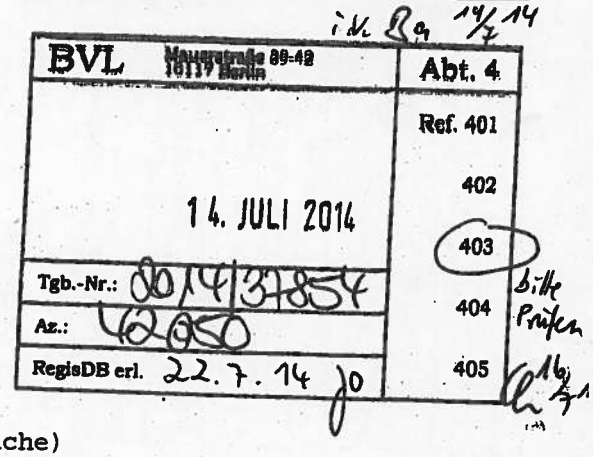
[redacted]

Von: Bartsch, Detlef
Gesendet: Montag, 14. Juli 2014 08:16
An: Gentechnik
Cc: Ref. 403
Betreff: WG: Request for opinion on RTDS

Anlagen: Request for an opinion 140711.pdf

Request for an opinion 140711...



Bitte GG (cc. 403 vorab m.d.B. um Rücksprache)

z.d.A.

Detlef Bartsch, apl Prof Dr.

BVL - Bundesamt für Verbraucherschutz und Lebensmittelsicherheit Referat 404: Koexistenz und GVO-Monitoring [Federal Office of Consumer Protection and Food Safety Unit 404: Coexistence and GMO Monitoring]
Besucher/Visitors: Mittelstraße 51-54
Post/Postal address: Mauerstrasse 39-41
D-10117 Berlin
Germany
Tel. +49-(0) 30-18445-6400
Fax: +49-(0) 30-18445-6099
Detlef.Bartsch@BVL.BUND.DE

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Freitag, 11. Juli 2014 18:32
An: Bartsch, Detlef
Cc: Patrick Rüdelsheim
Betreff: Request for opinion on RTDS

Dear Detlef,

Attached is the document that we like to submit for obtaining an opinion on how to proceed with field trials in Germany.
Of course, I need to stress that Cibus is willing to provide more information on any of the points mentioned in the report and would welcome any opportunity to discuss this.

Can you let me know how to proceed on this? Do we need to submit it in a hard copy or is this request sufficient?

Kind regards,

Patrick Rüdelsheim
[redacted]

PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel./Fax: +32 (0)9 321 07 05
[redacted]
Website: www.perseus.eu <http://www.perseus.eu/>

Ehlers, Dr. Ulrich

1)

Von: Ehlers, Dr. Ulrich im Auftrag von 403@bvl.bund.de
Gesendet: Mittwoch, 16. Juli 2014 16:01
An: [redacted]
Cc: 'detlef.bartsch@bvl.bund.de'; 'georg.leggewie@bvl.bund.de'
Betreff: WG: Request for opinion on RTDS

Dear Patrick,

Detlef asked me to answer your e-mail. [redacted]

With regard to the procedure I ask you to send us your request as a signed letter, either in hard copy, as a PDF, or both. This is a legal [redacted]

We already had a brief look at your request. Is it possible [redacted] the decisions by UK DEFRA, the Swedish Board of Agriculture, and [redacted] Gene Technology?

We understand that the request from Cibus is limited to the particular oilseed rape line BnALS-57 and all via traditional breeding derived material. In the PDF it says that Cibus has carried out a molecular analysis confirming the TGG to TTG change in the nucleotide sequence of line [redacted]. We will probably ask you for the data from this molecular characterization. We still have to check if in addition to that we need other information on line BnALS-57 or on the RTDS technique.

If you consider any information you send us as confidential (CBI), please identify that information clearly and provide a justification for the confidentiality. This justification can be given in a separate document.

The evaluation of your request by the BVL will not include any participation or active information of the public or involvement of other authorities. We will probably ask our national expert committee (ZKBS) for an opinion on the request. Although the ZKBS issued the position statement on new plant breeding techniques in 2012 we would like to back our decision by a ZKBS opinion on the particular oilseed rape line [redacted].

Do not hesitate to contact me in case you have any questions.

Kind regards,
Ulrich

Dr. Ulrich Ehlers

Federal Office of Consumer Protection and Food Safety (BVL) Unit 403 - Deliberate Release and Placing on the Market

403@bvl.bund.de
T +49-(0)30-18445-6300
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Mauerstrasse 39-42
D-10117 Berlin

Visitors address:
Mittelstrasse 51-54, Entrance 4
D-10117 Berlin

2) Kon, z.d.A. Q 16/7/14

42050

Regisdb. erl.)o 22/7/14

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Freitag, 11. Juli 2014 18:32
An: Bartsch, Detlef
Cc: Patrick Rüdelsheim
Betreff: Request for opinion on RTDS

Dear Detlef,

Attached is the document that we like to submit for obtaining an opinion on how to

proceed with field trials in Germany.
Of course, I need to stress that Cibus is willing to provide more information on any of the points mentioned in the report and would welcome any opportunity to discuss this.

Can you let me know how to proceed on this? Do we need to submit it in a hard copy or is this request sufficient?

Kind regards,

Patrick Rüdelsheim

PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel./Fax: +32 (0)9 321 07 05

Website: www.perseus.eu <http://www.perseus.eu/>

[redacted]

Von: Ref. 403
Gesendet: Freitag, 18. Juli 2014 12:40
An: Gentechnik
Betreff: WG: Request for opinion on RTDS

Anlagen: 110420 Letter UK DEFRA.pdf; CBS-14-001 Letter Perseus BVL 140718.pdf; 140124 Letter Fin Gene Tech Board.pdf; 120719 Letter Swedish Board Agriculture.pdf


110420 Letter UK DEFRA.pdf (33...


CBS-14-001 Letter Perseus BVL ...


140124 Letter Fin Gene Tech Bo...


120719 Letter Swedish Board Ag...

Bitte GG.

i.V. Gl. 21/7/14

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
Lebensmittelsicherheit (BVL)
Referat 403 - Freisetzung und Inverkehrbringen

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Mauerstraße 39-42
D-10117 Berlin

Besucheradresse:
Mittelstraße 51-54, Aufgang 4
D-10117 Berlin

BVL Mauerstraße 39–42 10117 Berlin

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18. Juli 2014

Tgb.-Nr.: 0614/37880
Az.: 42050
Regisdb. erl. 6.10.14 JO

i.V. n.R. Ba 22/7 14

Leggewie n.R.

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Freitag, 18. Juli 2014 12:12
An: Ref. 403
Cc: Bartsch, Detlef; Leggewie, Dr. Georg
Betreff: Re: Request for opinion on RTDS

Dear Ulrich,

I include a letter stating our request for an opinion. Please feel free to indicate if this requires further changes.

I also attach the letters from the 3 authorities.

The scientists at Cibus have been informed of the possible request on molecular characterization and possible other questions. As indicated before they are definitely available for addressing any request and pressed me again to confirm their willingness to present the information -if possible- in person.

Although there is no confidential information in what has been provided so far, we also understand that there will be no formal public involvement. Should this change, it would be of interest to Cibus to be informed so that they can be prepared for responding to questions.

Looking forward to your reaction. Kind regards, Patrick

Op 16-jul.-2014 om 16:01 heeft 403@bvl.bund.de het volgende geschreven:

> Dear Patrick,
>
> Detlef asked me to answer your e-mail.
>

> With regard to the procedure I ask you to send us your request as a signed letter, either in hard copy, as a PDF, or both. This is a legal requirement.
>
> We already had a brief look at your request. Is it possible that you send us copies of the decisions by UK DEFRA, the Swedish Board of Agriculture, and the Finnish Board of Gene Technology?
>
> We understand that the request from Cibus is limited to the particular oilseed rape line [redacted] and all via traditional breeding derived material. In the PDF it says that Cibus has carried out a molecular analysis confirming the TGG to TTG change in the nucleotide sequence of line [redacted] We will probably ask you for the data from this molecular characterization. We still have to check if in addition to that we need other information on line BnALS-57 or on the RTDS technique.
>
> If you consider any information you send us as confidential (CBI), please identify that information clearly and provide a justification for the confidentiality. This justification can be given in a separate document.
>
> The evaluation of your request by the BVL will not include any participation or active information of the public or involvement of other authorities. We will probably ask our national expert committee (ZKBS) for an opinion on the request. Although the ZKBS issued the position statement on new plant breeding techniques in 2012 we would like to back our decision by a ZKBS opinion on the particular oilseed rape line [redacted]
>
> Do not hesitate to contact me in case you have any questions.
>
> Kind regards,
> Ulrich
>
> Dr. Ulrich Ehlers
>
> Federal Office of Consumer Protection and Food Safety (BVL) Unit 403 -
> Deliberate Release and Placing on the Market
>
> 403@bvl.bund.de
> T +49-(0)30-18445-6300
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>
> Mauerstrasse 39-42
> D-10117 Berlin
>
> Visitors address:
> Mittelstrasse 51-54, Entrance 4
> D-10117 Berlin
>
>
> -----Ursprüngliche Nachricht-----
> Von: Patrick Rüdelsheim [redacted]
> Gesendet: Freitag, 11. Juli 2014 18:32
> An: Bartsch, Detlef
> Cc: Patrick Rüdelsheim
> Betreff: Request for opinion on RTDS
>
> Dear Detlef,
>
> Attached is the document that we like to submit for obtaining an opinion on how to proceed with field trials in Germany.
> Of course, I need to stress that Cibus is willing to provide more information on any of the points mentioned in the report and would welcome any opportunity to discuss this.
>
> Can you let me know how to proceed on this? Do we need to submit it in a hard copy or is this request sufficient?
>
> Kind regards,
>
>
> Patrick Rüdelsheim
> [redacted]
>
> PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
> Tel./Fax: +32 (0)9 321 07 05

Department for Environment, Food and Rural Affairs

GM Team
Area 8A, 9 Millbank
17 Smith Square
London SW1P 3JR

Telephone 08459 33 55 77
Website www.defra.gov.uk

defra
Department for Environment
Food and Rural Affairs

Dr. Peter Beetham
Cibus US LLC
6455 Nancy Ridge Road,
Suite 100,
San Diego, CA, 92121
USA

Date: 20th April 2011

Dear Dr. Beetham

Defra has considered the herbicide tolerant (HT) oilseed rape that was developed using your Rapid Trait Development System (RTDS™). We have concluded that these particular organisms are not within the scope of Part IV of the Environmental Protection Act 1990 or England's Genetically Modified Organisms (Deliberate Release) Regulations 2002. We therefore do not consider that you are required to notify or obtain a consent for your HT oilseed rape field trials.

Our expert Advisory Committee on the Deliberate Release into the Environment (ACRE) has advised us that RTDS™ is a form of mutagenesis that generates changes to the plant genome that could occur through traditional mutagenesis and by natural mutation. It has advised us that the oligonucleotide used as a mutagen (referred to as a GRON in this context) was present transiently in the original oilseed rape cell and is very unlikely to be inserted into its genome.

ACRE has also advised us that the GRON is not a recombinant nucleic acid. It is a chemically synthesised molecule comprising nucleic acids in a sequence that is identical to the target gene in the parental oilseed rape plant with the exception of one base. RTDS™ does not involve the use of a GMO.

Regulation 5(3) of the Genetically Modified Organisms (Deliberate Release) Regulations 2002 provides that "an organism shall be taken, for the purposes of Part IV of the Act, not to be a genetically modified organism if it is yielded from the techniques or methods listed in paragraph 2(i) or (ii) [which includes mutagenesis] provided that those techniques or methods did not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those made by techniques or methods listed in that paragraph". We therefore consider that this particular HT oilseed rape that was developed

using RTDS™ is not a genetically modified organism for the purposes of part IV of the Environmental Protection Act 1990 and therefore, you are not required to seek a consent from the Secretary of State prior to its release.

