European Coexistence Bureau

Summary of conclusions of the Sixth Plenary meeting of the Technical Working Group for Maize

29 - 30 November 2012, Seville, Spain

At the Sixth Plenary Meeting of the Technical Working Group for Maize (TWG maize) of the European Co-Existence Bureau (ECoB), which took place from 29 to 30 November 2012 in Seville, Spain, the experts from the following Member States (MS) and European Commission (EC) services were represented:

- Czech Republic (CZ), Denmark (DK), Germany (DE), France (FR), Spain (ES), Poland (PL), Sweden (SK);
- Directorate General for Health and Consumers (DG SANCO);
- Directorate General for Agriculture and Rural Development (DG AGRI);
- Joint Research Centre (JRC), Institute for Prospective Technological Studies (IPTS);
- Joint Research Centre, Institute for Health and Consumer Protection (IHCP).

The main topics for discussion were:

1. **Commission proposals for amendment of Council Directive 2001/110 in response to the European Court of Justice (ECJ) ruling on Case C442/09 (Bablok case)** (presentations by DG SANCO and DG AGRI)

2. **The effects of bee foraging behaviour on maize and its consequences for GM pollen dispersal, including in bee products** (presentations by Dr. Bernard Vaissiere)

3. **Verification report for extraction and analysis of GM pollen DNA in honey, steps forward for validation of analytical protocol** (briefing from JRC-IHCP)

4. **Best Practice Document (BPD) for coexistence of maize and honey production**: review of TWG maize contributions to the BPD: scope, structure and content of BPD (overall discussion)

5. **Priorities for ECoB until 2014, work program. Priorities and calendar for TWG Maize 2013** (presentations by DG SANCO and ECoB secretariat)

6. **BPD for monitoring efficiency of coexistence of GM maize production**: review of TWG contributions to the BPD: overview of comments received on the final draft (overall discussion)

7. **Update of analytical protocol for monitoring efficiency of coexistence measures of GM maize cultivation** (briefing from JRC-IHCP)

1. Commission proposals for amendment of Council Directive 2001/110 in response to the European Court of Justice (ECJ) ruling on Case C 442/09 (Bablok case)

DG SANCO presented the Commission's proposals for the amendment of Council Directive 2001/110 in response to the ECJ ruling on Case 442/09. DG AGRI provided information on the distribution of work on the issue, as agreed by the Cabinets of the two DGs.

The objectives of the proposal for a Directive of the European Parliament and of the Council to amend Council Directive 2001/110/EC relating to honey are:

- Identification of the delegated and implementing powers that should be conferred upon the Commission in line with the Treaty on the Functioning of the European Union (TFUE); and

- Clarification of the status of pollen in honey. The pollen in honey is not an ingredient in the sense of Article 6(4)(a) of Directive 2000/13/EC. In line with international standards, and in particular the Codex Alimentarius, the proposal defines pollen as a natural constituent of honey.

The Commission's proposal does not affect the conclusion of the ECJ as regards the application of the GMO legislation to GM pollen in food (in particular the application of Regulation (EC) No 1829/2003 on genetically modified food and feed). It does not alter the Court's conclusion that honey containing GM pollen can be placed on the market only if it is covered by an authorisation under this legislation. Furthermore, the labelling rules on GMOs in food will also be applicable.

In was confirmed that the TWG maize of ECoB should assess the current TWG maize recommendations for coexistence of GM maize production in respect to efficiently manage the possible presence of GM pollen in honey. If the current recommendations are not enough sufficient the TWG maize should propose, based on current scientific knowledge and agricultural practices, additional coexistence measures to limit GM maize pollen presence in honey taking into account the cost and burden for farmers as well as for beekeepers.

In that respect the following definitions were overviewed:

- Council directive 2001/110/EC of 20 December 2001 relating to honey, Annex II – composition criteria for honey:

  "When placed on the market as honey or used in any product intended for human consumption, honey must meet the following composition criteria:
  ……
  3. Water-insoluble content: in general - not more than 0.1g / 100g and for pressed honey - not more than 0.5g / 100g"

- Codex Alimentarius standard for honey – CODEX STAN 12-1981. In alignment with its point 3 for Essential composition and quality factors of honey, it was underlined that:

  "Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey…. No pollen or

constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter."

