

**Ministry of the Environment
Environmental Risks Department**

The Decision came into force on 15 July 2008.

Prague, July 11, 2008
According to Reference Number: 24722/ENV/08

D e c i s i o n

The Ministry of the Environment of the Czech Republic as the administrative body competent according to § 5 of the Act No.78/2004 Coll., on the use of genetically modified organisms and genetic products as amended by the Act No. 346/2005 Coll. (hereinafter the „Act“), and § 10 of the Act No. 500/2004 Coll., the Administration Code, as later amended,

has decided

on the basis of a request of Charles University in Prague – Faculty of Science, located in Albertov 6, 128 43 Praha 2 for the deliberate release of genetically modified tobacco into the environment in the Czech Republic pursuant to § 5 par. 8 of the Act:

**Charles University in Prague – Faculty of Science,
Albertov 6,
128 43 Praha 2**

i s g r a n t e d c o n s e n t

**for the deliberate release of genetically modified tobacco
into the environment in the Czech Republic**

Requirements of the consent according to § 18 par. 6 of the Act:

Authorised person

Name: Charles University in Prague – Faculty of Science

Identification Number (IČO): 00216208

Specification of the genetically modified organism

Genetically modified Virginia tobacco (*Nicotiana tabacum L. cv. Samsun*) transformed with fission yeast mitotic activator (gene *SpCdc25* from fission yeast *Schizosaccharomyces pombe*).

Specification of the genetic modification

The transformation was performed in the laboratory of Dr. Dennise Francise (Cardiff University, Great Britain).

Complementary DNA (cDNA) pCDC25-S9 originates from Professor Paul Nurse (Imperial Research Fund, Oxford, Great Britain). The size of *cdc25* gene is 1740 bp (McKibbin et al., 1998). A chimerical construct has been prepared by insertion of the coding sequence *cdc25* between 800bp promoter CaMV 35 S and 200bp terminator sequence of nopaline synthase gene. The chimeric gene has been inserted into the binary vector pBIN19 and electroporated into *Agrobacterium tumefaciens* strain LBA4404 in the way described by Shen and Ford (1989). For transformation the T-DNA of a vector pBIN19 (Bevan, 1984) with cDNA *cdc25* (pCDC25-S9), promoter CaMV 35S and terminator sequence of nopaline synthase have been used.

It has not been found out that the long-term and frequently used vector pBIN19 and other derived vectors do increase in any way a risk of the use of genetically modified organism (GMO). Orientation identification of transgenic plants is possible by testing the resistance to kanamycin. The unique identification of transgenic plants can be performed by molecular methods for detecting the presence of sequences of target genes in DNA of the plants: PCR method using specific primers to the inserted sequences and Southern hybridisation. Modified co-cultivation technique was used for *Agrobacterium*-mediated transformation. The inserted gene and the new phenotype showed stable integration and inheritance respectively. The phenotype stability has been found out within cultivation in green-house and in vitro conditions.

The transformed plants contain an *nptII* gene. With regard to the opinion of the European Food Safety Authority of April 2, 2004 this gene can be used in GMOs released into the environment. In nature there is no selective advantage for plants carrying *nptII* gene as there is no selective pressure for resistance to kanamycin.

Risk assessment results

On the basis of green-house experiments no increase of the resistance or invasiveness of transgenic lines in comparison with non-transgenic counterpart is expected. GM tobacco plants will be planted only to the stage before release of pollen and seed formation.

The deliberate release into the environment and cultivation of transgenic plants solely for research purposes as far as subject of the application will be performed on an experimental

plot of limited area at the premises of the Faculty of Science of Charles University in Prague (hereinafter "PřF UK"). Nearby in the Botanic Garden the following tobacco cross-pollinating varieties are cultivated:

Nicotiana rustica L., Nicotiana paniculata, Nicotiana alata, Nicotiana longiflora.

Since the experimental material *Nicotiana tabacum* is self-pollinating, it expresses a pollen incompatibility with above mentioned tobacco varieties and because of early terminated experiments with transgenic plants before the stage of pollen release there is practically no risk of cross-pollination.

Tobacco is neither commonly planted nor wild-growing variety on the territory of the Czech Republic. In traditional way of cultivation no harmful effects of tobacco and inserted gene on human and animals have been known. The plants shall not be used for food or feed. Since the transgenic plants are self-pollinating and thanks to rare occurrence of the cross-pollinating non-transgenic varieties there is no risk of gene escape.

The cultivation of genetically modified tobacco does not pose practically any risk because the particular construct does not contain a gene, which can itself or indirectly cause the toxicity of transgenic plants or its parts, it does not cause an increase in tobacco vitality in the natural environment (expected in accord with the results obtained in green-house experiments conducted under contained use conditions).

The plant material mentioned has been prepared and used solely for research purposes, and the planting in a large scale is not expected.

