European Coexistence Bureau

Summary of conclusions
of the 2nd meeting of
the Technical Working Group for Cotton

16 – 17 of April 2015,
Seville, Spain

The 2nd Meeting of the Technical Working Group for Cotton (TWG Cotton) of the European Co-Existence Bureau (ECoB) took place in Seville, Spain, from 16 to 17 of April 2015. Experts from the following Member States (MS), Liechtenstein and European Commission (EC) services were present:

- Bulgaria (BG), Croatia (HR), Finland (FI), Greece (GR), Hungary (HU), Liechtenstein (LI), Romania (RO), Spain (ES) and United Kingdom (UK);
- Directorate General for Health and Food Safety (DG SANTE);
- Joint Research Centre (JRC), Institute for Prospective Technological Studies (IPTS);
- Joint Research Centre, Institute for Health and Consumer Protection (IHCP).

The main topics of discussion were:

1. Priorities for ECoB 2015-2016;

2. Development of the Best Practice Document (BPD) for coexistence in cotton production on the basis of the Background Document (BD);

3. Review and analysis of the TWG Cotton contributions to the BD for coexistence in cotton production;

4. Information about analytical protocols for extraction and detection of GM presence in cotton harvests and honey;

5. Review and analysis of the TWG Cotton proposals for best practices for coexistence between GM and non-GM cotton;

The ECoB secretariat welcomed the participants of the 2nd meeting of TWG Cotton and briefly summarized its ongoing activities.

1. Priorities for ECoB 2015-2016

DG SANTE underlined the importance of ECoB for utilization and development of coexistence concepts in the EU and presented the main outlines of the ECoB work programme for 2015-2016:

Action/Deliverable	**Indicative timing**
2nd meeting TWG cotton	April 2015

Draft BPD cotton	2nd - 3rd Q 2015
Consultation of the BPD cotton with stakeholders and Member States	3rd Q 2015
Final version BPD cotton	3rd - 4th Q 2015
1st meeting TWG potato	3rd - 4th Q 2015
Contributions for the development of the background document for potato	4th Q 2015
First draft of background document for potato	1st Q 2016
2nd meeting TWG potato	2nd Q 2016
Draft BPD potato	July 2016

2. Development of the Best Practice Document (BPD) for coexistence in cotton production on the basis of the Background Document (BD).

The ECoB secretariat overviewed the steps for developing the BPD for coexistence in cotton production from the BD, which includes:

- Introduction of: EU legislative provision on which the BPD is based; the role of the ECoB; and scope of the BPD document;

- Revision and editing where needed of the information provided in the BD;

- Conclusion of: best practices for coexistence of GM cotton cultivation with conventional and organic farming; cost analysis of the proposed management practices.

The legal background, the role of the ECoB and scope of the BPD were extensively presented, discussed and agreed during the first meeting of TWG Cotton[1]. In this second meeting the ECoB secretariat reiterated that in line with the Commission Recommendation (2010/C 200/01)[2] the development of coexistence measures in the EU is the responsibility of the individual MS for the best reflection of their national agro-climatic, landscape and farm structures, as well as consumer preferences. Therefore the BPD of ECoB comprises a methodological tool to assist development of national coexistence measures, based on scientific evidence and practical experience.

It was also underlined that the main difference between coexistence and segregation regimes is that coexistence allows for the adoption of thresholds for adventitious GM presence (AGMP) to guarantee sustainable functioning of the GM and non-GM production systems. Hence, the *de facto* level of technically unavoidable GMO presence is below or equal to the threshold targeted by the coexistence measures in place; the agreed thresholds by the TWG on cotton are 0.1% and 0.9% and it is these thresholds that have been analysed in the BD and BPD.

[1] The summary of conclusions of the 1st meeting of TWG Cotton from 20-21st of October 2014 is published on ECoB webpage (http://ecob.jrc.ec.europa.eu/documents/Summaryofconclusions1stmeeting.pdf)
[2] Commission Recommendation of 13 July 2010 on guidelines for the development of national co-existence measures to avoid the unintended presence of GMOs in conventional and organic crops (2010/C 200/01), OJ C 200, 22.7.2010, p. 1-5

3. Review and analysis of the TWG Cotton contributions to the BD for coexistence in cotton production

The ECoB secretariat presented a review of the contributions of members of the TWG Cotton to the BD (second draft 17/03/2015) based on the written comments received from: ES, GR, HR, RO and UK. There were two types of comments: general and topic specific.

The general comments confirmed that the draft BD did not comprise any substantial issues for reading and understanding, and that it took into consideration the most up to date research results, using a comprehensive set of references

The topic-specific contributions were:

- Cotton production, EU demand and crop cultivation:

  The use of one uniform system of measurement units is recommended.

- Cotton seed production:

  The TWG recommended more detailed national data be provided.