Currently the EU Commission and the Member States are considering the extent to which organisms produced by oligo-directed mutagenesis should fall within the scope of the European deliberate release legislation. The regulatory position may therefore change in future. Furthermore, if you intend to trial these HT oilseed rape plants in any other Member State of the EU we recommend that you contact the relevant national authority for its view on the regulatory position in that Member State before proceeding.

In addition, if you intend to market these oilseed rape plants or products derived from them in the future, other controls including novel foods and plant protection legislation may apply.

Yours sincerely

pp Dr. SUE POPPLE
Deputy Director, Farming and Food Science and GM Policy and Regulation

perseus

biosafety and biotechnology regulatory services

Bundesamt für Verbraucherschutz
und Lebensmittelsicherheit
Abteilung 4: Gentechnik
Mauerstrasse 39-42
D-10117 Berlin
Germany

18/07/2014

Dear,

Re: Request for an opinion on field trials with oilseed rape material improved by Cibus using *RTDS*™

Cibus and its seed partners are evaluating performing field trials in Germany with crops developed by ***RTDS***™ [redacted] These field trials will be conducted according to normal breeding practices and will include performance testing, breeding trials as well as small seed productions for subsequent testing.

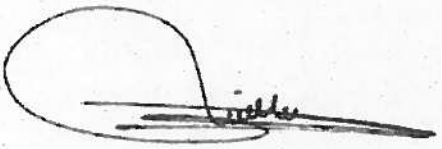
The material to be tested will be oilseed rape (*Brassica napus* L.), carrying a mutation induced via ***RTDS***™ that makes it tolerant to [redacted]
This request is limited to this particular material and all via traditional breeding derived material. More detailed information is provided in a background document.

Although several experts and authorities have confirmed that ***RTDS***™ should be considered a mutagenesis technique, in the EU ODM has been included in a list of New Breeding Techniques that are evaluated to determine if they are within the scope of the GMO legislation. This evaluation in the EU is prolonged and until a final interpretation is provided the status of products derived with these techniques remain undecided.

With this request Cibus would like to obtain an opinion:

- if the German CA requires additional information to confirm that this line is indeed exempted from the application of the GMO legislation, as indicated in the 2012 position statement of the German Central Commission for Biosafety (ZKBS)
- if such field trials can be conducted in Germany without requiring compliance with the relevant GMO deliberate release legislation.

Sincerely yours,



Dr. Patrick RÜDELSHEIM [redacted]

PERSEUS b.v.b.a • Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel / Fax +32-(0)9-321.07.05 • [redacted]

Jordbruks verket

Swedish Board of Agriculture

Plant Regulations Division
Tobias Olsson
Tel: (+46-36) 15 58 45
E-mail: tobias.olsson@jordbruksverket.se

INFORMATION Dnr 22-3666/12

[redacted]

Dr. Peter Beetham
Cibus US LLC
[redacted]
(only by e-mail)

Inquiry on oilseed rape trials

Dear Dr. Beetham,

The Swedish Board of Agriculture has considered your inquiry from [redacted] regarding oilseed rape breeding lines that were developed using your Rapid Trait Development System (RTDS). We have concluded that these particular organisms are not within the scope of Chapter 13 in the (SFS 1998:800) Swedish Environmental Code, or (SFS 2002:1086) Genetically Modified Organisms (Deliberate Release) Ordinance. We therefore do not consider that you are required to notify or obtain consent from The Swedish Board of Agriculture for the deliberate release of your oilseed rape field trials.

This conclusion refers to the organisms in your inquiry and not necessarily to any other organism produced by the RTDS technique.

Currently the EU Commission and the Member States are considering the extent to which organisms produced by oligo-directed mutagenesis should fall within the scope of the European deliberate release legislation. The regulatory position may therefore change in the future.

Yours sincerely

Tobias Olsson

[redacted]

Von: Ehlers, Dr. Ulrich
Gesendet: Dienstag, 22. Juli 2014 10:33
An: Gentechnik
Betreff: WG: Cibus request for an opinion on RTDS technique

Bitte GG.

i.V. Ba 22/7 14

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
Lebensmittelsicherheit (BVL)
Referat 403 - Freisetzung und Inverkehrbringen

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Mauerstraße 39-42
D-10117 Berlin

Besucheradresse:
Mittelstraße 51-54, Aufgang 4
D-10117 Berlin

BVL Mauerstraße 39–42 10117 Berlin

22. Juli 2014

Tgb.-Nr.: 20141328/42
Az.: 42050
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Von: Carina Knorpp [mailto:carina.knorpp@regeringskansliet.se]
Gesendet: Dienstag, 22. Juli 2014 10:29
An: Ehlers, Dr. Ulrich
Cc: staffan.eklof@jordbruksverket.se
Betreff: SV: Cibus request for an opinion on RTDS technique

Dear Ulrich,

As you correctly write this is a question that is handled by the Swedish CA, the Swedish Board of Agriculture. I work in the Ministry and am not involved in this type of more detailed and technical issues but I am happy to give you the names of the persons that will be able to help you.

Staffan Eklöf, staffan.eklof@jordbruksverket.se or Helene Ström, Helene.strom@jordbruksverket should be able to answer your questions.

However, be aware that July is the main vacation time in Sweden which could mean that the answer will take a little bit longer.

Kind regards

Carina

Carina Knorpp
Senior Advisor
Animal and Food Division
Ministry for Rural Affairs
Government Offices of Sweden
SE-SE-103 33 Stockholm
+46 84051517

+46 701453803
carina.knorpp@gov.se <mailto:carina.knorpp@gov.se> www.government.se
<http://www.government.se>

Från: Ehlers, Dr. Ulrich [mailto:ulrich.ehlers@bvl.bund.de]
Skickat: den 15 juli 2014 10:00
Till: louise.ball@defra.gsi.gov.uk; Carina Knorpp
Kopia: detlef.bartsch@bvl.bund.de; georg.leggewie@bvl.bund.de
Ämne: Cibus request for an opinion on RTDS technique

Dear Louise, dear Carina Knorpp,

BVL has received a request from Cibus for an opinion on field trials with plants that have been developed using the RTDS technique.

I understood that UK DEFRA and the Swedish Board of Agriculture were contacted by Cibus as well on this matter and decided that plants developed by this technique do not fall under the EU GMO legislation. For our evaluation of the Cibus request it would be very helpful if you could answer the following questions:

1. Are the decisions by DEFRA and by the Swedish Board of Agriculture publicly available?

2. Did ACRE or the Swedish CA ask for a more detailed description of the technique than is given in the attached PDF?

3. Did ACRE or the Swedish CA ask for more data on the plants or specific plant lines that were developed using RTDS?

4. Were the decisions by UK and Sweden issued for the RTDS technique in general or were they given for specific crops or plant lines?

Thanks a lot in advance!

Kind regards,

Ulrich

Dr. Ulrich Ehlers

Federal Office of Consumer Protection and

Food Safety (BVL)

Unit 403 - Deliberate Release and Placing on the Market

403@bvl.bund.de

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Mauerstrasse 39-42

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Visitors address:

Mittelstrasse 51-54, Entrance 4

D-10117 Berlin

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Request for an opinion
Field trials with Cibus' sulfonylurea tolerant oilseed rape (*Brassica napus* L.) improved with ***RTDS***™, an oligonucleotide-directed mutagenesis technique.

11th July 2014

Prepared and submitted by Perseus BVBA
Dr. Patrick Rüdelsheim
Technologiepark 3, 9052 Zwijnaarde Belgium

On behalf of Cibus Europe
Goessestraatweg 19, 4421 AD Kapelle, The Netherlands

1. Introduction

1.1. Background

Founded in 2001, Cibus Global is a privately-held global organization that produces products for the agricultural, industrial and healthcare markets. With its principal research lab based in San Diego, USA, Cibus established its first ex-US office in Europe (the Netherlands) in response to the interest of European developers and markets for products developed using its unique technology platform.

Cibus' proprietary technology, called the Rapid Trait Development System (***RTDS***™), is a specific application of oligonucleotide directed mutagenesis (ODM). While ***RTDS***™ products cannot be distinguished from products of other mutagenesis methods, the induced changes are extremely accurate thereby allowing precision breeding. Applications are envisaged in agriculture, industrial products and human health. Crops improved by ***RTDS***™ addressing crop quality as well as agronomic performance are advancing in development and the first products are ready for market introduction.

Seed companies and breeders are eager to have access to the technology and its products as this powerful technology fits perfectly with traditional breeding schemes. In consequence, extending field testing and breeding to locations in Europe is a recurrent and urgent request.

Although several experts and authorities have confirmed that ***RTDS***™ should be considered a mutagenesis technique, in the EU ODM has been included in a list of New Breeding Techniques that are evaluated to determine if they are within the scope of the GMO legislation. This evaluation in the EU is prolonged and until a final interpretation is provided the status of products derived with these techniques remain undecided.

Awaiting this interpretation, CA responsible for GMO regulations in EU Member States are being contacted to evaluate how field trials with this material can proceed. The UK, Swedish and Finish authorities informed Cibus that the first oilseed rape lines developed with ODM in their opinion are not GMOs and that we could proceed with field trials without complying with the GMO regulation. Accordingly field trials were performed in the UK and Sweden[redacted]

1.2. Request for opinion

Cibus and its seed partners are evaluating performing field trials in Germany with crops developed by ***RTDS***™ [redacted] These field trials will be conducted according to normal breeding practices and will include performance testing, breeding trials as well as small seed productions for subsequent testing.

The first material to be tested will be oilseed rape (*Brassica napus* L.), carrying a mutation that makes it tolerant to both imidazolinone and sulfonylurea herbicides. This request is limited to this particular line and all via traditional breeding derived material.

With this request Cibus would like to obtain an opinion:

- if the German CA requires additional information to confirm that this line is indeed exempted from the application of the GMO legislation,
- if such field trials can be conducted in Germany without requiring compliance with the relevant GMO deliberate release legislation.

2. Background information

2.1. *RTDS*™

Cibus has developed the ***RTDS***™ (Rapid Trait Development System) technology to achieve targeted mutations in plant genes (Beetham *et al.*, 1999). The ***RTDS***™ process provides a means to make targeted single nucleotide changes in specific genes without the incorporation of foreign DNA. This technology utilizes the naturally occurring repair machinery that exists in all living cells to specifically modify gene sequence *in situ*. It effects a precise change in the genetic sequence of the target gene and leaves the rest of the genome unaltered.

