



**OVERVIEW OF COMMENTS RECEIVED ON DRAFT
GUIDELINE ON THE QUALITY OF BIOLOGICAL ACTIVE SUBSTANCES
PRODUCED BY STABLE TRANSGENE EXPRESSION IN HIGHER PLANTS**

Table 1: Organisations that commented on the draft Guideline as released for consultation

Add name followed by link to individual received comment (upon publication by Web Services)

	Name of Organisation or individual	Country
1	Pharma-Planta	European Union

Table 2: Discussion of comments

GENERAL COMMENTS - OVERVIEW
<p>Thank you for the opportunity to comment on this draft document. We in the EU Pharma-Planta research programme are happy to offer you our expertise both now and in the future, as the need arises.</p> <p>As a general observation, we believe that the nature of transgenic plants and their cultivation will require particular emphasis to be placed on both the purification and definition of the pharmaceutical product, rather than in attempting to define the cultivation, harvesting and primary processing procedures with the degree of precision that is more practical in contained fermentation production systems (see comments below). This production component should however be embedded into a well-defined appropriate quality management system, consistent with the respective stage of the process. For cultivation and harvesting, we would suggest that the cGAP guidance is used, but developed into a formal regulatory requirement, backed up by site inspection and authorisation. The primary processing operations (including for e.g. extraction, filtration, and capture chromatography) should be viewed as a discrete transition stage between GAP-compliant cultivation and GMP-compliant downstream processing. Here, the principles of GMP will probably form the basis for an adequate quality system, but these may have to be modified to the peculiarities of plant processing on, or adjacent to, the cultivation site without compromising product quality and safety.</p> <p>Specific examples of current, well defined, germplasm banking systems are also offered for consideration.</p> <p>In our comments, we first respond to the specific requests in boxes in the Draft Guidelines and then offer comments on the different sections of the document.</p>

SPECIFIC COMMENTS ON TEXT			
GUIDELINE SECTION TITLE			
Line no¹. + paragraph no.	Comment and Rationale		Outcome
		Proposed change (if applicable)	
Box in 4.1 Terminology	<p>T0 - Primary transformant (initial transformed line)</p> <p>T1, T2, T3 Subsequent sexual generations from the primary transformant</p> <p>Tp – Production transformant – that used for</p>		The external commentator’s contribution to the nomenclature issue is noted. The guideline has been amended to mention the T0, T1, T2....., Tp system, while acknowledging that alternative systems can be used in MAAs. The definitions of terms (of “Elite Line” etc) have been studied, and where such terms are used in the guideline, these have been modified if considered

¹ Where available

	<p>pharmaceutical production.</p> <p>Elite line – a plant line selected for its production qualities (e.g. high yielding, pest / disease resistance, male sterile etc) that may be used for crossing with the transformed line to improve production of the product.</p> <p>Germ-line transformation – it is necessary to transform the germline of plants for the introduced gene(s) to be heritable.</p> <p>Cotransformation – where two gene constructs are inserted into a plant genome at the same time. They may or may not be genetically linked.</p> <p>We are happy to help defining further plant biology and plant genetic concepts, as the Guidance Document develops.</p>		<p>necessary.</p>
<p>Box in 4.1.5 Transgenic banking system</p>	<p>Figure 1 in the earlier EMEA “Points to consider” Document provided a useful basis for describing a plant development and production system and we recommend that it is included in the current guidance document. In the Developmental Genetics section of the Figure the line coming down from the “Host Plant” box needs to go directly to the “Primary Transformant (T0)” box. The intermediate “Transgenic Organism (Higher Plant Expression System)” should be deleted. The section on Transgenic Bank is suitably generic. The difficulty of seeking Master Gene Bank and Working Gene bank parallels is that the biology of different crops varies considerably. In some cases, plant seeds will live for up to 20 years under appropriate storage conditions, in other instances the life of seeds is much shorter. Some crops can only be reproduced vegetatively, so some form of vegetative maintenance (e.g. in vitro) may have to be</p>		<p><i>External commentator first paragraph</i></p> <p>The figure was included in the original Points to Consider document because it was instructive and served as a focus for comment at that point in the drafting process. Guidelines do not normally include diagrams, Therefore the Figure is not re-introduced into the guideline.</p> <p>The external comment on the banking system starts with a reference to it as “suitably generic”, and follows with some general comments on the diversity of plant life, including the fact that some plants reproduce only vegetatively. The external commentator is therefore assumed to support the existing text on banking, while discussing around the topic, and therefore no fundamental change is made to the guideline. The guideline also does not ever specify that only sexually reproducing plants are possible, so its generic approach accommodates the vegetative reproduction case too, in the event that a transgenic banking system can be developed for it.</p>

