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**DATUM** 14 juni 2011  
**KENMERK** CGM/110614-01  
**ONDERWERP** Teelt, import en verwerking van gg-zetmeelaardappel BPS-A1020-5

Geachte heer Atsma,

Naar aanleiding van een adviesvraag betreffende de vergunningaanvraag voor teelt, import en verwerking van de genetisch gemodificeerde zetmeelaardappel BPS-A1020-5 van BASF Plant Science Company GmbH (EFSA/GMO/SE/2010/88), deelt de COGEM u het volgende mee:

#### **Samenvatting**

De COGEM is gevraagd te adviseren over teelt, import en verwerking van de genetisch gemodificeerde zetmeelaardappel BPS-A1020-5. Deze aardappel bevat een construct waardoor het amylose-gehalte van de aardappel wordt verlaagd en het amylopectine-gehalte wordt verhoogd. Daarnaast brengt BPS-A1020-5 het *csr1-2* gen tot expressie waardoor BPS-A1020-5 minder gevoelig is voor imidazolinone herbiciden. Hierdoor kunnen deze herbiciden in het transformatieproces worden gebruikt voor het selecteren van transgene aardappelplanten.


Voor zijn verspreiding en overleving maakt Aardappel gebruik van zaden en knollen. Zaailingen zijn klein en zwak. Door hun geringe concurrentiekracht zijn zij in het algemeen niet in staat zich in een opvolgend gewas te handhaven en knollen te vormen of zaden te produceren. Aardappelknollen zijn vorstgevoelig. Eventuele opslagplanten uit zaad of knollen moeten bovendien in het kader van de verplichte bestrijding van *Phytophthora infestans* worden vernietigd.

Er zijn geen redenen om aan te nemen dat de ingebrachte eigenschappen het verwilderingspotentieel van Aardappel vergroten. Aardappel kan in Nederland wel uitkruisen naar andere aardappelrassen, maar niet naar wilde verwanten.

De COGEM is van mening dat moleculaire karakterisering van BPS-A1020-5 aan alle eisen voldoet en geen reden geeft om nadelige effecten te verwachten. Daarbij zijn er geen redenen om aan te nemen dat de ingebrachte eigenschappen een nadelig effect op niet-doelwitorganismen zullen hebben.

Gezien het bovenstaande acht de COGEM de risico's voor mens en milieu van de teelt van Aardappel BPS-A1020-5 verwaarloosbaar klein. De COGEM vindt echter wel dat het General Surveillance plan op een aantal punten verbeterd kan worden.

Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.



De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,



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Voorzitter COGEM

c.c.

Drs. H.P. de Wijs  
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*Met het oog op eventuele belangenverstrengeling is het COGEM buitenlid dr. P.M. Bruinenberg niet betrokken geweest bij de besluitvorming over dit advies.*

# **Cultivation, import and processing of genetically modified amylopectin potato BPS-A1020-5**

## **COGEM advice CGM/110614-01**

*This application concerns cultivation, import and processing of the genetically modified potato BPS-A1020-5. This potato contains a gbss RNAi construct which silences the endogenous GBSS gene. As a result of this modification, BPS-A1020-5 predominantly produces amylopectin. In addition, due to the expression of the introduced csr1-2 gene, BPS-A1020-5 is less sensitive to imidazolinone herbicides.*

*Previously, COGEM advised positively on a small scale (category 1) field trial with BPS-A1020-5. Furthermore, COGEM advised positively on the cultivation, import and processing of similar genetically modified amylose-free potatoes in 2004 and 2010.*

*In the Netherlands, potato (*S. tuberosum*) may give rise to volunteers. Outside the field, potato has difficulties to establish itself. There are no reasons to assume that the inserted trait will allow BPS-A1020-5 to establish feral populations. The field trials that were carried out by the applicant did not reveal any signs of an increased fitness of BPS-A1020-5. In addition, routine agricultural practices like ploughing, harrowing, herbicide application and compulsory measures to control potato blight, eliminate emerging volunteer plants in the field. In the Netherlands, wild relatives of *S. tuberosum* are present, but incompatibility barriers prevent hybridization between *S. tuberosum* and these wild relatives.*