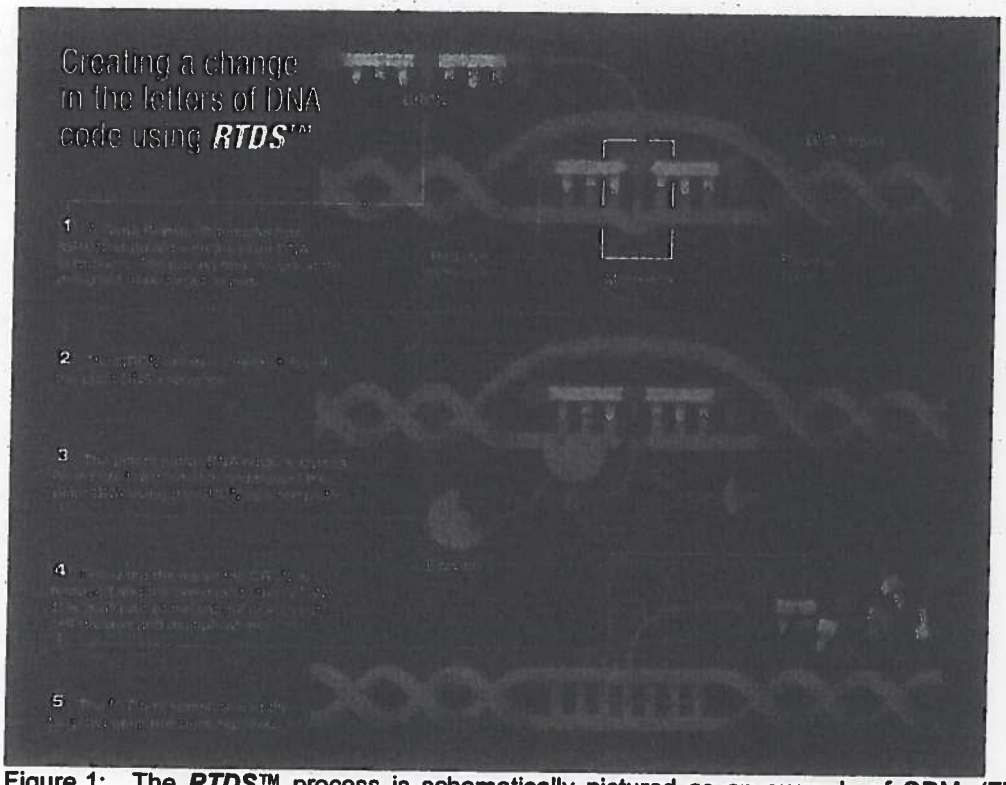

Figure 1: The ***RTDS***™ process is schematically pictured as an example of ODM. (Figure by courtesy of Cibus)

RTDS™ uses a specially designed element, a Gene Repair Oligonucleotide, called a "GRON", to direct a cell's own DNA-repair system to make a specific desired change in the targeted gene. The GRON that effects this change is a chemically synthesized non-recombinogenic oligonucleotide composed of DNA bases, as well as other non-naturally occurring chemical moieties, and is designed to hybridize at the targeted gene location to create a mismatched base-pair. This mismatched base-pair acts as a beacon to attract the cell's repair system to that site to correct the designated nucleotide(s) within the gene. The desired genetic mutation is induced directly and exclusively *via* the effect of the GRON itself, *i.e.* independent of any delivery vector system.

Once the correction process is complete the GRON is degraded and the corrected gene continues to be expressed under that gene's endogenous control mechanisms. Once this change is made it is heritable in each subsequent generation (Zhu et al., 1999). No foreign material is introduced in the organism's genome or remains in the cells. Consequently, the technique is limited to mutating functions already present in the organism. The action is comparable to what is known for mutagenic agents used in traditional forms of induced mutagenesis, adding precision and direction to the mutagenic process. Thus, ODM is a site-specific gene mutation system that makes precise changes in a specific gene sequence without the incorporation of foreign genes or control sequences.

2.2. Example of *RTDS*™ trait development – Improved imidazolinone tolerance

Acetohydroxyacid synthase[1] is the first enzyme in the biochemical synthesis of the branched chain amino acids valine, leucine and isoleucine (Singh, 1999). The enzyme is composed of two types of subunit, the catalytic and the regulatory subunit. The catalytic subunit in *Brassica napus* is encoded by 2 genes producing proteins that are substantially equivalent (99% identical), varying in only 8 amino acids out of 655 (*BnAHASIC*) and 652 (*BnAHASIIIA*). For the ALS/AHAS protein to become active, an active dimer needs to be formed. The dimer can be the product of either the *AHASIC* or the *AHASIIIA* gene, forming either a homodimer, if the structure consists of the product of only one of the two genes (A+A and C+C), or a heterodimer if both gene products dimerize (A+C). All three possible combinations show similar catalytic activity. The catalytic activity of this structure is modulated when combined with the smaller regulatory subunit that is encoded by separate genes (Ouellet et al., 1992).

Inhibition of ALS/AHAS results in plant death. A wide range of inhibitors, including imidazolinone and sulfonylurean herbicides, are widely used in modern agriculture due to their effectiveness at very low application rates and relative non-toxicity in animals. Yet, the ability to use spray-over techniques for selective weed control is dependent upon the presence of imidazolinone-tolerant varieties of the desired crop. Plants resistant to imidazolinones and sulfonylureas have been successfully produced using seed, microspore, pollen, and callus mutagenesis in *Zea mays, Arabidopsis thaliana, Brassica napus, Glycine max, Nicotiana tabacum*, and *Oryza*. In all cases, a single, partially dominant nuclear gene conferred resistance. Certain mutations render ALS/AHAS less susceptible to the herbicides: e.g. the early microspore mutants from the oilseed rape variety Topas, known as P1 (PM1) and P2 (PM2) mutations (Swanson et al., 1989), have been characterized in many organisms. In all cases the genomic mutations have arisen via mutagenesis.

The PM1 mutation results in a serine to asparagine amino acid change at a position known as 653 of the ALS/AHAS enzyme[2], respectively encoded by AGT and AAT in the *BnAHASIC* gene, which is located in the C genome of *B. napus*. The second mutation known as PM2, is a mutation at a position known as 574 and results in a tryptophan to leucine amino acid change due to the mutation encoded as follows – TGG to TTG in the *BnAHASIIIA* gene, located in the A genome of *B. napus*.

Both PM1 in the *BnAHASIC* gene and PM2 in the *BnAHASIIIA* gene have been introduced in oilseed rape lines via chemical mutagenesis or mutagenesis of microspores as part of herbicide tolerance trait development programmes. SMART® and Clearfield® varieties were generated by crossing two lines, each with different mutations (Tan et al., 2005). Although the level of conferred tolerance to each herbicide may vary depending on the presence of the PM1 and/or PM2 mutations, each mutation provides tolerance to the plant through similar strategies. The introduction of single base change in the nucleotide sequence at a specific location in each of the *BnAHASIC* and *BnAHASIIIA* genes produces an enzyme with a change in a single amino acid that reduces the ability of the herbicide to block the active binding site of that enzyme, allowing branched chain amino acid synthesis to occur even in the presence of the herbicide. Since 1995 the PM2 trait has been included in many commercial products grown on millions of acres with an excellent history of safety (Senior & Dale, 2002).

To make this weed control system more robust, Cibus aimed to develop an oilseed rape variety tolerant to both sulfonylurea and imidazolinone herbicide chemistries by replicating the PM2 mutation in both the *BnAHASIC* and *BnAHASIIIA* genes. While the PM1 mutation (AGT to AAT at position 653) has been shown to provide tolerance to imidazolinone, it does not provide commercial levels of tolerance to sulfonylurea herbicides (Tan et al., 2005). On the contrary, the PM2 mutation has been confirmed to provide tolerance to both chemistries and by duplicating the presence of the PM2 mutation in both genes from which the ALS/AHAS enzyme is derived, improved tolerance to both chemistries was expected (Swanson et al., 1989).

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[1] Acetohydoxyacid synthase (AHAS) is also described in the literature as the acetolactate synthase (ALS) enzyme. Throughout this document it will be referred to as ALS/AHAS.

[2] The numbering system used throughout this document is based on the amino acid sequence of *Arabidopsis* AHAS (At3g48560).

[redacted]

Providing tolerance to both chemistries, will offer farmers a superior weed control system for oilseed rape. The sulfonylurea chemistries offer farmers an alternative for their post-emergent weed control, allowing them better control of many broadleaf weeds. Importantly, this also provides an alternate mode of action for weed control in oilseed rape helping to prevent the development of herbicide resistant weeds.

2.3. Cibus' sulfonylurea tolerant oilseed rape

2.3.1. Mutation and selection

Cibus has developed an oilseed rape (*Brassica napus* L.) tolerant to both imidazolinone and sulfonylurea herbicides.

- [redacted]
- These tolerant microcalli were genotyped [redacted]

2.3.2. Early identification

- Following regeneration into plants, both *BnAHASIC* and *BnAHASIIIA* were sequenced in their entirety revealing that compared to these genes in the wild type check "Roper" there was only a single mutation in the TGG to TTG SNP, which causes the W574L amino acid substitution in *BnAHASIC*.
- No changes were detected in promoter regions or locations that would affect endogenous gene expression. This was confirmed by sequencing a 2500 bp region upstream of *BnAHASIC* which was determined to be identical with Genbank accession Z11524.
- In progeny and crosses, this SNP and its associated herbicide tolerant phenotype have been demonstrated to be inherited in a Mendelian fashion across multiple generations (Kochevenko & Willmitzer, 2003).
- Molecular analysis confirmed the TGG to TTG change in the nucleotide sequence causing an amino acid change resulting in a tryptophan to leucine change at amino acid position 574.

2.3.3. Performance testing

- [redacted]
- Molecular analysis on material from different generations confirmed the genetic stability of the mutation.
- ST oilseed rape was tested in the greenhouse [redacted] using multiple rates of ALS/AHAS active herbicides. [redacted]

[redacted]

- The combined location summary data indicate that ST oilseed rape and hybrids made using the ST oilseed rape are substantially equivalent to those of widely grown commercial oilseed rape. The PM2 mutation induced at the two AHAS loci will affect the plants ability to withstand applications of selected sulfonylurea and imidazolinone herbicides but these mutations do not affect the plants agronomic performance when no selective herbicide is applied.

2.3.4. Compositional analysis

- The compositional profile of Cibus' ST oilseed rape was compared to the commercially available CL oilseed rape line BY5525 planted in confined field trials conducted at 5 locations in Canada in the [redacted] The field trials were conducted using ST oilseed rape, several hybrid crosses with the ST oilseed rape variety and 3 commercially available oilseed rape lines as controls. One of the control lines included in the Canadian field trial program was BY5525, a CL variety which includes the PM2 mutation induced by conventional mutagenesis techniques.
- No statistically significant differences ($p<0.05$) were identified for crude nutrient comparisons conducted between Cibus' ST oilseed rape and BY5525.
- ST oilseed rape displayed a slightly lower range of total glucosinolates than the BY5525 line. However the total glucosinolate concentration was well below the established limits (sum <30 µmoles/gram) for oilseed rape products for both BY5525 and ST oilseed rape, as described in the Canadian Regulations for the Control and Regulation of the Sale of Feeds.
- Amino acid profiles were of particular interest due to the presence of the PM2 mutation in both the *BnAHASIIIA* and *BnAHASIC* genes in Cibus' ST oilseed rape. The enzyme product of these genes is ALS/AHAS which catalyzes the first reaction in the pathway for synthesis of the branched chain amino acids. These results show that, for the amino acids leucine, isoleucine and valine, the results are similar and compare favourably when ST oilseed rape is compared to the unmodified parent line BN2. Although the numbers are slightly higher than the reference range for most amino acids tested, the differences are relatively small and nutritionally insignificant
- When comparing the levels of the individual fatty acids between Cibus' ST oilseed rape with BY5525, statistically significant differences for several of the fatty acids were obtained. However the relative differences in ranges were minor and not biologically significant. For Cibus' ST oilseed rape the range in whole seed for erucic acid is 0.04 to 0.05%, well below the tolerable range required to achieve oilseed rape quality.
- None of the indicators for potential allergenicity (sequence homology, heat stability, resistance to digestibility) were observed for the modified ALS/AHAS protein.

2.3.5. Commercial deployment

- This line is first targeted for production as a grain crop in major oilseed rape growing areas[redacted]
- Further development and deployment in other oilseed rape growing areas is under consideration.

3. Regulatory status

3.1. Regulatory decisions

3.1.1. USA

- In 2004 US APHIS BRS determined that under their regulations, the agency has no authority to regulate products created by mutagenesis techniques. Cibus did not have to file a notification to conduct a field trial with products created with ***RTDS***.

3.1.2. Canada

- Subject to the Canadian approach of "Plants with Novel Traits", Cibus line 5715 was approved by Health Canada (November 2013) and by the Canadian Food Inspection Agency (December 2013).

3.1.3. UK

- In 2011, the UK DEFRA informed Cibus that based on an advice from ACRE (see below) they had concluded that Cibus' line 5715 could be tested in field trials in the UK without having to comply with the national GMO legislation. The conclusion indicates:
  - *We therefore consider that this particular herbicide tolerant oilseed rape that was developed using **RTDS**™ is not a genetically modified organism for the purpose of part IV of the Environmental Protection Act 1990 and therefore, you are not required to seek a consent from the Secretary of State prior to its release.*
- The opinion further refers to the ongoing discussion on new breeding techniques at EU level and the fact that this position may be adapted when this process would be concluded differently.

