



THE REPLACEMENT OF A CASE-BY-CASE SAFETY ASSESSMENT BY A QUALIFIED PRESUMPTION OF SAFETY (QPS) APPROACH FOR MICRO-ORGANISMS

FEFANA CONTRIBUTIONS TO EFSA'S REQUEST FOR COMMENT

FEFANA supports the use of Qualified Presumption of Safety (QPS) for the evaluation of micro-organism intended for fermentation and or as feed additives, as a replacement to individual case-by-case evaluations. FEFANA agrees that micro-organism, for which a large body of evidence already exists on their safety, should not be subject to additional and unnecessary extensive evaluations. FEFANA however considers that application of QPS should be consistent and the subject should not be isolated from the legislative, and thus risk management frameworks, and calls for the immediate integration of QPS in the various assessment tools with which EFSA undertakes risk assessments, and in particular within the guidelines for the evaluation of feed additives currently under negotiations.

FEFANA, the EU Association of feed additives and premixtures, has actively followed the initiation and subsequent discussions concerning Qualified Presumption of Safety (QPS). It has also followed through the concept in the light of the recent public consultation by the Scientific Committee published on the European Food Safety Authority website in January 2007 and has the following general comments. Detailed comments on the individual consultation annexes are in annexes 1 and 2 to this document.

Innovation and Existent Evaluated Products with regards to QPS

Though the QPS concept could introduce a discrepancy in approach towards development of innovative products in comparison to existing well known products, a priority of EU strategy and of our industry, FEFANA considers the approach to be helpful as long as it consistently streamlines the authorisation process in a fair, proportional and transparent manner. With this in mind, FEFANA strongly supports the use of the concept of a QPS in the evaluation of micro-organisms. It agrees that assignment of QPS status should be based on existing body of evidence regarding the safety of the micro-organism. FEFANA particularly appreciates the fact that it is now clear from this consultation, that the QPS concept is intended to apply evenly to live micro-organisms, including those used in fermentation processes, for food and feed use. This consistency in approach here is an important element for the credibility and thus applicability of QPS and no doubt furthers the Regulators' (Commission) upheld holistic farm to fork approach. FEFANA hopes that the approach will also increase consumer confidence and may even facilitate innovation across the board.

Scope of Application of the Currently Proposed QPS Micro-organisms

Despite the point made above, FEFANA is still concerned that the scope of application of the concept may further be restricted if it remains as in the proposed consultation documents. While QPS' value with regards to industry and assessment resources is beyond any doubt, we reckon that EFSA has secured the necessary level of robustness for the system. Being acclaimed to become part of an established regulatory process, it would be unconceivable that the scientific validity of the QPS be put into question once implemented and/or would subsequently be limited to specific cases. FEFANA is however assured that this is unlikely to be the case and or the intention. It is therefore for this reason that it considers that there seems to be no conceivable reason why the use of micro-organisms already assessed for their safety with regards to food and feed use, could not be extended to plant protection and or used as biocides. In other words, this work carried out by the Scientific Committee (*i.e.* the establishment of a list of suitable candidates for QPS status) subject to the present consultation, should already be seen as a practical test of the conceptual development that already preceded it (draft paper, workshop, *etc.*). In that respect, FEFANA believes that following the present consultation, the QPS should be established from the start as a general assessment procedure that applies across all the EFSA panels dealing with micro-organisms whatever their final application.

Integration of the QPS in the Regulatory Framework

FEFANA would wish to highlight an issue it considers of utmost importance regarding subsequent actions following this consultation. The QPS concept introduces an innovative albeit major change in the way the safety of micro-organisms is assessed in the EU legislature, in particular, for the micro-organisms (both deliberately introduced in the chain or otherwise) subject to a pre-marketing approval (*e.g.* feed additives). It is highly imperative that the QPS concept be immediately integrated in the legislative tools (*i.e.* Commission guidelines for the assessment of feed additives, for example) which are pivotal to the work of the Risk Assessor (EFSA) and of the Risk Manager (European Commission and Member States). It would therefore be unconceivable for operators to be bound to certain set of rules instigated by the legislator that is completely different to those put in place by the risk assessor. In this regard, we see a very urgent need to introduce the QPS concept without delay in the "Commission Regulation on Implementing rules and guidelines concerning applications for authorisation of additives for use in animal nutrition in accordance with Regulation 1831/2003", which is close to finalisation. Needless to say that a different approach would dramatically affect the transparency of the authorisation process, and practically hinder the implementation of QPS in general to the detriment of the Scientific Committee's intention(s).

