Ministry of the Environment
Environmental Risks Department

Prague, April 30, 2007
Reference Number: 20955/ENV/07

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., Administrative Code, as later amended,

has decided

on the basis of a request of the company AGRITEC, Research, Breeding & Services, located in Zemědělská 2520/16, 787 01 Šumperk, under § 5 par. 8 of the Act, on a consent for the deliberate release of genetically modified flax into the environment in the Czech Republic:

AGRITEC,

Research, Breeding & Services, Ltd.,

Zemědělská 2520/16,

787 01 Šumperk

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

for the deliberate release

of genetically modified flax

into the environment in the Czech Republic

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

Authorised person
Name: AGRITEC, Research, Breeding & Services, Ltd.
Address: Zemědělská 2520/16, 787 01 Šumperk
Identification Number (IČO): 48392952

Specification of the genetically modified organisms

According to § 18 par. 3 of the Act, the application for the deliberate release into the environment has been submitted for genetically modified flax (*Linum usitatissimum* L.*)* – T2 line and higher generations derived from Atalante, Biltstar, Hei Ya 11, Hermes, Ilona, Jitka, Lola, Nike, Recital, Venica varieties, and lines AGT 514 03, NLN 245
a) by insertional mutagenesis,
b) by introduction of *bar* gene for tolerance to phosphinothricin (an active ingredient of herbicidal preparations BASTA, Liberty etc.),
c) by introduction of SPI (proteases inhibitors) genes in order to increase the resistance of flax plants to fungal diseases and insect pests,
d) by introduction of αHMT1A and CP genes in order to increase an ability to accumulate heavy metals in flax plants and its parts (phytoremediation).

Specification of the genetic modification
1/ Insertional mutagenesis:
The DNA segment inserted by the binary vector GV3101 (pPM90RK)(pPCVRN4) – does not contain any gene with direct effect on the recipient properties. The result of transformation is the insertion of the construct into DNA of the recipient. The presence of a 35S tetramer enhancer fragment can cause in the plant an activation of the adjacent genes to the T-DNA insertion place (dominant mutation). On the contrary the T-DNA integration into an active gene area may cause an inactivation of this gene. This change is then expressed as a recessive mutation. Theoretically these mutations may result in changes of the plant metabolism and in specific cases of the morphological characteristics or the biological properties which may be useful for crop improvement. In glasshouse experiments the genotypes affected by insertional mutagenesis have been chosen that show differences in seed colour, powdery mildew resistance and plant height. In their progeny planted in field conditions the genotypes useful for crop improvement will be selected like as in previous phase of the experiment, and the presence of the construct will be proved using PCR method. Transformed genotypes contain a selectable marker *hpt* that confers resistance to hygromycin.
2/ Transformation with *bar* gene:
The inserted construct contain a *bar* gene expressing the PAT (phosphinothricin acetyl transferase) enzyme which detoxify phosphinothricin – an active ingredient of Basta herbicide. Although, the presence of a *bar* transgene in part of regenerants was proved using PCR method, the increased tolerance to Basta herbicide was not observed within glasshouse experiments, and therefore the aim of this project is to test the plant tolerance to this herbicide in the field conditions, it means within the scope of the deliberate release into the environment. Transformed genotypes contain, in addition to the *bar* gene, also a selectable marker *nptII* which confers resistance to kanamycin for easier selection of genetically modified tissues and plants.
3/ Transformation with *SPI-2* gene:
The inserted construct pBIN19-SPI contains a gene encoding SPI-2 (serine-type proteases inhibitor 2). This inhibitor should prevent plants under certain conditions from the attack of pathogens or pests. The selection on higher resistance to fungal diseases will be made within

the GM-flax material in the field conditions. Transformed genotypes contain also the selectable marker *nptII* conferring the resistance to kanamycin. In the next years the constructs with partially changed *SPI-2* gene codon will be used for flax transformation. The Ministry will be informed in advance about deliberate release of such materials, and at the same time the necessary documents must be submitted.