	<p>developed. A description of appropriate genetic banking systems will therefore need to be generic at this stage. Further guiding principles will emerge with further research and development on the use of different crops as pharmaceutical production platforms.</p> <p>It should be recognised that the banking of elite germplasm is already common practice in breeding / commercial agricultural practice. This is very much a worldwide practice and the banking systems are very sophisticated indeed. International organisations such as the International Maize and Wheat Improvement Center (CIMMYT), the Consultative Group on International Agricultural Research (CGIAR) and the International Rice Research Institute (IRRI) have all developed banking systems for a range of crops, which form the basis for conservation and distribution of defined genetic resources. We propose that a Master banking system for cGAP/GMP production of biologicals could be derived from such an established system, and we attach, as an example, the current working banking systems for Maize and Wheat as laid out by CIMMYT.</p> <p>http://www.cimmyt.org/english/docs/manual/gene_bank/manual.pdf</p> <p>(pdf attached)</p>		<p>However, the final version of the guideline has the following sentence added in Section 4.1.5 in recognition of the diversity of the range of possible transgenic plant production systems which might be proposed in the future: “Where possible a banking system should be included in the batch-to-batch consistency assurance strategy.</p> <p><i>External comment second paragraph</i></p> <p>The external commentator points out to the fact that “banking” for plant materials is well established in <u>agriculture</u>, and provides references to certain international organisations involved. However, the guideline is not in a position to commit to mentioning specific <u>agricultural</u> bodies.</p>
<p>Box in 4.2.1 General manufacturin</p>	<p>“The recommendation of the GACP should be taken into account” is a good proposal. We would propose that EMEA turn these recommendations</p>		<p><i>Applicability of GACP to transgenic plants production</i></p> <p>GACP is mentioned in the guideline, although GACP is of course targeted at non-transgenic medicinal plant</p>

<p>g strategy</p>	<p>into a quality system. This would involve an inspection process and authorisation, along the same lines that GMP production facilities are approved.</p> <p>In addition to GACP-based quality concepts applicable to cultivation and harvesting operations, the peculiarity and inherent variability of plants as biopharmaceutical production systems requires adequate addressing of quality issues in the primary processing stage which, in our opinion, should not only encompass cleaning, sorting and storage operations, but also unit operations like milling, maceration, extraction, biomass removal and filtration.</p> <p>The reason for this is because different potential types of raw material, e.g. seeds, leaves, fruit, storage organs, field grown vs. greenhouse-, hydroponically or closed-system grown plants etc., that will require different approaches/solutions for these primary processing steps. In some cases, it may be possible to spatiotemporally separate harvesting and primary processing, while in other cases post-harvest product deterioration may dictate immediate processing. In some cases, cleaning and sorting may be accomplished as a stand-alone operation while in other cases these operations may be feasible only in the context of further processing without hold-steps up to the stage of capture chromatography. We therefore suggest that a risk-based and process-oriented approach should be taken to define and implement appropriate points of transition between GACP and GMP quality management systems. As soon as the starting material enters the pathway leading to purification of the product, a GMP quality management system as described for e.g. in ICH Q7 should be in place to oversee and evaluate, in particular, the adequacy of plant-system specific</p>		<p>cultivation, and is therefore not alone sufficient to define good transgenic crop production. Also since the draft guideline was placed on the internet the GACP situation has been consolidated at EMEA level by the production of the EMEA/HMPC guideline on the topic. In view of this development, the text of the present transgenic guideline has been updated to mention this development, but to make it clear that GACP is not aimed at, or alone adequate for, the transgenic plants situation. The external commentator also suggests working with EMEA to develop GACP into a system with inspections and certification, but this is not practicable within the timeframe for finalising the guideline.</p> <p><i>Distinguishing between GMP and non-GMP operations</i></p> <p>The draft guideline already addresses this issue. The external commentator discusses the point. In order to enhance clarity, the relevant section in the guideline has been redrafted to include quality system/good practice guidance for a/ the banks, b/ “primary processing”, and c/ DSP.</p>
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	primary processing operations as described below.		
<p>Box 4.2.2 Cultivation, harvest, primary harvesting</p>	<p>Again, the development and adoption of GACP would be appropriate. The production of pharmaceutical products from non transgenic plants (e.g. opiates from poppy) involves defining agronomic procedures, pest control measures and harvesting protocols. These are subject to periodic inspection. It may be useful to use these protocols as a model for the development of GACP for pharmaceutical production in transgenic plants.</p> <p>We further suggest that it should be acknowledged that primary processing of plant material - in particular from plants grown in non-contained environments - requires a different approach to ensure product quality and consistency, than established fermenter-based production systems. We think that in most cases it is not feasible to introduce the starting material directly into a clean-room purification suite, and that the attempt to do so would imply risks for the integrity and hygiene status of the downstream processing facilities. We therefore suggest that, under the auspices of a GMP quality management system as described above, parameters should be defined that allow primary processing of plant material in a dedicated environment, with suitable equipment, by trained personnel and according to written procedures. The facilities and utilities should be designed to take into account the sense of proportion for the nature of the starting material and the process stage. The intermediate generated in this stage of the process should then be transferred, under appropriate conditions, into the GMP-compliant downstream processing facilities for product isolation and purification.</p> <p>We believe that the important aspects for such a</p>		<p>The very definition of “biological product” makes it clear that the production system and its control are important in determining the quality of biological products. It follows that GACP for poppies etc. is not adequate for transgenic plant production, although the EMEA/HMPC guideline can serve as a reference point for developing a good quality system. The guideline has been amended to make this clear. As already acknowledged by the external commentator, there is a great diversity of plants and cultivation methods, and obviously the good practice/quality system idea will have to be developed, and assessed, on a case-by-case basis.</p>