Transparency in the Process of Assignment of QPS Status

A critical aspect is accessibility to obtaining QPS status. The system would greatly benefit from clearly established procedures for submitting a micro-organism taxonomy to QPS status, and transparent criteria on the basis of which the decision is taken. The Scientific Committee began this work by identifying the first series of suitable candidates for QPS status on the basis of current scientific knowledge. The transparency of the process for granting, and of the elements considered before a product is put forward for QPS status are of paramount importance. The decision tree included (Fig. 2) in the EFSA summary report of the Scientific colloquium on QPS is an important element in this respect. FEFANA would therefore be grateful if the process of validation is identifiable in order to further demonstrate the concept of QPS as robust. In this regard, EFSA would do well to consider and integrate the points made in the attached annex 2 concerning *Pichia pastoris*, in its final position on this issue. The species, though identified in Annex I of the

consultation package as one of the species notified to EFSA, and does not seem to exhibit any properties that could lead to it being classified as unsafe, has however not been granted QPS status but the two most common species of this genus seemed to have scaled through.

Inconsistency in EFSA's approach for assigning QPS status

In a number of the annexes, and in particular the case mentioned above, we note a high level of inconsistency in approach from one annex to the other and even within annexes. This could be because more than an EFSA working group were charged and worked independently on the various development and evaluation of the aspects in the annexes without some level of exchanges and or co-ordination between the groups. FEFANA considers that it is only fair, consistent and proportionate if a similar approach is adopted in the evaluation of all microbial species for QPS status. EFSA may therefore wish to revisit some of the resulting evaluations undertaken by its working groups subject to this consultation to ensure some consistency across the board.

SPECIFIC COMMENTS ON ANNEXES 3 – 6 OF THE EFSA CONSULTATION PACKAGE

Comments on Annex 3 – Gram positive bacteria

FEFANA acknowledges the systematic approach taken in some of the assessment of Gram-positive, non-sporulating bacteria, but is nevertheless concerned by what seems to be an omission of a number of species from the evaluation and or lists of the taxonomic groups proposed for QPS status. These are:

- **Oenococcus:** this genus, although mentioned in the list of genera in the first paragraph of Annex 3 that “have been considered by WG QPS-GPNS with respect to QPS approach”, there seems to be no further mention of these group in the body of the text. The position of the Working Group on this genus is therefore not elaborated and or known.
- **Propionibacterium:** several dairy species (DPAB) are cited in points 9.1, 9.4 and 9.5 of Annex 3 where one of them states that “There is a long history of safe use of DPAB, particularly *P. freudenreichii* ...”. It is therefore surprising that the other dairy species which are acknowledged to have safe history of use, with some used as long as record exists in silage industry, such as *P. jensenii* and *P. acidipropionici*, failed to attain QPS status.
- **Corynebacterium glutamicum:** it seems inappropriate and even unfair that this species’ QPS is restricted to ‘production purposes only’ given that some of the species previously assigned to the genus *Brevibacterium* (e.g. *B. casei*, *B. iodinum*, *B. lactofermentum*) but now with improvement in technology re-assigned as *C. glutamicum*, appear to have attained QPS without restriction. Our understanding is that the basis for assignment of unrestricted status to the *Brevibacterium sp* is because of their extensive industrial use, and in particular, in cheese production and amino acid production. It therefore seems unfair that those *Brevibacterium sp* for which there appears to be some evidence of extensive use in food and feed industry will have their QPS status restricted because scientists decided to re-assign them to another genus or vice-versa. There does not seem to be supporting scientific evidence for such a position.

Comments on Annex 4 – Bacillus

FEFANA has no major comment on Annex 4 (Bacillus). EFSA may however wish to clarify the reference in the consultation package to the new restricted definition of the genus *Bacillus* referred to be in the second edition of the Bergey's Manual of Systematic Bacteriology, volume 3. The referenced link reads 2008 but in fact takes you to a volume dated 2006.

Comments on Annex 5 -Yeast

It could be said that yeast contains the species of micro-organisms that could be said to have the longest history of safe use. Evidence for this is the ubiquity of yeast in nature and or in use in the food and beverage industry in addition to direct use in human and animal nutrition.

