COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Cogem
P.o. box 578
3720 AN Bilthoven

State Secretary for Housing, Spatial
Planning and the Environment
Mr P.L.B.A. van Geel
P.O. Box 30945
2500 GX The Hague

Uw kenmerk	Uw brief van	Kenmerk	Datum
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Onderwerp
Advice effects on non-target organisms

Dear Mr van Geel,

Hereby I present you our advice *Guidelines on selecting non-target organisms for the environmental risk assessment of the introduction on the market of genetically modified crops* and the COGEM research report *Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies*.

Summary of the advice
All applicants for permission to cultivate genetically modified (GM) crops must provide certain information, including details about potential adverse effects of the GM crop on organisms that are not intended to be affected by the expression product of the inserted gene, called 'non-target organisms'.

COGEM is of the opinion that testing on non-target organisms is necessary only in those cases in which it is likely that the expression product of the transgene could lead to an adverse effect on non-target organisms.

Should tests on non-target organisms be considered necessary, COGEM recommends the following procedure for selecting relevant non-target arthropods.

For each individual crop an ecological food web should be compiled that consists of arthropods found in and near the crop and which are not target organisms. COGEM has had crop-specific ecological food webs drawn up for oilseed rape, maize and potato for North-West European conditions. From these lists of non-target arthropods, arthropod species should then be selected that meet one or more of the following criteria (see Annex 1): 1) sensitive to or exposed to high concentrations of the compound produced by the inserted gene, 2) of ecological or economic importance, or 3) rare or in danger of extinction. The selected arthropods must also represent a larger group, commercially available or easy to rear, and where possible relevant for other crops and other geographical areas within Europe. If it is likely that non-target arthropods could be adversely affected by the GM crop, a final group of from four to six arthropods should be selected for testing per crop/gene combination.

 Telefax: 030 274 4476 **E-mail:** info@cogem.net **Internet:** www.cogem.net

This advice was prepared in response to a request for advice (031009-MG01) from your ministry in which COGEM was asked to make concrete proposals for improving the methodology used for testing effects of genetically modified plants on non-target organisms, and specifically on arthropods.

To provide an evidence-based answer to this question COGEM commissioned a study by two entomological experts: Dr E.J. Scholte and Professor Dr M. Dicke of Wageningen University and Research Centre. Their findings are recorded in the research report cited above and served as the basis for our advice.

Our advice is concerned mainly with the procedure for selecting non-target organisms. The COGEM has not looked into the question of how the tests on the selected arthropods should be carried out or which organisations should do these tests. A possible follow-up study could throw more light on these issues.

Before any further study is carried out, COGEM considers it desirable to hold consultations with the Ministry of Housing, Spatial Planning and the Environment (VROM) on the details of the research to be carried out. The Ministry's opinions on the selection procedure and the choice of non-target organisms will be important for the design of this follow-up research because the details of standardised tests could depend on the chosen non-target organisms.

Further, in its request for advice your ministry also indicated that it wishes to present this advice to the European Commission and the other member states with a view to initiating a European discussion on this topic. The European context can also be of importance in determining the nature and direction of further research.

The text of the advice, the grounds on which COGEM has reached its conclusions and the resulting recommendations are set out in the enclosure.

Yours sincerely,

Professor Bastiaan C.J. Zoeteman
Chair of COGEM

c.c. Dr B.P. Loos
Dr I. van der Leij

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

1. Introduction

As required by the European GMO legislation, all applicants for permission to cultivate genetically modified crops must provide information about potential adverse effects of the genetically modified plant on human health and the environment. This information must include details about the possible effects of the genetically modified plants on organisms that are not intended to be affected by the expression product of the inserted gene, called 'non-target organisms'.

In the past, COGEM has raised doubts about the quality and the relevance of the scientific information submitted by applicants about effects on non-target organisms, especially arthropods. On several occasions, when testing on non-target organisms applicants have used organisms that do not occur in the crop or are never exposed to the expression product of the gene. COGEM therefore considers that conclusions on potential adverse effects based on such test results are unsound.

In previous advisory reports (CGM/030822-01 and CGM/030919-04) it was noted that this problem arises from the fact that the European procedure contains no standard criteria or guidance when carrying out studies on non-target organisms.

The Ministry of Housing, Spatial, Planning and the Environment (VROM) asked COGEM (031009-MG01) to specify its comments and to develop concrete proposals which will improve the quality of the information provided and therefore lead to better environmental risk assessments. Further, the ministry indicated that it wishes to present this advice to the European Commission and the other member states with a view to initiating a European discussion on this topic.

2. The environmental risk assessment

Prior to the release into the environment of genetically modified crops, the applicant must submit a scientifically sound environmental risk assessment, as described in Annex II of the European Directive 2001/18.