	<p>primary processing area would be; access control, pest control measures, easy clean ability of premises, use of closed systems where possible and appropriate, risk-based equipment design and qualification, adequate cleaning verification or validation (or the preferential use of disposable equipment) as well as recording and documentation of key process parameters.</p> <p>In-process controls should be defined on a case-by-case basis considering both the properties of the starting material and product-specific criteria.</p>		
Exec Sum	Replace the first sentence of the Executive Summary with the following sentence:	“While plants have been used for centuries as a source of pharmacologically active compounds, the use of transgenic plants is emerging as a new route to their production.”	The Executive Summary has been redrafted so that it takes the external comment into account and to form a better summary of the amended guideline.
Exec Sum line 5	Reword last sentence to avoid ambiguity with the agricultural meaning of “field.”	“The emphasis is on guidance specific to the production of pharmacologically active proteins from transgenic plants”	The editorial comment is taken on board and the Introduction has been amended accordingly. The opportunity is also taken to introduce a mention of chloroplast transformations into the guideline.
Exec Sum Line 6	Clarify sentence	“Since the use of transgenic plant technology for this purpose is an emerging one, there.....”	See two boxes above for the response to this comment.
Exec Sum General comment	It may be helpful to make reference here to the current guidelines that cover medicinal plants used for making alkaloids for human pharmaceuticals.		As already outlined in the boxes above, GACP guidance is mentioned in the guideline, but under “Manufacturing”, and its proper role and context.
Introduction General comment	Although N-linked complex glycosylation in plants differs from that in mammalian cells, this is not a plant-specific problem, and is an important consideration for all heterologous protein	Delete the section of the Introduction from: “ which in turn may impact..... that do not occur in humans.”	As explained in boxes above, the Introduction has been rewritten. The level of detail about plant glycosylation has been reduced, as this will be very much case-by-case for MAAs.

	expression systems. To devote half of the first paragraph of the Introduction to plant glycosylation is inappropriate.		
Introduction Line 2	Replace “possible complement” by	“..possible alternative”...	“Complement” refers to the <u>range</u> so the word is retained. It is in any case not envisaged that the existing production systems will be <u>replaced</u> .
Section 3 Legal basis Line 7	The first sentence is not clear. The term ‘Containment’ has a particular meaning for transgenic plant cultivation. Rework.	“Cultivation measures for the production of medicinal products in transgenic plants need to take account of product quality maintenance (by endeavouring to protect transgenic material from variations in the environment) and environmental protection (by protecting the environment from transgenic material).” <i>Perhaps a better term would be ‘confinement’ and not ‘containment’ in this context</i>	Since the word “containment” is not always the best one to describe the measures which might be used, the word is replaced by “containment/confinement” in response to the external comment.
4.1.2 Line 2	Not sure of the relevance of the “source of the cell” from which nucleotide sequence was originally obtained”. Rework.	“This should include the identification and source of the plant genotype from which the nucleotide sequence was originally obtained.”	This section has been rewritten for clarity and to ensure that it is suitably generic. In any case the proposed wording in the external comment is not valid, since the origin of the transgene sequence will not normally be a plant.
Line 8	Reference to viral vectors. Note in Section 2 “the production using transiently transfected plants” was excluded from the scope of this document. It would be helpful to the reader if there could be reference to another guidance document or working group activity covering transiently transformed plants.	Delete	The external comment is noted. The reference to vectors has been removed, in order to avoid giving the impression that <u>transient</u> infections are involved. The section 4.1 has been rewritten overall to accommodate the external comments where valid.