With respect to both the character of the inserted transgene and current experience (green-house experiments within the contained use) negative effects on human and animal health or the environment are not expected. Effects on human health arising from the genetic modification of *Nicotiana tabacum* have not been described.

Thus the deliberate release of this GMO into the environment has been considered as non-risky or minimally risky regarding the harmful effects on human and animal health, the environment or biological diversity.

Conditions for the use

This genetically modified organism shall be used only in the way described in the application Reference Number 24722/ENV/08 submitted to the Ministry of the Environment (hereinafter "MoE") on March 31, 2008, and supplemented with the submission delivered to MoE on June 21, 2008, when keeping all given conditions as follows:

- Every handling with the genetically modified material shall be under conditions minimizing a possibility of transgene escape into the environment.
- Persons responsible for resolution to an accident are stated in the emergency plan.
- All staff that is getting into contact with GM tobacco shall be trained every year about rules for the use of genetically modified organisms by the professional consultant.
- GM tobacco shall not be used as food or feed.
- The material pre-planted in the contained use conditions will be assessed in field trials.
- Maximally 100 genetically modified plants shall be planted in particular year (within two consecutive experiments). If the procedure requires the assessment of very young plants characteristics, which consequently enable to carry out three consecutive experiments in one year, the total number of plants cultivated in one season is allowed to reach 150.
- , The transgenic line C shall be used preferentially, and the particular results will be if needed verified using the other independently transformed lines A, B, F (the experiments were already conducted with them within the contained use conditions). The planting plan will vary according to particular experiment and it shall be a part of work protocols.

- Material released into the environment was obtained from the laboratory of Dr. Francis (Cardiff University, Great Britain) in seeds form.
- The obtained seeds are placed into closed Eppendorf micro test-tubes and stored in a laboratory dedicated to the use of GMOs. The seeds intended to be sown and subsequently planted at the plot shall be transferred in the contained Eppendorf micro test-tubes (which are placed into the rigid packaging) from GMO laboratory to a green-house dedicated to the use of GMOs. Then the cultivated seedlings in cultivation containers will be packed into labeled transporting basket and closed transferred into the experimental plot where they will be grown.
- Every year approximately in the middle of April (according to climatic conditions) the seeds will be sown and seedlings produced in the green-house dedicated to the use of GMOs. Within one season the seeds shall be sown and the seedlings planted in two cycles under the scheme as follows:

1st cycle: At the beginning of May the experimental plot will be planted with the seedlings pre-cultivated in the green-house. The cultivation will continue depending on climatic conditions approximately up to the early August.

2nd cycle: At the beginning of June pre-cultivated seedlings are planted, and the cultivation will continue by early September.

Two seedlings are planted on the area of 1 square meter. Generally all planted seedlings grow up to maturity.

- The seedlings will be planted up to the stage of early building of flower structures. Plants shall be harvested before the stage of pollen release and seed formation. The sowing, cultivation and harvesting of GM tobacco will be carried out in the same way as in the case of non-transformed material.
- Since the transgenic plants derived from various events will be used for “field” trials, even several week's differences in entering of plants to the generative stage are expectable. A removal of the inflorescence and subsequent plucking (depending on a real need for the sampling) of transgenic plants seems to be the most effective method of flowering prevention.
- If the control non-transgenic plants will be cultivated on the experimental plot together with the genetically modified tobacco plants they shall be handled as if they were transgenic including the method of inactivation.
- During vegetation the particular tested lines shall be monitored in the interval 2-3 days and compared with control plants which were sown simultaneously.
- The course of the observation and carrying out of the field trial shall be recorded. A photo-documentation shall be carried out as well. All staff shall be properly instructed how to use the transgenic material.
- After termination of the experiment the plants shall be harvested by hand. Particular parts of the plants intended for further processing shall be transferred in closed containers or using liquid nitrogen if appropriate into greenhouse or laboratory both dedicated to GMO.
- The extent of the field trials in subsequent years will depend on a work progress and on research projects obtained. The current list of tested lines including information about the phase of the field trial shall be specified always before every vegetation season in a letter addressed to the Ministry of the Environment.
- The applicant shall in accordance with § 19, letter c) of the Act submit to the Ministry data on the amount of GM tobacco and on handling with it in written and electronic form annually, always by February 15 in the calendar year, and pursuant to § 19, letter d) of the Act submit within 60 days from termination of the use of genetically modified organisms a final report on

the course and consequences of this activity, particularly with regard to risks for human health and the environment. The final report shall be submitted also in English pursuant to the Annex of the Commission Decision 2003/701/EC.