- Identity cottons:

  The aims of the organic schemes should be presented more extensively to ensure they clearly appear as aims and not achievements that have already been made.

  The identity cottons systems which don't deal with GM varieties' segregation should be outlined, but not described in detail.

- Seed-mediated gene flow:

  The TWG explicitly recommended that it should be clearly noted that in their natural state cotton seeds are tightly packed by both lints and linters in the boll. Without deliberate human intervention to remove lints and linters this packing causes a low germination rate.

- Occurrence of cotton pollen in honey:

  The TWG recommended that the international and EU quality requirements for marketed honey in respect of pollen content should be more comprehensively quoted.

- Detection of GM events in cotton crop and honey:

  The TWG highlighted that the development of methods for GMO detection in food and feed through the use of genomic DNA extracted from seeds is a common practice; and that

  the information about the detection of newly induced proteins and transcript DNA in cotton fibres should be placed in the appropriate section of the BD (i.e. chapter 4.2.3 - seed-mediated gene flow)

4. Information about analytical protocol for extraction and detection of GM presence in cotton harvests and honey

JRC-IHCP presented an overview about the worldwide trends in GM cotton detection with particular focus on the European Union Reference Laboratory for GM food and feed (EU-RL GMFF) activities.

The European Network of GMO Laboratories (ENGL) reported that:

- GM cotton is rarely included in MS control plans;
- Tests are only conducted by a few MS;
- Most analyses are carried out on raw materials (seed, leaf) and cotton seeds as feed (cotton meal);
- Cotton fibres can be analysed with methods available (CTAB + PCR);
- There is no evidence of testing on oil.

The limit of detection (LOD) and the limit of quantification (LOQ) of EURL GMFF validated PCR, DNA-based methods, determined on DNA extracted from cotton seeds are:

- LOD: 0.023 – 0.05%;
- LOQ: 0.1%.

However, the practical LOQ (LOQ_{pract}) is likely to be greater than this. For example if the number of target taxon-specific copies in PCR is e.g. 2000, the LOQ_{pract} will be 5%. Therefore, the sensitivity and the capacity to quantify depend to a large extent on the sample, even though validated PCR methods are very sensitive and accurate.

The LOD for currently available on market lateral flow strip tests (protein-based) is about 0.25% for bulk cottonseed; some commercial kits only guarantee a result expressed as "presence or absence" in leaf or single seed tissue.

For ELISA GM cotton test kits the LOD, depending on the targeted protein, could reach 0.1%; also in this case, some commercial kits only guarantee a result expressed as "presence or absence" in leaf or single seed tissue (qualitative tests).

Direct comparison between PCR and protein-based tests results should be done carefully considering the different nature of the provided information. The main drawback of protein-based methods are the limited specificity (trait-specific rather than event-specific) and the limited or non-applicability to processed materials, due to degradation of proteins.

The results of the activity conducted by the ENGL working group for "Seed Testing" about the cost and targeted LOD of a given test plan were also reported. To achieve 0.05% LOD the tested sample must comprise 6096 seeds, divided into three sub-samples, and the cost will be equal to the reference value – cost for achieving LOD of 0.1% in maize testing. An LOD of 0.1% can be achieved by testing 3066 seeds, in two sub-samples, at a cost of 69% of the maize reference value.

5. Review and analysis of the TWG Cotton proposals for best practices for coexistence between GM and non-GM cotton;

Before the meeting the ECoB secretariat received seven written proposals for best practices for coexistence between GM and non-GM cotton from: BG, ES, GR, HR, HU, RO and UK. The ECoB secretariat summarised these proposals and highlighted below are their main elements:

1. Spatial isolation is a feasible and effective coexistence measure to reduce adventitious presence of GM cotton in conventional and organically produced cotton even if it is the only measure applied (worst case scenario). The differences in the experimental designs and the field conditions for the evidence published to date resulted in considerable variance in the reported width of buffer zone needed to reduce adventitious presence of GM cotton from cross-pollination to the two target thresholds of 0.9% and 0.1%. Consequently the TWG considered that buffer zones between 1 and 10m in width would be needed to meet 0.9% and between 10 and 20m width to meet the 0.1% target.

To limit this imprecision, it was agreed that the ECoB secretariat will perform additional comparative analyses of the available studies with the aim of better explaining variation in the reported results.

The efficiency of isolation distances to limit the cross-pollination between GM and non-GM cotton was also reviewed in the literature. The data suggested that the isolation distance needs to be about twice the width of a buffer zone to give comparable reductions in magnitude of AGMP.

ECoB secretariat will carry out additional analyses of the available data to attempt to more precisely estimate this conversion factor.

It was agreed that the BPD will underline the possibility for choosing buffer zones or isolation distances to achieve the desired thresholds of admixture based on their economic performance under particular conditions.

