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The Minister of Housing
Spatial Planning and the Environment
Dr J.M. Cramer
P.O Box 30945
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DATE 17 February 2009
REFERENCE CGM/090217-02
SUBJECT Letter of advice on standardising laboratory tests on non-target organisms

Dear Mrs Cramer,

Hereby I present you the research report '*Designing experimental protocols to investigate the impact of GM crops on non-target arthropods'* (CGM 2008-01). The report contains recommendations for improving and standardising laboratory experiments to identify potential effects of genetically modified (GM) crops on non-target organisms. This letter comments on these recommendations.

Applicants for permission to cultivate a GM crop must provide information about the potential effects the crop could have on 'non-target organisms'. Non-target organisms are those organisms (amongst others insects) that should not suffer adverse effects from a GM crop, as opposed to organisms like insect pests, which certain GM crops are designed to suppress. GM crops are allowed on the European market only when the information supplied contains evidence that the risks to non-target organisms are negligible.

The 2912th session of the Environment Council, which met on 4 December 2008, concluded that the guidance on assessing the potential effects on non-target organisms should be further developed and updated. In the past, COGEM has raised doubts about the quality and the relevance of the scientific information submitted by applicants about the effects on non-target organisms. COGEM noted that this problem has its roots in the lack of standard criteria or guidance for studies on non-target organisms in the European licensing procedure.

The Netherlands Ministry of Housing, Spatial Planning and the Environment (VROM) asked COGEM to make concrete proposals for improving the methodology used for identifying potential effects of GM crops on non-target organisms (031009-MG01). To provide a sound, evidence-based proposal COGEM commissioned two research projects. The first research project, which was carried out in 2005, concerned the selection of non-target organisms for the investigation of potential effects of GM crops. This research project was carried out by Dr E.J. Scholte and Professor Dr M. Dicke of Wageningen University and Research Centre.
The results are recorded in the report '*Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies'* (CGM2005-06).

In response to this report COGEM issued an advice containing guidelines for selecting non-target organisms (CGM/051020-01). The report and accompanying advice provide an essential first step towards compiling guidelines for studies of the potential effects of GM crops on non-target organisms.

As a follow-up to this research project COGEM commissioned Dr D.S. Charleston and Professor Dr M. Dicke of Wageningen University and Research Centre to examine how laboratory tests should be performed on non-target organisms. The results of this study, which was carried out in 2007 and 2008, are described in the attached research report.

The majority of studies into the potential effects of GM crops on non-target organisms are carried out according to protocols based on the mode of action of synthetic pesticides. The toxicity of synthetic pesticides is determined by establishing the dose at which 50% of the tested organisms are killed. This LD_{50} or LC_{50} value is also used as a measure of the toxicity of a GM crop.

The authors argue that exposure of non-target organisms to the compounds present in insect-resistant GM crops is different in nature from exposure to synthetic pesticides. In this respect they mention the following aspects:

1. When plants are sprayed with an insecticide they are rarely completely covered. The parts of the plants not covered by the insecticide provide refuges where some non-target organisms are able to survive. Transgene expression products in GM crops, on the other hand, are present throughout the plant. This makes it more difficult for organisms to avoid these compounds.
2. After an insecticide is applied to a crop it immediately starts to decompose, whereas the compounds produced in a GM plant are continuously produced always present.
3. The mode of action of sprayed synthetic insecticides is acute and immediate. The mode of action of the compounds present in insect-resistant GM crops is not acute and does not have to be immediate.

These differences see to it that non-target organisms are chronically exposed to the compounds present in insect-resistant GM crops.

It is due to this chronic exposure, say the authors, that tests on non-target organisms should not only investigate the mortality of the insects but also sublethal effects. Sublethal effects include changes in fecundity and the developmental time of the insect. If only the mortality of the insects is measures (as in the current tests), sublethal effects such as sterility are ignored, whereas they may have an important influence on the size of the population.

The growth rate of a population provides information about the survival, development and reproduction of a population. The authors maintain that determining the growth rate of a population is a good way of detecting sublethal effects and mortality.
The report contains laboratory protocols for determining the rate of population growth for an organisms. Specific protocols have been drawn up for four groups of non-target organisms (predators, parasitoids, pollinators and soil-dwelling organisms).

