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Safety of synthetic *trans*-resveratrol as a novel food pursuant to Regulation (EC) No 258/97

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked for an opinion on the safety of synthetic *trans*-resveratrol as a novel food with a purity of $\geq 99\%$ (w/w). The Panel considers that the information provided on the composition and specifications of the novel food is sufficient. The applicant intends to market the novel food as a food supplement in capsule or tablet form at daily doses up to 150 mg/day. The Panel considers that resveratrol does not have a nutritionally relevant role in the human diet and that the consumption of the novel food is not nutritionally disadvantageous. In accordance with the EFSA Scientific opinion on genotoxicity testing strategies, the Panel considers that the negative *in vivo* genotoxicity assay is sufficient to rule out the concern based on the positive *in vitro* chromosomal aberration tests. Reduced body weight gain is seen consistently in animal studies. On the basis of the BMDL₀₅ of 344 mg/kg body weight (bw) per day derived from body weight data of female rats in a subchronic toxicity study and the intended intake of 150 mg/day, the margin of exposure is 172. For pregnant rats, it is below 62. Considering the weight of evidence, the Panel concludes that the intended intake level of 150 mg/day for adults does not raise safety concerns. The Panel notes that diarrhoea or other gastrointestinal symptoms were reported in four uncontrolled intervention studies at doses of 1 g resveratrol/day or higher. The Panel considers that the metabolite *trans*-resveratrol sulfate could inhibit CYP enzymes in humans and may interact with medicines which are mainly metabolised by CYP2C9. The Panel concludes that the novel food, synthetic *trans*-resveratrol, is safe under the proposed conditions of use.

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Keywords: novel food, resveratrol, synthetic

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Summary

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked for an opinion on the safety of synthetic *trans*-resveratrol as a novel food in the context of Regulation (EC) No 258/97, taking into account the comments and objections of a scientific nature raised by Member States.

The Panel considers that the information provided on the composition and specifications of the Novel food is sufficient.

The novel food with a purity of at least 99% *trans*-resveratrol is produced by a chemical process, consisting of a Wittig reaction of 3,5-dimethoxybenzylphosphonate ester with 4-methoxybenzaldehyde leading to trimethoxyresveratrol. This intermediate is purified and then dealkylated with aluminium chloride and diisopropylamine to yield resveratrol which is crystallised from isopropanol/water and recrystallised from ethanol/water. The Panel concludes that the production process is sufficiently described and does not raise safety concerns.

The applicant intends to market the novel food as an ingredient for food supplements in capsule or tablet form at daily doses up to 150 mg/day. The target population is adults. Resveratrol occurs naturally in grapes, grape juice and wine with low amounts found in peanuts, pistachios, and in blueberries. The Panel notes that the intended maximum supplemental intake of 150 mg/day value is about 50 times higher than the high percentile background intake from foods.

The Panel considers that resveratrol does not have a nutritionally relevant role in the human diet and that the consumption of the novel food ingredient is not nutritionally disadvantageous.

A *Salmonella* Typhimurium reverse mutation assay compliant with good laboratory practice (GLP) and OECD guideline 471 provided negative results. An *in vitro* chromosome aberration study with *trans*-resveratrol using human lymphocytes was conducted according to OECD guideline 473 and in compliance with GLP. There were significant increases in structural chromosome aberrations at doses **of 10, 20 and 30 µg/ml without activation and at 40 and 50 µg/ml with activation.** An *in vivo* mammalian erythrocyte micronucleus test was conducted in compliance with GLP and according to OECD guideline 474 using Sprague–Dawley rats given 0, 500, 1,000 or 2,000 mg/kg body weight (bw) per day *trans*-resveratrol for 2 consecutive days by gavage. There was no change in the proportion of immature erythrocytes. There was no increase in micronucleated erythrocytes at any dose of resveratrol thus the test indicates the absence of clastogenic activity *in vivo*. In accordance with the European Food Safety Authority (EFSA) Scientific opinion on genotoxicity testing strategies (EFSA, 2011), the Panel considers that the negative *in vivo* genotoxicity assay is sufficient to rule out the concern based on the positive *in vitro* chromosomal aberration tests considering the endpoints structural and numerical chromosome aberrations (clastogenicity and aneugenicity).