The ECoB secretariat, with the assistance of experts from DG AGRI and other honey production experts, clarified that the measurement of water-insoluble content is designed to detect impurities in honey that are higher than the permitted maximum. There was a time when a significant portion of world honey was harvested by pressing the combs. However, nowadays almost all commercial honey is harvested by centrifugation. The permitted maximum of water-insoluble content according to the Codex Alimentarius and European standards is 0.1 g/100 g. Insoluble matter in honey includes[1] pollen, honeycomb debris, bee and filth particles. It is measured by the filtration of a honey solution in a glass crucible with a pore size of 15 to 40 µm. The maize pollen grains have an average diameter of 94 to 103 µm[2]. Therefore all maize pollen grains present in honey will remain in the crucible and will be measured as part of its water-insoluble content, which should not exceed 0.1% of the total mass.

The threshold of 0.5% for water-insoluble content in pressed honey reflect the specificity of the harvesting technique utilized, which mainly results in the increased content of honeycomb debris (in the form of wax) in the final product - pressed honey.

2. The effects of bee foraging behaviour on maize and its consequences for GM pollen dispersal, including in bee products[3]

The presentation covered the following main topics:

- Honeybee (*Apis mellifera L.)* and other bee race (in total 2500 species of apiformes) in Europe, of which only *Apis cerana cerana* and *Apis cerana indica* are domesticated;
- Beekeeping in the EU: pollen vectors for crops in Europe;
- Morphology of bees – branched hairs;
- Honeybees' foraging behaviour, equivalent to resource collection (nectar and pollen). Honeybees are social foragers;
- Pollen morphology and viability: pollen collectability;
- The effects of honeybee foraging behaviour on maize;
- Foraging distances;
- Maize pollen in beekeeping products;
- Beekeeping products: pollen, royal jelly, propolis:
- Honey: formation of honey;
- Pollen intake in honey: nectar (primary means). Primary intake of pollen from wind-pollinated plants (maize) in nectar and honeydew;

[1] Bogdanov, S., Lüllmann, C. , Martin, P. , von, der, Ohe, W. , Russmann, H. , Vorwohl, G. , Persano, Oddo, L. , Sabatini, AG. , Marcazzan, GL. , Piro, R. , Flamini, C. , Morlot M., Mitt Lebensm Hyg. 90, 1999, 108-125

[2] Baltazar, B.M., de Jesus Sanchez-Gonzalez, J., Cruz-Larios, L., Schoper, J.B.. Pollination between maize and teosinte: an important determinant of gene flow in Mexico. *Theoretical and Applied Genetics,* 2005, 110, 519-526

[3] Part of Dr. Bernard Vaissiere presentation is kindly contributed by Dr. Werner von der Ohe

- Secondary means of pollen intake in honey – bees can lose pollen by passing in front of cells with nectar or unripened honey, but it is marginal;

- Third means of pollen intake in honey – by the beekeepers;

- Relative frequency and occurrence of some pollen species in honey;

- Investigation of GM pollen versus total pollen;

- Conclusions:

  - Honeybees store pollen separately, in specific pollen combs for preparation of bread for larvae, but nevertheless very small amounts of pollen are always present in the honeycombs of the frame.

  - The maize pollen content in honey is always very limited since maize plants do not produce nectar and maize pollen has large and smooth grains which are filtered out of the honey crop (not regurgitated) before the honeybee arrives at the hive and unloads the remaining contents of its honey crop for the use of other bees in the hive. The filtering process is particularly efficient in the case of large pollen grains, as is the case for maize pollen.

  - Furthermore by law (CODES STAN 12-1981 and Directive 2001/110/EC relating to honey), honey must contain ≤ 0.1g/100g of water-insoluble content (filtered at 15 microns ≈ mainly pollen and wax debris). Therefore the total pollen content and, by consequence, GM maize pollen content is always below the 0.1% limit.

