



Service of Biosafety and Biotechnology

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**DISCUSSIONS OF THE WORKING GROUP ON
THE PROTOCOL FOR GROWING GM BRASSICA IN FIELD RELEASES**

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Introduction

1. Demand of the Cabinet

In 2002, the Cabinet of the Minister of Social Affairs, Public Health and Environment, Magda Aelvoet, demanded to the Service of Biosafety and Biotechnology (SBB) to address the following topics in the protocol for growing genetically modified Brassica (reference: 02/AB/JT/MV/KJ/KV/grn017):

- specific containment measures towards organic and conventional farmers,
- specific containment measures towards nature reserves,
- specific containment measures towards bees and produced honey.

Within this framework, the biosafety dossiers B/BE/00/VWSP9 (spring and winter oilseed rape with male sterility, fertility restorer, herbicide resistance to glufosinate) and B/BE/02/VW4 (oilseed rape with pod shatter resistance, herbicide resistance to glufosinate) had to be used as case studies.

2. Objectives of the protocol

The protocol prescribes different acts and operations that the notifier needs to follow when carrying out a field trial with genetically modified oilseed rape (*Brassica napus*) or Indian mustard (*Brassica juncea*). These include the attribution of responsibilities, the keeping of a logbook, the isolation distance and/or isolation border that should be respected, the communication of the sites of release, different operations concerning sowing, planting, harvest, transport, analysis and destruction of the transgenic crop and the submission of different reports to the competent authority at the end of the release. After harvest of the transgenic crop, some guidelines need to be respected as well. These guidelines indicate which crop can or cannot be cultivated on the trial site in the following rotation, how the potential volunteer plants must be destroyed and how the site of release will be monitored during the following years. These measurements try to limit biological and physical mixing.

3. History of the protocol

The Belgian Brassica protocol is in place since 2000. During a meeting of the Biosafety Advisory Council in March 2000 Aventis CropScience (now Bayer CropScience) was asked to make a first proposal for a protocol. Since, the General Inspectorate of Raw Materials and Processed Products, has taken the lead for the co-ordination of the drafting of this protocol, assisted by experts of the Biosafety Advisory Council, the SBB and in consultation with the concerned

stakeholders. The protocol has been upgraded different times over the last years and always been evaluated and approved by the Scientific Committee "Transgenic plants" and the Biosafety Advisory Council.

The consent of field trials with genetically modified oilseed rape and Indian mustard always clearly stipulates that the trial needs to be carried out in conformity with the Brassica protocol valid for that cultivation season. The different protocols can be downloaded from the Belgian Biosafety Server <http://www.biosafety.be/TP/partB/protocols.htm>.

4. Creation of the Working Group

For the upgrade of the protocol the SBB created a Working Group (WG) grouping the experts of the Scientific Committee "Transgenic plants" of the Biosafety Advisory Council with specific expertise in agronomy and breeding of oilseed rape and other *Brassicaceae*, weed science, outcrossing to wild relatives, gene flow modelling, volunteer management, impact on biodiversity, nature conservation, phytopharmacy, population genetics, entomology and ecotoxicology.

In addition, the SBB took the opportunity to draft a report reviewing all the available information on vertical gene flow in oilseed rape. This report formed the basis for the discussions of the WG. In order to have access to all the available information the SBB also created a private password protected website for the experts of the WG.

5. Meetings

In agreement with the Federal Service of Public Health, Safety of the Food Chain and Environment, the SBB invited stakeholders concerned by the minister's questions. The beekeeper association CARI, the umbrella organisation of organic farming in Belgium, Bioforum as well as the notifier Bayer CropScience were invited to present their opinions on the actual protocol for growing GM Brassica. An open discussion was held with the experts of the WG, stakeholders, Competent Authorities (CA) and the SBB on **November 14th, 2002** and **January 7th, 2003**. The meetings were chaired by the SBB.

Report of the meeting of November 14th, 2002

1. Isolation distance towards other cropping systems and nature reserves

The protocol valid for the cultivation season 2002 obliges the notifier to ensure an isolation distance of 1000m to neighbouring commercial Brassica fields. This recommendation ensures that the genetically modified Brassica crop and the commercial Brassica fields are not grown side-by-side and will limit the spread of the bulk of pollen by wind and insects to a very low and acceptable level.

The experts agreed on the fact that this isolation distance is larger than those recommended for standard certification of seeds or used in other European member states. This stringent measure takes into account the limited knowledge about the ecological consequences of gene flow in oilseed rape. These consequences are dependent on a two-step model: (a) dissemination and cross-pollination from transgenic plants to non-transgenic crops or to wild relatives, and (b) reproductive fitness of the hybrids created from this cross-pollination. For the moment most of the scientific data focus on the first part of the equation (dissemination) and only address pollen movement, seed movement, outcrossing rate, contamination rate and hybridisation. Little information on the other hand addresses the second part of equation being the consequences of this gene flow e.g. the ability to persist and produce progeny. The ability of a volunteer or a hybrid to persist and produce progeny will depend on the nature of the transgenic trait, the genetic and environmental background in which the transgene is expressed, the stability of the transgenic trait and the levels of continued gene flow from the crop.