4.1.3 Line 2/3	Not clear why a distinction is made between the initial and final transformant. It is surely the transformation event actually used for production that is being assessed?		The section 4.1 has been rewritten overall to accommodate the external comments where valid.
Line 5	Reword the final sentence	“... residues of material remaining from the transformation process”...	The commentator’s proposal for rewording has been accepted and the guideline modified accordingly.
4.1.1 to 4.1.3 General comment	Many of these points are covered within the 2001/18/EC regulations for both experimental field and commercial release of GM plants. It may be appropriate to cross refer to these regulatory requirements.		Directive 2001/18 is the GMO Directive. This is not legally relevant to Module 3 in MAA in the usual situation where the active substance is a recombinant protein, and therefore not a GMO.
4.1.4 General	There is reference to the concept of “initial transformant” and “final transformant”. This implies that they are different transformation events (different gene insertion events). Often in plant transformation a gene construct is first inserted into an easily transformable plant genotype to produce many independently transformed plants. These plants, each containing a different transgene insertion event, are analysed for transgene expression. Those plants that have acceptable temporal and spatial levels of transgene expression within the plant are selected. The selected plants may then be crossed with elite high yielding plant genotypes to increase the yield of the plant and of the pharmaceutical product (e.g. seed). To avoid the implication that the terms “initial transformant” and “final transformant” refer to different transformation events this section needs rewording. In plant transformation studies initial transformants are called “primary transformants” or the T0 generation. We suggest that the term “production transformant” or Tp be used to describe a transgenic line that has undergone further breeding to produce a transgenic	Replace the terms “initial transformant” and “final transformant” with the terms “primary transformant” and “production transformant” respectively and indicate that they contain the same transgene insertion event, but have undergone plant breeding to improve the production characteristics.	The section 4.1.4 has been rewritten overall to accommodate the external comments where valid. In particular most of the external commentator’s advice on 4.1.4 with regard to terminology has been adopted.

	line for pharmaceutical production. The abbreviation T1, T2, T3 refer to the number of sexual generations removed from the primary transformant i.e. the seeds from the primary transformant would be the T1 generation.		
4.1.4 Line 12 (last line)	Reword	Replace “culture” by “cultivation”.	The section has been reworded including the replacement of the word “culture” in the guideline by “cultivation”.
4.1.5 Line 6	We should avoid being too prescriptive in what we mean by “well characterised” because the genetics of different crops will vary. Genetical terms have very specific meanings, for example: homogeneous in its strict sense means that all plants will have identical genotypes in every way. Also, if we use the term homozygous – this could mean homozygous for the transgene only or homozygous for the background genotype or both. Reword sentence.	“Manufacture should begin with well characterised and stable transgenic plant material	The final version of the guideline has the following sentence added in Section 4.1.5 in recognition of the diversity of the range of possible transgenic plant production systems which might be proposed in the future: “Where possible, a banking system should be included in the batch-to-batch consistency assurance strategy. One of the most important attributes of an effective banking system is that it is based on well characterised material. Therefore no change is made in response to the external comment. The words “homogeneous” is retained for the reason that a principle of banking is that the material should be homogeneous. Owing to the diversity of plant material, it cannot be predicted what materials might be proposed for banking in any particular case, so the extent and nature of the homogeneity achieved must be presented in MAAs and assessed on a case-by-case basis. The word “homozygous” does not in fact appear in the guideline draft.

Line 8	Again, we cannot be too prescriptive because the genetics of different crops can vary considerable; also some crops can be vegetatively propagated only. Reword.	“Consequently manufacturers need to establish a method of producing a consistent supply of the production transgenic line (Tp). The methods adopted should involve appropriate protocols for long term storage or propagation. Whatever system is adopted it should be capable of providing consistent and sufficient starting material for a large number of production runs. The method of achieving this should be defined and clearly described.”	The proposed sentence is not adopted because it would dilute and/or contradict what is written in the guideline for banking. As mentioned above, the nature of the banking will be case-by-case.
4.1.5. Line 12	Too prescriptive because the genetics of crops varies considerably. Reword the sentence.	“The approach applied to characterising and testing the banked transgenic plant material should take into account”	The general principles stated in the draft are maintained for the reasons described in the boxes above, and therefore the guideline is not modified to include this external comment. Also, the genetic stability of production crops is an issue dealt with under the appropriate heading in the guideline.
Line 15	Reword	Replace “master transgenic bank” by “production transgenic line”	The banking section of the guideline has been rewritten, and the piece of text referred to by the commentator is no longer in the guideline
Line 27	Plants that cannot be maintained as seeds may need to be kept as vegetative material. Reword.	“Specification for maintaining banked stocks e.g. seeds, in vitro plants”.	The text of the guideline on the banking issue is sufficiently general to accommodate any type of plant material, if it can be validated as suitable for banking. Therefore no change is made to the guideline on this