4/ Transformation with metallothionein genes:

Constructs pBIαMT and pBICP should increase the production of proteins in plants which are able to bind heavy metals and thereby inactivate it (metallothioneins). In field trials the effect of the inserted constructs on the agricultural properties of transformed plants will be observed. Transformed genotypes contain a selectable marker *nptII* conferring the resistance to kanamycin as well.

Risk assessment results

When carrying out the insertional mutagenesis no target genes are introduced into flax, but natural plant genes are silenced or activated. The used selectable marker *hpt* conferring the resistance to hygromycin has no adverse effects on human health. The *nptII* and *hpt* genes have been by the European Food Safety Authority (EFSA) categorised into the first group of selectable marker genes conferring the resistance to antibiotics with no or very low potential risks on human and animal health (EFSA 2004).

The inserted *bar* gene does not induce either the toxicity of plants or increased alergenicity thereof. Aventis company has carried out many experiments with rice with the inserted *bar* gene, which proved that transgenic rice fulfils the health criteria. The supposed effect of the flax genetic modification is the tolerance to glufosinate in a similar way to the rice and other crops (particularly oilseed rape), and any other effect is expected. The selectable marker *npt* conferring the resistance to kanamycin was already used in past for other plant species, and no adverse effects on humans were reported.

Although, the first constructs for *SPI-2* gene transformation have constitutive 35S promoter in the future the creation of tissue specific constructs is expected. To be significant for plant protection against fungi diseases the *SPI-2* gene must be expressed in plant roots, stems or leaves. To be significant for transformed flax protection against insect pests, the inserted *SPI-2* gene must be expressed in cotyledon leaves after germination. These parts are not used in human nutrition. The construct with 35S promoter has been already available, and it will be used for studying purposes to find out possible effects on the insect pests and fungal diseases. The similar situation as for the construct with 35S promoter is the case of constructs with genes for metallothioneins.

The cultivation of genetically modified flax pose practically no risk because the used constructs do not contain genes that could either itself or vicariously cause the toxicity of plants or its parts, and they do not cause the increased vitality of a flax in the natural environment (verified in the glasshouse experiments within the contained use, and in case of the insertional mutagenesis in field trials within the previous deliberate release of GM flax into the environment), and there is no risk of the flax escape into the environment (under the common farming technique the flax is not able to survive on field in the following year after the sowing, and it is not able to survive and reproduce itself in the environment without human help, and has no sexually compatible counterpart in the nature of the Czech Republic.

With regard to cultivation demands, the type of reproduction, non-existence of wild sexually compatible counterparts in the vicinity of the cultivation areas in the Czech Republic and impossibility of becoming a wild variety the cultivated flax is considered as a quite safe plant species as it has minimal potential for spreading which can thus be easily managed.

In this case the risk of the deliberate release into the environment lies more likely in the presence of the active selectable markers. The *nptII and hpt* genes were commonly assessed as safe in GMOs and food derived from them. In addition to it, the tested genetically modified lines are not intended for food or feed, and thereby the introduction of such genes into the food chain is *de facto* minimal. Further, the risk assessment depends on the right handling with GM plants. The elaborated Operational Rules an Emergency Plan serve for this purpose, and all persons authorised for handling with GMOs shall be acquainted with them. Therefore, the deliberate release of GM flax into the environment has been assessed, under given conditions, as safe or with minimal risk with respect to the adverse effect on human and animal health, the environment or biodiversity.

Conditions for the use

The genetically modified organism above mentioned shall be used only in the way described in the application of the company Agritec, Research, Breeding & Services s.r.o., located in Zemědělská 2520/16, 787 01 Šumperk, Ref. No. MŽP 20955/ENV/07 submitted to the Ministry of the Environment (hereinafter "MoE") on March 19, 2007, and supplemented by the submission delivered to MoE on April 24, 2007, and only when keeping all given conditions as follows:

- Every handling with the genetically modified organism shall be under conditions minimising a possibility of transgene escape into the environment.
- The measures taken for setting, keeping and harvesting of the field trials, and also for further handling with harvested genetically modified flax shall be in accord with the precautionary principle rules, and its compliance shall fully guarantee the safe use within the deliberate release of the genetically modified flax into the environment.
- Approximate number of genetically modified higher plants: 1000 seeds are sown per 1 m^2, and usually approximately 700 plants will get into ripening phase.
- Parts of the plots sown with regenerants after the transformation with the *bar* gene shall be treated by the herbicide Basta. The effect of the various herbicide concentrations in various flax development phases on the weed occurrence or flax itself shall be tested.
- Genotypes carrying the *spi* gene shall not be treated by insecticides, and as early as the first month after flax emergence the feeding damage caused by flax flea beetles shall be evaluated.
- Samples for analyses shall be taken from genotypes which will be distinct from the original one and interesting for crop improvement simultaneously. The presence of inserted construct shall be proved, then.
- Record shall be kept on the course of observance and carrying out of the experiment. In addition to this, the photo documentation of the field trial shall be done.
- Preparation and treatment of the plot before planting of the genetically modified higher plants: the common procedure as for non-genetically modified flax. With regard to actual land conditions the operations shall be used at the plots as follows: stubble ploughing, medium-deep ploughing, land levelling, the use of harrows, the use of presowing combinator, and basic NKP fertilisation.
- The way of transportation of genetically modified organisms: seeds for sowing shall be transported to the field in sacks or in glass bottles used in Hege sowing machine, and placed in plastic boxes with cover (without the holes – Manutan CL605005). Seeds shall be transported to the field in the contained space by the vehicles owned by the company Agritec. Moreover, the boxes shall be placed in the vehicle into firm contained packaging to prevent possible seed spillage into the vehicle shipping space in case of the inner packaging damage.

- Hand-harvested plants shall be labelled and placed in dense-netted sacks into the firm closed packaging. It shall be transported by the closed vehicle to the company premises for further processing. Combine-harvested seeds shall be transported in the high-density woven sacks placed into the plastic boxes closed with a lid (without holes – Manutan CL605005).
- The way of cultivation of genetically modified higher plants on a plot: every year approximately in middle April (according to climatic conditions) the seeds will be sown of genotypes chosen for the evaluation in the field trials. First year the sowing should be carried out only after receiving the Consent of the Ministry. In compliance with the common flax farming technique the crop stands shall be protected from pests (flea beetles) and weeds (monocotyledonous or dicotyledonous).
- For sowing the sowing machine of Hege type for sowing without residuals shall be used. Sowing shall be carried out by trained staff in the presence of responsible person Dr. E. Tejklová. After the end of sowing the sowing machine shall be checked, the remaining seeds shall be removed, packaging used for the transport and sowing of seeds shall be cleaned, and the remaining seeds transported to the workplace for destruction by composting.
- Plant treatment during vegetation will be the same as for non-transgenic flax: the treatment against flea beetles (Vaztak etc.) after flax emergence, post-emergent treatment against monocotyledonous weeds (Pantera and others) and dicotyledonous weeds (Glean 75 WG, Basagran and others). The particular treatments of the crop stands, where the effects of *bar* or *spi2* genes will be assessed, should be modified in accordance with the field trial plan.
- The way of genetically modified higher plants harvest: harvesting shall be carried out in the same way as for non-transgenic flax. After ripening the plants shall be hand-harvested by plucking or in the case of oil flax being evaluated in the performance tests the harvest shall be carried out by the small-plot combine harvester.
- Further handling description: GM flax shall be processed separately from other flax non-transgenic material. Harvested plants and seeds shall be stored to finish the drying. The selected plants (distinct genotypes) shall be analysed and hand-deseeded individually. Other plants shall be deseeded by a sheller, bunch-by-bunch. The seeds shall be stored at the dedicated place waiting for further analyses. The workplace shall be perfectly cleaned up of the rests of plants and seeds by sweeping and using a vacuum cleaner. The waste shall be burned at the dedicated place or destroyed by composting. According to the used construct the seeds will be further assessed and used for testing within the contained use: the assessment of colour, size, health, oil content, sowing on the plates in glasshouse for the use of GMOs for sampling for PCR analyses, setting *in vitro* cultures for testing a tolerance to heavy metals etc.
- Deadline and the way of the evaluation of the deliberate release into the environment of genetically modified higher plants: every year the seeds will be sown in the second half of April (depending on climatic conditions), the plants will be harvested during August (depending on climatic conditions), the harvested plants and seeds will be processed during the period from October to December or within first months in the following year. Every year in January the report on the use of GMOs shall be submitted to the Ministry of the Environment of the Czech Republic pursuant to the Act No. 78/2004 Coll.
- The road no less than 3 m wide shall surround the area where GM flax will be planted, here the flax plants emerged from possible seeds spillage during sowing shall be destroyed.
- A leguminous, cereal and other crops but never flax shall be sown on the neighbouring areas. Non-genetically modified flax shall be sown in minimum distance of 300 m from genetically modified flax.
- Description of the methods of a plot treatment after the end of the field trial: after harvest the plot shall not be treated until volunteer emergence from harvest losses, these plants shall be treated with the herbicide (Roundup), and the further steps shall follow a common farming technique.