Within this framework pest resistance was presumed to induce a selective advantage in natural habitats, which could potentially make that population more invasive. There is often a mixture of many plant species in natural populations and the density of any one species may be too low for a pest to make a significant impact on selective advantage, but the added selective advantage may be sufficient to affect the balance between wild species. At low frequencies random genetic drift will be another important factor that needs to be taken into account in the ability to persist. In the case of herbicide tolerance the survival in natural habitats should not be a problem because herbicides are not used in these locations, herbicide drift will be limited to the close surrounding of the field, and therefore the plants would not be at a selective advantage in the wild. In the field on the other hand these plants could cause a weed problem if non-appropriate management measures are taken. For these reasons the experts of the WG suggested to limit the scope of the protocol to herbicide tolerant Brassica and to maintain the protocol as a minimal requirement for other traits. The Scientific Committee "Transgenic plants", the Biosafety Advisory Council or the CA can still request additional

conditions on a case by case basis. In the case of an oilseed rape resistant to a pest, uncertainties remain and therefore more stringent measures could be requested case by case.

One of the experts of the WG proposed to develop a model on gene flow in oilseed rape based on the different parameters described in the report. The obtained estimates based on the available data and different tested scenarios could give guidance to the experts of the WG in this matter. Another expert indicated that the range of results obtained would be too large and would not be very useful.

The experts agreed on reducing the isolation distance to 600 or 400m if an effective isolation border of the same variety (of minimum 10m) would surround the trial. This proposal will aim to reduce gene flow to an equivalent level as the 1000m isolation distance without border. The plants used in the isolation border need to flower synchronously with the transgenic oilseed rape and have the same height as the GM one. The border will increase the distance pollen has to travel and will introduce competing pollen which is more likely to reach the receptor crop than that from the emitter area. In addition insects are more likely to deposit pollen grains from the emitting crop on the border before moving to a receptive area. The border needs to be large enough to reduce cross-pollination by the emitter to an acceptable level. To maintain the purity of the transgenic crop in the case of hybrid production and thus avoid contamination of the transgenic plants the isolation border has to be composed of male sterile plants of the same variety.

Most of the experts supported the idea to adapt the isolation distance and if present the size of the isolation border in function of the trial size. Small plots would not generate as much pollen as bigger ones. For this reason it was suggested to propose different isolation distances and if present variable sizes of the isolation border depending of the trial size. For dossier B/BE/02/VW4 the foreseen trial size ranged from 12000 to 13000m² (with a total surface of 1490m² transgenic material). For the spring experiments of 2002 described in dossier B/BE/00/VWSP9 field trial size ranged from 1000 to 10000m² with an average of 3685,7m².

The isolation distance to neighbouring commercial Brassica fields will be of application for all the actual cropping systems including organic farming. This rule will only be of application for Brassica that flowers synchronously with the transgenic oilseed rape or Indian mustard and for which spontaneous hybridisation occurs under field conditions. Sexually compatible species that are harvested before bloom of the transgenic oilseed rape or Indian mustard or which are used as green fertiliser are exempted of this rule. In addition, data obtained from BLIK and ECOCERT indicate that the organic farming with oilseed rape or Indian mustard is a very rare practice in Belgium.

An additional source of concern towards organic farmers is the potential for the deposition of GM pollen on their crops even if fertilisation does not occur. The experts explained that the viability of pollen is short and that the amount of pollen quickly declines with an increasing distance from the source. Due to the short

viability of pollen and quick decline of pollen concentration with distance, a negligible fraction could be taken into account in this context. In this framework the effect of rain events in removing pollen on leaves, wind intensity, wind direction or the density of hairs on leaf surfaces were discussed. In the case of low hair density e.g. it is probable that pollen grains do not adhere well to the leaf surface and move around on the leaf in response to gravity, wind, or leaf movement, causing pollen to fall off. Differences in the position of the leaves were addressed as well as the distance of sampling compared to the source, the position of the plant in the field (field edge or center) etc.

The representative of the CA and SBB explained that it is not always possible for a notifier to guarantee the requested isolation distance. Due to the authorisation procedure, which counts without asking additional information 90 or 120 days respectively without and with the consultation procedure of the public, the notifier needs to know the exact locations of the field trials 3 to 4 months before sowing. At that time the notifier can contact all the farmers present in the isolation distance and ask which crop they will sow for the following cultivation season. In addition they could submit a map of the trial and surrounding of the field trial at parcel level containing the foreseen cultures around the GM trial for the whole isolation distance. But how can it be guaranteed that a contacted farmer will not change his mind? How can the situation that occurred in Chimay be avoided? In that case a commercial Brassica field was sown at 800m of GM OSR field already in place. Both fields were destroyed and the notifier had to remunerate the farmer for the destruction of the commercial Brassica field. The experts estimated that in the isolation zone of 1000m a zone of 350ha would have to be checked, which at Belgian agricultural level would correspond with contacting 10 to 15 farmers.

Checking the presence of organic farmers growing Brassica species that flowers synchronously with the transgenic oilseed rape or Indian mustard and for which spontaneous hybridisation occurs under field conditions induces an additional problem. It was suggested that the notifier or CA could contact BLIK and ECOCERT to obtain the data of organically grown Brassica in Belgium and thus determine if these are present in the surrounding of the GM field trial. For the moment this measure is not workable because BLIK and ECOCERT collect the requested data for the beginning or middle of March, which is much too late if you take the duration of the authorisation procedure into account. To fasten this process BLIK and ECOCERT could on the other hand immediately give the information to the concerned actors (CA, SBB and notifier) as soon as available.

The isolation distance will not only be of application towards neighbouring commercial Brassica (including all cropping systems) but also towards nature reserves. The WG still needs to determine what is meant with the term nature reserve and it is not yet clear who will be responsible for mapping these nature reserves. The spontaneous hybridisation potential towards sexually compatible wild relatives in these areas or the establishment of GM volunteers in these areas forms the basis for this request.