3.1.4. Sweden

- The Swedish Board or Agriculture concluded in 2012 in a similar way that the oilseed rape breeding lines developed using ***RTDS***™ are not in the scope of Chapter 13 in the (SFS 1998:800) Swedish Environmental Code, or (SFS 2002:1086) Genetically Modified Organisms (Deliberate Release) Ordinance. Cibus was not required to notify or obtain consent for the deliberate release of its oilseed rape field trials.
- Reference was made to the EU process and the possibility that this opinion might change in future.

[redacted]

3.2. Opinion from advisory committees

3.2.1. EU New Techniques Working Group

- At the request of the Competent Authorities under Directive 2001/18/EC, a working group was established (October 2007) to analyse a non-exhaustive list of techniques for which it is unclear whether they would result in a GMO. In 2012 a final report was issued.

- A minority of experts felt that ODM falls under the scope of Directive 2001/18/EC and Directive 2009/41/EC is similar to techniques listed in Annex IA, Part 1, because ODM is a recombinant nucleic acid technique that (i) leads to a new combination of genetic material resulting in a heritable change in the DNA sequence and (ii) it involves the direct introduction of heritable material prepared outside of the organism.
- However, the majority of experts considered that ODM is not captured by Annex IA Part 1 on the grounds that oligonucleotides introduced into the cell are not recombinant nucleic acid molecules capable of continued propagation and they are not heritable material. Furthermore, they indicated that the resulting organisms from ODM are captured by Annex IB because the technique entails mutagenesis. Mutagenesis is listed as one of the techniques yielding organisms to be excluded from the application of Directive 2001/18/EC and Directive 2009/41/EC.
- All experts agreed that ODM results in changes in organism that can be obtained with other forms of mutagenesis.

3.2.2. Germany

- The German Central Commission for Biosafety (ZKBS) issued a position statement on new plant breeding techniques in 2012. In particular for Oligo Directed Mutagenesis the following is concluded:
  - *The oligonucleotides which are introduced into cells do not represent new combinations of genetic material because their sequence is determined by the target sequence (Watson-Crick base pairing or Hoogsteen base pairing), in some cases with a deviation of one or a few nucleotides. The inserted oligonucleotides are not recombinant nucleic acids according to § 3(3a)(a) GenTG. The same assessment results according to Annex I A, Part 1, No. 1 of Directive 2001/18/EC and Annex I, Part A, No. 1 of Directive 2009/41/EC.*
  - *The oligonucleotides including the chemically modified nucleic acids and derivatives do not constitute genetic material or heritable material according to § 3(3a)(b) GenTG. The same assessment results according to Annex I A, Part 1, No. 2 of Directive 2001/18/EC and Annex I, Part A, No. 2 of Directive 2009/41/EC.*
  - *The oligonucleotides act as mutagens inducing mutations of one or a few NPs as can also occur spontaneously or following the application of mutagens, and can therefore not be differentiated from spontaneous mutations or from mutations induced by mutagenesis. Genetic variants produced by mutagens are not GMOs according to point (a) of the second sentence of § 3(3b) GenTG (mutagenesis). The same assessment results according to Annex I B of Directive 2001/18/EC and Annex II Part A, No. 1 of Directive 2009/41/EC.*
  - *Organisms which have been generated using the ODM technique are not GMOs.*

3.2.3. Belgium

- In 2007 the Belgian Biosafety Advisory Council issued an advice on the use of "Targeted Gene Repair" as a strategy to develop novel organisms. This covers the use of RNA/DNA or DNA/DNA molecules, single stranded DNA oligonucleotides containing phophorotioate linkages at the 5' and/or 3' ends or triplex-forming oligonucleotides.
- The main conclusion are that the BBAC:
  - *Notes that the terminology "Targeted Gene Repair" covers a range of different techniques and applications and is therefore not ideal for specific statements or rules.*
  - *Concludes that the technology must be considered as leading to genetic modification.*
  - *Notes that the technology is not used for introducing new genes in organisms but for altering chromosomal or episomal sequences in situ in their natural genetic background.*
  - *Notes that the technology does not use integrative vectors and thus the risk of insertional mutagenesis associated with them is eliminated.*
  - *Concludes that the targeted gene repair technology is a form of mutagenesis, a technology which is excluded from the scope of the regulation.*
  - *Notes that the technology makes use of oligonucleotides which should not be considered as being recombinant nucleic acid molecules.*
  - *Notes that the targeted gene repair is in many cases more precise than other mutational techniques such as irradiation, or chemical treatment, which should lead to less unintended effects.*

- *Notes however that the reliability and reproducibility of the technology show great variability and that further studies are still needed to gain a better knowledge on the mechanisms of action at the molecular level.*
- *Notes that the risks of potential side effects in the genome of the recipient cells should not be neglected.*
- *Notes that organisms developed through the targeted gene repair technology can not be distinguished at molecular level from those developed through "traditional" mutation technology.*

3.2.4. The Netherlands

- In 2010 the Dutch Commission on Genetic Modification (COGEM) issued an advice and report on "The status of oligonucleotides within the context of site-directed mutagenesis". This is an important position as part of the discussion on the GMO status concerns the criterion if recombinant nucleic acids are used in the technique.
- The conclusions of this report include:
  - *According to COGEM the most important consideration is whether there is a combination of sequence orders in the oligonucleotide that do not naturally occur next to each other. Furthermore, it is dependent on the environment (the cell) into which the oligonucleotide is inserted. If a sequence is identical to the sequence order in the genome (of nucleus, chloroplast or mitochondrion) of the recipient cell, this sequence is not a recombinant nucleic acid. This is in line with the previous advice of COGEM on mutagenesis using oligonucleotides coupled to a mutagen (chemical or radioisotope). The sequence of the oligonucleotide is identical to the genome sequence. Accordingly, this is a type of classical mutagenesis, which is exempt from GMO legislation.*
  - *Targeted mutagenesis (without chemical or radioactive mutagens) uses nucleotides that contain mutations relative to the known genome sequence. COGEM points out that differences do exist between sequences of individuals of one species. Additionally, several points mutations in a sequence cannot be seen as a sequence originating from a different source, and therefore no recombination takes place. COGEM is therefore of the opinion that the sequence of an oligonucleotide does not need to be fully identical to the known natural sequence to be regarded as non-recombinant. As an arbitrary limit at which an oligonucleotide should not be considered a recombinant nucleic acid, a difference of one in twenty nucleotides could be used.*

3.2.5. UK

- March 2011 the UK Advisory Committee on Releases to the Environment issued the following advice on ***RTDS™***, a plant breeding technique involving oligo-directed mutagenesis:
  - *ACRE considers that herbicide tolerant (HT) oilseed rape plants produced by Cibus LLC have been developed using a form of mutagenesis. It considers that this technique does not involve the use of recombinant nucleic acid molecules. Consequently, the HT oilseed rape plants could be excluded from the GMO Deliberate Release legislation in accordance with Annex 1B of Directive 2001/18/EC.*
- This opinion is further confirmed in an advice on New Breeding Techniques (ACRE, 2013);
  - *...the ODM technique does not fall in the category of techniques that are not considered to result in genetic modification (Annex I A, Part 2 of Directive 2001/18/EC). It is not captured by Part 1 of Annex 1A in that it is not a recombinant nucleic acid technique (the oligonucleotide is a synthetic molecule not generated by recombinant techniques, and does not involve the insertion of foreign DNA into a genome).*
  - *However, Part 1 of Annex 1A is not comprehensive and ODM is a form of mutagenesis, which is referred to in Annex 1B. ACRE"s view is that if ODM is defined as a GM technique, then the organisms produced should be excluded from the legislation. ACRE advises that oligonucleotides that are used in site-directed mutagenesis should not be considered as being recombinant nucleic acid molecules and thus ODM is captured by Annex 1B.*

3.3. Publications

- In a 2009 commentary, Breyer et al. describe regulatory and safety issues associated with the use of oligonucleotide-mediated mutagenesis to develop novel organisms. They present scientific arguments for not having organisms developed through this technique fall within the scope of the EU regulation on GMOs:
  - *... ODM must be considered as leading to genetic modification in the meaning of the EU Directives. However, it is important to note that the technique does not involve homologous recombination, and is not used for introducing new genes in organisms, but for altering chromosomal or episomal sequences in situ in their natural genetic background.*
  - *ODM uses oligonucleotides, which should not be considered as being recombinant nucleic acid molecules.*
  - *ODM should be considered as a form of mutagenesis, a technique which is excluded from the scope of the EU regulation.*
  - *ODM is more specific than recombinant DNA technology and other mutational techniques, such as irradiation or chemical treatment, which makes the risk to generate unintended effects in the genome of the recipient cells very unlikely, provided that the oligonucleotide structure and chemistry are properly designed.*
  - *The reliability, efficacy and reproducibility of ODM show nevertheless a great variability, and further studies are still needed to improve the efficiency of mediating mutations, the effectiveness of their detection, and the knowledge on the mechanisms of action at the molecular level.*
  - *Organisms developed through ODM can in many cases not be distinguished at the molecular level from those developed through "traditional" mutation techniques or from wild-type organisms, thus challenging the enforcement of the EU detection and identification rules for GMOs.*

4. References

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- ZKBS (2012) Position statement of the ZKBS on new plant breeding techniques Ref. No. 402.45310.0104

[redacted]

Von: Ehlers, Dr. Ulrich
Gesendet: Freitag, 1. August 2014 15:27
An: Gentechnik
Cc: Leggewie, Dr. Georg
Betreff: WG: Cibus request for an opinion on RTDS technique
Anlagen: RE: Cibus Field Trials; Swedish CA - RTDS description March 19 2012.pdf; USDA Dossier updated - Jan 2011.pdf

f.d. Bg 4/8/14

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Bitte GG.

U. Ehlers

-----Ursprüngliche Nachricht-----
Von: Staffan Eklöf [mailto:Staffan.Eklof@jordbruksverket.se]
Gesendet: Freitag, 1. August 2014 13:22
An: Ehlers, Dr. Ulrich
Cc: Carina Knorpp
Betreff: SV: Cibus request for an opinion on RTDS technique

Dear Ulrich,
I attach an e-mail conversation that covers the additional requirements and a pdf-file that was an attachment. It seems as if we had a bit different material to start with when I read the pdf-file you sent and compare it to our material. I therefore attach also a document that originally is the application to the USDA, a document that we received early.
Please return with questions if you like Ulrich.
I hope you can have some holidays to, best regards, Staffan

Staffan Eklöf, PhD.
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SE-551 82, Jönköping, Sweden
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e-mail: staffan.eklof@jordbruksverket.se

Thanks a lot!

It is not urgent, beginning of August would be fine with me. If possible I would like to get information about what additional data on the plants/plant line you asked for.

Enjoy your holiday!

Ulrich

Dr. Ulrich Ehlers

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-----Ursprüngliche Nachricht-----
Von: Staffan Eklöf [mailto:Staffan.Eklof@jordbruksverket.se]
Gesendet: Mittwoch, 23. Juli 2014 21:22
An: Ehlers, Dr. Ulrich
Betreff: SV: Cibus request for an opinion on RTDS technique

Dear Ulrich,
I answer the questions.
1. There is no decision. There is the letter to Cibus. I understand that you have it allready.
2. No.
3. Yes, we did.

Please tell me if you would like to know more. If you need documents I can send them 1/8. Bur if it is urgent I will try to do it earlier.

Have a nice sumer, best regards,
Staffan

Från: Ehlers, Dr. Ulrich [ulrich.ehlers@bvl.bund.de]
Skickat: den 22 juli 2014 10:49
Till: Staffan Eklöf; Heléne Ström
Kopia: 400@bvl.bund.de; georg.leggewie@bvl.bund.de
Ämne: WG: Cibus request for an opinion on RTDS technique

Dear Staffan, dear Helene,

I would appreciate if you could answer the questions (# 1 to # 3) I sent to Carina Knorpp on July 15th (see my email below). I have attached the document we received from Perseus on behalf of Cibus.