3. Verification report for extraction and analysis of GM pollen DNA in honey, steps forward for validation of analytical protocol

At present, real-time PCR methods, specific to GM events, can be used to detect and quantify DNA extracted from the total pollen in honey. The sensitivity of such methods, though maize pollen is usually a small fraction of the total pollen in honey, seems to allow quantification at the proposed thresholds. For the moment this analytical approach has been tested and is reliable only for quantification of GM maize pollen in relation to the total maize pollen.

Waiblinger et al., 2012[4], published a multi-laboratory validated method and the EU-RL reported intra-laboratory validated method[5].

The EU-RL GMFF performed an in-house study on the extraction method for isolation and analysis of the pollen DNA present in honey, including the isolation and analysis of isolated genomic pollen DNA using real-time PCR on commercial honey samples and honey samples spiked with various levels of GM MON810 pollen. The study verified that:

- The honey pollen extraction protocol developed at the EU-RL GMFF is suitable for reproducibly extracting PCR-grade DNA from genetically modified maize pollen present in honey samples;

[4] Waiblinger HU, Ohmenhaeuser M, Meissner S, Schillinger M, Pietsch K, Goerlich O, Mankertz J, Lieske K, Broll H (2012) In-house and interlaboratory validation of a method for the extraction of DNA from pollen in honey, Journal of Consumer Protection and Food Safety, 7(3):243-254

[5] EU-RL GMFF: verification report, 2012, http://gmo-crl.jrc.ec.europa.eu/doc/GM_Honey_REPORT.pdf

- The lowest spiked amounts tested in this study were 5 mg 1% GM pollen in 10 g honey corresponding to 119 GM maize pollen grains (taking 0.21 μg as the average weight of a maize pollen grain). Such level was reproducibly detected using the EU-RL GMFF protocol and the EU-RL GMFF validated real-time PCR methods;

- In honey samples purchased from the retail market, the presence of maize, rapeseed and soy pollen could be demonstrated when applying the EU-RL GMFF protocol combined with EU-RL GMFF validated/verified real-time PCR methods;

- Taking into account the average content of total pollen and maize pollen in real honey samples (Stawiarz and Wrolewska, 2010), it is foreseeable that the protocol presented in this report is suitable for the detection of GM pollen in honey.

4. Best Practice Document (BPD) for coexistence of maize and honey production

The ECoB secretariat presented a summary of proposals for the BPD for coexistence between GM maize and honey production based on the contributions and comments of 10 Member States (MS): AT, CZ, DK, FR, DE, IE, LT, NL, ES and UK. The main highlights are:

1. All professional and amateur beekeepers supplying products for further distribution in the food chain should be considered if new coexistence measures are established in addition to the already existing measures. Only honey production for own consumption could be excluded from them;

2. When honey is considered a single ingredient food, the maize pollen content is extremely unlikely to exceed the threshold of 0.9%, as well as 0.1% of the total mass of the product. Therefore no additional measures for coexistence of GM maize and honey production need to be applied.

This low level presence of maize pollen in honey is determined by biological and technological methods:

a) The biological method is extensively described in the submitted background document:

- the maize pollen could not access the primmer intake of pollen in honey by contaminating harvested nectar, because maize is not a nectar-producing plant;

- the morphology and size of a maize pollen grain additionally prevent its random contamination of collected nectar, which is stored in the bee's honey crop. There a high proportion of pollen grains are filtered out before the bee arrives at the hive and unloads the remaining contents of its honey stomach to other bees for use in the hive. This filtering process is particularly efficient in the case of large pollen grains, as is the case for maize pollen;

b) For honey harvesting, centrifugation of combs is currently the most common method, during which additional separation of solid particles (such as pollen) from the honey fraction takes place. This technological specificity is reflected in European and international quality standards for honey, as a low level threshold for water-insoluble content of honey has been adopted.

3. Utilization of temporary measures: beehive mobility (isolation distances restricted in time); establishment of pollen traps at beehives and/or artificial supplementation of

honeybee colonies during maize flowering periods is not appropriate with respect to decreasing possible adventitious presence of GM maize pollen in honey.