2. Measures towards wild relatives, hybrids and volunteers

The actual protocol obliges the notifier to check during bloom of the transgenic crop the presence of the following eight related sexually compatible species: *Brassica nigra*, *Brassica oleraceae*, *Brassica rapa*, *Brassica carinata*, *Brassica juncea*, *Raphanus raphanistrum*, *Hirschfeldia incana*, *Sinapis arvensis* in a surveillance zone of 40m surrounding the transgenic field. This zone is extended to 100m in case of abundant presence of one of these species. The abundant presence of one of these species still needs to be determined by the WG. The detected wild relatives have to be destroyed.

The experts of the WG agreed on limiting the number of wild relatives that needs to be checked in the **surveillance zone** of 40m to the following 3 species: *Brassica rapa*, *Brassica juncea* and eventually *Raphanus raphanistrum*. Scientific literature clearly indicates a potential for spontaneous hybridisation and introgression for these wild relatives. In this framework distribution maps of the sexually compatible species should be kept over all Belgium to determine if some of the species are abundantly present in the vicinity of the foreseen trials. In the case one of these wild relatives is abundantly present special containment measures, surveillance or monitoring conditions could be requested.

To avoid the transfer of the transgenes to these 3 wild relatives they will not be allowed to set seed. As flowering is requested to clearly determine the species they will be allowed to flower but not allowed to set seed. An expert was not sure that it is necessary to wait for flowering to identify the species. In case of doubt, the expert explained that it is easier to destroy the plant and that avoiding flowering is better to maximise precautions.

The notifier should foresee that the surveillance zone of 40m is part of the field trial (within the same property of the concerned person(s)) and avoid the presence of (a) field margin(s) in this zone. The destruction of the wild relatives mentioned above in field margins could not be accepted by some of the experts of the WG.

A **monitoring zone** of 60m will surround the surveillance zone of 40m. The status of the 3 wild relatives mentioned above, potential hybrids and volunteers have to be monitored in this monitoring zone using counting methods and detection techniques.

The monitoring will be done in the foreseen zone outside the field and can not be compared with the different foreseen follow up measures to minimise the number of GM volunteers and their control in subsequent crops depending on the type of authorisation procedure and gained knowledge. The follow up measures are only carried out in the field and ensure a maximum germination before completing weed and volunteer control measures (e.g. shallow cultivation, no use of deep soil inversion, application of appropriate herbicide, etc.). Depending on the authorisation procedure and gained knowledge a follow up of 1 or 3 years has to be respected by the notifier.

The following section contains a first proposal of monitoring, which is based on the monitoring plan submitted to the Belgian CA by Bayer CropScience to market the oilseed rape transformation events MS8, RF3 and MS8/RF3.

As the monitoring zone will contain field margins of irregular dimension it will be difficult to walk through following a W-shaped pattern and to randomly choose spots along this pattern where number of wild relatives, potential hybrids and volunteers can be scored in quadrates of 1m². For this reason it will be easier to determine individual sampling locations for each site. Any permanent visual means outside of the field (trees, electric fence, roads, etc.) as well as metal stakes can be used to mark permanent quadrates in the monitoring zone. A yet to be determined number of quadrates of 1m² can be selected per monitoring zone and followed up during XX year(s). The quadrates need to be numbered at the beginning of the monitoring period and the same numbering need to be used each year of the monitoring period. Their location will be accurately reported on a map of the site.

The inspections of the selected plots will be done at XX separate visits during the growing season. Wild relatives and oilseed rape can be monitored during the first year of the trial. This way the initial presence of wild relatives and non-transgenic oilseed rape volunteers susceptible to outcross with the transgenic crop can be determined. The monitoring of the hybrids and volunteer will start the first year after cultivation and harvest of transgenic crop. This monitoring will continue during the XX subsequent years corresponding to the actual follow up period of 3 years of the field for a simplified procedure or an entire crop rotation cycle (next OSR growing).

To confirm the presence of the herbicide tolerance trait in the potential hybrids and volunteers the LL Leaf Test Kit can be used in the field. The test allows a qualitative (yes/no) detection of the PAT protein in plant tissue. The assay uses a double antibody sandwich format. If a lot of hybrids or volunteers are present in the monitoring plots a fraction could be analysed using PCR analysis. A protocol will have to be determined by the WG. As this method will be used outside the field the WG will have to determine a protocol for sampling for confirmation of a GM trait in a single plant (spot sampling). That protocol should provide the recommended procedures for sampling, packing, labelling and dispatch of single plant samples for testing a GM trait. By assessing the draft minutes an expert mentioned that the measures regarding the identification of the trait used by LL Leaf Test Kit and PCR analysis are not dispositions of precaution but measures to realise new kind of studies.

In the proposed monitoring plan monitoring will not be conducted in both transgenic and non-transgenic oilseed rape fields. This will not allow the establishment of a baseline for comparisons. The plan will not indicate differences between normal and transgenic oilseed rape. The proposal will only allow determining the initial presence of wild relatives and non-transgenic oilseed rape volunteers susceptible to outcross with the transgenic crop, to estimate outcrossing rates and to assess the persistence of hybrids and volunteers.

Enough data and samples will have to be collected to draw statistically relevant conclusions. The questions of time and costs should clearly be discussed on this point. The experts suggested that an independent body should carry out the monitoring in collaboration with the notifier. The costs of the monitoring should be attributed to the notifier and government.

In the future some conditions of the protocol for growing GM Brassica could be modified in function of the obtained data.

