Safety Assessment of Resveratrol (resVida®)

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Novel Food Classification: 6

Introduction

An application for the authorisation of resveratrol (resVida®) as a novel food ingredient was submitted to the Food Safety Authority of Ireland (FSAI) by DSM Nutritional Products Ltd. in accordance with *Article 4* of the Novel Food Regulation (EC) No. 258/97 and accepted by the FSAI on December 11th, 2012.

Resveratrol is a polyphenol that belongs to a group of substances known as phytoalexins, which are low molecular weight secondary metabolites produced by hundreds of plant species in response to microbial infection and abiotic stress. Resveratrol occurs naturally in a number of food plants, the highest food levels being in red wine and grapes, with lower levels in peanuts and mulberries. The highest known level of resveratrol in any plant occurs in the roots of the Japanese knotweed (*Polygonum cuspidatum*). The applicant reports that daily intake of naturally occurring resveratrol in Europe is associated primarily with the consumption of wine or grapes and estimated to be 0.01 – 0.4 mg/day, with upper intake levels at 1.5mg/day.

DSM Nutritional Products Ltd. proposes to market resveratrol as an ingredient in food supplements to adults at levels of up to 450mg/day. The novel ingredient is produced in a multi-step chemical process to a final purity of at least 99%. The by-products of the novel process have been identified and have known toxicological profiles.

The applicant classifies resVida® as novel in line with *Article 1(2)(f)* of the Novel Food Regulation (EC) No. 258/97; (foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to

significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances). For the purposes of the safety assessment, resveratrol is placed in Class 6 (foods produced using a novel process), in line with Commission Recommendation 97/618/EC.

I. Specification of the novel food

The novel ingredient (resVida®) is described as an off-white to beige crystalline substance, with a *trans*-resveratrol content of ≥99 (w/w). It has low solubility in water and is reported by the applicant to be stable for at least 24 months in unopened containers at temperatures below 25ºC. Undesirable substances are controlled by specifications relating to microbial content and heavy metals such as lead and arsenic, which are supported by batch test results. The applicant has identified possible process by-products and controlled their individual limits to <0.1% (<0.5% in total).

The applicant states that the novel food ingredient is packaged in 1kg, 5kg or 20kg lots using standard packaging materials and is stable for at least 24 months at temperatures less than 25ºC. In response to a request from the FSAI, the applicant has clarified that an older production process yielded resveratrol that was stable for 36 months and that stability tests for resveratrol produced by this new process were ongoing since 2012. Given the high purity of the resveratrol from both processes, the applicant does not foresee any changes in stability which should be borne out by the ongoing study.

Also in response to a request for clarification from FSAI, the applicant has confirmed that the novel ingredient is to be marketed directly to supplement manufacturers and that initial stability data relates only to the primary product marketed to supplement manufacturers. The applicant provided additional information which indicated that the stability of resVida® at varying concentrations (up to 48 months) was not affected during storage as supplements formulated with other ingredients which were not identified.

II. Effect of the production process on the novel food

The applicant detailed the chemical process that underlies the production of their resveratrol. The raw material specifications are presented along with the identity of

residual impurities (solvents) and by-products that may be present at low levels in the final product. The applicant has controlled the limits of the individual by-products in the final product to <0.1% (<0.5% in total) and no concerns are identified. Process solvents are within permitted limits in the final product. One solvent used is diisopropylamine, a known respiratory, skin and eye irritant but which does not have an established maximum regulatory limit in foods. However, the applicant applies the Threshold of Toxicological Concern (TTC) scheme and assigns it to Cramer Class III, with a threshold for human exposure of 90µg/day. At a controlled maximum concentration of 50 ppm in resVida®, a supplement dose of 450 mg/day corresponds to 22.5µg/day diisopropylamine which is within the threshold for human exposure. Each step of the production process is controlled to ensure a consistent purity and yield of the end-product.

III. History of the source organism

The novel ingredient is produced by a chemical process and therefore no information is required under this section.

IX. Anticipated intake/extent of use of the novel food

The novel ingredient (resVida®) is intended for use by adults in the form of food supplements in which resveratrol is the sole nutrient or is combined with certain vitamins. Intake of the supplement (one capsule) is estimated to deliver up to 450 mg/day of resveratrol, though the applicant concludes that this level is not likely to be achieved by most people as many users do not consume supplements regularly. Current EU intake data suggests that the average dietary intake of naturally occurring resveratrol (excluding supplements) can range from 0.3 mg/day to 1.5 mg/day. Therefore, supplementation as recommended by the applicant would represent a 1500-fold and 300-fold increased intake for average and high consumers respectively.