The ECoB Secretariat emphasised the difference in aims between segregation and coexistence, based on their different economic incentives, which results in the different approaches for isolation, including for spatial separation. For example, the isolation strategy applied to seed production is based on segregation because it has a completely different economic basis (goals and revenue) from that of bulk crop production, for which coexistence is sustainable.

For both buffer zone and isolation distance, it was underlined that all of the available data about the rate of cross-pollination refers to within-row estimates of adventitious GM presence at the specific distance from the pollen source and does not therefore take into account the dilution factor which would occur during the harvesting of the whole field. The dilution factors start from one (i.e. no dilution, for single row cultivation), going to above 1000 depending on the size of the field. Even in the smallest EU farms the dilution factor is likely to be above 100.

2. The replacement of isolation distances with temporary isolation achieved by planting of different maturity classes of cotton is not viable due to European climatic conditions and quite narrow gene pool of cultivated cotton varieties.

3. Taking into account:

- the empirical evidence from palynological analysis[3] on the content of *Gossypium hirsutum* pollen in commercial cotton honeys (which range from 1.2 to 16.5% of the total pollen with 2/3 of the samples containing <10%); and

[3] Karabournioti (2000); Tsigouri et al. (2004) and Hamdy et al. (2009)

- the quality requirements (*Codex Alimentarius* standard for honey - CODEX STAN 12-1981 and Council Directive 2001/110/EC) for honey marketed in the EU, in particular the mandatory limit for water-insoluble content,

the TWG Cotton agreed that it is very unlikely that presence of GM cotton pollen in honey could exceed 0.1%. The current practices in honey production and marketing in Europe are sufficient to ensure that adventitious presence of GM cotton pollen in honey is far below the legal labelling thresholds and even below 0.1 %, as was concluded in the BPD for coexistence of GM maize and honey production[4].

During the discussion about the coexistence between GM cotton cultivation and honey production, there was extensive debate about the risk of misleading BPD users on the subject of adventitious or technically unavoidable GM cotton pollen presence in honey. This concern arose because we are not going to recommend any additional coexistence measure to the current practices in honey production and marketing.

It was proposed to include a specific appendix or paragraph in the BPD, repeating the information from topic-specific chapters of the BPD (and which TWG agreed during the first meeting) and reiterating the conclusion that in honey from beehives located in the vicinity of GM cotton fields some traces of GM pollen may occur, although this will be far below 0.1%.

The ECoB secretariat extensively explained that the coexistence analysis of the different farm production systems (different crops or crop and honey) follows uniform methodology for identification of the admixing factors and the practices limiting their impact below certain thresholds. These thresholds are applied universally to guarantee coherency of the BPD. It was reiterated that to have coexistence there must be a tolerance threshold of admixing and that the efficacy of the coexistence measures used to limit potential intermixing to below this threshold defines what is "adventitious or technically unavoidable" for open-space farm activities. Therefore, there are no grounds to adopt different approaches for presentation of results from coexistence analyses between different cropping systems.

Despite these methodological explanations two of the TWG members retained their doubts about the assumption that the possible traces of GM cotton pollen in cotton honey from beehives located in close vicinity of GM cotton fields are adventitious or technically unavoidable.

4. Quantitative PCR-based approaches such an EU-RL GMFF validated methods should be used as reference method for analysis/detection/quantification of GM cotton presence including GM cotton pollen in honey

For monitoring of the production process the flow strip or ELISA based test could be applied.

5. Additional agricultural and/or management practices are recommended for handling of seeds, storage, cleaning of used machinery and equipment. These measures could complement the spatial isolation but not to substitute it.

To limit GM cotton admixture to 0.9% threshold, cleaning of planters, harvesters, trucks and trailers, etc. after use in GM-plots and before use in non-GM plots was recommended as sufficient. Paying attention to the order in which machinery is used, i.e. conventional before GM, could also help to avoid admixture and reduce the number of cleaning operations needed to meet the target threshold.

To limit GM cotton admixture to 0.1% threshold, utilisation of dedicated equipment was considered an effective strategy. Cleaning of complex equipment is time and labour costly and often would not be sufficient to achieve a 0.1% threshold as seeds and/or debris can remain in

[4] Rizov, I. and Rodriguez-Cerezo, E. (2013)

small cavities that are almost impossible to access for cleaning and provide a source of constant admixture.

Working procedure for the BPD for coexistence in cotton production

After the extensive discussion on the BD and proposals for the BPD by members of the TWG Cotton, the next steps for finalisation of the BPD for coexistence in cotton production were agreed:

- the first draft of the BPD for coexistence in cotton production will be circulated among the members of the TWG Cotton by the end of May;
- the consultation process will take place up to the end of June and, if needed, the final consultation will be undertaken in the first half of July in order to submit the draft BPD by 15th of July 2015 to DG SANTE.