Perseus meanwhile sent us the letter of 2012-07-19 from the Swedish Board of Agriculture, so it is clear that it was issued for a specific plant line and not for the RTDS technique in general.

Kind regards and best wishes,
Ulrich

Dr. Ulrich Ehlers

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[redacted]

Von: Peter Beetham [redacted]
Gesendet: Mittwoch, 18. April 2012 02:24
An: Jenny Andersson
Cc: Patrick Rüdelsheim; Staffan Eklöf; Jim Radtke
Betreff: RE: Cibus Field Trials

Dear Jenny,

Thanks for your email. Please see my answers to your questions set out below with your questions. As always if you need more information please don't hesitate to email or call me.

Kind regards

Peter

From: Jenny Andersson [mailto:Jenny.Andersson@jordbruksverket.se]
Sent: Friday, April 13, 2012 4:27 AM
To: Peter Beetham
Cc: Patrick Rüdelsheim; Staffan Eklöf
Subject: SV: Cibus Field Trials

Dear Peter,

I have to ask for some clarification of the information in the message below and in the attachment, regarding which lines you want to use in the field trial. I'm sorry to come with these questions at this time point, but I think that they should be easy to answer.

In the attachment you describe the development of the line [redacted]

Then you describe that Cibus canola lines [redacted]

In the E-mail you state that the [redacted]

Was the [redacted] developed by the RTDS technique? If yes, describe [redacted]

[redacted]

Is Cibus canola [redacted]

[redacted]

Is Cibus canola [redacted] derived from traditional breeding methods not involving the RTDS technique?

[redacted]

[redacted]

[redacted]

Kind regards,

Jenny

.....

Jenny Andersson

Swedish Board of Agriculture

Plant and Environment Department

Plant Regulations Division

SE-551 82 Jönköping, Sweden

+46 36 15 57 22

From: Jenny Andersson [mailto:Jenny.Andersson@jordbruksverket.se]
<mailto:[mailto:Jenny.Andersson@jordbruksverket.se]>
Sent: Thursday, February 23, 2012 6:17 AM
To: Peter Beetham
Cc: Patrick Rüdelsheim; Jim Radtke; Staffan Eklöf
Subject: SV: Cibus Field Trials

Dear Peter,

We will have to assess whether your plants fall within the scope of Directive 2001/18/EC. If they do not, there is nothing to approve, hence no procedure exists.

In order for us to assess this, I would like you to provide some information on the plants that you would like to use in the trial. Specifically, information on how the RTDS technique was applied in this particular case. [redacted].

This will enable us to decide on your application of the technique, and will not require a statement from us on all potential applications of the technique.

Kind regards,

Jenny

Från: Jenny Andersson
Skickat: den 1 februari 2012 16:56
Till: 'Peter Beetham'
Kopia: Patrick Rüdelsheim; Jim Radtke; carina.knorpp@rural.ministry.se <mailto:carina.knorpp@rural.ministry.se> ; marie.nyman@genteknik.se <mailto:marie.nyman@genteknik.se> ; Staffan Eklöf
Ämne: SV: Cibus Field Trials

Dear Peter,

Thank you for your message. We will discuss this matter and will contact you as soon as possible.

Best regards,

Jenny

. . . . . .

Jenny Andersson

Jordbruksverket

Växt- och miljöavdelningen

Regelenheten

551 82 Jönköping

036-15 57 22

Från: Peter Beetham [redacted]
Skickat: den 30 januari 2012 21:29
Till: Jenny Andersson; Staffan Eklöf; marie.nyman@genteknik.se <mailto:marie.nyman@genteknik.se> ; carina.knorpp@rural.ministry.se <mailto:carina.knorpp@rural.ministry.se>
Kopia: Patrick Rüdelsheim; Jim Radtke
Ämne: Cibus Field Trials

Dear Jenny, Staffan, Marie and Carina,

As discussed with you in December, I have attached the USDA letter (the PDF is low resolution from the original) as you will see it is very brief and to the point! This letter was addressed to [redacted]

The second document is an updated version of the dossier we submitted to the USDA. This dossier was created in response to the USDA panels questions about our RTDS technology. Essentially the three major questions were – 1. Are the oligonucleotides incorporated into the genome?, 2. How long do the oligonucleotides last? and 3. Please explain the current understanding of the mechanism and why we believe it is a mutagenesis technology.

[redacted]

Based on the information provided previously, the discussions we have had and this attached documents, is it possible for you and your team to guide us on the next steps for us to receive approval (if needed) for the unconfined release of our herbicide spring oilseed rape in Sweden?

[redacted]

Please do not hesitate to call or email me if you have some initial feedback.

Kind regards and thank you so much for your consideration.

Peter

Peter Beetham Ph.D.

Cibus

6455 Nancy Ridge Dr.

San Diego, CA 92121

USA

[redacted]

Website: www.cibus.com <http://www.cibus.com>

Cibus

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Phone 858-450-0008 Fax 858-450-2626

RTDS Method for Oilseed Rape

At Cibus we have developed the ***RTDS***™ (Rapid Trait Development System) technology (described in detail at www.cibusllc.com) to achieve targeted mutations in plant genes (Beetham *et al.*, 1999; Zhu *et al.*, 1999).

[redacted]

The ***RTDS*** process provides a means to make targeted single nucleotide changes in specific genes without the incorporation of foreign DNA providing a non-transgenic approach to achieving the desired trait. This technology utilizes the naturally occurring repair machinery that exists in all living cells to specifically modify gene sequence *in situ* and without inserting foreign DNA and control sequences. This process effects a precise change in the genetic sequence of the target gene and leaves the rest of the genome unaltered.

RTDS uses a specially designed element, a Gene Repair Oligonucleotide, called a "GRON", to direct a cell's own DNA-repair system to make a specific desired change in the targeted gene. The GRON that effects this change is a chemically synthesized oligonucleotide composed of DNA bases as well as other non-naturally occurring chemical moieties, and is designed to hybridize at the targeted gene location to create a mismatched base-pair. This mismatched base-pair acts as a beacon to attract the cell's repair system to that site to correct (replace, insert or delete) the designated nucleotide(s) within the gene. Once the correction process is complete the GRON is degraded and the corrected gene continues to be expressed under that gene's endogenous control mechanisms. Once this change is made it is heritable in each subsequent generation (Zhu *et al.*, 1999).

[redacted]

Cibus

6455 Nancy Ridge Drive, #100, San Diego, CA 92121, USA
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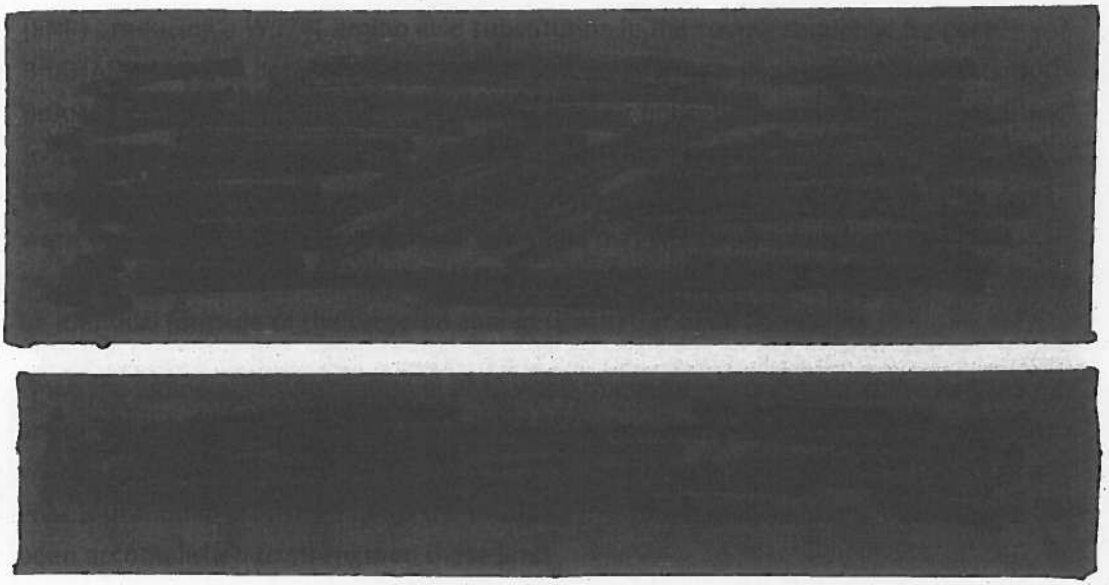

References

Beetham, P.R., Kipp, P.B., Sawycky, X.L., Arntzen, C.J. and May, G.D. (1999). A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause in vivo gene-specific mutations. *Proc. Natl Acad Sci. USA,* 96, 8774-8778.

Dovzhenko, A. and Koop, H.U. (2003). Sugarbeet (Beta vulgaris L.): shoot regeneration from callus and callus protoplasts. *Planta* 217, 374-381.

Zhu, T., Mettenberg, K., Peterson, D.J., Tagliani, L., and Baszczynski, C.L. (2000). Engineering herbicide resistant maize using chimeric RNA/DNA oligonucleotides. *Nature Biotechnology* 18, 555-558.

Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
Dienststelle Berlin • Postfach 11 02 60 • 10832 Berlin

1. Dr. Patrick Rüdelsheim
PERSEUS b.v.b.a
Technologiepark 3
B-9052 Zwijnaarde

Belgium

Dr. Ulrich Ehlers
Head of Unit

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E-MAIL 403@bvl.bund.de

IHR ZEICHEN
IHRE NACHRICHT VOM 18/07/2014

AKTENZEICHEN 42050 Regisdb. erl. 20/8/14
(bitte bei Antwort angeben)

DATUM 8. August 2014

Request for an opinion on field trials with oilseed rape material improved by Cibus using RTDS™

Dear Dr Rüdelsheim,

In response to your letter of 18/07/2014 we have identified the need for further information to be able to conclude on the request by Cibus. We, therefore, ask Cibus to provide the following information:

- [redacted]

If Cibus considers any information sent to BVL as confidential (CBI), that information should be clearly identified and a justification for the confidentiality should be provided.

Sincerely yours,

8/8 14

Dr. Ulrich Ehlers

2. Reinschrift als PDF vorab an [redacted], cc. AL4 i.V. Erledigt 11/8/14
3. Poststelle: Bitte Reinschrift verschicken. 11. AUG. 2014
4. 401, z. d. A.

Ehlers, Dr. Ulrich

1)

Von: Ehlers, Dr. Ulrich
Gesendet: Freitag, 8. August 2014 14:37
An: [redacted]
Cc: Bartsch, Detlef; 'georg.leggewie@bvl.bund.de'
Betreff: Request for opinion on RTDS
Anlagen: BVL_to_Perseus_14-08-08.pdf

Dear Dr. Rüdelsheim,

As a response to your request of 18/07/2014 please accept the attached letter asking for additional information on the RTDS technique and on oilseed rape[redacted]

Sincerely yours,

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
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D-10117 Berlin

9) z.d.A. Ch 8/8/14

[redacted]

Von: Leggewie, Dr. Georg
Gesendet: Mittwoch, 3. September 2014 11:22
An: Gentechnik
Betreff: WG: Request for opinion on RTDS
Anlagen: CBS-14-001 Letter Perseus BVL 140903.pdf; Cibus request for an opinion - addit. info 140903.pdf

i.V. Ba 3/9 14

Bitte in den GG.
Danke

Georg

BVL	Mauerstraße 39–42 10117 Berlin	Abt. 4
		Ref. 401
		402
	03. Sep. 2014	403
		404
Tgb.-Nr.:	2014 38139	
Az.:	42 050	405
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Bitte übernehmen 4/9 14

Leggewie legg 4/9 14
Matthies M 9/9/14

401: z.d.A.
Leggewie
ue. jo

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim[redacted]
Gesendet: Mittwoch, 3. September 2014 10:46
An: Ehlers, Dr. Ulrich
Cc: Bartsch, Detlef; Leggewie, Dr. Georg
Betreff: Re: Request for opinion on RTDS

Dear Dr. Ehlers, Dear Ulrich,

I enclose our answers to your letter of early August.
Can you confirm that you received the two files in good order?
Let us know if we can provide any further support to this process.