3. Honey bees and contamination of honey

The protocol valid for the cultivation season 2002 obliges notifiers to check the presence of beehives of professional beekeepers at the site of the field trial and within the zone of the isolation distance because beekeepers regularly place beehives in the vicinity of oilseed rape fields for their honey production. These data need to be recorded by the notifier in the activity report, which is requested at the end of the growing season by the CA. Thus, before bloom of oilseed rape the notifier needs to contact concerned beekeepers associations, which contain the list of all professional beekeepers for Belgium. Checking the presence of beehives will allow tracing the source of the contamination of the honey in the case it would occur. Taking the scale of activity of beekeepers into account it was suggested to limit the control to professional beekeepers. The number of beehives they are using is much more important compared to non-professional beekeepers.

After contacting the beekeeper association CARI it became clear that only 5% of all the beekeepers are registered in Belgium. The experts therefore suggested that the notifier would demand the information of the registered beekeepers collected by these beekeeper associations in due time. The non-registered beekeepers will on the other hand be informed about the presence of GM oilseed rape in certain areas through the foreseen public information and consultation procedure at the level of the commune according to the draft Royal Decree that will implement the European directive 2001/18/EC. With this information non-registered beekeepers will have the opportunity to contact the notifier and ask the location of the trial.

In order to stimulate pollination between the two transgenic lines (production of seed hybrids) the notifier will use bees and thus place beehives in the field containing the transgenic oilseed rape. In the future the notifier will be obliged to destroy these beehives after flowering of the crop. To obtain more certainties about this topic the notifier could be asked to take some samples from the honey in order to monitor the presence of DNA in the honey before destroying these beehives.

Regarding the impact of the pollen of herbicide tolerant oilseed rape on bees most of the studies were not able to detect an effect. To obtain more information on this topic Prof. Frans Jacobs of the University of Gent will be contacted.

4. Destination of harvested seeds

The protocol should clearly ask the purpose of the trial and the notifier should clearly explain the final destination of the harvested vegetal material. In the case of oilseed rape the final destination of the seeds needs to be clarified. Without this justification it was suggested that the harvested material should be destroyed by default. An expert suggested the destruction on the field. The method used and the place to destroy this material need further discussion.

The quantity of vegetal material needed for each purpose should be addressed for each lot of seeds (taking the number of the seed lots into account).

- Give the obtained yields per seed lot (with the number of the seed lot)
- Explain what shall be done with the harvested seeds
 - Research (research on the seeds, quality control, indoor (e.g. greenhouse) or outdoor cultivation of the hybrids for research purposes, within this framework the location of the activities should also be indicated: Wallonia, Flanders, EU, other country, etc.)
 - Non-research (oil production, etc.)
 - Multiplication (indoor or outdoor in Wallonia, Flanders, EU, other country, etc.)
- Give the quantity used for each purpose

5. Legal basis of the protocol

The legal basis of the protocol was questioned. The SBB explained that the consent of field trials with genetically modified oilseed rape and Indian mustard always clearly stipulate that the trial needs to be carried out in conformity with the Brassica protocol valid for that cultivation season. Inspectors are in charge of inspecting the trials for compliance with the conditions specified in the consent and specific protocol and to investigate potential breaches of the consent. In case where mismanagement or fraud is identified specific sanctions are imposed.

6. General topics

The experts agreed on asking that the farm staff and all the people that would come in contact with the GM material must be adequately informed about the content of the protocol that will concern them. All these persons need to be aware of the considerations required to grow genetically modified Brassica (e.g. bookkeeping, isolation distance, cleaning of machinery), of the possible risks and problems involved, and recognise the importance of the proposed protocol. This will strengthen the information and stimulate them to follow the recommendations of these guidelines. Therefore, the notifier must provide information on all aspects of the management of the GM crop and the possible consequences of failing to meet management guidelines. Within this framework the protocol will explain "why" certain measures need to be taken and "why" certain recommendations need to be followed.

In the introduction of the protocol it will also clearly be mentioned that the existing principles of "good agricultural practices", including all relevant codes of practices and pesticide regulations, must be followed when growing and harvesting GM Brassica crops.

Report of the meeting on January 7th, 2003

1. CARI

1.1 Foraging behaviour of bees

CARI's representative explained that bees should be avoided around GM Brassica fields because they are strongly attracted by Brassica flowers. In some honey, the analysis of the pollen diversity taken as a probe of visited flower species shows that Brassica nectar/pollen is often a major food component of the bees. Bees play an important role in and mediate pollen flow over distances proportional to the size of the colony. Bees can move up 1 to 6km with an average action radius of 3km and with most movements occurring close to the hive and between neighbouring plants. Foraging ranges of 1 to 6km of honeybees have indicated that forage sources lying within an area of up to 100km² could be exploited supporting the idea that honeybees do not only forage on their own doorsteps. The behaviour of these bees is affected by the size of the source field, the distance between source fields, the density of beehives, the quality of nectar, the environmental conditions as well as the amount of rewards.

The related literature documents that optimally foraging bees will spend more time within large populations than small populations, because they are able to collect full pollen/nectar loads and have no need to move between plots in larger plots. This will favour short flights and affect the proportionately more intra-population matings of the plants. If, however the nectar rewards (smaller plot sizes, end of the flowering season, high density of bees, etc) are small and larger distances separating the plot, greater movement is required to obtain a full pollen load. This will favour longer flights. The amount of rewards will vary over a season within a species, which will inevitably influence the pollinator behaviour. CARI told that the decreasing amount of rewards at the end of the flowering period would not cause a major problem because it is a common practice for beekeepers to remove their beehives at the end of the growing season. Within this context CARI explained the different foraging strategies addressing the foraging behaviour of moving apiary and scout bees.