*The molecular characterization of BPS-A1020-5 potato does not give any reason to expect adverse effects. It is adequately performed and meets the criteria laid down by COGEM. There are no reasons to assume that the traits introduced in BPS-A1020-5 will affect non-target organisms adversely.*

*Based on the considerations described above, COGEM is of the opinion that cultivation of BPS-A1020-5 potato poses a negligible risk to the environment. The general surveillance plan can, however, be improved.*

*COGEM points out that a food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.*

### **Introduction**

The scope of the present notification (EFSA/GMO/2010/88) by BASF Plant Science Company GmbH concerns cultivation, import and processing of potato BPS-A1020-5 (the unique identifier assigned to AM04-1020). BPS-A1020-5 will be cultivated and processed into starch and by-products such as pulp and fruit juice.

The genetically modified potato BPS-A1020-5 contains a *gbss* (granule bound starch synthase) RNAi construct which greatly reduces the amount of endogenous GBSS protein. As a result the tuber starch composition is altered. The components of starch are amylose and amylopectin. Due to the genetic modification BPS-A1020-5 predominantly produces amylopectin. In addition to the

*gbs*sRNAi construct, BPS-A1020-5 expresses the *csr1-2* gene which confers tolerance to imidazolinone herbicides.

### **Previous COGEM advice**

COGEM has previously issued a positive advice on a small scale (category 1) field trial with BPS-A1020-5.<sup>1</sup> In addition, COGEM issued positive advices on cultivation, import and processing of other GM-potatoes with reduced amylose content.<sup>2,3</sup> One of these GM-potatoes is the Amflora potato, which was authorized for cultivation and industrial use in the European Union in 2010.<sup>4</sup> In addition, it was authorized for use as feed, as well as adventitious or technically unavoidable presence of the potato in food and other feed products.<sup>5</sup>

### **Aspects of the crop**

Potato (*Solanum tuberosum*) belongs to the *Solanaceae*. This family also includes tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*) and pepper (*Capsicum annuum*). *Solanum tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is cultivated in Europe.<sup>6</sup>

Potato (*S. tuberosum*) is not able to form feral populations. In the Netherlands, wild relatives of the potato are present, including black nightshade (*Solanum nigrum*), bittersweet (*Solanum dulcamara*), hairy nightshade (*Solanum physalifolium*) and cutleaf nightshade (*Solanum triflorum*).<sup>6,7</sup> Outcrossing between *S. tuberosum* and these wild relatives is not possible because incompatibility barriers prevent hybridization.<sup>8</sup>

Potato can disseminate and survive through pollen, seed and tubers. Potato pollen is relatively heavy and is spread via wind and insects (mainly bumblebees). Potato can reproduce sexually by cross- or self-pollination. Depending on the variety, 80 to 100% of the seeds is formed due to selfing.<sup>9</sup> Outcrossing to other cultivated potato species is possible but reduces markedly with increased distance. Several studies have shown that outcrossing barely occurs beyond ten meters.<sup>10,11,12</sup>

Viable seeds can stay behind after harvest. A small part of these seeds may survive winter and germinate. Potato seed can remain viable in the soil for up to 10 years.<sup>14</sup> Potato seedlings are fragile and are in competitive disadvantage with other plants.<sup>13</sup>

Tubers are sensitive to frost and will not survive temperatures below -3°C.<sup>14</sup> In the Netherlands, during mild winters or in the presence of an isolating cover of snow, tubers might be able to survive and generate volunteers in the next year.<sup>8</sup>

Routine agricultural practices like ploughing, harrowing and herbicide application eliminate emerging volunteer plants. Therefore, they usually do not survive. Furthermore, volunteers will be eliminated due to compulsory measures to control potato blight (*Phytophthora infestans*).<sup>15</sup>