			point.
4.1.6. Paragraph	<p>The meaning in this paragraph is not clear as some concepts seem to have been taken from microbial production systems. For example – it is not clear what is meant by “global strategy” in the way it is used.</p> <p>Also it is not clear what “establishment of the expression construct” means in the case of production in plants. The expression construct (transgene construct?) would not have a phenotype at its establishment (following its construction as DNA?).</p> <p>Similarly it is not clear what is meant by “supportive data obtained from in-process controls during culture, and controls of the active substance”</p>		<p>The Manufacturing section of the guideline has been rewritten for clarity, and the term “global strategy” is no longer used.</p> <p>The supportive data term derives from the requirement for release and stability specifications to be set taking into account data obtained from a range of parameters, including characterisation, in process testing, etc. Therefore the requirement is maintained in the guideline.</p>
4.2.1 Line 9	Reword to clarify	“For the first phase of the production process, a quality control system and / or good practice system should be defined and fully described.”	The manufacturing section of the guideline has been rewritten to specify exactly which are the quality system/GMP requirements for each phase.
Line 18	Additional sentence for end of paragraph.	“The precise nature of the quality system will be influenced by the genetics and characteristics of the transgenic crop being used. The quality and consistency of the pharmaceutical product will be the primary criterion for determining the appropriateness of a quality control system”.	The manufacturing section of the guideline has been rewritten to specify exactly which are the quality system/GMP requirements for each phase
4.2.2 Line 6.	Concepts from the contained production of pharmaceutical substances in microbial culture will need to be revised for pharmaceutical production in plants. There is extensive		The measures in place to ensure satisfactory quality of herbal active substances are simply not alone sufficient for controlling transgenic plant production, as explained in

	<p>experience of the production of pharmaceuticals in cropped plants (e.g. opiates from poppy, cannabis from hemp), which can be drawn upon. In these production systems where climate, season, and soil type varies from farm to farm, the main emphasis of the quality control system needs to be on the quality and consistency of the harvested raw material and the purified product, rather than in attempting to define the growth environment with a degree of precision that is largely impractical for most methods of plant cultivation.</p>		<p>several boxes above.</p> <p>The MAA needs to include details on the production conditions, and therefore the bullet points describing the minimum details needed are retained in the guideline.</p>
4.2.2	<p>Sites of cultivation, procedures for cultivation and harvest.</p> <p>As stated above, because of difficulty of defining the nature of the environment during plant cultivation (under field and often even under glasshouse production) the emphasis of the quality control will need to be placed on assessing the quality and consistency of the purified product (as is done for the production of opiates from poppy, for instance).</p> <p>It will be important to reflect on the value of some of the information being requested in the list on page 8. For example, what is the relevance of detailed description of soil type? What is the value of giving the prevailing meteorological conditions?</p>		<p>The manufacturing section of the guideline has been rewritten to specify exactly which are the quality system/GMP requirements for each phase.</p> <p>See also the boxes immediately above for an explanation of why details of the manufacturing conditions are needed in the MAA.</p>
4.4.1	<p>Ensuring the absence of infestation is already part of GAP. If this comment is specific for the plant production phase and harvest, the comment should specify this.</p>		<p>The Adventitious Agent section is about having zero or defined bioburden levels in the active substance. Controls during cultivation/harvest, including GACP, in-process controls, and the Quality System, can contribute to bioburden control at the early stages only. Therefore no change is made to the guideline at this point</p>
Definitions	<p>Transgene: ‘heterologous DNA’</p>	<p>Why is homologous DNA excluded from this definition</p>	<p>Homologous DNA would be a DNA segment native to the plant, which would not normally qualify as “trans” in the “transgene” sense. Therefore no change is made to the</p>

	Transgenic bank:	Would be useful to add the term 'replenishable' – for example if seed stocks are chosen as the banking system	guideline.. Adding "replenishable" would be somewhat contrary to the banking concept, and is therefore best avoided in the context of a simple definition. Therefore no change is made here.
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