1.2 Economical impact of GM-pollen presence in honey for producers of "natural honey":

CARI explained the nature of the different brands of honey available on the Belgian/EU/USA markets. Mixed honeys, filtered to remove pollen and particles, are seen by CARI as having the lowest quality and this is reflected by a basal world market price of 1 E/Kg. Unfiltered/unmixed honeys can range from 4 to 9 E/Kg, the highest price corresponding to unfiltered plant species-specific, flavour- or

ecosystem-specific honeys, but non-contaminated by GM pollens. While recognising that Americans were eating GM-contaminated honeys for years without apparent sufferings, CARI explained that the European consumers, who are generally seeking for traditional, flavour-specific and GM uncontaminated honeys, would not accept GM-stuff in "natural honeys". CARI's opinion is that in the case of contamination with GM pollen, the GM pollen content has to be given in function of the total pollen content in honey (% of GM pollen to total amount of pollen). Bayer CropScience however supported the idea to estimate the amount of transgenic pollen in function of honey. Depending on which interpretation is followed the economical impact and labelling provisions will be different.

CARI explained that the majority of beekeepers are private persons, unregistered and that their incomes would be severely lost (1 E/kg versus 4-9 E/Kg in case of GM-stuff contamination). CARI expressed the idea to the attention of the companies that adventitious contamination of natural honeys by GM pollen should somehow be covered by a fund or directly reimbursed.

The chairman concluded that CARI's report is a concrete report about economical risks about which the concerned and CA should be informed.

1.3 Proposed apiaries, GM-fields co-existence measures

CARI clearly asked that beekeepers should be informed before inadvertently placing their beehives in the surrounding of GM Brassica. CARI proposed an inventory of all the hives in a radius of 1,5km, apiaries of 3-10 hives in a radius of 3km or apiaries of >10 hives in a radius of 5km. In addition, CARI requested that no moving apiary should be allowed in a radius of 3km surrounding the GM field. An expert specialised in plant-insect interactions and particularly honeybee ecology explained that despite the large amount of non-registered beekeepers and the complexity of the beekeeping organisation in Belgium, an accurate inventory of beekeepers could be accessible to the notifier. Beehives locations are moreover predictable since most beehives are located each year at the same place by the same people. Additionally, the hives are often located close to the beekeeper's house or around the same type of fields. The only problem regards the practice of moving beehives during the plants cultivation season. This practice mostly concerns the Northern part of Belgium. In Walloon almost all beekeepers keep the beehives at the same place. This means that the notifier could easily contact the beekeeper associations and thus the beekeepers in order to obtain the requested information. Taking the purpose of the demand into account (to set up a GM field test) an expert indicated that these beekeeper associations would not be restrained to give a list of all the beekeepers (professional and amateur). Moreover, beekeepers always put their name on their beehives, which could be useful information for the notifier and facilitate the preliminary information task of the notifier.

Due to the clear role of bees as the main mechanism of oilseed rape pollen dispersal and the variability of bee foraging and beekeeper behaviour, the above mentioned expert further proposed to delocalise the beehives of beekeepers

present around the GM fields for a remuneration paid by the notifier. If the average action radius of swarming bees is taken into account the beehives would be transported 2-3km away from the GM fields. The expert was of opinion that this could easily be done and that beekeepers would not be contradictory if the notifier would explain the reasons of this measure and provide remuneration. Natural hives will on the other hand not be transportable, but the proposed measure is already a step forward.

Bayer CropScience explained that if an accurate list of all the beekeepers and requested information are provided by the beekeepers associations and/or CA (professional and amateur), feasible according to the experts opinion (but in contrary of last year's information that only 5% of the beekeepers are registered), the company can engage itself to contact the beekeepers in the vicinity of the field trial (up to 3 km) with the request to replace the hives outside the region. Bayer CropScience indicated on the other that they could not provide them a place to go to, nor oblige them to do so. Thereby, it is possible that a beekeeper is situated outside the region but that his hives are placed in the vicinity, so hardly to control or hardly to contact the beekeeper in advance. The notifier stressed that they can only agree with this measurement if their responsibility ends by contacting the beekeepers from the list in the range of 3km around the field trial.

CARI did not support the proposal of delocalising the beehives within a radius of the 3km around the GM field trials. According to CARI this measure will be at the cost of the beekeepers.

1.4 Pollen and GM-pollen traceability, eco-toxic contaminants

The SBB questioned CARI and the competent experts about pollen and GM-pollen traceability in honeys. CARI explained that honey is mainly composed of sugars with small amount of waxes and pollen (0,1% or 10.000-40.000pollen/10gr). In a letter that the SBB received from MELI, it was written that tests have indicated that only 1% pollen is present in honey and that amount of pollen in oilseed rape honey corresponds with 1mg/kg. The SBB also informed that this amount of pollen provides a large amount of gene copies for genetic traceability. Taking a contamination level of 0.01% as detection limit, 30 copies of GM-specific markers would be present in 3gr of honey (a normalized size for laboratory samples) and consequently well above the limit of detection of standard methods but very closed to the limit of quantification. However, filtered honeys could well be not so easily traceable genetically.

Microscopic analysis allows to determine the type of pollen present in the honey, to differentiate between the types of pollen and thus to determine the origin of the honey. Only the pollen fraction can identify the presence of GM pollen. In the case of GM contamination CARI was in favour of destroying the contaminated honey as well as the beehive.