Kind regards, Patrick

Patrick Rüdelsheim
[redacted]

PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel./Fax: +32 (0)9 321 07 05
[redacted]
Website: www.perseus.eu <http://www.perseus.eu/>

Op 8-aug.-2014, om 14:37 heeft Ehlers, Dr. Ulrich <ulrich.ehlers@bvl.bund.de <mailto:ulrich.ehlers@bvl.bund.de> > het volgende geschreven:

Dear Dr. Rüdelsheim,

As a response to your request of 18/07/2014 please accept the attached letter asking for additional information on the RTDS technique and on oilseed rape[redacted]

Sincerely yours,

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
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<BVL_to_Perseus_14-08-08.pdf>

perseus

Bundesamt für Verbraucherschutz
und Lebensmittelsicherheit
Abteilung 4: Gentechnik
Mauerstrasse 39-42
D-10117 Berlin
Germany

biosafety and biotechnology regulatory services

03/09/2014

Dear,

Re: Request for an opinion on field trials with oilseed rape material improved by Cibus using *RTDS*™ – additional information (your reference 42050)

Attached we submit additional information provided by Cibus on the points formulated in your letter of 8 August 2014. We hope that this information allows you to proceed with the evaluation of the request for an opinion, but irrespectively Cibus staff remains available for further inquiries. They asked me to confirm their willingness to present the information in person if such an occasion would be presented.

We like to point out that some of the information in this document is detailed and has not been shared publicly. It is considered by Cibus as confidential since it provides unpublished information that could guide competitors to enhance their own technologies. Based on this, a general claim on confidentiality is indicated. While the authorities are given access to obtain a complete picture on all relevant information, it is understood that access remains limited to this use.

Looking forward to your reply.

Sincerely yours,

Dr. Patrick RÜDELSHEIM

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CONFIDENTIAL

Cibus Global

Memorandum – Additional information on the request for an opinion

Prepared by the Cibus Technical Team
24 August 2014

Background

On 18 July, Perseus submitted on behalf of Cibus a request for an opinion on field trials with oilseed rape material improved by Cibus using ***RTDS***. The request was supported by an initial information package.

Following a first review, the authority identified certain points, which required further information. These were specified in the letter from Dr. U. Ehlers dated August 8th 2014.

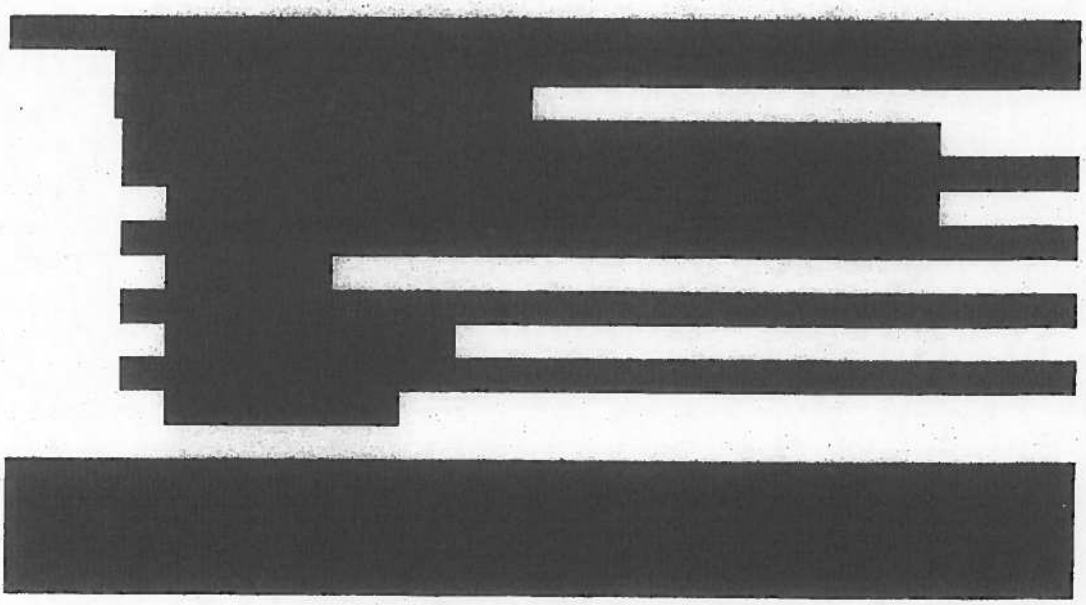
This document provides further information on each of the points raised in the letter and as such complements the initial information package. Some of the information in this document is detailed and has not been shared publicly. It is considered by Cibus as confidential since it provides unpublished information that could guide competitors to enhance their own technologies.

Based on this, a general claim on confidentiality is indicated on the following pages. While the authorities are given access to obtain a complete picture on all relevant information, it is understood that access remains limited to this use. In case, the authority would consider certain information to be of more general public interest, then Cibus wishes to have the opportunity to reconsider submission of such information.

CBI DELETED

1. Results of the molecular analysis of oilseed rape (OSR) line [redacted]

[redacted]



6455 Nancy Ridge Drive, Suite 100, San Diego, CA 92121
Phone: 858.450.0008 Fax: 858.450.2630

AHAS genes, showing clearly that the 574 mutation is the only location changed in these lines.

2. Information on the possibility that the use of [redacted] GRON may have caused heterotopic or off-target changes.

Heterotopic changes have only been seen when RNA/DNA chimeric oligonucleotides are used to direct mutagenesis. This type of oligonucleotide is not a component of the GRONs used in our experiments. The [redacted] GRON targeting 574 has shown no heterotopic or off-target changes in 15 independent experiments that were assayed using DNA sequencing.

3. Information on the half-life of GRON [redacted] in plant cells.

The half-life of a GRON is very important with respect to its ability to undergo recombination and persist in plant cells. Researchers studying ***RTDS*** in animal systems have shown that the half-life of a GRON is relatively short, but perhaps longer than that in plant cells. While these studies were not designed to measure the half-life of the GRON they do provide some evidence that GRONs are rapidly degraded. These studies showed that GRONs survived for less than 72 hours in mouse muscle. Other studies from animal systems show that GRONs are rapidly degraded within 48 to 72 hours *in vivo* and *in vitro*.

During the development of ***RTDS***, Cibus' scientists conducted several studies to measure the half-life of GRON in plants and in cell free extracts. [redacted]

[redacted]

The samples were extracted, precipitated overnight and run on a denaturing FAGE gel (A FAGE gel is a highly denaturing gel using formamide in the acrylamide gel). These gels are designed to ensure the GRONs run as linear nucleic acid molecules and produce clearly resolved bands on a gel, when stained with ethidium bromide. Figure 2 shows a photo of a gel from one of these experiments. [redacted]

[redacted] The additional bands are attributed to the various DNAs from the OSR cell extracts as clearly shown by the gel photo in Figure 2. This experiment has been repeated several times with the same results.

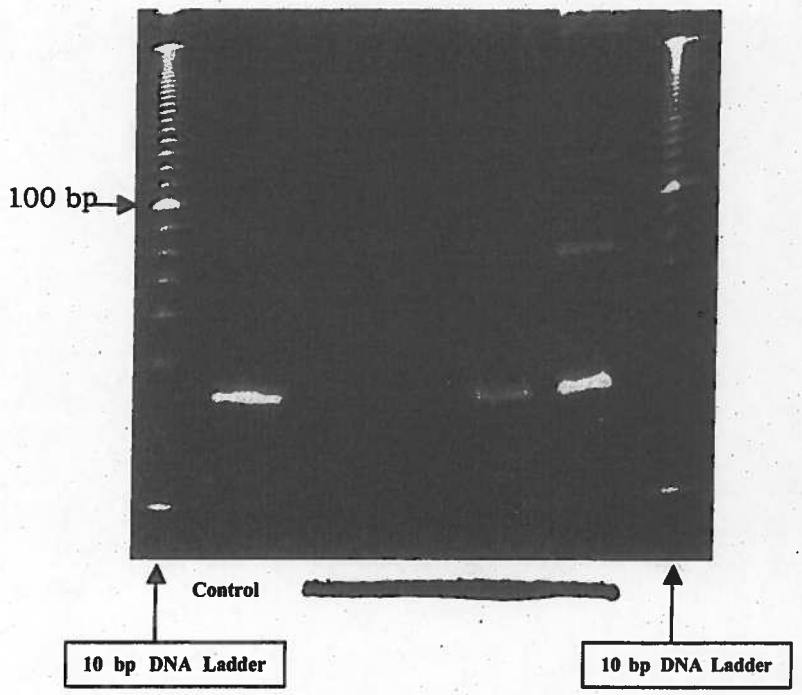


Fig. 2. GRONs on a denaturing FAGE (formamide acrylamide gel electrophoresis) gel showing the breakdown in OSR cells.

[redacted]

Another example is shown in Figure 3 where GRONs of the RNA/DNA structure where were collected over time just in the presence of small aliquots of an *Arabidopsis* cell suspension. Once again over time the GRONs quickly degrade.

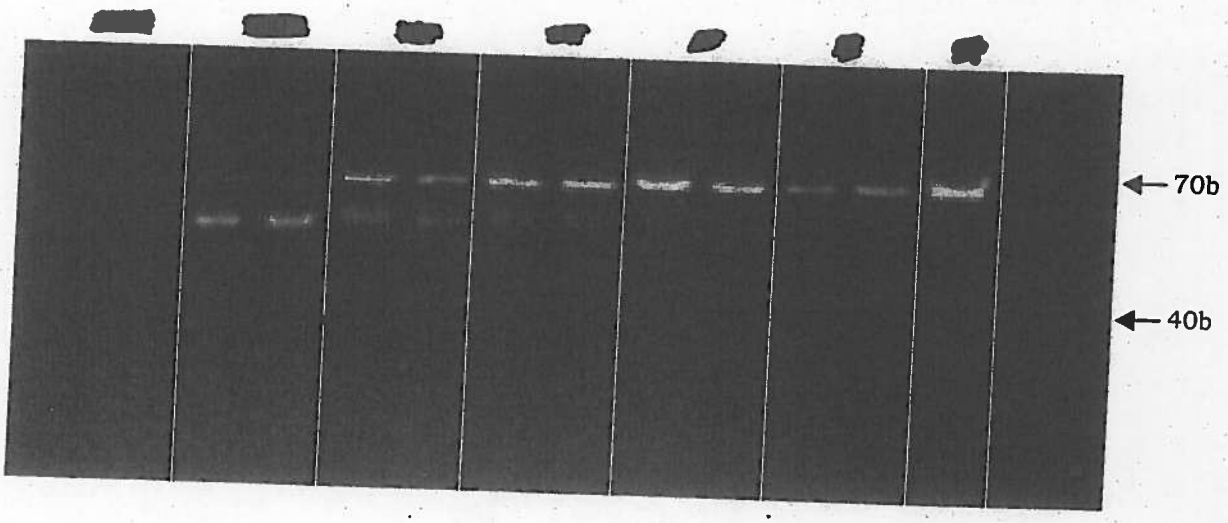
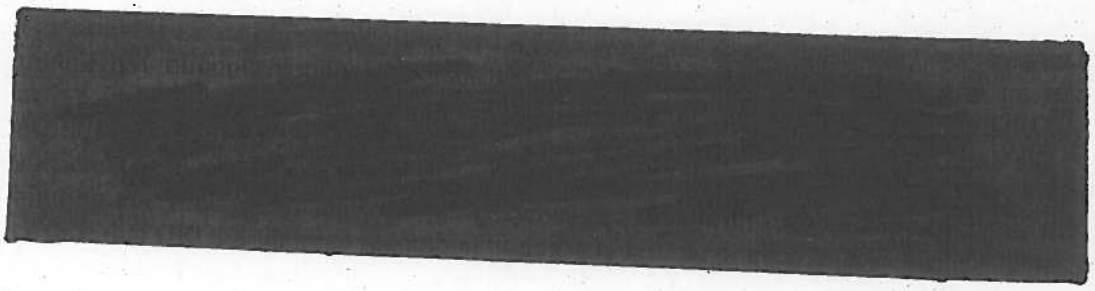


Fig. 3. This gel photo (ethidium bromide stained formamide acrylamide gel) shows the degradation of GRONs over time in the presence of a plant cell suspension. The GRONs are similar to the design in Figure 1A (this document) and the cell suspension is from *Arabidopsis*. The numbers on top of the gel photo represent the time of incubation of GRONs and cells in minutes. The arrows represent the size estimates based on the 10 bp DNA ladder on the far right of the photo.

