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To the Minister for
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REF CGM/161012-02

SUBJECT Advisory letter on the 'Representativity of non-target organisms'

Dear Minister,

When permit applications are made for the cultivation of insect-resistant genetically-modified (GM) crops, the assessment of potential effects on non-target organisms[1] forms an important part of the environmental risk assessment. For this purpose, laboratory experiments are conducted to investigate whether the insects concerned will be affected by the GM crop. By necessity, such a study is carried out using a small number of species because it would be impossible to investigate all the hundreds of species which may be found in a crop.

To provide useful information for the Netherlands' own risk assessment, the insects investigated must be found in the GM crop here, or be representative of the non-target organisms which may be exposed to the GM crop in the Netherlands.

However, for practical reasons, applicants often carry out the investigation using species which are not found in Europe. This leads to discussions with risk assessors, like COGEM, who raise concerns about the representative nature of the species investigated in relation to the species exposed in the Netherlands.

At the moment, just one insect-resistant GM crop has been authorised for cultivation in Europe. Shortly, a vote will be taken to decide whether three insect-resistant GM crops will be admitted to the EU for cultivation. Based on the new national cultivation competence (Directive 2015/412), it is expected that the number of permit applications for the cultivation

[1] Non-target organisms: all organisms which are directly or indirectly exposed to the GM crop during cultivation with the exception of the pest organism(s) targeted by the trait introduced into the GM crop.

of GM crops will start to rise again, and the discussion about the representativity of the non-target organisms investigated will again resurface.

Uncertainty about the representativity of the non-target organisms investigated in the laboratory makes it more difficult to draw conclusions about the environmental safety of GM crops. This leads to delays in the permit application process because applicants are asked to supply additional studies with European non-target organisms. To prevent such problems arising with future permit applications COGEM would hereby like to establish its requirements concerning the selection of representative non-target organisms.

On the basis of the considerations set out below, COGEM concludes that the species investigated in the laboratory must be found in the crop in the Netherlands or in Europe. If this is not the case, they must at least belong to the same genus as the species in Europe that will be exposed to the GM crop. Only then will the research results obtained be sufficiently representative and appropriate for the purpose of the risk assessment.

COGEM's advice to the Minister is that its considerations and conclusions should be brought to the attention of the European Food Safety Authority (EFSA) so that they may be used to improve the guidance on the risk assessment of GM plants.

Considerations

Present practice in the selection of test species

At the moment the ecological functions of species are taken as the most important criteria when selecting species for investigation.[2,3] EFSA states in its guidance that at least one species in each of the relevant functional groups (e.g. herbivores, natural enemies, pollinators, detrivores and symbionts) should be investigated.[2]

Experiments should ideally be carried out with species representing different functional groups and which are found in the crop in Europe. There are various sources available which may be consulted for this purpose. For example, EFSA had a database set up with information on the arthropods found in field margins and in various crop fields (maize, beet, potato, rapeseed, soy, etc.) in Europe.[4] COGEM had the ecological food webs of various crops in North-West Europe identified and a list drawn up of non-target organisms (e.g. predators, parasitoids, pollinators and detrivores) that play an important role in maize, potato

[2] EFSA (2010). Scientific Opinion. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 8(11): 1879

[3] COGEM (2005). Guidelines on selecting non-target organisms for the risk assessment of the introduction on the market of genetically modified crops. COGEM advice CGM/051020-01

[4] Riedel J *et al.* (2016). Update and expansion of the database of bio-ecological information on non-target arthropod species established to support the environmental risk assessment of genetically modified crops in the EU. EFSA supporting publication 2016: EN-956

or rapeseed in North-West Europe.[5] COGEM has previously recommended an approach in which permit applications for these crops would require that four to six of the species on this list should be investigated. [3]

Besides ecological function there are also other aspects which are important when selecting species, e.g. whether the species could be exposed, directly or indirectly, to the GM crop, and whether there are indications that the species may be sensitive to the GM crop.[2,3,6]

In practice, however, practical matters such as suitability for laboratory study and the difficulty of rearing a species, often play a decisive role in the selection of species for research. As a result, in practice, most of the species investigated in permit applications are either not found in the crop concerned or not in Europe.