With over 780,000 tons per year (source: COFALEC, <http://www.cofalec.com/>) European production of yeast and diversity of application in mind, a reported number of 92 pathogenic cases involving *S. cerevisiae* is indeed very low. None of these reported cases concern the healthy

average population but concern patients who were either pre-disposed (intravenous catheter, previous antibiotic therapy, *etc.*) or immuno-compromised.

It is for this reason that FEFANA questions some of the positions taken concerning, in particular, *Saccharomyces cerevisiae*. For a start, (i) it would be helpful if further attention is paid to the taxonomic references and methods of identification referred to in the text in order to ensure that they are accurate. (ii) Some restricting conditions associated to the QPS status for *S. cerevisiae* (*i.e.* should not grow at 42°C and are not filamentous - EFSA may need to define 'growth', which in the case of micro-organisms depend on a number of factors.) do not seem appropriate and notably, are inconsistent and disproportionate if you considered the stance EFSA has taken with some bacterial genus. For example, FEFANA would expect that such a restriction is unnecessary with regards to *S. cerevisiae* where an even lower (in contrast to the high level of use) 'frequency of infection, most often associated with people having predisposing factors' is reported. Pathogenic yeasts are not known to harbour virulence determinants associated to filaments and pseudo-hyphae therefore EFSA's position is puzzling. In addition, *S. cerevisiae*'s long history of safe use is widely known, a fact acknowledged by the EFSA's scientific panel.

Comments on Annex 6 – Filamentous fungi

The working group on filamentous fungi seemed to have concluded that some given species are ineligible for QPS status on the basis that some of their isolates have been reported to produce mycotoxin(s) under certain conditions. However some bacterial strains seem to have been accorded conditional QPS status despite probable capacity to produce toxins. This seems inconsistent and disproportionate.

The QPS approach taken with regards to filamentous fungi is on species level and some species only were considered. It was then concluded that all assessed species were unsuitable for QPS status because the taxonomic groups of the species were too broad and diverse to allow for an assessment of the entire group. FEFANA is concerned with this generic approach and is aware that the evaluation could be open for finer evaluations perhaps via defined groups (*i.e.* a subset of strains from a given species or even a pedigree of strains all originating from the same mother strain, a so called 'safe strain lineage'). For example, demonstration that a strain or lineage does not have the ability to produce toxins should lead to a QPS, albeit conditional in some cases, assigned to the strain concerned. With fermentation products, defined process conditions (defined media components, *etc.*) where non-production of adverse extrolytes has been proven could be considered for QPS status. FEFANA would therefore urge EFSA to revisit this group with the aim of re-evaluating for QPS status on one of the above suggested criteria.

PROPOSED ADDITIONS TO CANDIDATES FOR QPS STATUS' LIST

Summary

The yeast, *Pichia pastoris*, had been notified to EFSA (in Annex 1 of the consultation package). FEFANA therefore questions why this species has not been included in the list of taxonomic units proposed for QPS status (Annex 2) given *Pichia pastoris* is known to be safe with no known toxigenic or pathogenic properties. It has a long history of safe use, and is easily identifiable. Further scientific supporting evidence is attached in appendix 1.

Introduction

Pichia is identified as one of the yeasts considered, with *P. angusta* and *P. anomala* proposed, for QPS status (EFSA, 2007b). FEFANA considers that *P. angusta* and *P. anomala* should not be the only species in *Pichia* that should be proposed for QPS status. *Pichia pastoris* is known to be safe with no known associated toxigenic or pathogenic properties, has a long history of safe use, and can easily be identified.

Taxonomy

The best targets for identifying species of the genus *Pichia* are the sequences of D1/D2 (26S) or PCR and restriction of 5.8S-ITS (EFSA, 2007b).

The general taxonomy of *P. pastoris* is as follows:

Name:	<i>Pichia pastoris</i>
Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Saccharomycetes
Order:	Saccharomycetales
Family:	Saccharomycetaceae
Genus:	<i>Pichia</i>
Species:	<i>pastoris</i>

According to the definitive source of yeast taxonomy (Kreger-van Rij, N.J.W., 1984) as well as a thorough literature search, there are no indications that *Pichia pastoris* has been associated with animal or human illness.