4. A validated method for DNA pollen extraction from honey is available. In combination with the EU-RL real-time PCR detection methods already in place, specific for GM events, it provides a promising opportunity for the quantification of GM maize pollen in relation to the total maize pollen. The calculation of the results should be extended for quantification of GM maize pollen content in total mass of honey.

The PCR method could be supplemented by microscopic pollen species analysis, which would comprise an efficient analytical approach for quantification of GM maize pollen in honey.

Summary of framework conclusions for proposal of coexistence measures of GM maize and honey production

1. The proposal for a BPD for coexistence of GM maize and honey production is complementary to the proposal for a Directive of the European Parliament and of the Council amending Council Directive 2001/110/EC relating to honey.

2. The submitted background document comprehensively and extensively reviews the information available on appearance and management of adventitious presence of GM maize pollen in honey and other beehive products. It should be adjusted accordingly with the proposal for a Directive of the European Parliament and of the Council amending Council Directive 2001/110/EC relating to honey, during its extension to the BPD for coexistence of GM maize and honey production.

3. The scope of the BPD for coexistence of GM maize and honey production covers: single event GM maize; coexistence only between maize crop and honey production in the EU; GM maize pollen adventitious presence in honey with the labelling threshold of 0.9% or with an alternative threshold in the markets of 0.1% of the total mass of honey; focus on possible measures that GM maize farmers can take, but also a review of measures that beekeepers can take without compromising their economic efficiency; monitor the efficacy of coexistence for honey production, including a state of the art of methods for detection of GM maize pollen in honey.

4. The main coexistence measures which were agreed are:

- Separation distance is not required, when honey is considered as a single ingredient food, because maize pollen content is unlikely to exceed the threshold of 0.9% as well as 0.1% of the total mass of the product. This very low level presence of maize pollen in honey is determined by: the maize plant, which does not produce nectar; maize pollen size and morphology; bee filtering pollen phenomenon; and European and international quality criteria for placing the product on the market, reflecting the most commonly adopted honey harvesting technique.

- The PCR method supplemented by microscopic pollen species analysis comprises an efficient analytical tool for quantification of GM maize pollen in honey.

Working procedure for the BPD for coexistence of GM maize and honey production

The experts of the TWG for maize were asked to submit additional scientific and technical information (data and best practice proposals) related to the topic by the end of 2012.

The ECoB secretariat will facilitate consultation on the BPD for coexistence of GM maize and honey production by circulating a draft version among the TWG for maize by the middle of January 2013.

The ECoB secretariat will prepare and submit a consolidated draft of the BPD for coexistence of GM maize and honey production to DG SANCO by the middle of February 2013.

DG SANCO will be responsible for stakeholder consultations and feedback to the ECoB.

5. Priorities for the ECoB until 2014, work program. Priorities and calendar for TWG Maize 2013 (presentations by DG SANCO and ECoB secretariat)

DG SANCO summarised the priorities of the ECoB for 2013:

- BPD for coexistence of GM maize and honey production and BDP for monitoring of efficiency of coexistence measures for GM maize cultivation;

- Report on the coexistence of GM crops with conventional and organic farming based on MS contributions;

-Set-up and kick-off of the TWG Soy Bean and the TWG sugar beet and potato.

The general timeframe is confirmed although some specific deadlines are still to be bilaterally agreed between DG SANCO and the ECoB secretariat.

6. BPD for monitoring efficiency of coexistence of GM maize production

The ECoB secretariat presented a summary of proposals for the BPD for monitoring the efficiency of coexistence measures in maize crop production based on the contributions and comments of 12 Member States (MS): AT, DK, FR, DE, IE, LT, NL, PL, PT, SK, ES and UK. The main highlights are:

1. The monitoring of the efficiency of coexistence measures in respect to their potential to reduce adventitious presence of GM maize could be done at the field level.
The efficiency of coexistence measures for reduction of adventitious presence of GM maize should be evaluated based on:

- location of the monitored fields in relation to the GM maize area, and their size;
- envisaged efficiency of applied coexistence measures;
- regional specificities.