Other conditions on the use of GMOs under § 5 par. 10 of the Act

- Every year at least 30 days before the plot is planted with GMOs the applicant shall notify the Ministry of the environment of information on deliberate release into the environment, plots where it will be carried out and about amendments in the original request (point 7 and following, part B of the Annex No. 2 of Decree No. 209/2004 Coll., on detailed conditions for the use of genetically modified organisms and genetic products).
- The remains of harvested plants after the analyses realized in GMO laboratories shall be destroyed in an autoclave. The remains of plants on the experimental plot shall be dried and composted on the spot.
- Since the plant vegetative material is from technical reasons destroyed on the spot by composting and not in an autoclave the compost shall serve only for material from the field trials in question, shall be properly labeled and be under surveillance.
- The applicant shall on request of the Ministry of the Environment or of the laboratory if appropriate pursuant to § 28 par. 1 letter f) of the Act to provide with samples of tested genetically modified higher plants (GMVR) or their genetic material whenever during the trial.
- In case of an accident the person responsible for resolution to an accident shall contact laboratory where the control samples of tested genetically modified material are deposited and which has approved methodologies for the unique detection of particular GMO.

Purpose of the release

Realisation of field trials will serve only for research purposes at the plot of ground in the area of the Department of Plant Physiology of PšF UK which is located in a city residential territory, and it will be carried out in order to verify biological characteristics of transgenic tobacco plants (*Nicotiana tabacum*) with a transgene influencing cell cycle regulation. The transformation with the gene *cdc25* significantly speeds up plant development and flowering earliness. The changes in meristem structure before flowering (detailed study on anatomy of the changes) and changes in saccharide metabolism (observation of changes in spectra and content of saccharides and its distribution and allocation in plant organs) will be studied. The observation of the mentioned features will be carried out during the vegetative phase of plant development. The planting in free soil (the deliberate release into the environment) is necessary for the research of the impact of transformation with *cdc25* gene on morphological features and biological characteristics of tobacco. The use either for food, feed or other purposes (e.g. cosmetics) is not a subject of the planned field trials with GM tobacco lines.

Other requirements for labelling

For deliberate release of GMO the common conditions for labelling of genetically modified organism have been laid down in law. In addition to that the plot itself where the GM tobacco will be cultivated shall be signed as follows: “Experimental plot of the Department of Plant Physiology, No entry! Genetically modified tobacco”, and plus warning sign “Biohazard”.

Place of the deliberate release into the environment

Charles University in Prague – Faculty of Science, Department of Plant Physiology, Viničná 5, 128 44 Praha 2. The experimental plot is located in cadastral territory of the Capital of Prague, parcel No. 1586.

The experimental plot is located in the bounded area of the Department of Plant Physiology PřF UK that is a part of a built-up city area, which is closed and secured overnight. Since the experimental plot is very small (25 m²) GM tobacco plants can be easily observed individually. The experimental plot, compost for destruction of vegetative material, laboratories (seed storage, analyses of plant material) and a greenhouse (sowing, planting of seedlings) for the contained use of GM tobacco are located in the area of the Faculty of Science of UK.

Size of the site: 25 m² in particular years. The use of the insulation strip has not been planned. The vicinity of the site has a character of park arrangement and includes also greenhouses belonging to the Department of Plant Physiology. The buffer strip is not necessary because the field trials on the experimental plot will be terminated before flowering of plants, and therefore any risk of pollen spread and formation of GM tobacco seeds is eliminated.

Requirements for monitoring and reporting of monitoring results

Monitoring within vegetation period:

With respect to a low number of the experimental plant individuals (maximum 150 for one season) the plants shall be observed individually, records kept regularly in written, and photo documented. The close vicinity of the experimental plot shall be also observed.

Post-harvest monitoring of the experimental sites:

Although, an occurrence of the plants after harvesting at the experimental plot is not expected, the plot will be monitored regularly in the same way by the next season.

Monitoring will be carried out on the plot where genetically modified tobacco was planted, and in the closed vicinity.

The area planted with GM tobacco shall be monitored in particular years always from harvesting to the autumn in the subsequent year.

Monitoring shall be performed in the first month after harvesting and in the spring of the subsequent year.

After sanitation of the plot all vicinity shall be regularly monitored for the presence of particular species of plants (in one week intervals to the end of a season, and at the beginning of the following season). Records on monitoring shall be kept in written.

The plants shall be observed in 2-3 days intervals but at least once a week.

Validity

Total time of GMO's use: 10 years

I n s t r u c t i o n s

Within 15 days from the date of the notification of this Decision there is an opportunity by the submission to the Ministry of the Environment, Vršovická 65, 100 10, Praha 10, make representations to this Decision according to § 152, par. 1 of the Act No. 500/2004 Coll., on administrative proceedings (Administrative Code), whereupon the Minister for the Environment will decide.

Ing. Pavel Forint
Department Director

This Decision shall be received by:

- A. Participant in the proceedings for personal delivery:
Charles University in Prague – Faculty of the Science, Albertov 6, 128 43 Praha 2

- B. For information:
 - 1. Ministry of Health
 - 2. Ministry of Agriculture
 - 3. MHMP (Prague City Hall), Department of the Crisis Management