Based on current natural intake levels from foods, along with data from the UK NDNS food consumption surveys on patterns of supplement use, predicted total intakes of resveratrol range from 50 -160 mg/day (1.0 to 2.1 mg/kg bw/day) up to 451 mg/d (8 – 13 mg/kg bw/day) by high users. Data presented by the applicant indicate that a NOAEL of 750 mg/kg/day, and an Acceptable Daily Intake (ADI) of 7.5 mg/kg bw/day (equivalent to 450 mg/day for a 60 kg person or 525 mg/day day for a 70 kg person) are within intake estimates for high level users and therefore not a concern.

X. Information from previous human exposure to the novel food or its source

Resveratrol extracted from the roots of the Japanese knotweed plant (*Polygonum cuspidatum*) has a significant history of consumption in food supplements within the EU prior to May 15 1997 according to an application for substantial equivalence submitted to the Food Safety Authority of Ireland (FSAI) in 2011. In 2012, the FSAI provided a positive opinion on the substantial equivalence of resveratrol produced *via* fermentation in comparison with that from Japanese knotweed. Both products are marketed in supplement form and contain greater than 98% trans-resveratrol, with a maximum recommended intake of 500mg/day.

The novel resveratrol (resVida®) has been on the US market as a dietary supplement since June 2008, with GRAS status for multiple food uses since 2012. Similarly in Russia, resVida® is marketed as a dietary supplement since July 2008. The applicant indicates that a number of foods such as grape seed extracts that naturally contain resveratrol are on the EU market, but that the precise resveratrol content of these products can be difficult to establish.

XI. Nutritional information on the novel food

The applicant states that resveratrol has no nutritional value and that there is no evidence to suggest that its consumption would have an effect on the bioavailability of any dietary nutrients. The novel resveratrol is intended to compete with other resveratrol-containing dietary supplements on the EU market and should not have a significant impact on overall resveratrol intakes in the EU population.

XII. Microbiological information on the novel food

Microbiological test results of three batches of resVida® demonstrate compliance with the product specifications.

XIII. Toxicological information on the novel food

Resveratrol from a variety of sources has been consumed within the EU and in non-EU countries for a considerable time, with no adverse events being reported. Regardless, the applicant has provided a considerable suite of toxicological information in relation to chemically synthesised resveratrol, some of which is derived from studies on resVida® itself and more from published literature.

ADME (Absorption, Distribution, Metabolism & Excretion)

The ADME of resveratrol was described in a number of studies on either ^{14}C-resveratrol or on resVida®. In addition, relevant peer-reviewed scientific publications were reviewed and 13 studies on the kinetics of resveratrol in humans were also identified, including a study on resVida®.

Resveratrol is rapidly and extensively absorbed in both rats and humans, with uptake and bioavailability being dose related and also influenced by the food matrix. Maximum plasma concentrations are biphasic, indicating enterohepatic circulation.
Resveratrol undergoes low level distribution to a number of organs, especially those involved in absorption and elimination such as the stomach, liver, kidney and intestine, but also the heart, lung and brain. In human studies, it was found that up to 50% of metabolites were bound to plasma proteins. There is no evidence of bioaccumulation from either rat or human studies. Significant metabolism occurs in the liver and intestinal epithelia via glucuronidation and sulfation conjugation pathways which do not become saturated, even at high oral doses. In humans, only trace amounts of the parent compound were detectable in plasma one hour after the consumption of 25 mg of resveratrol. Resveratrol-3-sulfate is the main metabolite in humans, while glucuronidation to resveratrol-3-glucuronide and resveratrol-4'-glucuronide predominates in the rat. Dihydro-resveratrol and its sulfate and glucuronide conjugates were also identified in both rats and humans. The half-life of resveratrol in rats is 7-10 hours for high oral doses and 8-12.5 hours for low doses. Also in rats, approximately 65% of resveratrol was eliminated almost evenly between urine and faeces within 24 hours, with a further 12% recovered from the GI tract. Radioactivity was not detected in expired air. The mean elimination half-life in humans is approximately 9.2 hours. In a human study, 77% of 500 mg resveratrol, taken either as a single oral dose or repeat doses for 7 or 29 days, was excreted within four hours.

General toxicity

Acute toxicity studies on resVida® were not supplied. LD_{50} values in rats, mice and dogs of >1000 mg/kg were quoted from the literature. Tests for skin and eye irritation, sensitisation (local lymph node assay) and photo-toxicity/photo-mutagenicity were all negative.