Sensitivity to Bt toxins and representativity

GM crops which have been made insect-resistant are often termed Bt crops. Bt crops produce proteins (Bt proteins or Bt toxins) which are naturally produced by the *Bacillus thuringiensis* bacteria. In an insect that is susceptible, these Bt proteins (protoxins) are dissolved in the midgut following which proteases convert the Bt proteins into δ-endotoxins. These δ-endotoxins bind to specific cell membrane receptors in the midgut which ultimately leads to the death of the insect.[7]

B. thuringiensis can kill insects and nematodes and is found in the soil as well as other environments, such as in plants (rhizosphere, leaves, endophytic) and in water.[8] Because *B. thuringiensis* is found in a very wide range of environments, there is discussion about its ecological role. Some scientists see *B. thuringiensis* as an obligate pathogen of insects or nematodes,[9] while others hold the view that *B. thuringiensis* is a saprophyte or endophyte that is also an opportunistic pathogen.[10] It appears however that *B. thuringiensis* does not thrive in the vicinity of plants or in the soil. **Error! Bookmark not defined.** Based on all the scientific data COGEM considers *B. thuringiensis* to be a pathogenic organism.

As indicated, the pathogenicity of *B. thuringiensis* and the toxic effect of the Bt toxins lies in its binding to specific receptors. It is reasonable to assume that species which are genetically closely-related will have similar receptors and be equally sensitive to Bt toxins. Taxonomists

[5] Scholte E-J & Dicke M (2005). Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies. COGEM research report CGM 2005-06

[6] Romeis J *et al.* (2011). Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res. 20: 1-22

[7] Xu C (2014). Structural insights into *Bacillus thuringiensis* Cry, Cyt and parasporin toxins. Toxins 6: 2732-2770

[8] Ruan L *et al.* (2015). Are nematodes a missing link in the confounded ecology of the entomopathogenic *Bacillus thuringiensis.* Trends Microbiol. 23(6): 341-346

[9] Raymond B *et al.* (2010). *Bacillus thuringiensis:* an important pathogen? Trends Microbiol. 18(5): 189-194

[10] Argôlo-Filho RC & Loguercio LL (2014). *Bacillus thuringiensis* is an environmental pathogen and host-specificity has developed as an adaptation to human-generated ecological niches. Insects 5: 62-91

place species which are genetically most closely related to one another in the same genus. Sensitivity to Bt toxins may therefore be expected to be most similar between species found in the same genus.

So far little research has been conducted on the extent to which non-target organisms that are more or less closely-related, differ in their sensitivity to Bt toxins. Such research would be desirable because this would increase our understanding of the sensitivity of species and provide insight into the representativity of species.

Conclusions and advice

Practical considerations often play a key role when selecting species to investigate the potential effects of a GM crop and, quite wrongly, little or no consideration is given to the importance of functional (i.e. physiological, biochemical and molecular) similarities when selecting representative species.

To be able to obtain information which may be used to assess the potential effects of the GM crop on non-target organisms, experiments must be carried out with non-target organisms which will be exposed to the GM crop in the field or which are sufficiently representative of these non-target organisms.

COGEM considers species that belong to the same genus as species that are found in the crop in Europe to be sufficiently representative as it may be expected that species which are closely-related genetically will be functionally similar to one another and react in a similar way to toxic substances. Species belonging to the same genus as the non-target organisms that will be exposed to the GM crop, therefore, will generally provide sufficient information to be able to assess the risks to European non-target organisms.

This position implies that investigations with species which belong to genera that are not found in the crop in Europe will not be suitable for assessing the risks to European non-target organisms.

COGEM takes the view that the EFSA guidance on the risk assessment of GM plants should include a requirement that the species investigated in the laboratory must be found in the crop concerned in Europe or must belong to the same genus as the species that will be exposed to the GM crop in Europe.

COGEM recommends that you bring the above considerations and conclusions to the attention of EFSA to further improve its guidance.

Hoogachtend,

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Voorzitter COGEM

c.c. Drs. H.P. de Wijs, Head GMO Office
Mr. J.K.B.H. Kwisthout, Ministry of Infrastructure and the Environment