### **Molecular characterization**

The genetically modified potato BPS-A1020-5 was produced by *Agrobacterium tumefaciens* strain LBA4404 mediated transformation of the potato variety Kuras. An overview of the introduced sequences is given below:

- Right T-DNA border region from *A. tumefaciens*;
- *gbss* promoter, from the granule bound starch synthase (*gbss*) gene from *S. tuberosum*;
- RNAi*gbss*450, fragment of the *gbss* gene from *S. tuberosum* in sense direction;
- *gbss* spacer, spacer composed of *gbss* gene sequence from *S. tuberosum*;
- RNAi*gbss*450, fragment of the *gbss* gene from *S. tuberosum* in antisense direction;
- *nos* terminator, polyadenylation sequence derived from the nopaline synthase gene from *A. tumefaciens*;
- Transposon Tn5 fragment, derived from *Escherichia coli*;
- *nos* promoter, derived from the nopaline synthase gene from *A. tumefaciens*;
- *csr1-2* CDS, coding region of the acetohydroxyacid synthase large subunit gene derived from *A. thaliana* containing a S653N mutation;
- *nos* terminator, polyadenylation sequence derived from the nopaline synthase gene from *A. tumefaciens* (truncated).

### *Molecular analysis*

Southern blot analysis using *ApoI*, *AseI* or *NcoI* digested genomic DNA and two backbone probes DNA showed that no backbone elements were introduced in BPS-A1020. Thus, the spectinomycin resistance gene *aadA*, which is located on the vector backbone is not present in BPS-A1020-5.

In addition, Southern blot analysis using *ApoI*, *AseI* or *NcoI* digested genomic DNA and three probes targeting different regions of the T-DNA showed that BPS-A1020-5 contains one copy of the T-DNA at a single locus.

The sequence of the T-DNA insert (5,212 bp) and its flanking regions (1,112 bp of the 5' flanking region and 2,444 bp of the 3' flanking region) was determined. This analysis shows that the sequence of the T-DNA inserted in BPS-A1020-5 is identical to the corresponding region of the transformation vector, that forty-one nucleotides from the right border region are present, and that the left border region and thirty-eight nucleotides of the adjacent *nos* terminator, which is located on the T-DNA, are missing.

The applicant used a *S. tuberosum* database (assembled BACS) and a *Solanum phureja* database (scaffolds) to perform bioinformatic analysis of the sequences flanking the T-DNA region (1076 bp of the 5' and 1090 bp of the 3' flanking region). Based on the results the applicant concluded that the flanking regions are genomic sequences from potato.

The junctions between the insert and its flanking regions were analysed to determine if new open reading frames (ORFs) that could produce novel chimeric proteins were created. An ORF was defined as a string of amino acid-encoding codons located between two stop codons. In total, twelve putative ORFs were identified. The amino acid sequences of these putative ORFs were

deduced and used for bioinformatics analysis. None of the deduced amino acid sequences showed homology to known allergens. Three of the deduced amino acid sequences showed some homology to known protein sequences, but none of these three amino acid sequences shared significant homology to known toxins.

COGEM is of the opinion that the molecular characterization of BPS-A1020-5 has been adequately performed and meets the criteria laid down by COGEM.<sup>16</sup>

#### **Properties of the *gbss* RNAi construct introduced in BPS-A1020-5**

BPS-A1020-5 contains a sense (457 bp) and an antisense fragment (457 bp) of the *gbss* gene from *S.tuberosum*. These two fragments are separated by a short spacer composed of *gbss* gene sequences (66 bp). Together these sequences form an inverted repeat. The RNA originating from this inverted repeat forms a hairpin which is subsequently cleaved into small RNA fragments. These RNA fragments will not be translated into a protein, but will lead to RNA interference which results in the degradation of the endogenous *GBSS* mRNA. Due to this degradation process the expression of *GBSS* protein is greatly reduced.