History of Safe Use

Pichia pastoris, a methylotrophic yeast, has been used for the production of over 300 recombinant proteins since the mid-1980's (Cereghino, J.L. *et al.*, 2000). The majority of these proteins have been expressed as secreted proteins using the *Saccharomyces cerevisiae* α -mating factor pre-pro-peptide as a secretion signal. One of the advantages of using *P. pastoris* as a production host is that heterologous protein purification is enhanced due to the low levels of endogenous proteins secreted (Lin Cereghino, G.P. *et al.*, 2001). Many human genes have been expressed in *P. pastoris* for pharmaceutical use. Two such recombinant proteins, Angiostatin and Endostatin, have completed Phase I clinical trials and are entering into Phase II clinical trials (Herbst, R.S. *et al.*, 2002). *P.*

pastoris is also used for the production of vaccines. *Enivac-HB* is a recombinant hepatitis B vaccine expressed in *P. pastoris*; a recent clinical study demonstrated that *Enivac-HB* is safe and provides effective titers against hepatitis B (Hussain, Z. *et al.*, 2005). A Phase I clinical trial has been completed for an AMA1-C1 malaria vaccine, whose proteins have been expressed in *P. pastoris*. In this clinical trial, the AMA1-C1 malaria vaccine proved to be safe and able to induce a significant immune responses in malaria-naïve individuals (Malkin, E.M. *et al.*, 2005). *P. pastoris* is also used as a production host for the food industry. A recent publication is available concerning the safety of a lipase enzyme preparation expressed in *P. pastoris* for the food industry (Ciofalo, V. *et al.*, 2006). The U.S. FDA regards this lipase enzyme preparation as Generally Regarded As Safe (FDA Center for Food Safety and Applied Nutrition, 2007). Finally, *P. pastoris* is a U.S. FDA approved animal feed protein source and is approved for use in broiler feed up to 10% of the total feed (FDA, 1993). The organism has also been used as a production host for the animal feed additive Quantum™ Phytase, which is currently in review at EFSA under Regulation (EC) No 1831/2003 (EFSA, 2006).

Absence of pathogenicity and toxicity

As discussed in Pariza and Foster (Pariza, M.W. *et al.*, 1983) and Pariza and Johnson (Pariza, M.W. *et al.*, 2001), the two papers that set forth the gold standard used by the enzyme industry for assessing the safety of enzyme products, the primary consideration in the evaluation of microbial enzyme preparations to be used in food is the safety of the production organism. Yeasts are not known to produce toxins that are active by the oral route (Pariza, M.W. *et al.*, 2001) and *Pichia pastoris* has been placed in the Biosafety Level 1 (BL-1) class by the ATCC organization, a category reserved for well-characterized agents not known to cause disease in healthy human adults and to be of minimal hazard to laboratory personnel and the environment (Center for Disease Control, 1999). *Pichia pastoris* does not produce any killer toxins, which inhibit the growth of sensitive yeasts and pathogens (Banerjee, H.N. *et al.*, 2000). In addition, *P. pastoris* itself has been approved by FDA as a source of animal feed protein for use in broiler feed up to 10% of the total feed (FDA, 1993). Toxicity studies done in support of the above-referenced *P. pastoris*-approved animal feed (including a pathogenicity study in mice, an acute oral toxicity study in rats, a sub-acute oral toxicity study in rats, and a two generation teratology study in rats) also demonstrated - per FDA's review in 1993 - that *P. pastoris* is neither pathogenic nor toxigenic (FDA, 1993). Toxicity studies conducted for the animal feed additive Quantum™ Phytase (EFSA, 2006), included genotoxicity studies in bacteria and mammalian cells *in vitro* and *in vivo* and sub-chronic oral toxicity study in rats using both refined feed additive enzyme and unrefined material from the fermentation broth. The results of these studies indicated that there was no toxicity or genotoxicity observed in these tests. Furthermore, results from toxicology and genotoxicity tests of the lipase enzyme produced by *P. pastoris* demonstrate that this material does not contain toxic or genotoxic substances (Ciofalo, V. *et al.*, 2006)

Conclusion

The information provided above demonstrates that *Pichia pastoris* is a safe production host with no known toxigenic or pathogenic properties. Based upon a history of safe use, published literature, and previous U.S. FDA approval as a feed additive (FDA, 1993), *Pichia pastoris* meets the criteria for a safe, non-toxigenic, and non-pathogenic production organism as described by Pariza and Foster (Pariza 1983), International Food Biotechnology Council (International Food Biotechnology Council, 1990), Organization for Economic Cooperation and Development (OECD, 1992), and most recently by Pariza and Johnson (Pariza 2001). *Pichia pastoris* should be proposed for QPS status along with *P. angusta* and *P. anomala*.