Where possible, these protocols are consistent with the standards developed by the European and Mediterranean Plant Protection Organization (EPPO) and the US Environmental Protection Agency (EPA).

Before a laboratory test to establish whether a GM crop has an effect on a non-target organism can be carried out, two variables have to be determined. The first variable is the duration of the experiment. The experiment must be long enough to cover the period during which the organism produces the most offspring, called the 'peak reproduction' period. The experiment can be ended shortly after this point. Information about the period during which a certain organism produces the most offspring can be obtained from the literature or should be determined before the start of the test.
The second variable is the test concentration of the test substance. Laboratory tests can be used to identify whether there are any effects for only a limited number of non-target organisms. Moreover, the sensitivities of organisms to an insecticide may vary. To be on the safe side, therefore, a higher concentration (from ten to a hundred times higher) than the in-field predicted environmental concentration (in-field exposure) is used. The authors state that a lower concentration can be used in tests for sublethal effects, for example a test concentration of five times the expected exposure concentration in the field.

Furthermore, the authors are of the opinion that all experiments should meet the following conditions:

- To avoid any variation among test organisms, the test organisms should be the same age and come from the same identified source.
- The rearing history of these test organisms must be known and the organisms should be available to third parties to allow them, if desired, to verify the data with organisms from the same source.
- Experiments should be conducted under controlled environment rooms and the temperature and humidity continuously monitored. If the GM crops being tested for potential effects are going to be grown in a temperate climatic region, the lighting should be set at a 16:8 L:D cycle.
- The plants should be grown under controlled conditions (light, temperature, humidity, nutrients and substrate) which should reproduce field conditions as closely as possible.
- The transgenic plants to be used in the experiment should be tested to establish the presence of the insecticidal protein and determine its concentration in the plants.
- At least 40 replications should be performed per test.
- The mortality in the control group must be lower than 15%; if not, the experiment must be repeated.

Recommendations by COGEM
COGEM endorses the report, with a few qualifications. COGEM believes that data on potential sublethal effects of GM crops on non-target organisms are important for the environmental risk assessment because sublethal effects can have a significant influence on the population size of non-target organisms.

COGEM considers determining the growth rate of a population to be the preferred method for testing for potential effects of GM crops on non-target organisms. If effects are observed during laboratory tests, field tests will be necessary to investigate whether these effects also occur in the field. Any observed effects should always be compared with baseline data from 'standing agricultural practice' that is the yield, phytosanitary measures, tillage and measures before and after harvest as practised in agriculture according to the latest scientific insights (CGM/021017-06).

Using the proposed protocols requires knowing the moment of peak reproduction. COGEM considers the protocols proposed in the research report to be feasible if the moment of peak reproduction is known. COGEM notes, however, that in many cases additional research will be needed to identify this moment. The moment of peak reproduction has to be determined only once per test organism.

COGEM has reservations about the conditions stated by the authors. The researchers state that it is important to include sufficient replication in the experiments to identify any variation. They suggest 40 replications as a guideline. COGEM shares the opinion of the researchers that the number of replications should be high enough to establish with sufficient certainty that any effects that occur will actually be observed. The measure of this is the 'statistical power' of a study. COGEM considers that the laboratory studies should have a statistical power of at least 0.8, which means that in at least 80% of the cases an effect will be identified. The number of replications needed to achieve a certain statistical power depends on the size of the effect which can be detected and the distribution of observations across the groups to be compared. Although, in practice, 40 replications will often be enough to achieve a statistical power of 0.8, COGEM points out that a different number of replications may be necessary to achieve a sufficiently high statistical power.

At the moment the EFSA is revising its guidance for the risk assessment of GM crops. COGEM is of the opinion that the research reports mentioned in this letter and the accompanying COGEM advices contain several scientifically sound recommendations of considerable importance for the revision of the EFSA guidance. Accordingly, COGEM will also send these documents to the EFSA and other European advisory bodies. I recommend that you also bring these reports to the attention of the EFSA.

Yours sincerely

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Chair of COGEM

cc. Drs. H.P. de Wijs
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