*GBSS* is one of the key enzymes catalyzing the formation of amylose. If the amount of *GBSS* enzyme is reduced, the tuber starch composition is altered. The components of starch are amylose and amylopectin. Because of the transformation, BPS-A1020-5 predominantly produces amylopectin (>98% of the starch fraction in the tuber). Pure amylopectin is used in, amongst other things, the paper and chemical industries.

#### **Properties of the introduced selection marker**

BPS-A1020-5 expresses the *csr1-2* gene which encodes a S653N mutated acetohydroxyacid synthase (AHAS) protein which is also known as acetolactate synthase. AHAS catalyses the first step in the synthesis of branched-chain amino acids (valine, leucine and isoleucine).<sup>17</sup> Imidazolinone herbicides inhibit AHAS resulting in a lack of branched-chain amino acids, which may lead to plant death.<sup>18</sup>

The S653N mutated AHAS protein which is produced by BPS-A1020-5 is less sensitive to imidazolinone herbicides<sup>17</sup> which allows the use of these herbicides for the selection of transformants.<sup>18</sup> The applicant investigated the tolerance of BPS-A1020-5 to imidazolinone herbicides. The study confirmed that BPS-A1020-5 is less sensitive to imidazolinone herbicides and showed that the reduced sensitivity is of no practical use under field conditions.

#### **Environmental risk assessment**

The agronomic characteristics and plant development and phenotype of BPS-A1020-5 have been studied in 2007 and 2008 at a total of 15 locations in Germany, Sweden and the Czech Republic in regions that are representative for commercial cultivation of starch potatoes. These field trials showed that emergence of BPS-A1020-5 is slightly delayed. A delay in flowering was also observed, but the time of emergence to flowering was nearly the same when compared to its mother variety Kuras. No statistically significant differences were observed for other plant development parameters or plant phenotypic parameters. No meaningful statistical analysis could

be carried out on the frequency of flowering and fruiting, due to the low frequency of flowering or fruiting. BPS-A1020-5 was found to be no different for disease and pest susceptibility when compared to its mother variety Kuras.

The tubers from potato are sensitive to frost and will not survive temperatures below -3°C.<sup>14</sup> The applicant studied the frost sensitivity of BPS-A1020-5 under laboratory conditions. This study showed that the cold tolerance of BPS-A1020-5 is comparable to its mother variety Kuras and its capability to give rise to volunteers after overwintering in the field is, depending on the conditions, equal or somewhat reduced. The results from the study carried out by the applicant thus confirmed the results from previous experiments which showed that a reduced amylose content has no effect on frost sensitivity of *S. tuberosum*<sup>19</sup>, and support COGEM's previous conclusion that a reduced amylose content does not influence the frost sensitivity of these GM-potatoes.<sup>20</sup>

### **Non-target organisms**

BPS-A1020-5 has an increased amylopectin content and has a reduced sensitivity to imidazolinone herbicides. Although there is no reason to assume that the introduction of these characteristics would lead to an effect on non-target organisms, the applicant provided studies to check whether BPS-A1020-5 has an unintended effect on non-target organisms. COGEM is of the opinion that studies on the effect of a GM-crop on non-target organisms are only necessary when there is a reason to assume that the expression product of the transgene could lead to an adverse effect on non-target organisms.<sup>21</sup> In the case of BPS-A1020-5 COGEM is of the opinion that studies on the effect on non-target organisms are not necessary. However, since these studies are part of the application COGEM assessed them.

The applicant performed two *in planta* laboratory studies and one field study with three different locations. In the laboratory studies reproduction and mortality of the Colorado potato beetle (*Leptinotarsa decemlineata*) and the peach aphid (*Myzus persicae*) were assessed. The applicant also studied the effect of BPS-A1020-5 on earthworm (*Eisenia fetida*) survival, biomass development and reproduction in soil. In these laboratory studies no statistically significant effects were detected.