In a 90-day study conducted on synthetic *trans*-resveratrol produced by the applicant, Wistar rats received diets containing *trans*-resveratrol at doses of 0, 120, 300 or 750 mg/kg bw per day. The study was performed in compliance with GLP and was based on OECD guideline 408. Body weight and body weight gain of high-dose animals were lower than that of controls throughout the study for males and from week 4 for females, and remained lower during the recovery period. The reductions were approximately 10% but were not always statistically significant. Body weight gain of the intermediate group was slightly lower than controls for both sexes and body weight of females from this group was lower from week 4. The reduced body weight at the mid and highest dose was associated with reduced food consumption. Food consumption returned to control levels during the recovery period. No effect was observed on the body weight and food consumption of the 120 mg/kg bw per day treatment group. A benchmark dose approach provided a BMDL05 of 344 mg/kg bw per day derived from body weight data of female rats.

Chronic toxicity/carcinogenicity studies performed in accordance with OECD Guidance 451 and 452 or 453 were not carried out on *trans*-resveratrol. Despite the limitations of a provided study with a p53-knockout mouse model for carcinogenicity testing (i.e. short duration, not generally accepted as an alternative to carcinogenicity testing in accordance with OECD Guidance), the Panel notes that this study does not indicate a carcinogenic effect.

In a GLP-compliant embryo–fetal toxicity study with *trans*-resveratrol, fetal examination for soft tissue and skeletal abnormalities showed no treatment-related effects, thus the no observed adverse effect (NOAEL) for fetal development was at the highest dose (750 mg/kg bw per day) tested. The only maternal effect observed was reduction in body weight, however, this occurred at all doses. The Panel considers 120 mg/kg bw for maternal toxicity as a lowest-observed-effect level (LOEL). The Panel considers that the studies provided do not raise concerns regarding developmental toxicity.

Member States expressed concern that *trans*-resveratrol has potential for oestrogenic activity, due to its stilbene structure and structural similarity to the potent oestrogen diethylstilbestrol. In an *in vitro* study, resveratrol was found to bind to oestrogen receptors and to act as a weak oestrogen antagonist in human breast cancer cell lines. In another experiment, resveratrol induced the expression of progesterone receptor in a mouse mammary organ culture model, when administered alone, but expression was suppressed in the presence of estradiol (1 nM). In another *in vitro* study, it was shown that resveratrol binds to human oestrogen receptor and stimulate MCF-7 cell proliferation in a dose-dependent manner at concentrations ranging from 10 nM to 10 mM. No signs for an oestrogenic effect were found in the histological examination of ovaries and in the organ weight measurements of ovaries, testes and uterus in rats receiving resveratrol at doses up to 1,000 mg/kg per day for 3 months. When administered to female weanling rats, *trans*-resveratrol at dose levels up to 100 µg did not exhibit oestrogenic effects as indicated by uterine weight and epithelial cell height of the uterus. In a second experiment of this study, 1,000 µg *trans*-resveratrol administered for 6 days antagonised the E2-induced cholesterol lowering effect in weaning rats. In two uterotrophic assays with *trans*-resveratrol, no oestrogenic effects were found. The Panel considers that these data do not indicate concerns with respect to oestrogenic activity of resveratrol *in vivo*.

The dossier of this application contains 23 articles on human studies and one review on resveratrol which covered additional 14 human studies.

The Panel considers that no conclusions with regards to the safety of resveratrol at the intended dose levels can be drawn on fourteen pharmacokinetic studies and clinical studies with a maximum duration of 96 h, on eight studies with dose levels of below 40 mg/day, on three studies with cancer patients and on four studies investigating supposed beneficial effects.