Appendix

References

- Banerjee, H.N. and Bussineau, C.M. *Search for a novel killer toxin in yeast Pichia pastoris*. Plasmid 43, pp.181-183 (2000).
- Center for Disease Control. *Biosafety in Microbiological and biomedical Laboratories*. 17-53 CDC Office of Health and Safety (1999).
- Cereghino, J.L. and Cregg, J.M. *Heterologous protein expression in the methylotrophic yeast Pichia pastoris*. FEMS Microbiol.Rev. 24, pp.45-66 (2000).
- Ciofalo, V., Barton, N., Kreps, J., Coats, I., and Shanahan, D. *Safety evaluation of a lipase enzyme preparation, expressed in Pichia pastoris, intended for use in the degumming of edible vegetable oil*. Regulatory Toxicology and Pharmacology 45, pp.1-8 (2006).
- EFSA *Quantum™ Phytase: Application to the European Food Safety Authority for two phytase preparations for use as a zootechnical additive for chickens for fattening, laying hens, ducks, turkeys for fattening and piglets (weaned)*. Website, Last Accessed 02/20/2007, Available from: http://www.efsa.europa.eu/en/science/feedap/an_applications/summaries/feedap_applications_quantum.html
- EFSA *Annex 1: List of microorganism already notified to EFSA* Website, Last Accessed 02/15/2007a, Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/sc_comitee/sc_consultations/qps.Par.0002.File.dat/Annex1.xls
- EFSA *Annex 5: Assessment of Yeasts with respect to a Qualified Presumption of Safety* Website, Last Accessed 02/15/2007b, Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/sc_comitee/sc_consultations/qps.Par.0006.File.dat/Annex5.pdf
- FDA *21 CFR Part 573. [Docket No. 87F-0221] Food additives permitted in feed and drinking water of animals: Pichia Pastoris dried yeast*. Federal Register 58, pp.59169-59170 (1993).
- FDA Center for Food Safety and Applied Nutrition Agency Response Letter GRAS Notice No. GRN 000204 Website, Last Accessed 02/15/2007, Available from: <http://www.cfsan.fda.gov/~rdb/opa-g204.html>
- Herbst, R.S., Hess, K.R., Tran, H.T., Tseng, J.E., Mullani, N.A., Charnsangavej, C., Madden, T., Davis, D.W., McConkey, D.J., O'Reilly, M.S., Ellis, L.M., Pluda, J., Hong, W.K., and Abbruzzese, J.L. *Phase I study of recombinant human endostatin in patients with advanced solid tumors*. J.Clin.Oncol. 20, pp.3792-3803 (2002).
- Hussain, Z., Ali, S.S., Husain, S.A., Raish, M., Sharma, D.R., and Kar, P. *Evaluation of immunogenicity and reactogenicity of recombinant DNA hepatitis B vaccine produced in India*. World J.Gastroenterol. 11, pp.7165-7168 (2005).
- International Food Biotechnology Council *Chapter 4: Safety evaluation of foods and food ingredients derived from microorganisms*. Regul.Toxicol.Pharmacol. 12, p.S114-S128 (1990).

Kreger-van Rij, N.J.W. *The Yeasts, a taxonomic study*, third revised and enlarged ed. Elsevier: Amsterdam, pp.353-354 (1984).

Lin Cereghino, G.P., Sunga, A.J., Lin, C.J., and Cregg, J.M. *Expression of foreign genes in the yeast Pichia pastoris*. Genet.Eng (N.Y.) 23, pp.157-169 (2001).

Malkin, E.M., Diemert, D.J., McArthur, J.H., Perreault, J.R., Miles, A.P., Giersing, B.K., Mullen, G.E., Orcutt, A., Muratova, O., Awkal, M., Zhou, H., Wang, J., Stowers, A., Long, C.A., Mahanty, S., Miller, L.H., Saul, A., and Durbin, A.P. *Phase I clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for Plasmodium falciparum malaria*. Infect.Immun. 73, pp.3677-3685 (2005).

OECD *Safety considerations for biotechnology 1992* Organization for Economic Co-operation and Development, Website, Available from: <http://www.oecd.org/dataoecd/8/3/2375496.pdf>

Pariza, M.W. and Foster, E.M. *Determining the safety of enzymes used in food processing*. J.Food Prot. 46, pp.453-468 (1983).

Pariza, M.W. and Johnson, E.A. *Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century*. Regul.Toxicol.Pharmacol. 33, pp.173-186 (2001).