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

- Every year at least 30 days before planting the company Agritec, Research, Breeding & Services s r.o. shall notify the Ministry of the Environment of an information on deliberate release into the environment and plots concerned with it, where the amendments has been done to the original request (point 7 and the following, part B of the Annex No. 2 to the Decree No. 209/2004 on detailed conditions for the use of genetically modified organisms and genetic products).
- Minimum isolation distance of **300 m** between the place of the experimental release of GM flax into the environment and neighbouring plot with commercially grown non-GM flax shall be kept.
- The company Agritec, Research, Breeding & Services s r.o. shall on request of the Ministry of the Environment or of a laboratory if appropriate pursuant to § 28 par. 1 letter f) of the Act to provide with samples of genetically modified organisms above mentioned or genetic materials thereof at any time during the use of GMOs.
- Post harvest residuals (stems and stubble after combine-harvesting, broken branches and seed capsules after hand-harvesting), which remain on the field, shall be together with the emerging volunteers treated by Roundup herbicide. Treated material shall be subsequently ploughed under the soil. Deseeded plant residuals after hand-harvest, and the residuals from seed cleaning after combine-harvest shall be either burned at a designated place or destroyed in a composting site dedicated to GMOs.

Purpose of the release

Field trials are intended to verify biological characteristics and cultivation parameters of selected transgenic flax lines that were obtained:

a) by insertional mutagenesis,
b) by introducing *bar* gene for tolerance to phosphinothricin (an active ingredient of herbicidal preparations BASTA, Liberty etc.),
c) by introducing of SPI (proteases inhibitors) genes in order to increase the resistance of flax plants to fungal diseases and insect pests,
d) by introducing αHMT1A and CP genes in order to increase an ability to accumulate heavy metals in flax plants and parts thereof (phytoremediation).

The flax responds very sensitively to the environmental conditions under which it is planted. Therefore it is not possible, under glasshouse conditions, to assess some properties important for the selection of a material for crop improvement (e.g. the plant height, number of capsules per plant, fibre content etc.). In a similar way, the expression of target inserted genes may be influenced. The deliberate release into the environment is necessary for the realisation of a research on the effect of the constructs and target genes insertion on morphological and biological characteristics of the flax, the search for morphological and other variations of T2 and higher plant generations after transformation (insertional mutagenesis), testing the plant endurance to biotic or abiotic stresses (tolerance to Basta herbicide of genotypes modified by *bar* gene, the resistance of genotypes modified by *SPI-2* genes to insect pests and fungal diseases, the ability of the *αHMT1A* bearers to accumulate heavy metals (the part of a gene sequence encoding the human metallothionein) and *CP* (the short nucleotide sequence

encoding synthetic peptide), and the selection of a material that may be useful for crop improvement, picking out of certain genotypes for the process of the crop improvement, and the assessment of qualitative and yield parameters of the newly developed variety.
The expected result of the deliberate release into the environment: acquirement of the material useful for crop improvement that may exceed the original varieties and lines in its properties. Development of new varieties of fibre and seed flax.