Considering the behaviour of bees, the diversity of pollen markers gathered in a batch of honey, the SBB asked whether honey could not be exploited as an

environmental tracer, or in other words that honey could be seen as an environmental integrator offering a picture of the state of the environment in which the bees are foraging. CARI found it interesting to identify the DNA in the honey. Analysing honey chemical and biological parameters would indeed allow to describe the presence of GM material, antibiotics, pesticides, heavy-metals, etc. The audience recognised as a realistic issue a research exploiting honeys as probes of environmental quality. CARI reminded that some beekeepers filter their honey with rough filter to extract the pollen and that the amount of pollen extracted will vary in function of the type of filter (micro/ultra filtrated honey) being used. This practice will have an impact on the quality of the honey, detection of GM material and on the determination of the origin of the honey. This treatment would also have its impact on the price of the honey.

Studying the potential impact of GM pollen as potential bees eco-toxicants was further discussed. In the case of toxin presence in the GM-pollen the bees that may be affected by the produced toxins are the larvae and younger bees (up to 10 days of age) that feed from it and for which pollen is collected. The larvae and the younger bees would be the targets of adverse effects. The experts explained that the bees are collecting the pollen feed from floral nectaries (that is also collected for the production of honey). Nectar is produced from the phloem, where these toxins are not produced hence these bees are not likely to ingest the toxins. It was suggested that the toxicity should thus be determined for larvae and young bees of up to 10 days old.

1.5 Organic honey production in Belgium

The SBB asked whether honey could also be produced organically and if organic honey does exist on the Belgian market. The experts explained that in theory it is possible but that it would be very difficult for Belgium. CARI said that it would not be possible in Flanders, but if beekeepers would organise themselves it could be possible in Walloon. Bioforum also supported the fact that it is not possible for the moment, but that the possibility could be addressed. So, for the moment there is no organic honey produced on the Belgian market.

After the meeting Bioforum confirmed that it would be difficult to have organic honey production in Belgium and referred to the provisions on beekeeping, beekeeping products, characteristics of hives and materials used in beekeeping of the Council Regulation (EC) N° 1804/1999 of 19 July 1999 supplementing Regulation (EEC) N° 2092/91 on organic production of agricultural products on foodstuffs to include livestock production. The sitting of the apiaries e.g. must:

- (a) ensure enough natural nectar, honeydew and pollen sources for bees and access to water;
- (b) be such that, within a radius of 3km from the apiary site, nectar and pollen sources consist essentially of organically produced crops and/or spontaneous vegetation, according to the requirements of article 6 and Annex I of this Regulation, and crops not subject to the provisions of this Regulation but treated with low environmental impact methods such as, for

- example, those described in programs developed under Regulation (EEC) No 2078/92, which cannot significantly affect the qualification of beekeeping production as being organic;
- (c) maintain enough distance from any non-agricultural production sources possibly leading to contamination, for example: urban centres, motorways, industrial areas, waste dumps, waste incinerators, etc. The inspection authorities or bodies shall establish measures to ensure this requirement.

The above requirements do not apply to areas where flowering is not taking place, or when the hives are dormant.

2. BIOFORUM

2.1 Bioforum's opinion

Bioforum explained that according to the Council Regulation N° 2092/91, supplemented by Regulation N° 1804/1999, identity criteria for organic farming and products require that an organic product should be produced without the use of genetically modified organisms and/or any products derived from such organisms. Organic farmers are prohibited from using transgenic organisms in their cultivations. In such context of zero tolerance, the consequences of GM contamination would be economically significant because the purity and marketability of the organic crop is put at risk. GM pollen can also be defined as an indirect contaminant even if fertilisation does not occur, so the deposition of GM pollen on organically grown crops has to be taken into account. To allow an effective protection of organic and conventional farming, Bioforum explained that the GM producers/testers should be kept responsible for contamination of non-GM crops and this point should be formulated in the protocol.

The SBB reminded that the adventitious presence of GMOs in conventional or organic crops is one among many other sources of GM contamination, and it is already established that processing and transport are the main causes of contamination. Furthermore, the adventitious presence of GMOs in conventional or organic crops does not imply that the presence of GMOs renders them harmful. The risks for the environment and human health are thoroughly assessed before allowing a deliberate release, even if anti-GMO organisations claim not to believe in such assessments. Dr. Moens however declared that living altogether in the same society, organics agro-industries have the right to develop according to their quality standards and consequently to be correctly protected. And this is not restricted to the GMO-organics co-existence problem. So the present working group is happy to welcome representatives of Bioforum and to build together ways of peaceful management.

2.2 Contamination threshold and isolation distance, detection and quantification

The experts explained that the use of isolation zones, border areas and thresholds are some of the strategies, which are actually employed in field trials to confine the

spread of (non)-transgenic pollen and seeds and to reduce contamination. To ensure successful co-existence of organic, conventional and genetically modified oilseed rape crops, industry and growers will need to accept similar standards of purity to those currently used for oilseed rape seed production crops worldwide. In the regulation of seeds grown for sale statutory measures exist to minimise genetic contamination and maximise varieties purity. EU seed marketing directives specify separation distances as well as time intervals between seed crop and any other crop of the same species grown on the plot. For organic farmers similar standards practices are known in order to exclude contamination by synthetic chemicals. According to the United Kingdom Register of Organic Food Standards (UKROFS) organic farmers provide an effective windbreak to minimise the risk of spray-drift contamination and ensure equipment is free of non-permitted substances. The representative of Bioforum was not aware of this type of measures.

