PART II
SUMMARY
VITAMIN B12 WITH RECOMBINANT HUMAN INTRINSIC FACTOR EXTRACTED FROM *ARABIDOPSIS THALIANA*

MANUFACTURED BY COBENTO A/S

SUMMARY OF APPLICATION FOR GM PLANT DERIVED FOOD

A. General Information
1. Details of application

a) Member state of application	Denmark
b) Application number	Not available on the date of application
c) Name of product (commercial and other names)	B_{12} with recombinant human intrinsic factor (rhIF)
d) Date of acknowledge of valid application	Not available on the date of application

2. Applicant

a) Name of applicant	Cobento A/S
b) Address of applicant	Gustav Wieds Vej 10 C 8000 Århus C Denmark
c) Name and address of the person in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant.	CEO Poul Nørgaard Poulsen Cobento A/S Gustav Wieds Vej 10 C 8000 Århus C Denmark

3. Scope of the application

New vitamin B_{12} food supplement containing an ingredient derived from GM plants

4. Is the product being simultaneously notified within the framework of another regulation?

No

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and /or Directive 90/220/EEC?

No

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC and /or Regulation (EC) 258/97?

No

7. Has the product been notified in a third country either previously or simultaneously?

Yes	
Application submitted in Ukraine	

8. General description of the product

a) Name of the recipient or the parental plant and the intended function of the genetic modification	The recipient plant belongs to the species, *Arabidopsis thaliana* and the genetic modification results in the ability of the plant to produce the human protein intrinsic factor, rhIF
b) Types of products planned to be placed on the market according to the authorisation applied for	Food supplements with vitamin B_{12} and rhIF as facilitator for the absorption of B_{12}
c) Intended use of product and types of users	The food supplement is intended to alleviate lack of B_{12} in people lacking the ability to produce intrinsic factor. A failure of secretion of intrinsic factor leads to a deficiency of vitamin B_{12} which plays an important role in a number of processes in the body. 10 – 30 % of the population over 60 years secretes less or no intrinsic factor.
d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for	Tablet taken daily. Labelled according to Directive 2002/46/EC of The European parliament and of the council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplement and Regulation (EC) 1829/2003.
e) Any proposed packaging requirements	Packaging in plastic container.
f) A proposal for labelling on accordance with Articles 13 and Articles 25 of Regulation (EC) 1829/2003. In case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC	Food Supplement Contains: Vitamin B_{12} 2 microgram, Intrinsic factor 76 microgram (produced from genetically modified *Arabidopsis thaliana*), Recommended daily dosage: 1 tablet Keep out of reach of children. Do not exceed the recommended daily intake. Food supplements are intended to supplement the diet and should not be regarded as a substitute for a varied diet.
g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)	Not applicable as the application concerns food.
h) If applicable, geographic areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited	Not applicable as the genetically modified plants are only grown in designated greenhouses.

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

The supervising authority will be informed immediately if plants or seeds escape the premises or if genetically modified plants are identified outside the production areas. In case of breach of barriers towards the surrounding greenhouse area, the damages will be repaired as soon as possible and if necessary the plants in the production area will be covered. If plants/plant material or seeds are spread to the surrounding areas, they will be collected and the areas burned with a gas burner. If sprouting is observed at a later time, the area will be sprayed with a simple herbicide such as round-up. All accidents are described in the log. Waste, which may contain viable genetically modified plants are inactivated prior to disposal. Waste is collected and

put in designated locked containers. The waste is disposed of in a specific municipal plant where it is emptied directly into the facility's waste trench. No measures for misuse or treatment are suggested. We have no knowledge of or have found any references to misuse of vitamin B_{12} products. As both B_{12} and intrinsic factor goes into the natural metabolism there will be no accumulation of either.

B. Information relating to the recipient or (where appropriate) parental plant
1. Complete name

a) Family name	Brassicaceae
b) Genus	*Arabidopsis*
c) Species	*Arabidopsis thaliana* (L.) Heynh
d) Subspecies	Landsberg *erecta*
e) Cultivar/breeding line or strain	*Gi-6*, N183
f) Common name	Thale cress

2a. Information concerning reproduction

(i) Model(s) of reproduction	Self-pollinating
(ii) Specific factors affecting reproduction	None
(iii) Generation time	5 to 12 weeks

2b. Sexual compatibility with other cultivated or wild plant species

A.thaliana is not sexually compatible with any other cultivated or wild plant species.