In general, COGEM is of the opinion that laboratory studies that examine the effect of a GM crop on non-target organisms should mention the statistical power and the effect size that the applicant aims to detect. COGEM is of the opinion that laboratory studies should have a statistical power of 0.8 or more.<sup>22</sup> The reproduction assessment of Colorado potato beetles was carried out with only four to five replicates per treatment, each including 26 to 32 adult beetles. The studies on earthworms were carried out with only four replicates per treatment, each including 10 adult earthworms. The statistical power of the non-target organism studies is not mentioned, but COGEM doubts whether the above mentioned numbers of replicates will be sufficient to obtain a statistical power of 0.8.

Another important aspect when assessing non-target organism studies is mortality in control groups. COGEM is of the opinion that mortality in control groups should not exceed 15%, because a high mortality in the control groups could mask effects of the treatment.<sup>22</sup>

In the laboratory study on the Colorado potato beetle the mortality in the controls was higher than 15%, namely between 20 and 22.5%.

In addition, COGEM points out that the applicant concludes that ‘no statistically significant effects on the survival or reproduction of the Colorado potato beetle were observed’ although no statistical analysis was carried out on the reproduction assessment results. COGEM emphasizes the necessity for a correct reflection of the performed analysis.

For the earthworm study artificial soil was supplemented with rasped potato tubers (150 g rasped potato tubers per kg dry soil). The applicant did not motivate why this amount of material was chosen. COGEM is of the opinion that the rationale for the chosen amount should be given.

The field study was carried out on three locations and focused on selected soil and plant dwelling non-target organisms: Colorado potato beetles, potato aphids, ladybugs, mites and springtails.

The number of lady bugs was too low to allow conclusions on the effect of BPS-A1020-5 on the abundance and diversity of lady bugs. No statistical differences were detected between BPS-A1020-5 and the mother variety Kuras with regard to the abundance of soil micro-arthropods, Colorado potato beetles and aphids. In addition, no significant differences were detected in the Collembola species composition. The dossier does, however, contain conflicting statements on the difference in aphid species abundance. On one hand the applicant states that there was no difference between BPS-A1020-5 and other potato varieties in the abundant aphid species. On the other hand it is stated that densities of *Aphis frangulae* were higher in the mother variety Kuras when compared to BPS-A1020-5 ( $p=0.03$ ). In COGEM’s view the number of detected individuals is too low to allow conclusions to be drawn. In addition, COGEM wants to emphasize that the information in the application should be consistent.

The applicant investigated whether BPS-A1020-5 has an effect on carbon transformation. No adverse effects were observed. BPS-A1020-5 had a 16.22% deviation from the mother variety Kuras, which is in the normal range of variation observed with other potato varieties. This study was carried out with 50 g of rasped potato tubers per kg dry soil. No explanation for the amount of material used is given. In COGEM’s view the chosen amount of material should be explained.

Although COGEM has some remarks on the studies which were carried out, the results from these studies do not give an indication that BPS-A1020-5 has an adverse effect on non-target organisms.

### **General surveillance**

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified crops or its use on human health or the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The authorization holder assumes responsibility for general surveillance in the areas where BPS-



A1020-5 is grown and monitored for any potential adverse effects of its cultivation on farm level (farm and its immediate surrounding). A general surveillance plan is presented. The main elements of the general surveillance plan are farm questionnaires, selected existing networks, an 'identity preservation system' and publications (scientific literature, internet sites of official bodies and monitoring programs, expert reports).

The general surveillance plan does not pay attention to the observation of unexpected adverse effects during import and processing. Since import and processing is part of the scope of the application, COGEM is of the opinion that the general surveillance plan should also include measures that allow the detection of unexpected adverse effects during import and processing.<sup>23</sup>

The BPS-A1020-5 potato will be cultivated under a contract system. The farmers will be asked annually to complete the questionnaire and will receive assistance from either consultants experienced in GMO monitoring or from trained staff of the authorization holder to complete the questionnaire.