2. The number of monitored fields should be selected in a cost-efficient manner for achieving a representative data set.

3. A stratified monitoring strategy on field is recommended. It could be substituted by random online sampling in a cost-efficient manner during the harvesting, or immediately afterwards - from trailers.

4. The sampling should be performed at the harvesting stage for maize crop production in the field or on the trailer. For the monitoring of silage maize production, only adoption of one pre-sowing sampling approach is possible because of technical and cost-efficiency reasons.

5. A standardized sampling procedure is recommended: the ISTA sampling methodology, JRC Recommendation 2004/787/EU and sampling approach for KeLDA protein analysis could be utilized.

6. Utilization of the standard Real-Time PCR method validated by the JRC is recommended. Results could be presented as number of copies (recommendation of the EC) or percentage of GM-DNA in terms of the haploid genome.

7. Additional input from the members of the TWG for maize is required for: comparison of results; analysis of results and possible follow-up and formatting and communication of monitoring results and data exchange between countries.

The PRICE project activities in the development of a monitoring aid tool were briefly presented.

Summary of framework conclusions for proposal of BPD for monitoring efficiency of coexistence measures in maize crop production

1. The draft of the BPD for monitoring the efficiency of coexistence measures in maize crop production of 14.02.2011 should be updated following recent advances in certain relevant areas. In the revision the following topics should be specifically addressed:

- Cost of applied measures. Feasibility of use of coexistence measures;

- Overview of coexistence monitoring activities carried out by EU Member States;

- Identification and selection of non-GM fields to be sampled. Sampling at various steps of maize crop production;

- Quantification of GMO content.

2. In the BPD the agreed proposals for best practices for monitoring the efficiency of coexistence measures in maize crop production should be included.

Working procedure for the BPD for monitoring efficiency of coexistence measures in maize crop production

The experts of the TWG for maize were asked to submit additional scientific and technical information (data and best practice proposals) related to the topic by the middle of January 2013.

The IPTS' Unit J4 will contribute to the update of the chapter for "Cost of applied measures. Feasibility of use of coexistence measures", with a deadline of mid-January 2013.

The ICHP will revise and propose updates for sampling strategy and analytical protocol for monitoring efficiency of coexistence measures of GM maize cultivation, again with the mid-January 2013 deadline.

The ECoB secretariat will facilitate consultation on the BPD for monitoring of coexistence measures in maize crop production by circulating a draft among the TWG for maize by the middle of February 2013.

The ECoB secretariat will prepare and submit a consolidated draft of the BPD for monitoring the efficiency of coexistence measures in maize crop production to DG SANCO by the middle of March 2013.

DG SANCO will be responsible for stakeholder consultations and feedback to the ECoB.

Cross-border issues

The ECoB secretariat will examine the possibility of incorporating available information on cross-border issues in the EU in the Report on the coexistence of GM crops with conventional and organic farming based on MS contributions. Three case studies are identified: Germany-Denmark; France-Switzerland and Spain-Portugal.

In addition, the experience of Member States producing GMOs or performing field trials on a large scale (Spain, Czech Republic, Portugal, Romania, France, Germany and UK) will be essential. The main difficulties for the establishment of common coexistence measures for border areas stem from differences in national and regional policy and are not at the technical level.

It is clear that cross-border coexistence is more of an administrative and legislative issue than a technical one. For that reason it is mostly outside the scope of the ECoB. The target for the ECoB will be the examination of technical measures to be adopted and the possibility of their harmonization among the Member States. A possible example could be the existing technical agreement between Spain and Portugal for border farms for utilization of common coexistence measures.

Working procedure for collection and analysis of cross-border experiences and approaches

The experts of the TWG for maize were asked to submit additional scientific and technical information related to the topic by the end of January 2013.

The ECoB secretariat will facilitate consultation on cross-border experiences and approaches by circulating a draft among the TWG for maize by the middle of March 2013.

The ECoB secretariat will prepare and submit a Report on the coexistence of GM crops with conventional and organic farming based on MS contributions, focusing on cross-border issues, to DG SANCO by the end of April 2013.

DG SANCO will be responsible for stakeholder consultations and feedback to the ECoB.