3. Survivability

a) Ability to form structures for survival dormancy	*A. thaliana* is an annual plant and as such it does not survive seed production. Fully matured seeds can survive in nature and germinate when the conditions are favourable.
b) Species factors affecting survivability	None

4. Dissemination

a) Ways and extent of dissemination	The plant is harvested before flowering and only the leaves are used for the extraction of rhIF. 95 % of the cultivation area is used for vegetative growth. Seed production is performed in an insect proof containment in a greenhouse in order to avoid pollen spread by insects. The risk of pollen spread is therefore negligible. As the seeds are very small area dedicated protective cloth is worn when working with the seed harvest, seed cleaning, and sowing to avoid spread.
b) Specific factors affecting dissemination	None

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

A. thaliana is an annual plant native to Europe, Asia, and north western Africa, from British Isles south to the Azores and Morocco, east to Japan, and southeast to northern India.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

A. thaliana is a weed. It grows on coarse sandy soils, in areas with low vegetation, arable land and in gardens.
A. thaliana is widely used as one of the model organisms for studying plant sciences, including genetics and plant development.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem, where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

As the cultivation is constricted to greenhouses it will have no effect on biodiversity of the surrounding ecosystems. To our knowledge there are no toxic effects of *A. thaliana* on humans, animals or other organisms.

C. Information relating to the genetic modification

1. Description of the methods used for the genetic modification

Wild type *A. thaliana* was transformed using *Agrobacterium tumefaciens*

2. Nature and source of the vector used

Transformations was performed with *A. tumefaciens* strain GV3101 carrying a binary vector

3. Source of the donor DNA, size and intended function of each constituent fragment of the region intended for insertion

The donor DNA was cDNA for human intrinsic factor coding for the vitamin B_{12} binding protein intrinsic factor and the NPTII gene that confers kanamycin resistance to the transformed cell

D. Information relating to the GM plant

1. Description of the trait(s) and characteristics which have been introduced or modified

The ability to produce recombinant human intrinsic factor

2. Information on the sequence actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial	The plant, GIF, contains three complete copies of the insert
b) In case of deletion(s), size and function of the deleted region(s)	There are no deletions
c) Chromosomal location(s) of the insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination	The inserts are located on chromosome 1 (two inserts) and chromosome 3, as verified by sequencing of the flanking regions
d) The organisation of the inserted genetic material at the insertion site	No relevance as the manufactured product only contains the protein of interest, rhIF

3. Information on the expression of the insert

a) Information on the developmental expression of the insert during the life cycle of the plant	Not applicable as the protein is extracted from the aerial plant parts at the emergence of inflorescence
b) Parts of the plant where the insert is expressed	As the promoter used is constitutive, expression of rhIF is expected in all plant parts

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction	We have observed no differences in the morphology or growing features between GIF and N183.
b) Dissemination	Not applicable
c) Survivability	We have observed no difference
d) Other differences	None that we have observed

5. Genetic stability of the insert and phenotypic stability of the GM plant

The inserts have been stably integrated through several generations. The GM-plant, GIF is phenotypically stable

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer	The plants are grown in greenhouses in designated areas and are not in contact with other plants. Not applicable as the soil used for cultivation of GIF is either destroyed in an incineration plant or inactivated by steam treatment, therefore any bacteria which have been in contact with the GM plant will be killed.
b) Plant to plant gene transfer	No plant to plant gene transfer can take place because the plant is grown in an insect proof greenhouse during seed production and the plant is self pollinating with no detectable airborne pollen.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator	No comparator exists

7.2 Production of material for comparative assessment

a) Number of locations, growing seasons, geographical spread and replicates	Not applicable as no comparator exists
b) The baseline used for consideration of natural variations	Not applicable as no comparator exists

7.3 Selection of material and compounds for analysis

Not applicable as there is no natural non-GM comparator. *A. thaliana* is purely a research plant which is not consumed.

7.4 Agronomic traits

Not applicable since non-GM *A. thaliana* is not grown for commercial use.

7.5 Product specification

rhIF coupled to vitamin B_{12}

7.6 Effect of processing

Not applicable. The applied product is extracted via affinity chromatography in an aqueous solution. The rest of the plant is not used for food or feed.

