

## SCIENTIFIC OPINION

### Regulat® and “the immune system”

#### Scientific substantiation of a health claim related to Regulat® and “enhancement/modulation/improvement/regulation of the activity of the immune system” pursuant to Article 13(5) of Regulation (EC) No 1924/2006<sup>1</sup>

#### Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2009-00453)

Adopted on 2 July 2009 by written procedure

#### PANEL MEMBERS

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#### SUMMARY

Following an application from Dr. Niedermaier Pharma GmbH submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to Regulat® and “enhancement/modulation/improvement/regulation of the activity of the immune system”.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.

The food/constituent that is the subject of the claim is Regulat®, a liquid concentrate derived from a stepwise fermentation of 17 vegetable and fruit species by five different fermentation steps involving five different strains of *Lactobacillus*, which are inactivated by heat and removed after the fermentation process. The bacterial cultures used are not clearly identified and characterised. The types of bacteria may have an impact on the fermentation outcome and the metabolites present in the final product. The Panel considers that Regulat® has not been sufficiently characterised.

The claimed effect is “enhances the activity of the immune system by immune-stimulation,

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antioxidative and enzyme regulating effects and modulates the innate unspecific immune system improving the immune-cell function and immune system performance. Moreover, it down regulates cell adhesion molecules stabilising cell/cell contacts and interactions". The target population is the general population. The Panel notes that the applicant has not provided any evidence that the measured changes in the biomarkers of the activity of the immune system represent beneficial physiological effects.

One human intervention study was provided, claimed as proprietary and reported as pertinent by the applicant. The study was a randomised double-blind placebo-controlled design of 4-weeks intervention investigating the effect of Regulat® in 24 healthy human male volunteers aged from 20 to 48 years.

With regard to immune parameters, no significant changes were reported in TNF-alpha response (Tumour Necrosis Factor-alpha), concentrations of prostaglandin metabolites, and natural killer (NK) cell activity. Following stimulation with Interleukin 2 (IL-2), the NK cell activity was significantly increased in the Regulat® group when volunteers with no increase in lytic activity after IL-2 activation had been excluded for this statistical evaluation. Glutathione values in lymphocytes and monocytes increased significantly in the Regulat® group while the increase in the placebo group was not significant. Levels of reduced glutathione in NK cells increased significantly in both groups, but when excluding the subjects with high TNF-alpha values the increase was no longer significant in the placebo group while remaining significant in the Regulat® group. A significant reduction of total oxidation status was reported for the Regulat® group, while no change could be observed in the total antioxidative status of subjects in both groups. Levels of soluble VCAM (Vascular cell adhesion molecule-1) and ICAM (intercellular adhesion molecule-1) were significantly reduced in the Regulat® group after the intervention. The Panel notes that upregulation of VCAM may occur in reaction to a high TNF-alpha response, and that the subjects excluded for this reason earlier were not excluded from this analysis.

The Panel notes the weaknesses of the study which include the small sample size, absence of power calculations and inconsistent exclusions of subjects for the statistical analyses. Moreover, frequent symptoms of cold and/or influenza were common among the subjects of both groups (with about 50% reporting such symptoms during the trial) which is considered to limit the significance of the results in a study measuring immune parameters.

The Panel considers that studies provided on potential antioxidant and immune stimulating properties of Regulat® using *in vitro* and *ex vivo* models provide little evidence to support the claimed effect.

In weighing the evidence the Panel took into account the lack of information to sufficiently characterise Regulat®, the lack of evidence that the measured changes in the biomarkers of the activity of the immune system represent beneficial physiological effects, the weaknesses of the human intervention study, and the limited evidence provided by the *in vitro* and *ex vivo* studies to support the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of Regulat® and the claimed effect, relating to enhancement/modulation/improvement/regulation of the activity of the immune system.

**Key words:** Regulat®, fermented fruit, vegetables, organic, nuts, immune modulation, antioxidant, anti-inflammatory

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## **BACKGROUND**

Regulation (EC) No 1924/2006<sup>2</sup> harmonises the provisions that relate to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of that Regulation and are authorised in accordance with this Regulation and included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of that Regulation lays down provisions for addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of that Regulation, an application for inclusion in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, who will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

### **Steps taken by EFSA:**

- The application was received on 09/03/2009.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.
- The scientific evaluation procedure started on 09/03/2009.
- On 02/07/2009 the NDA Panel, after having evaluated the overall data submitted, adopted by written procedure an opinion on the scientific substantiation of a health claim related to Regulat® and “enhancement/modulation/improvement/regulation of the activity of the immune system”.