This directive on the deliberate release of GMOs into the environment applies to both market authorisation and field testing. The aim of the environmental risk assessment is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO – in this case the genetically modified plant – on human health and the environment. The results are used to determine if there is a need for risk management, and if so the most appropriate methods to be used.

The environmental risk assessment must take account of the inserted gene, the organism and the environment into which the genetically modified organism is released, as well as the interaction between these. When assessing the interactions between the GM plant and the environment one of the aspects that must be established is the effect of the GM plant on non-target organisms.

The directive prescribes that the information provided by the applicant must include an: 'identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction.' The directive gives no further details on how this should be done.

It is therefore unclear 1) which organisms have to be tested, 2) which tests have to be carried out, 3) who should carry out these tests, and 4) how the results of these tests should be interpreted.

In practice this means that when determining the potential effects on non-target arthropods, applicants use organisms that are routinely used for toxicity and other tests, such as water fleas (*Daphnia*), or rely on studies used for risk assessments. As a result, toxicity tests are often performed on arthropods that are used in studies for determining the effects of conventional insecticides on non-target organisms.

But whereas conventional insecticides are often directed at a broad group of organisms, the expression products of transgenes are often directed at just one or a limited number of pests. And whereas conventional insecticides are often applied by spraying and are therefore present on the surface of the plant, genetically modified toxins are produced within the plant. Therefore only organisms that feed on the transgenic plants or their products, such as pollen, or their natural enemies can come into contact with these toxins.

3. Research brief

To be able to give an informed and reasoned answer to the research question by the Ministry of VROM, COGEM commissioned a research study. Two entomological experts, Dr E.J. Scholte and Professor Dr M. Dicke of Wageningen University and Research Centre, were asked to carry out a desk study and to suggest improvements to the methodology. Their findings can be read in the research report *"Effects of insect-resistant transgenic crops on non-target arthropods: first steps in pre-market risk assessment"*.

Briefly stated, the authors recommend a case-by-case approach to the selection of non-target arthropods. Both the inserted gene and the crop plant into which it is introduced should be considered when selecting the non-target arthropods. They also recommend, from a practical point of view, identifying key species for each crop/gene combination that are representative of a larger number of arthropods or ecological groups.

4. Reasoning and advice

COGEM agrees with the outline of the selection procedure set out by the authors in the report cited above. The proposed selection procedure results in a selection of relevant non-target arthropods to be tested. The choice of using a selection of arthropods that represent a broad range of species leads to a method that is feasible and workable in practice. This is examined in more detail below.

4.1 When should non-target organisms be tested

COGEM is of the opinion that testing on non-target organisms will not always be necessary. Testing on non-target organisms should be required only in those cases in which it is likely that the expression products of the transgene could have adverse effects on non-target organisms. This means that in the absence of any demonstrable reasons for there being any potential adverse effects, the applicant would not have to submit any information on such effects. For example, COGEM considers that testing on non-target organisms of possible adverse effects will be necessary for the insertion of a *Bacillus thuringiensis* (Bt) gene, which renders the plant toxic to certain species of insect pests. However, it is arguable that testing of potential adverse effect on non-target organisms will not be required for the insertion of a gene that gives the plant increased tolerance to herbicides.

4.2 Ecological food webs

When selecting non-target arthropods, COGEM considers that the species to be included should be based not only on the expression product of the gene, but also on the crop ecosystem. To make a selection of non-target arthropods to be tested it will therefore be necessary to identify which arthropods are found both in and near the crop. In their report, Scholte and Dicke clearly show that a crop-specific ecological food web can be compiled for each crop and they have compiled ecological food webs for oilseed rape (*Brassica napus*), potato (*Solanum tuberosum*) and maize (*Zea mays*). In compiling these food webs they limited themselves to the most important insect pests, the natural enemies of these insect pests (predators and parasitoids), pollen/nectar-feeding insects and soil organisms.

Organisms belonging to these ecological groups can come into contact with the expression products of the transgene. Insect pests and insects that feed on pollen or nectar can be exposed to the transgene product by ingesting the pollen or nectar. Soil organisms are directly exposed to the transgene product when they feed on decaying plant material. Their natural enemies are then indirectly exposed to the transgene product.

The research brief was limited to the three crops mentioned above: oilseed rape, potato and maize. These crops are currently considered to be the most relevant in the Netherlands and Europe in relation to the development of genetically modified varieties. However, comparable ecological food webs can be compiled for other crops

for which genetically modified varieties are likely to be introduced in future, such as sugar beet, fodder beet and chicory.