7.7 Anticipation intake/extent of use

76 – 342 microgram rhIF per day given together with 2 to 9 microgram vitamin B_{12}. Intrinsic factor is not anticipated to be otherwise consumed.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins	Humans are born with the ability to secrete intrinsic factor. For healthy persons this happens throughout life the ability normally declines with age. Normal secretion of intrinsic factor is in the range of 3.1 – 19.4 microgram/hour. Intrinsic factor has only one function, to make a complex with vitamin B_{12} in the intestine to become recognisable by the intestinal receptor cubilin. After internalisation into the intestinal epithelial cell, intrinsic factor is degraded and only vitamin B_{12} pass into the blood circulation. The present application concerns a rhIF which is a copy

	of the native human protein. The amino acid sequence is identical to that of human intrinsic factor which is already present in the body and no accumulation occurs as it goes into the normal metabolism. rhIF is only used in VERY SMALL quantities and it is only for oral use. It is only intended and needed for use in very small quantities, 76 – 342 microgram per day corresponding to an ingestion of 1 gram of rhIF in 38 to 9 years. With the current process we are sure that no plant parts but only the pure rhIF is in the product and this is not toxic. We have therefore not made further safety studies with the protein.
7.8.2 Testing of new constituents other than proteins	Only rhIF is of interest. When this is extracted – the remainder of the plant is destroyed. Testing for new constituent other than the protein is not relevant.
7.8.3 Information on natural food and feed constituents	The plant is not to be consumed – only the extracted rhIF. It is therefore not relevant to look at other constituents
7.8.4 Testing of the whole GM food/feed	The plant is not to be consumed. Testing of the whole plant is not relevant.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein	An allergenicity assessment of rhIF has been performed by the Danish company DHI. DHI made the following conclusion: It is very improbable that the rhIF produced by Cobento A/S could be allergenic. The few uncertainties that still exist are due to lack of scientific knowledge on individual allergens and not due to properties of the rhIF amino acid sequence.
7.9.2 Assessment of allergenicity of the whole GM plant or crop	The plant is not to be consumed so testing of the whole plant is not relevant

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food	Not applicable as only micrograms of the recombinant human intrinsic factor is being ingested. It will therefore have no relevant nutritional effect.
7.10.2 Nutritional assessment of GM feed	Not applicable as the application is not about feed

7.11 Post-market monitoring of GM food/feed

No specific post-marketing monitoring plan but in relation to Cobento's authorisations we have standard operating procedures including handling of complaints and recall plan.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

Not applicable. There is no target organism.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

9.1 Persistence and invasiveness	The plants are grown in greenhouses and after extraction of the protein – the reminder of the plant is non viable. The soil used for cultivation is either destroyed in an incineration plant or inactivated by steam treatment. Therefore the risk of uncontrolled spread of the GMO is negligible.
9.2 Selective advantages or disadvantages	Not applicable as the plants are grown in greenhouses.
9.3 Potential for gene transfer	Not applicable as the plants are grown in greenhouses and are not in contact with other plants
9.4 Interactions between the GM plant and target organisms	Not applicable as the plants are grown in greenhouses and there are no target organism
9.5 Interactions of the GM plant with non-target organisms	Not applicable as the plants are grown in glasshouses and are not in contact with other plants.
9.6 Effects on human health	We have worked with the plant for 4 years. The plant is a well know research plant and as far as we know no adverse effects on human health have been reported.
9.7 Effects in animal heath	Not applicable as the plant is grown in greenhouses and not consumed by animals
9.8 Effects on biogeochemical processes	Not applicable as the plant is grown in greenhouses
9.9 Impacts of the specific cultivation, management and harvesting techniques	The plants are grown in greenhouses and are not in contact with other plants. Since the GM plant is only used in research it is not relevant to compare the growing conditions of the present plant with that of a non-GM *A. thaliana*

10. Potential interactions with the abiotic environment

The plants are grown in greenhouses and are not in contact with other plants.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produces from GM plants and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

11.1 General (risk assessment, background information)	Applications concerning only food/feed or ingredients will normally not be required a detailed environmental monitoring plan. In January 2004 The Danish Protection Agency gave Cobento an authorization for production of vitamin B_{12} binding protein using genetically modified plants.
11.2 Interplay between environmental risk assessment and monitoring	Please see 11.1
11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)	Please see 11.1

11.4 General surveillance of the impact of the GM plant (approach, strategy, method of analysis)	Please see 11.1
11.5 Reporting the results of monitoring	Every 3 months, the company must perform a visual inspection of the insect net in the seed production area and enter the results into the log. Once a year the company must perform a field examination the aim of which is to clarify whether genetically modified plants have been spread to the surroundings near the growth block and the waste container area. The company must convey the result to the supervising authority.

12. Detection and event-specific identification techniques for the GM plant

The public will not be exposed to the GM-plant nor DNA from the GM-plant, hence we have not submitted an identification technique