Within this framework the SBB raised the point of the *de minimis* threshold for unavoidable contamination foreseen in article 13 of Regulation (EEC) N° 2092/91. The SBB asked if Bioforum already established a trace threshold below which the organics activities/products are preserved and stressed the importance of determining a trace level because this level will help to determine the isolation distances in this working group. Bioforum spoke about a detection level of 0,1%. The discussions around the table and information from various participants reported that the *de minimis* threshold level for organics was being discussed around 0.1%. The representative of the competent authority indicated that since 1999, the Commission should have defined such threshold of contamination, but that the discussions are still ongoing. The SBB informed that the German "Association Organic Food Industry" demanded to admit an adventitious GMO-presence up to 0.5% for organic farming. The parliament fraction of the Christian Democrats welcomed this proposal and rated the demanded 'zero tolerance' by the German Federal Minister of Consumer Protection, Food and Agriculture as scientifically unfounded and unrealistic.

An expert explained that the detection level of 0,1% and a threshold level of 0,1% are not synonymous. A threshold level is a level measured quantitatively and implies more than simply detecting the presence of GM material. For documentation, the detection of GM material at the level of 0,01% is possible, but the limit of quantification is around 0.1% for technical reasons and because the certified reference material do not exist below 0.1%. From European ring trials organised by the European Network of GMO laboratories of the European Commission, there is still 40% variability between laboratories carrying out the analysis of samples containing 1% of GMO at 95% confidence level. It is the present limit of trustable quantification given the lack of certified reference material below 0.1% (Source JRC-IRMM Geel).

In such a context, the importance of the sampling methods becomes very important and was discussed. How many samples are needed to allow the requested detection or quantification? It was concluded from various reports that the problem

is still immature even for seeds, which are the simplest matrices to evaluate among food/feed/environmental matrices.

Within this framework, the SBB asked Bioforum to give a definition of 'GM contamination' and to make a proposal of threshold. The SBB explained that the rational method of working of the experts of the Biosafety Advisory Council is always based on consensus criteria and norms.

Bioforum was in favour of maintaining the isolation distance of 1000m towards commercially grown Brassica because frequencies of cross-pollination have been recorded at distances of up to 4km from the source field. The experts accepted such compromise although firmly declaring that a 400m isolation would not bring less safety against contamination. One expert estimated the levels of outcrossing to be not significantly different for an isolation distance of 400 or 1000m. Given that the difference would be very small, an isolation distance of 400m could be chosen, but a 0% contamination level would not be possible. The SBB explained that the isolation distance of 1000m was put in place in order to take the active transport by pollinators into account and not only passive pollen movement through wind. Research and the report of CARI indicate that bees play an important role and mediate gene flow over a longer range of distances (see previous chapter). The notifier indicated to be in favour of reducing the isolation distance, but also explained that the isolation distance of 1000m can be maintained if there is a real argument to do so and not only on ideological grounds.

The SBB asked Bioforum if ECOCERT and BLIK could contact the notifier as soon as they know whether a farmer is going to grow Brassica organically. Knowing the exact locations of that (these) field(s) will allow the notifier to locate field tests in order to carry out an experiment far from any kind of conflicting interests. Bioforum supported this idea. This proposal is workable because growing Brassica organically is not a common practice. According to Bioforum there is only one farmer cultivating Brassica organically for the moment and always on the same location. In this context Bioforum stressed that organic farming should stay possible in the future.

Management of co-existence, brain storming about fixed dedicated research areas: The proposal of grouping all the GM trials on the fields of the governmental institutes: CLO (Gent) and CRA (Gembloux) as examples was discussed. This would create a predictable situation for all the concerned actors, research would be allowed and co-existence would be possible because there would be no interference with other interests. The single company present - but also the main research actor in Belgium - regretted such option because it narrows the agronomical possibilities (regional variability). The reluctance of the notifier was also linked to the agronomic consequences like spreading the risks of diseases, storms, etc. The homogeneity of the soil is of importance, type and history of the soil. The notifier explained that performing field trials outside Belgium can solve the regional variability. However, if it would be the single option left in Belgium then the company would necessarily comply with such a scenario. The company explained that it is only feasible to carry the field trials in this kind of regions for new

events, put in the field for the first time. Trials would be small and the monitoring will be extensive. For traits nearby commercialisation, however, the trials will be up-scaled and more agronomic factors will influence the outcome of it. For this reason the notifier suggested to apply the case-by-case approach: for some trials it might be advisable to work in close relationship with universities/institutions but for other cases there is no reason to do so, year after year.

The SBB did not agree with the company on the point of narrowing agronomical possibilities. Indeed each GMO development carried out by the company along the past 10years (and this is published on the JRC-Ispra web site) always occurred in various member states in parallel. The various assays aimed at collecting different ecosystems-related information about the GMO under research. To allow a stop of the destruction of authorised field tests, the companies and responsible of dedicated agronomic research areas have to share the building of a social deal first. The hypothesis of dedicated research area is an example of organised co-existence. The company would have anyway the choice to carry out several research aspects outside Belgium at it was already the case before.

In conclusion, the idea of dedicated research areas seemed to attract positively the interest of all participants. Given the complexity of the set up of such an option the concerned participants declared to bring back the point to a next meeting with less hypothetical data.

3. BAYER CROPSCIENCE

3.1 Bayer CropScience's opinion

Bayer CropScience's questions, opinions, uncertainties about agricultural management by the State, request of guarantee about future authorised field tests: Bayer CropScience wanted more clarification about the legal basis of the protocol. On one hand, the protocol is elaborated in this working group foreseeing different measures to ensure that cross-pollination stays below specific tolerance thresholds and detection limits. On the other hand, the competent minister supports a zero tolerance and uses quotes of this protocol in the discussions to not deliver the requested permit. In addition, one of the consent conditions obliges the notifier to carry out the field in accordance with the protocol for growing GM Brassica in order to perform a scientific trial without risks to human health and environment. Working in compliance with the protocol is not a guarantee to obtain the permit. It was explained that it was not the adequate group to discuss this topic. A social agreement was repeated to be the preliminary condition in order to secure the protocol.

