respectively.

34. Besides the main fermentation end products, certain lactobacilli produce variable amounts of other metabolites such as, acetaldehyde ("yoghurt aroma"), formic acid and H_2O_2. Although lactobacilli are not known to produce actual antibiotics, production of protein or peptide bacteriocins active against other Gram positive bacteria is relatively common. Some species and strains also produce some low molecular weight antimicrobial substances, such as "reuterin" or 3-hydroxypropanal (produced by *L. reuteri*). Most of these are, however, chemically poorly defined. Production of biogenic amines by lactobacilli has been occasionally reported.

35. Lactobacilli are fastidious organisms requiring a milieu rich in nutrients, especially fermentable sugars, for growth. Consequently, their natural habitats include the mouth, intestinal and urogenital tract, decaying plant material and milk. No actually pathogenic lactobacilli are known, although they can be occasionally indicated in opportunistic infections, usually in cases where there has been a severe underlying disease.

36. Because common lactobacilli used in dairy applications can be readily identified to the species level, and because the dairy species or strains have only extremely rarely, if ever, been indicated even in the rare opportunistic infections caused by lactobacilli, species such as *L. delbrueckii* and *L. helveticus* could be reasonably considered for QPS-status. The only qualification that might be attached is evidence of the absence of acquired antibiotic resistance.

Bacillus subtilis and related bacteria

37. The guidelines for the delineation of a bacterial species require strains within a species to share more than 70% chromosomal DNA hybridisation and between species less than 70% hybridisation. The *B. subtilis* group traditionally comprises four species*: B. amyloliquefaciens, B. licheniformis, B. pumilus* and *B. subtilis* itself. These taxa all conform to the DNA hybridisation guidelines for bacterial species. More recent ecological studies have identified some very close relatives of *B. subtilis*, notably *B. atrophaeus, B. mojavensis* and *B. vallismortis.*

38. The 16S rRNA gene sequences differ between representative species of the *B. subtilis* group, but such data are not available for the "ecological" group. Species of the traditional group can be distinguished phenotypically, but *B. mojavensis, B. subtilis* and *B. vallismortis* are indistinguishable and can only be identified by molecular means while *B. atrophaeus* is distinguished from *B. subtilis* only by pigmentation. One of the main implications of the inability to distinguish the members of the ecological group is that strains of "*B. subtilis*" being used by industry may actually belong to *B. mojavensis, B. vallismortis* or to other species.

39. The taxonomic status of the traditional members of the group is well established and allows connection to a sizeable body of information on their biology. *B. subtilis* was one of the first organisms to be fully sequenced and its genome is now extensively annotated. Although the species traditionally included in the *B.subtilis* group could be considered as a group for QPS purposes, in the first instance it would seem prudent to deal with them on an individual species basis.

40. Member of the "ecological group", may have considerable genetic similarity to the more generally recognised members, but little is known about their biology. It is unlikely that these species would be proposed for QPS unless a reassessment of taxonomic status led to the inclusion of an existing production strain

41. Strains of *B. licheniformis, B. pumilus* and *B. subtilis* have occasionally been reported as causative agents in food poisoning. Both diarrhoeal and emetic types of outbreaks have been recorded, but the nature of the toxins associated with these species is not fully understood. In particular, it is not clear if the enterotoxins are the same as those of *B. cereus*, the common cause of *Bacillus* food poisoning, or if other enterotoxins are involved. The indirect evidence of the presence for genes similar to the *B. cereus* haemolytic toxin (*Hbl*) provided by PCR, and for Hbl and the non-haemolytic toxin (Nhe) by the commercial ELISA kits is not conclusive. Without purification of toxins and sequencing of the PCR products it is impossible to be sure about the presence of similar or identical virulence factors. However, *B. licheniformis* has been shown to produce a toxin that shows similar physico-chemical properties to cereulide although with a different pattern of biological activity.

42. Other indications of pathological conditions associated with the *B. subtilis* group are rare. However, in many clinical reports on opportunistic infections, the causative *Bacillus* has not been identified to species level. *B. pumilus* stains have been implicated in infections mimicking listeriosis. *B. licheniformis* is also associated with bovine toxemia and abortions, although it is evident that this species is only

weakly virulent and usually will multiply freely only in animals which, for various reasons, are immune compromised.

43. *Bacillus* species are commonly isolated from gut contents but their presence appears to be due to constant re-inoculation rather than outgrowth and clonal expansion. Consequently, both animals and humans are constantly exposed to those *Bacillus* species encountered in the environment with no apparent ill effects. The lack of evidence for retention in the gut also reduces the likelihood of any genetic transfer occurring and any adverse consequences in the very unlikely event that such a transfer occurs.

44. The possible pathology of these organisms essentially is limited to a few strains able to produce symptoms of mild food poisoning and there is sufficient knowledge to allow this risk to be substantially reduced. Consequently, with the possible exception of *B. pumilus,* potential pathogenically is not a barrier for QPS at the species level.

45. Approximately half of the present commercial production of bulk enzymes derives from strains of bacilli, most from the *B. subtilis* taxonomic group. These include proteases and α-amylases (from *B. amyloliquefaciens, B. licheniformis).* Strains of *B. subtilis* are used for the preparation of nucleic acid bases such as inosine which are precursors of flavour enhancing nucleotides for use in the food industry. These bacteria also produce lipopeptide surfactants and a diversity of polypeptide "antibiotics" with activity against bacteria and fungi. Some of these *Bacillus* species (*B. subtilis*, *B. licheniformis*) have also found use in the animal feed industry as live feed additives and have been used as probiotic preparation for humans.

46. Tools exist that allow the identity of strains to be established, the species comprising the *B. subtilis* group are familiar and their biology well understood and sufficient is known about their pathogenicity to exclude problem strains. Consequently, *B. subtilis B. amyloliquefaciens and B. licheniformis* might reasonably be considered individually for QPS status. Strains falling within these taxonomic units could then be presumed safe providing the following qualifications were met:

    - Provision of PCR-based evidence of the absence of a toxigenic potential and, because of doubts about the homology existing between genes encoding enterotoxins, evidence of an absence of effects in cytotoxicity assays.

    - For production strains only in which the live organism is excluded from the final product, evidence of a capacity for toxin production would not necessarily exclude a strain from QPS. However, it would have to demonstrate that the strain failed to produce detectable levels of toxin under the production conditions employed. There would also be a requirement for the same evidence to be produced each time there was a change to the production system. This would not be necessary in the absence of a toxigenic potential.

    - The strain should be shown to be free of any acquired resistance to antibiotics of importance in human and veterinary medicine. Again, the presence of antibiotic resistance would not exclude its safe use for production purposes but would exclude it from use as a live organism likely to enter the food chain.

    - The absence of a capacity to produce antibiotics with structural similarities to those of importance in human and veterinary medicine likely to encourage development of resistance.