4. Are data available to demonstrate that due to modifications of GRON ssDNA (e.g. 5'CY-dye and 3' reverse base idC) recombination of GRON into the plant genome is prevented?

Genes or DNA sequences used in recombination do have specifically designed ends that make them compatible for "splicing" into a plant's DNA with recombination. GRONs do not contain these primer sequences and so would not be incorporated into plant genes via recombination.



Aktenzeichen 42 050

Ehlers, Dr. Ulrich

1)

Von: Ehlers, Dr. Ulrich
Gesendet: Dienstag, 16. September 2014 11:00
An: 'Patrick Rüdelsheim'
Cc: Abt. 4; Matthies, Dr. Anastasia; Leggewie, Dr. Georg
Betreff: AW: Request for opinion on RTDS
Anlagen: BVL_to_Perseus_14-09-16.pdf

Dear Dr. Rüdelsheim, Dear Patrick,

I enclose a letter asking for further clarification of two points concerning the request by Cibus.

Kind regards,
Ulrich

Dr. Ulrich Ehlers

Federal Office of Consumer Protection and Food Safety (BVL) Unit 403 - Deliberate Release and Placing on the Market 403@bvl.bund.de

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Visitors address:
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D-10117 Berlin

7) z.d.A. Ch 16/9/14

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Mittwoch, 3. September 2014 10:46
An: Ehlers, Dr. Ulrich
Cc: Bartsch, Detlef; Leggewie, Dr. Georg
Betreff: Re: Request for opinion on RTDS

Dear Dr. Ehlers, Dear Ulrich,

I enclose our answers to your letter of early August.
Can you confirm that you received the two files in good order?
Let us know if we can provide any further support to this process.

Kind regards, Patrick

Patrick Rüdelsheim
[redacted]

PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel./Fax: +32 (0)9 321 07 05
[redacted]

[redacted]

Website: www.perseus.eu <http://www.perseus.eu/>

Op 8-aug.-2014, om 14:37 heeft Ehlers, Dr. Ulrich <ulrich.ehlers@bvl.bund.de <mailto:ulrich.ehlers@bvl.bund.de> > het volgende geschreven:

Dear Dr. Rüdelsheim,

As a response to your request of 18/07/2014 please accept the attached letter asking for additional information on the RTDS technique and on oilseed rape[redacted]

Sincerely yours,

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
Lebensmittelsicherheit (BVL)
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<BVL_to_Perseus_14-08-08.pdf>

[redacted]

Von: Ehlers, Dr. Ulrich
Gesendet: Montag, 27. Oktober 2014 09:16
An: Gentechnik
Betreff: WG: Request for opinion on RTDS
Anlagen: CBS-14-001 Letter Perseus BVL 141027.pdf; Cibus request for an opinion - addit. info 141027.pdf

i.V. Bo 27/10 14

BVL Mauerstraße 39–42 10117 Berlin | Abt. 4 | Ref. 401 | 402 | 403 | 404 | 405
27. Okt. 2014
Tgb.-Nr.: 2014/35938
Az.: 42050
Registr. erl. 15. 1. 15. Jo

28/10 14

Bitte GG.

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Montag, 27. Oktober 2014 09:00
An: Patrick Rüdelsheim
Cc: Ehlers, Dr. Ulrich; Abt. 4; Matthies, Dr. Anastasia; Leggewie, Dr. Georg
Betreff: Re: Request for opinion on RTDS

Matthies } AM 30/10 14 m.d.B.um Prüfung
Leggewie n.R. } Legg 29/10 14

Dear Dr. Ehlers, Dear Ulrich,

Please find attached the reply (letter + additional information) following your letter from 16/9.
Can you confirm that you received the 2 files in good order?

401: z.d.A ✓ Leggewie 31.10

We remain available for any further clarification.

Kind regards, Patrick

Patrick Rüdelsheim
General Partner

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Tel./Fax: +32 (0)9 321 07 05
[redacted]
Website: www.perseus.eu <http://www.perseus.eu/>

Op 16-sep.-2014, om 14:04 heeft Patrick Rüdelsheim [redacted] > het volgende geschreven:

Well received, Ulrich.

I will transmit and let you know ASAP.
I don't have a copy of all communications with the Swedish authority so I also look forward to see the consolidation of this information.

Kind regards, Patrick

Op 16-sep.-2014, om 11:00 heeft Ehlers, Dr. Ulrich <ulrich.ehlers@bvl.bund.de <mailto:ulrich.ehlers@bvl.bund.de> > het volgende geschreven:

Dear Dr. Rüdelsheim, Dear Patrick,

I enclose a letter asking for further clarification of two points concerning the request by Cibus.

Kind regards,
Ulrich

Dr. Ulrich Ehlers

Federal Office of Consumer Protection and Food Safety (BVL)
Unit 403 - Deliberate Release and Placing on the Market
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Visitors address:
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D-10117 Berlin

-----Ursprüngliche Nachricht-----
Von: Patrick Rüdelsheim [redacted]
Gesendet: Mittwoch, 3. September 2014 10:46
An: Ehlers, Dr. Ulrich
Cc: Bartsch, Detlef; Leggewie, Dr. Georg
Betreff: Re: Request for opinion on RTDS

Dear Dr. Ehlers, Dear Ulrich,

I enclose our answers to your letter of early August.
Can you confirm that you received the two files in good order?
Let us know if we can provide any further support to this process.

Kind regards, Patrick

Patrick Rüdelsheim
[redacted]

PERSEUS bvba, Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel./Fax: +32 (0)9 321 07 05
[redacted]

[redacted]
Website: www.perseus.eu <http://www.perseus.eu/> <http://www.perseus.eu/>

Op 8-aug.-2014, om 14:37 heeft Ehlers, Dr. Ulrich <ulrich.ehlers@bvl.bund.de <mailto:ulrich.ehlers@bvl.bund.de> <mailto:ulrich.ehlers@bvl.bund.de> > het volgende geschreven:

Dear Dr. Rüdelsheim,

As a response to your request of 18/07/2014 please accept the attached letter asking for additional information on the RTDS technique and on oilseed rape[redacted]

Sincerely yours,

Dr. Ulrich Ehlers

Bundesamt für Verbraucherschutz und
Lebensmittelsicherheit (BVL)
Referat 403 - Freisetzung und Inverkehrbringen

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<BVL_to_Perseus_14-08-08.pdf>

<BVL_to_Perseus_14-09-16.pdf>

perseus

Bundesamt für Verbraucherschutz
und Lebensmittelsicherheit
Abteilung 4: Gentechnik
Mauerstrasse 39-42
D-10117 Berlin
Germany

biosafety and biotechnology regulatory services

27/10/2014

Dear,

Re: Request for an opinion on field trials with oilseed rape material improved by Cibus using *RTDS*™ – additional information (your reference 42050)

Attached we submit additional information provided by Cibus on the points formulated in your letter of 16 September 2014.

[redacted] We hope that the attached provides clarity on the nature of the material as well as on intended activities. In this respect, I was asked to stress again that Cibus' staff remains available for further inquiries and presentations.

We like to point out that some of the information in this document is detailed and has not been shared publicly. It is considered by Cibus as confidential since it provides unpublished information that could guide competitors to enhance their own technologies. Based on this, a general claim on confidentiality is indicated. While the authorities are given access to obtain a complete picture on all relevant information, it is understood that access remains limited to this use.

Looking forward to your reply.

Sincerely yours,

Dr. Patrick RÜDELSHEIM [redacted]

PERSEUS b.v.b.a • Technologiepark 3, B-9052 Zwijnaarde, Belgium
Tel / Fax +32-(0)9-321.07.05 • [redacted]

CONFIDENTIAL

Cibus Global

Memorandum – Additional information on the request for an opinion

Prepared by the Cibus Technical Team
27 October 2014

Background

On 18 July, Perseus submitted on behalf of Cibus a request for an opinion on field trials with oilseed rape material improved by Cibus using ***RTDS***. The request was supported by an initial information package.

Following an initial exchange providing additional information, further clarifications were requested in the letter from Dr. U. Ehlers dated September 16th 2014 with reference 42050.

This document provides further information on each of the points raised in the letter and as such complements the initial information package. Some of the information in this document is detailed and has not been shared publicly. It is considered by Cibus as confidential since it provides unpublished information that could guide competitors to enhance their own technologies.

Based on this, a general claim on confidentiality is indicated on the following pages. While the authorities are given access to obtain a complete picture on all relevant information, it is understood that this access is limited to this use. In case, the authority would consider certain information to be of more general public interest, then Cibus wishes to have the opportunity to reconsider submission of such information.

6455 Nancy Ridge Drive, Suite 100, San Diego, CA 92121
Phone: 858.450.0008 Fax: 858.450.2630

1. [redacted]

In the course of a programme of experiments Cibus assembled a collection of rapeseed lines with mutations in the AHAS gene that can influence tolerance to various ALS inhibitors. The collection comprises mutations at several locations within the AHAS gene, a number of different mutations at each location and examples of these various mutations in both the A and the C genome (BnAHAS IIIA and BnAHAS IC respectively). Our testing programme evaluates these mutations alone and in combination, within the same genome and in different genomes (*i.e.* A & C) and in homozygous and heterozygous form.

The majority of the mutations in the collection are the direct result of ***RTDS*** GRON targeted mutagenesis experiments. A small number of somatic mutations were also isolated from our programme using ALS inhibiting herbicide selection. [redacted]

Within the collection of rapeseed lines we have examples of the same mutation generated independently by GRON targeting and via somatic mutation. These lines cannot be

CF (Clearfield) represents the SP Cougar Clearfield variety, which has a PM1 mutation (AGT to AAT) in BnAHAS I C and a PM2 mutation in BnAHAS IIIA. [redacted]

[redacted]

[redacted]

3. **Glossary of Terms**

Term	Meaning
[redacted]	[redacted]
BnAHAS I C	Gene for the catalytic subunit of acetohydroxyacid synthase located on the *Brassica napus* C genome
BnAHAS III A	Gene for the catalytic subunit of acetohydroxyacid synthase located on the *Brassica napus* A genome
BnAHSY I	Synonym of BnAHAS I C
BnAHSY III	Synonym of BnAHAS III A
[redacted]	
GRON	Gene Repair OligoNucleotide
ODM	Oligonucleotide Directed Mutagenesis
[redacted]	[redacted]
[redacted]	[redacted]
RTDS	Rapid Trait Development System
[redacted]	[redacted]
WT	Wild type

Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Entwurf

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
Dienststelle Berlin • Postfach 11 02 60 • 10832 Berlin

1.) Geschäftsstelle der ZKBS
Dr. Inge Kruczek

Im Hause

Dr. Ulrich Ehlers
Referatsleiter

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TELEFAX +49 (0)30 18445-6099
E-MAIL gentechnik@bvl.bund.de
INTERNET www.bvl.bund.de

IHR ZEICHEN
IHRE NACHRICHT VOM

AKTENZEICHEN 42050 Regisdb. erl. 10 3/6 15
(bitte bei Antwort angeben)

DATUM 24.11.2014

Stellungnahme der Zentralen Kommission für die Biologische Sicherheit

Feststellungsantrag der Firma Cibus Europe (Kapelle, Niederlande) vom 18.07.2014

Sehr geehrte Frau Dr. Kruczek,

mit Schreiben vom 18.07.2014 hat die Firma Cibus Europe (Kapelle, Niederlande) beim BVL einen Antrag gestellt, in welchem festgestellt werden soll, dass die mittels RTDS™ entwickelten Rapslinien nicht als GVO im Sinne des GenTG einzustufen sind und die geplanten Feldversuche daher ohne den für GVO erforderlichen Genehmigungsantrag durchgeführt werden können. RTDS™ gehört zu den Neuen Techniken in der Pflanzenzüchtung, über deren regulatorischen Status in der EU noch nicht abschließend entschieden wurde. Im Juni 2012 hat die ZKBS eine Stellungnahme verfasst um zu klären, ob die aus den Neuen Techniken der Pflanzenzüchtung resultierenden Organismen im Sinne der bestehenden Rechtsvorschriften gentechnisch verändert sind. Vor diesem Hintergrund bitten wir Sie um eine Bewertung der RTDS™-Technologie sowie um eine Stellungnahme, ob die damit hergestellten Rapslinien gentechnisch veränderte Organismen im Sinne des GenTG sind.