## **TERMS OF REFERENCE**

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: Regulat® and “enhancement/modulation/improvement/regulation of the activity of the immune system”.

## **EFSA DISCLAIMER**

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of Regulat®, a positive assessment of its safety, nor a decision on whether Regulat® is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

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<sup>2</sup> European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

## **ACKNOWLEDGEMENTS**

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## 1. Information provided by the applicant

**Applicant's name and address:** Dr. Niedermaier Pharma GmbH, Taufkirchner Straße 59, 85662 Hohenbrunn, Germany

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006.

### 1.1. Food/constituent as stated by the applicant

Regulat® is a food made by fermentation from fresh and organically grown fruits, nuts and vegetables.

### 1.2. Health relationship as claimed by the applicant

According to the applicant, the supplementation of Regulat® shows significant physiological effects on immune cell modulating, antioxidative and anti-inflammatory parameters.

### 1.3. Wording of the health claim as proposed by the applicant

Regulat® is a liquid concentrate for the regulation and modulation of the immune-system.

It enhances the activity of the immune system by immune-stimulation, antioxidative and enzyme regulating effects and modulates the innate unspecific immune system improving the immune-cell function and immune system performance. Moreover, it down regulates cell adhesion molecules stabilizing cell/cell contacts and interactions.

### 1.4. Specific conditions of use as proposed by the applicant

20 mL Regulat® is the recommended daily dose and should be consumed in two parts per day (10 mL in the morning and 10 mL in the evening), diluted in half a glass of water.

## 2. Assessment

### 2.1. Characterisation of the food/constituent

The food/constituent that is the subject of the health claim is Regulat®. Regulat® is a liquid concentrate derived from a stepwise fermentation of 17 vegetable and fruit species by five different fermentation steps involving five different strains of *Lactobacillus*, which are inactivated by heat and removed after the fermentation process. The fermentation is a patented process, which includes several fermentation steps with a bacterial culture which appears to be *Lactobacillus casei* in one step. The strain(s), their identification or culture collection numbers or other information for the ones used in other fermentation steps are not provided. The types of bacteria may have an impact on the fermentation outcome and the metabolites present in the final product. The other components used are certified organic fruits, vegetables, nuts, beans and water. At the end of the process a raw concentrate is obtained, a mixture of spices, saffron and herbal glycerol is added and the product is thermally treated and packaged in bottles.

The Panel considers that Regulat®, which is the subject of the health claim, has not been sufficiently characterised, because the bacterial cultures are not clearly identified and characterised.

## 2.2. Relevance of the claimed effect to human health

The claimed effect is “enhances the activity of the immune system by immune-stimulation, antioxidative and enzyme regulating effects and modulates the innate unspecific immune system improving the immune-cell function and immune system performance. Moreover, it down regulates cell adhesion molecules stabilising cell/cell contacts and interactions”. The target population is the general population.

A well functioning immune system is important for maintaining physiological integrity and thus health. Even if it is possible to modulate certain aspects of immune function, the Panel notes that the applicant has not provided any evidence that the measured changes in the biomarkers of the activity of the immune system represent beneficial physiological effects.

The Panel considers that the applicant has not provided any evidence that the claimed effect, relating to enhancement/modulation/improvement/regulation of the activity of the immune system, is beneficial to human health.

## 2.3. Scientific substantiation of the claimed effect

The applicant has conducted a literature search applying the inclusion criteria which were the physiological effects of polyphenolic compounds, including antioxidative, immunomodulatory and anti-inflammatory effects. Regulat®-specific published and unpublished and proprietary/confidential data were provided by the applicant.

According to the applicant, the pertinent human studies consisted of only one product-related human intervention trial (Schulz et al., unpublished, proprietary data). Five other identified published clinical studies with other polyphenolic compounds were not evaluated in detail by the applicant because they were not considered to be pertinent due to the great variability of the polyphenolic profiles in plant foods.

The product-related study had a randomised double-blind placebo-controlled design (Schulz et al., unpublished, proprietary data): 48 healthy human male volunteers of normal BMI (20-28 kg/m<sup>2</sup>) aged from 20 to 48 years consumed 10 mL twice daily of the test product (Regulat®) (n=24) or the same amount of a placebo product (watery solution of essence of vinegar with yellow food colour) (n=24) for four weeks. The aim was to assess the immunomodulatory effects of Regulat® in the context of antioxidative status and parameters of inflammation. Blood samples were taken once prior to the intervention period and immediately at the end of the intervention period. The statistical analysis focused mainly on comparison within groups over time. Differences between groups appear small.