Other requirements for labelling

For deliberate release of GMO the common requirements for labelling of genetically modified organism have been laid down in law.

Place of the deliberate release into the environment

The experimental plot is located at the municipality of Vikýřovice, the Olomoucký region. The genetically modified flax will be sown in every single year under the crop rotation plan on the plots No. 1670, 1712 and 1715 at the cadastral territory of Vikýřovice. The company Agritec rents this plot from its user – The Research Institute for Cattle Breeding (RICB) Rapotín. The plots with GMOs shall be placed according to the cropping, and therefore in every single year they will be located in the different places of the land plot. The copy of a cadastral map shall be made for every single year, where the GMOs plot shall be highlighted. The area sown by GM flax will be in every single year approximately of 300 m^2 (according to the amount of the seed material produced).

Requirements for monitoring and reporting of monitoring results

The monitoring shall be carried out as follows:
- Repeatedly after harvesting until the volunteers emerge (sufficient soil moisture is necessary for plants emergence – it depends on the weather),
- Approximately 14 days after the post emergent treatment of volunteers by the total herbicide,
- In the following year in spring, approximately 3 weeks after the sowing of a subsequent crop,
- After the harvest of the subsequent crop.

The monitoring shall be carried out on a plot, where the genetically modified flax was planted, and its vicinity (up to 20 m from the plot). On the areas sown by GM flax the monitoring shall be carried out every single year always in the period from harvest to the autumn in the following year.
<u>Monitoring during the vegetation period:</u>

The monitoring plan is based on the results of the GM flax risk assessment related to the environment, and its objective is to early observe and identify the events, which would have expected and unexpected effect of the deliberate release of GM flax plants into the environment. It has been proven that there is minimal or no risk of the GM flax escapes into the environment when keeping given minimum distance between the GM flax and non-GM flax plots. Nevertheless, the measures have been taken to reduce potential risks of the GM

flax use to minimum. The plot itself and its vicinity should be monitored regularly by a responsible person accompanied with the head of the department or an expert adviser if appropriate during the vegetation period at least once every two weeks. The written records of the monitoring results shall be kept. All facts not only about GM flax crop stand but also about potential effects on the neighbouring areas and the other crops in its vicinity shall be recorded. Based on the current agricultural practice the common crop characteristics (UPOV) shall be recorded relating to the agronomic properties of GM flax lines (e.g.: the resistance to pathogens, the infestation of pests etc.). The records shall be updated during the whole period of the deliberate release of GM flax into the environment. The behaviour of genetically modified flax lines shall be compared with the behaviour of a recipient (non-GM) variety planted simultaneously at the same experimental place.

The post-harvesting monitoring of the experimental areas:

During the vegetation period the individual testing lines derived from the regenerants shall be studied and compared with the original varieties which will be sown simultaneously as control variants. Morphologically and in other way distinct genotypes from the original materials shall be searched for and identified. Volunteer emergence from harvest losses shall be monitored weekly after the harvest. The volunteers shall be destroyed by the treatment with the total herbicide Roundup. In the following vegetation season the occurrence of the volunteers in the subsequent crop planted at the experimental area and in its vicinity shall be monitored. If the volunteers occur, they should be removed by plucking, and in case of its mass occurrence this shall be destroyed by the application of relevant selective herbicide depending on the type of the crop. However such cases have not occurred in the agricultural practice in the Czech Republic yet. When the common farming technique is applied the flax can not survive the winter either in the vegetative stage or in seed form. It is expected that GM flax cultivated in the field trials will have no additional effect on the ecosystem in comparison to the non-GM flax cultivation.

Validity

This Consent shall apply for a period of 10 years from the date of its notification.

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:
Agritec, Research, Breeding & Services s.r.o.,
Zemědělská 2520/16, 787 01 Šumperk,

B. For information:
1. Ministry of Health
2. Ministry of Agriculture
3. The Regional Authority of the Olomoucký Region
4. The Municipal Authority of Vikýřovice