- The absence of any treatment-related effects from a repeated dose 28-day oral toxicity study of resVida® in rats yielded a NOAEL of 500 mg/kg bw/day, the highest dose tested.
- The NOAEL from a 90-day oral toxicity study in rats using resVida® was 750 mg/kg bw/day, the highest dose tested. Decreases in body and organ weights along with feed intake in rats were reported as due to palatability problems associated with the administration of resVida® via supplemented feed. This was also recorded in the prenatal development toxicity study in rats. Reductions in organ weights were not accompanied by histopathological, clinical chemistry or other changes.
- In a 90-day oral study in rats, significant reductions in body weight and body weight gain observed in females at doses of 400 and 1000mg/kg bw/day were not associated with any gross pathological or histopathological changes. Significant increases in absolute liver weight, also in female rats at 1000 mg/kg bw/day, are of unknown significance as clinical chemistry parameters were not discussed.
- Mild to moderate hydronephrosis was recorded in a 6-month gavage study on p53-deficient mice at doses of ≥1000 mg/kg bw/day, while hyperplasia of the bladder epithelia was recorded at 2000 mg/kg bw/day. Histopathology confirmed these effects were treatment-related and a NOAEL was not established in this study. Dose-dependent increases in liver weight and serum cholesterol were also recorded at these doses which were not corroborated by histopathology.
- Reversible nephrotoxicity recorded in a 28-day oral study of resVida® (500 mg/kg bw/day) in rabbits was not corroborated by the results of a six month study using the same resveratrol formulation (SRT501) which improved bioavailability. The NOAEL for this study was 500 mg/kg bw/day, the highest dose tested. The only effect recorded with this formulation in a 28-day oral study in rats was slight haemolytic anaemia in males at 1000 mg/kg bw/day, which was not associated with any effects on bone marrow or spleen. A chronic toxicity study in rats with this formulation did not record effects that were attributed to resveratrol. The NOAEL from the 28-day oral study in dogs with SRT501 was the highest dose tested. Nephrotoxicity was recorded in human patients with multiple myeloma who were administered 5000 mg/day of a highly bioavailable resveratrol formulation alone, and in combination with the anti-cancer drug bortezomib.

However, the specific damage (cast nephropathy) is associated with multiple myeloma and therefore not attributable to resveratrol intake.

- A 90-day dog feeding study recorded inflammatory infiltrates in the bladder and kidney at 1000 mg/kg bw/day of resveratrol but this was not associated with other clinical toxicity signs. At necropsy, all organ weights were comparable to controls and histopathology was unremarkable.

Genotoxicity and Carcinogenicity

Mutagenic potential was not associated with resVida® even when spiked with production impurities or homo-resveratrol. There was evidence of clastogenicity from *in vitro*, but not *in vivo* studies. This was attributed to resveratrol-induced H_2O_2 formation which is largely prevented by protective mechanisms in living systems and thus less likely to occur *in vivo*.

Focussed chronic toxicity/carcinogenicity studies on resVida® were not carried out due to the absence of any evidence of bioaccumulation potential or pre-neoplastic changes. There was no evidence presented to indicate that resveratrol is carcinogenic, with some of the studies actually suggesting possible anti-carcinogenic effects.

Reproductive toxicity

A dietary embryo-foetal toxicity study was carried out in rats using resVida®, with no treatment related effects recorded in the dams or pups. The maternal NOAEL was set at the highest dose tested of 750 mg/kg bw/day, which was also the NOEL for embryo-foetal development.

A 90-day rat study with resVida® up to 750 mg/kg bw/day did not identify treatment-related effects on sperm parameters, oestrous cycle length or the histopathology of the reproductive organs. The diameter of the seminiferous tubules was found to be significantly reduced as a result of a 90-day oral gavage study of male rats administered 20 mg/kg bw/day of resveratrol. However, it was noted that the density of the seminiferous tubules was significantly increased, as was the sperm count and plasma levels of certain sex hormones (LH, FSH, and testosterone). Testes weight and sperm quality were unaffected. The reason put forward for these changes was that oral administration of resveratrol stimulates the hypothalamic-pituitary-gonadal axis, leading to elevated production and release of sex hormones with increased sperm

output, but without any adverse health effects in male rats. A NOAEL of 1000 mg/kg bw/day for developmental effects was quoted from a rat study in which resveratrol formulation SRT501 was administered on days 7 through 17 of gestation.

Effects of resveratrol on cytochrome P450 (CYP) enzymes

The applicant presented an overview of the published literature (up to 2010) concerning the potential for resveratrol to modulate the expression of cytochrome P450 isoenzymes, which are involved in metabolising xenobiotics. Some *in vitro* evidence suggests that resveratrol (especially resveratrol-3-O-sulfate) may result in weak interference with the metabolism of pharmaceuticals, particularly at high doses (~1000 mg/day). Depending on the metabolic pathways affected, this feature may have positive implications by way of anti-carcinogenic effects, but may also impede xenobiotic metabolism, with possible ramifications for instance in pharmaceutical therapies.

Conclusions

This application seeks to authorise the marketing of a novel resveratrol (resVida®) to EU adults in food supplements at doses of up to 450 mg/person/day (7.5 mg/kg bw/day). These supplements will be marketed in competition with existing food supplements containing resveratrol from different sources and therefore should not affect the overall intake of this ingredient. Resveratrol does not have role in nutrition and the information provided in this application did not identify any toxicological concerns for the proposed level of supplementation.

Therefore, the Food Safety Authority of Ireland is of the opinion that based on the information provided, resveratrol (resVida®) marketed in food supplements at a maximum recommended intake of 450mg/day meets the criteria set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.