The basic principles of case-by-case and step-by-step for risk assessment and management were reminded and have to be better addressed in the protocol. The variability in function of the type of trial should be addressed. The SBB explained that the protocol is a minimal requirement and that additional provisions can be defined on a case-by-case basis.

The scientific basis of some of the proposed containment measures were challenged and left without answers (e.g. isolation distance of 1000m, surveillance zone of 40m, monitoring zone of 60m etc.) as well as a practical definition of thresholds, methods to measure the isolation distance, liability in the case of changing situations (changing locations, unexpected neighboring cultures), the reason to include specific measures towards organically grown crop and nature reserves, the definition of nature reserves, the determination of the abundant present of wild relatives, etc.

The proposal of reducing the isolation distance to 600 or 400m if an effective isolation border of the same variety (of minimum 10m) would surround the trial will not always be workable according to the notifier. In the case of hybrid production no contamination of the border can be allowed. In addition, Bayer CropScience stated that using a male sterile conventional oilseed rape cultivar to avoid this contamination is not feasible. The company however was in favour of implementing the 400m, with a transgenic male sterile border if required and proposed a case-by-case approach in the protocol.

3.2 Respect of isolation distance, measure the isolation distance, capacity to consult the farming neighbourhood

The company discussed the problem guaranteeing the requested isolation distance. The method used to measure the isolation distance should also be discussed and be identical for the competent authority, inspectors and notifier. In addition, the notifier should be given serious documentation means by the administration in order to contact all the farmers or owners who cultivate land in the surrounding of the field test location in order to know which crop neighbouring farmers or owners will sow for the following cultivation season. Moreover, the notifier could also submit a map of the trial and surrounding of the field trial at parcel level containing the foreseen cultures around the GM trial for the whole isolation distance. But how can it be guaranteed that the contacted farmers will not change their mind and create a business situation after the sowing period? Indeed, at time of introducing the regulatory dossier, most surrounding farmers do not know what they will cultivate the next growing season. And how should the responsibilities be determined in the case of unforeseen location and crop changes resulting of the surrounding farmer who do almost what they want in terms of crop choice and locations? All these questions are based on a real case that occurred in 2002 and where the company was submitted to very big economical harm as a result of the unpredictable contextual agricultural management. A predictable situation should therefore be worked out in the protocol in agreement with the control and authorisation authorities. The actions that need to be taken by the notifier in the case the isolation distance is not guaranteed have to be foreseen in the protocol e.g. destruction of the GM field, destruction of the GM and other field. The notifier explained that the required actions should be clear in advance. Within this framework the possibility to adjust the situation or on the other hand to end and destroy the trial if infringements are found was questioned.

3.3 About thresholds of adventitious contamination

Bayer CropScience explained that the containment measure towards conventionally and organically grown Brassica would remain the same as the GM events are seen as not authorised. For that reason the thresholds are the same.

3.4 About co-existence and nature reserves

The measures towards nature reserves and wild relatives are not well understood in the case of herbicide tolerance. The definitions used in the protocol are still unclear and are still not accurately clarified. Bayer CropScience is of opinion that the hybrids will only be formed at very low levels and that these hybrids will not have a selective advantage. Bioforum told that these hybrids could produce pollen and contaminate organic crops. In the context of escaping transgenic oilseed rape out of the cultivation fields an expert also indicated that for the moment no oilseed rape was found in nature reserves and that these escaped oilseed rape plants are not more invasive than the conventional counterpart. For oilseed rape it seems extremely difficult to establish an oilseed rape populations into established vegetation, and populations which are experimentally established on disturbed ground tend to go extinct after only a few generations. Data highlights the importance of competition-free gaps for successful recruitment of oilseed rape from seed in natural habitats. An expert indicated that in most nature reserves specific management measures are taken and these habitats are also regularly disturbed.

3.5 About monitoring

The notifier was not in favour of carrying out the proposed monitoring at that stage of development. A post-marketing monitoring plan will be done at the marketing stage. The value to do this each year for each field trial was questioned. According to the notifier it is not feasible to carry out the monitoring for each field trial (part B), each year again. Once proven, the authorities should take general measures. In connexion with the idea of dedicated research areas, the company supported the multidisciplinary approach and the idea to let an independent body do the monitoring. The company however suggested that in the case a certain aspect wants to be studied it is more appropriate to organise a specific trial in this regard (see biosafety trials with CLO). In addition, the company explained that anything can be studied but that a difference should be made in data that are nice-to-know and need-to-know. The notifier as well questioned the costs. The notifier did not want to carry all the costs by its own. Literature and the obtained data will allow refining the containment measures and can confirm their utility

3.6 About co-existence with beekeepers

The containment measures proposed towards bees, beekeepers and honey were questioned. See above for details. As a general attitude, the company was considering this aspect as being a logical component of agricultural management

by the authorities and that the company is willing to comply with rules, which are scientifically or legally justifiable.

3.7 About the harvested material

The notifier explained that it would not be a problem to give the requested information on the final destination of the harvested vegetal material.

List of experts

Within the framework of the SBB, which is the Secretariat of the Biosafety Advisory Council (the Belgian advisory board on GMOs), the WG on the protocol for growing GM Brassica has contributed to the development of this document.

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1 = Meeting of 14-11-2002

2 = Meeting of 07-01-2003

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Comments or questions on this report

If you have any comment on the proposed report or wish to obtain additional information on the protocol for growing GM Brassica, please contact us at the following address.

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