Mit freundlichen Grüßen

im Auftrag

24/11/14

U. Ehlers

Anlage: Kopie des Antrages

2) Original per Hauspost an Adressatin
3) z. d. A.

Dienstsitz Braunschweig
Bundesallee 50, Geb. 247
38116 Braunschweig
Tel: +49 (0)531 21497-0
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Stellungnahme der ZKBS zu mittels RTDS (Rapid Trait Development System) hergestellten herbizidresistenten Rapslinien der Firma Cibus.

Anlass

Die Firma Cibus Europe (Kapelle, Niederlande) beabsichtigt, Feldversuche mit herbizidresistenten Rapspflanzen (*Brassica napus L*) durchzuführen, die mittels der sogenannten RTDS™-Technik entwickelt wurden. Im Vordergrund stehen dabei Leistungsprüfungen und die Saatgutproduktion für weitere Untersuchungen.

Firma Cibus hat das Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) um eine Stellungnahme gebeten, in welcher festgestellt werden soll, dass die mittels RTDS™ entwickelten Rapslinien nicht als GVO im Sinne des GenTG einzustufen sind und die geplanten Feldversuche daher ohne den für GVO erforderlichen Genehmigungsantrag durchgeführt werden können.

RTDS™ gehört zu den Neuen Techniken in der Pflanzenzüchtung, über deren regulatorischen Status in der EU noch nicht abschließend entschieden wurde. Im Juni 2012 hat die ZKBS eine Stellungnahme verfasst um zu klären, ob die aus den Neuen Techniken der Pflanzenzüchtung resultierenden Organismen im Sinne der bestehenden Rechtsvorschriften gentechnisch verändert sind. Vor diesem Hintergrund bittet das BVL die ZKBS um eine Bewertung der RTDS™-Technologie sowie eine Stellungnahme, ob die damit hergestellten Rapslinien gentechnisch veränderte Organismen im Sinne des GenTG sind.

Die Firma Cibus hat auch bei den zuständigen Behörden des Vereinigten Königreichs, Schwedens und [redacted] diesbezüglich Stellungnahmen eingeholt und diese vorgelegt. Übereinstimmend stufen die o.g. Behörden die mittels RTDS™-Technologie entwickelten Rapslinien nicht als GVO ein.

Beschreibung der RTDS™-Technologie

Die RTDS™-Technologie erlaubt es, zielgerichtete Mutationen im Pflanzengenom herbeizuführen. Dabei kommt es zum Austausch einzelner Nukleotide in den Zielgenen, ohne dass die Fremd-DNA in das Pflanzengenom integriert wird. Bei der Anwendung der RTDS™-Technologie werden zelleigene DNA-Reparaturmechanismen ausgenutzt. Das Schlüsselelement dieser Technologie ist ein chemisch synthetisiertes Oligonukleotid, das sogenannte Gene Repair Oligonucleotide (GRON). Die Sequenz dieses Nukleotids ist, mit Ausnahme von einem oder zwei Nukleotiden, komplementär zu der Zielsequenz im Genom und führt zur ortsspezifischen Hybridisierung des GRONs mit der Ziel-DNA in der Zelle. Die

im GRON eingebauten, von der Zielsequenz abweichenden Nukleotide führen dabei zu einer DNA-Fehlpaarung. Wird diese Fehlpaarung erkannt, so kommt es nach allgemeiner Vorstellung zur Veränderung der Ziel-DNA, indem die fehlgepaarten Basen im Zielgen ausgetauscht werden, wobei GRON als Template dient. Nach dem erfolgten Austausch wird GRON abgebaut. [redacted] Gleichzeitig verhindern diese Modifikationen vermutlich die Interaktion von GRON mit den für die Rekombination notwendigen DNA-bindenden Proteinen und Enzymen und damit die Integration von GRON in das Pflanzengenom. [redacted] Die hohe Spezifität des GRONs liegt in seiner Basensequenz begründet, welche sich nach der Zielsequenz des Zielgens richtet. Nach [redacted] Die hohe Spezifität von RTDS wurde in einer Studie mit Tabakpflanzen demonstriert (3).

Beschreibung der zu testenden Rapslinien

Bei den zu testenden Rapslinien handelt es sich um imidazolinon- und sulfonylharnstoffresistenten Raps. Die Herbizidresistenz basiert auf einer Mutation im Acetolactatsynthase-Gen. Acetolactatsynthase (ALS) ist ein Schlüsselenzym in der Synthese der verzweigten Aminosäuren. Das für ALS kodierende Gen (*AHAS*) ist sowohl im A-Genom (*AHASIIA, AHASIIIA, AHASIVA*) als auch im C-Genom (*AHASIC, AHASVIC*) des Rapses lokalisiert, wobei nur *AHASIIIA* und *AHASIC* konstitutiv exprimiert und für die katalytische Aktivität des Enzyms essentiell sind (4). Die katalytische Untereinheit der ALS bildet ein Dimer, welches als Homodimer (Produkt von *AHASIC*, C+C oder von *AHASIIIA*, A+A) oder als Heterodimer (Produkt von *AHASIC* und *AHASIIIA*, C+A) vorliegen kann (5). Die Inhibierung von ALS durch Imidazolinone und/oder Sulfonylharnstoffe führt zum Absterben der Pflanze, da die Wirkstoffe die Bindungsstelle im katalytischen Zentrum des Enzyms blockieren und damit die Synthese der verzweigten Aminosäuren verhindern. Mutationen in den katalytischen Untereinheiten des Gens können jedoch Toleranz gegenüber diesen Wirkstoffen bewirken. So führt der Austausch von Serin zum Asparagin an der Position 653 (PM1-Mutation) zur Resistenz gegenüber Imidazolinon, der Austausch von Trypthophan zum

Leucin an der Position 574 (PM2) führt zur Resistenz gegenüber Imidazolinon und Sulfonylharnstoffen. In der Vergangenheit wurden bereits imidazolinonresistente Rapslinien entwickelt, in welchen die Mutationen PM1 im Gen *AHASIC* bzw. PM2 im Gen *AHASIIIA* z.B. durch chemische Mutagenese eingeführt wurden (4). [redacted]

Bewertung

RTDS™ stellt eine Technologie dar, bei der keine integrativen Vektorensysteme genutzt bzw. neue Kombinationen des genetischen Materials in das Pflanzengenom integriert werden oder in der Zelle verbleiben. RTDS™ führt zu ortspezifischen Punktmutationen im Pflanzengenom, wobei endogene Reparatur- und Regulationsmechanismen genutzt werden. Das bei RTDS™ eingesetzte Nukleotid GRON ist keine rekombinante Nukleinsäure; seine Eigenschaften wie Struktur, chemischer Aufbau und kurze Halbwertszeit verhindern dessen Integration in das Pflanzengenom. Das GenTG und die RL 2001/18/EG und RL 2009/41/EG definieren rekombinante Nukleinsäure als neukombiniertes genetisches Material. In ihrer Stellungnahme vom Juni 2012 vertritt ZKBS die Meinung, dass ein Segment mindestens 20 Nukleotidpaare (NP) umfassen muss, um zu einer rekombinanten Nukleinsäure zu führen. Eine absichtliche Änderung von weniger als 20 NP kann von dem zufälligen Vorkommen dieser Sequenz nicht hinreichend sicher unterschieden werden. Bestimmte Sequenzen von weniger als 20 NP können zwar nachgewiesen werden, eignen sich jedoch nicht zur Bestimmung ihrer Herkunft. Sie sind nicht von den durch konventionelle Mutagenese oder durch zufällige natürliche Mutation entstandenen genetischen Veränderungen zu unterscheiden. Bei der RTDS™-Technik werden Mutationen in das Pflanzengenom eingeführt, die über 1 oder 2 NP nicht hinausgehen und damit von der spontanen Mutation nicht zu unterscheiden sind. Die durch Mutagenese-Verfahren induzierten Mutationen gelten gemäß § 3 Nr. 3b. Satz 2 Buchst. a GenTG (Mutagenese) nicht als gentechnische Veränderungen.

Die RTDS™-Mutagenese ist als eine Oligonukleotid-gesteuerte Mutagenese (OgM) einzuordnen. In ihrer Stellungnahme vom Juni 2012 bewertete ZKBS die durch die OgM entstandenen Organismen mit folgenden Begründungen als Organismen, die nicht gentechnisch verändert sind:

- *Die Oligonukleotide, die in die Zellen eingebracht werden, sind keine neuen Kombinationen genetischen Materials, denn ihre Sequenz richtet sich nach der Zielsequenz (Watson-Crick-Basenpaarung oder Hoogsteen-Basenpaarung), ggf. mit einer Abweichung von einem oder wenigen Nukleotiden.*
  Bei den eingebrachten Oligonukleotiden handelt es sich nicht um rekombinante Nukleinsäuren gemäß § 3 Nr. 3a. Buchst. a GenTG. Die gleiche Bewertung ergibt sich gemäß Anhang I A Teil 1 Nr. 1 RL 2001/18/EG bzw. Anhang I Teil A Nr. 1 RL 2009/41/EG.
- *Die Oligonukleotide, einschließlich der chemisch veränderten Nukleinsäuren und Derivate, sind gemäß § 3 Nr. 3a. Buchst. b GenTG kein genetisches Material oder Erbgut. Die gleiche Bewertung ergibt sich gemäß Anhang I A Teil 1 Nr. 2 RL 2001/18/EG bzw. Anhang I Teil A Nr. 2 RL 2009/41/EG.*
- *Die Oligonukleotide wirken wie Mutagene und rufen Mutationen von einem oder wenigen Nukleotidpaaren hervor, wie sie gleichermaßen auch spontan oder nach Anwendung von Mutagenen auftreten können und sind damit nicht von spontanen Mutationen oder von Mutationen, die durch Mutagenese hervorgerufen werden, unterscheidbar. Durch Mutagene erzeugte genetische Varianten sind gemäß § 3 Nr. 3b. Satz 2 Buchst. a GenTG (Mutagenese) keine GVO. Die gleiche Bewertung ergibt sich gemäß Anhang I B Nr. 1 RL 2001/18/EG bzw. Anhang II Teil A Nr. 1 RL 2009/41/EG.*

Unter Berücksichtigung der aktuellen Rechtsgrundlage und der vorgelegten Daten kommt die ZKBS daher zum Schluss, dass die durch die RTDS™-Technologie entwickelten Rapslinien nicht als GVO zu bewerten sind.

Berlin, den 6. Februar 2015

Prof. Dr. Pfister

Vorsitzender der ZKBS

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