The questions in the farm questionnaire refer to 'the usual situation', but COGEM points out that the usual situation is not well defined. It would be better to rephrase the questions to acquire data that can be used to detect negative or positive trends in populations of organisms relevant to the monitoring scheme.

In the farm questionnaire (among others) questions are included on plant development, plant health and the occurrence of wildlife, i.e. mammals, birds and insects. In COGEM's view the inclusion of questions on the occurrence of other animals and on the presence of dead animals would enhance the practical value of the farm questionnaire.<sup>23</sup>

COGEM notes that the questions in the farm questionnaire relate to aspects associated with cultivation of BPS-A1020-5 on the farmer's field. COGEM is of the opinion that the questionnaire should also contain questions about unexpected effects of BPS-A1020-5 on other parts of the farmer's premises (e.g. storage areas).<sup>23</sup>

Although the applicant mentions existing monitoring networks as one of the elements of the general surveillance plan, according to the applicant most existing monitoring systems and networks are unlikely to provide relevant data because these programs have been designed for other purposes. Therefore, according to the applicant, the design of these programs (targets; time, frequency and scale of data collection; sampling; analysis; reporting) will not suit general surveillance. The applicant does consider it feasible that established routine surveillance practices such as the monitoring of agricultural plants (plant protection and seed certification services; soil surveys) could complement the information collected by the applicant.

COGEM is aware of the problems associated with the use of data from existing monitoring networks for other purposes, but is of the opinion that the applicant should not exclude these networks as a possible tool for general surveillance. In an advice on post-market monitoring in the Netherlands, COGEM has listed several monitoring networks that could be used for general surveillance in the Netherlands.<sup>24</sup>

According to the applicant the ‘identity preservation system’ allows the participants of the value chain to capture any deviations in the characteristics of BPS-A1020-5 that might lead to adverse effects. COGEM notes that the ‘identity preservation system’ seems to be focused on limiting the possibility for admixture of different potato varieties. Therefore, it appears to be less suitable for the detection of possible adverse effects. For example, the ‘identity preservation system’ does not mention the necessity to report unusualities other than defective products which need to be checked. COGEM is of the opinion that the applicant should explain why the ‘identity preservation system’ is considered suitable for general surveillance purposes.

### **Advice**

COGEM was requested to advice on the cultivation of BPS-A1020-5 potato. This potato contains a *gbss* RNAi construct which silences the endogenous GBSS gene. As a result of this modification, BPS-A1020-5 predominantly produces amylopectin. In addition, BPS-A1020-5 expresses the *csr1-2* gene, which results in a reduced sensitivity to imidazolinone herbicides. The reduced sensitivity is too low to be of practical use under field conditions.

Potato (*S. tuberosum*) does not establish outside cultivated areas. There are no reasons to assume that the inserted traits have an effect on the persistence or invasiveness of BPS-A1020-5. The field trials that were carried out by the applicant did not reveal any signs of an increased fitness of BPS-A1020-5.

In the Netherlands, outcrossing between BPS-A1020-5 and its wild relatives including black nightshade, bittersweet, hairy nightshade and cutleaf nightshade is not possible. COGEM considers the molecular analysis of BPS-A1020-5 adequate.

COGEM is of the opinion that studies on the effect of non-target organisms are only necessary when there is a reason to assume that the expression product of the transgene could lead to an adverse effect on non-target organisms. In the case of BPS-A1020-5 studies on non-target organisms are therefore not required by COGEM. However, since these studies are part of the application COGEM examined them. Although COGEM has some minor remarks on these studies, as expected the results do not give an indication that BPS-A1020-5 has an adverse effect on non-target organisms.

Based on the above described considerations, COGEM is of the opinion that cultivation of BPS-A1020-5 potato poses a negligible risk to human health and the environment. The general surveillance plan can, however, be improved.

A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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