4.3 Selection of non-target organisms

As it is unrealistic to demand that all non-target arthropods identified by compiling ecological food webs should be tested, it will be necessary to make a selection. Crucial factors to be considered when making this selection are the expression product of the inserted gene and where in the plant the gene is expressed.

Selection criteria

In the first instance, the most relevant non-target organisms are those that are highly sensitive to or exposed to high concentrations of the transgene porduct. However, it will also be necessary to determine the extent to which the organisms are 1) of ecological importance (e.g. pollinators, natural enemies of pest species), 2) of economic importance (e.g. bees that produce honey, pollinators of fruit-bearing crops) or 3) rare and/or in danger of extinction.

COGEM is of the opinion that the selected non-target organisms must be representative of a larger group of non-target organisms. From a more practical point of view, COGEM also considers it important that the selected non-target organisms 1) are commercially available or are easy to rear and keep, and 2) where possible can be used as test organisms for various crops and for the different geographical areas within Europe.

COGEM's opinions are in agreement with the selection criteria described by Scholte and Dicke. By way of illustration, Scholte and Dicke applied the selection criteria to the ecological food webs they drew up for potato, oilseed rape and maize. They concentrated on the insertion (in these cropt) of various *Bt* genes coding for the endotoxins Cry1, Cry2, Cry3 and Cry5, which are toxic to certain insect pests. Which insect pests are affected depends on the inserted Cry gene.

Scholte and Dicke's procedure eventually resulted in a list of non-target arthropods which are found in North-West Europe, are common, are of demonstrable ecological or economic value, and are easy to rear or are commercially available. For each crop/gene combination from four to six non-target arthropods should be tested. A flow diagram with questions about the crop and the inserted gene helps the applicant to make the right selection of non-target organisms. To illustrate the proposed procedure the list of non-target arthropods is given in Annex 1 and the flow diagram is reproduced in Annex 2 to this advice.

COGEMs preference for drawing up a Europe-wide list of non-target arthropods was outside the scope of this study; the literature review for the purposes of compiling the ecological food webs and the selection of non-target organisms was limited to North-West Europe. However, to avoid applicants being presented with lists of specific

organisms for each member state or region, which could result in a dramatic increase in the number of non-target arthropods to be tested, COGEM suggests that the member states cooperate on the preparation of a consolidated list.

As for North-West Europe, ecological food webs and lists of relevant non-target organisms can be drawn up for the other regions of Europe. By comparing these lists it will be possible to ascertain the possibilities for compiling a list containing a limited number of non-target organisms. Of course, it will be necessary to obtain the consent of the member states for the procedure that is adopted in this report.

5. Recommendations

To summarise, COGEM recommends the following procedure for selecting relevant non-target arthropods. For each individual crop an ecological food web should be compiled that consists of organisms found in or near the crop. These food webs should be limited to relevant arthropods, consisting of the most important insect pests, the natural enemies of these insect pests (predators and parasitoids), pollen/nectar-feeding insects and soil organisms.

From this list of non-target arthropods, organisms are then selected that are highly sensitive to or exposed to high concentrations of the transgene product, are ecologically or economically important, or are rare or in danger of extinction. These selected arthropods must also be representative of larger groups of arthropods, commercially available or easy to rear, and where possible applicable to other crops and the other European geographical areas.

The final list should consist of four to six non-target arthropods to be tested for each crop/gene combination.

The designation of non-target organisms is just the first step in the process of obtaining a validated assessment on the effects on non-target organisms of the release into the environment of transgenic plants. It does not answer the following questions: 1) which types of tests should be carried out, 2) who should do these tests, and 3) how the results of these tests should be interpreted.

In their report Scholte and Dicke say that in their opinion laboratory experiments should include toxicity tests, behavioural studies and physiological studies. They mention the following important parameters of the non-target organisms that should be tested for in toxicity tests: developmental time, adult longevity, body weight and fecundity. In addition, they recommend standardising these tests and studies per non-target organism and having them carried out by renowned laboratories/institutes that have sufficient experience to guarantee completion of these tests to the required standards.

COGEM concurs with the recommendations that the tests should be standardised and performed by laboratories with proven expertise. However, at this stage COGEM does not think it is opportune to expand on these recommendations. Further research

will be needed in order to come to a well-considered judgement on the tests to be performed and their standardisation.

Before any further study is carried out, COGEM considers it desirable to hold consultations with the Ministry of Housing, Spatial Planning and the Environment (VROM) on the details of the research to be carried out. The Ministry's opinions on the selection procedure and the choice of non-target organisms will be important for the design of this follow-up research because the details of any standardised tests could depend on the chosen non-target organisms. Further, in its request for advice your ministry also indicated that it wishes to present this advice to the European Commission and the other member states with a view to initiating a European discussion on this topic. The European context can also be of importance in determining the nature and direction of further research.