No significant changes in routine blood clinical parameters including C-reactive protein were reported during the study.

With regard to immune parameters, no significant changes were reported in TNF-alpha response (Tumour Necrosis Factor-alpha), concentrations of prostaglandin metabolites, and natural killer (NK) cell activity with or without IL-2 (Interleukin 2) stimulation. Following stimulation with IL-2, the NK cell activity was significantly increased in the Regulat® group when volunteers with no increase in lytic activity after IL-2 activation had been excluded for this statistical evaluation (6 from the placebo and 5 from the Regulat® group). Glutathione values in lymphocytes and monocytes increased significantly in the Regulat® group while the increase in the placebo group was not significant. Levels of reduced glutathione in NK cells increased significantly in both groups, but when excluding the subjects with high TNF-alpha values (4 from the placebo and 1 from the Regulat® group) the increase was no longer significant in the placebo group while remaining significant in the Regulat® group. A

significant reduction of total oxidation status was reported for the Regulat® group after setting a cut-off value. In contrast no change could be observed in the total antioxidative status of subjects in both groups. Levels of soluble VCAM (Vascular cell adhesion molecule-1) and ICAM (intercellular adhesion molecule-1) were significantly reduced in the Regulat® group after the intervention. The Panel notes that upregulation of VCAM may occur in reaction to a high TNF-alpha response, but the subjects excluded for this reason earlier (4 from the placebo group and 1 in the Regulat® group) were not excluded from this analysis. Food frequency analysis did not indicate differences in fruit and vegetable consumption. Self-evaluation of the quality of life using a visual analogue scale, for which no reference to validation was made, indicated small changes in the Regulat® group during the intervention.

The Panel notes the weaknesses of the study which include the small sample size, absence of power calculations and inconsistent exclusions of subjects from statistical analyses. Moreover, it was reported that the study was conducted during the autumn and frequent symptoms of cold and/or influenza occurred in the subjects of both groups (with about 50% reporting such symptoms during the trial) which is considered to limit the significance of the results in a study measuring immune parameters.

The Panel also notes that this study has been discussed in a publication by Schoen et al (2009), in which the authors conclude that the results need to be confirmed in more volunteers with a more prolonged application to assess significant beneficial effects of Regulat® in the general population.

The other five human studies identified in the literature review have been conducted with different material (fruit juices and vegetable juices), but not with the fermented fruit and vegetable product with fermentation bacteria removed. Therefore, they cannot be used to support the claimed effect and the applicant also regarded them not to be pertinent to the claimed effect.

Hippeli et al. (2007) characterised the potential antioxidant and immune stimulating properties of Regulat® with the aid of *in vitro* and *ex vivo* model reactions. The Panel considers that these studies provide little evidence to support the claimed effect.

In weighing the evidence the Panel took into account the lack of information to sufficiently characterise Regulat®, the lack of evidence that the measured changes in the biomarkers of the activity of the immune system represent beneficial physiological effects, the weaknesses of the human intervention study, and the limited evidence provided by the *in vitro* and *ex vivo* studies to support the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of Regulat® and the claimed effect, relating to enhancement/modulation/improvement/regulation of the activity of the immune system.

## CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- Regulat®, which is the subject of the health claim, is not sufficiently characterised.
- The claimed effect is “enhances the activity of the immune system by immune-stimulation, antioxidative and enzyme regulating effects and modulates the innate unspecific immune system improving the immune-cell function and immune system performance. Moreover, it down regulates cell adhesion molecules stabilising cell/cell contacts and interactions”. The target population is the general population.



- The applicant has not provided any evidence that the claimed effect, relating to enhancement/modulation/improvement/regulation of the activity of the immune system, is beneficial to human health.
- A cause and effect relationship has not been established between the consumption of Regulat® and the claimed effect, relating to enhancement/modulation/improvement/regulation of the activity of the immune system.

#### **DOCUMENTATION PROVIDED TO EFSA**

Health claim application on Regulat® and “regulation and moderation of the immune system” pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 244\_DE). March 2009. Submitted by Dr. Niedermaier Pharma GmbH.

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#### **GLOSSARY / ABBREVIATIONS**

ICAM-1	Intercellular adhesion molecule-1
IL-2	Interleukin 2
NK cells	Natural killer cells
TNF-alpha	Tumour Necrosis Factor-alpha
VCAM-1	Vascular cell adhesion molecule-1