Regarding the other studies, the Panel notes that diarrhoea or other gastrointestinal symptoms were reported in four uncontrolled intervention studies at doses of 1 g resveratrol/day or higher, corresponding to about 14 mg/kg bw per day, which is by a factor of 10 lower than the LOEL of the animal studies. No treatment-related effects were reported for doses of below 1 g/day in studies up to 3 months of duration. Taking into account the limitations of the available human studies, i.e. low number of subjects, short duration, limited safety-relevant endpoints studied, no study reports available, the Panel considers that these studies per se do not provide sufficient evidence of safety. Considering the weight of evidence, the Panel concludes that the intended intake level of 150 mg/day for adults does not raise safety concerns.

The Panel considers that the metabolite *trans*-resveratrol sulfate could inhibit CYP enzymes in humans and may interact with medicines which are mainly metabolised by CYP2C9.

The Panel concludes that the NF, synthetic *trans*-resveratrol is safe under the proposed conditions of use.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 11 December 2012, the company DSM Nutritional Products Ltd. submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No 258/97 to place on the market resveratrol as a novel food ingredient.

On 28 June 2013, the competent authority of Ireland (FSAI) forwarded to the Commission their initial assessment report, which came to the conclusion that resveratrol meets the criteria for acceptance of a novel food defined in Article (3)1 of Regulation (EC) No 258/97.

On 4 September 2013, the Commission forwarded the initial assessment report to the other Member States. Several Member States submitted comments or raised objections.

In consequence, a decision is now required by the Commission under Article 7(1) of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- As regards the specifications provided by the applicant states that the impurities arising from the production process total no more than 0.5%. Given that point 3.2 of the specifications refers to *trans*-resveratrol content of no less than 99%, we would like the applicant to clarify this difference and inform us of the possible presence of other manufacturing components or by-products such as *cis*-isomers.
- The applicant states that 'the residual solvents are covered by normal defined regulatory maximum levels in food except for diisopropylamine'. It should be clarified to which regulatory levels this refers as not all reported solvent residuals meet the criteria of Directive 2009/32/EC.
- While it is stated in the dossier that synthetic resveratrol is currently produced using a new process, no details are provided by the applicant about this new process and how it differs from the previous manufacturing process. This information is important because the test materials used in various safety studies were produced with the 'old' process and it is essential to know how this might differ from the current product.
- The stability studies and the applicant's reply to a question asked by the Irish authorities refer to two methods of manufacturing the ingredient, namely the new one and the old one. The applicant is asked to clarify which of these methods the stability data set out in Annex II (on capsules and tablets) correspond to. The applicant is also asked to submit the final data of the stability study of the ingredient (resveratrol in crystalline form) produced using the definitive method (new process), which began in June 2012. Long-term stability tests (36 months) are currently being conducted, but have not yet been concluded.
- There is incomplete information about the influence of such parameters as light or pH on the stability of the synthetic *trans*-resveratrol.
- **It is not stated in the application documents whether that applicant's laboratory** has been accredited under an internationally recognised scheme for the analysis in question. DE
- There is no information on the validation of the HPLC procedures used. It is unclear whether the tests conducted meet the requirements of an Hazard Analysis and Critical Control Points (HACCP) concept.
- The proposed maximum daily intake of resveratrol equals exactly the proposed acceptable daily intake (ADI) at 450 mg/day. Any other intake of resveratrol (e.g. from natural sources) would exceed this ADI. Consequently, it is recommended to provide more information on the impact of the combined intake of resVida[®] with natural sources of resveratrol and/or other polyphenols.

- It should be made clear whether the toxicological studies carried out with resVida® took place with the product manufactured using the definitive production method (new process) or a different one.
- Studies have been made that set a no observed adverse effect (NOAEL) level for *trans*-resveratrol in quantities of 200 or 300 mg/kg body weight (bw) per day (Crowell et al., 2004; Elliot et al., 2009; Johnson et al., 2011). The result of the subchronic toxicity study of resVida® gives an NOAEL of 700 mg/kg bw per day (Edwards et al., 2007a). The applicant states, however, that this is likely to be 750 mg/kg bw per day based on the results of other studies. The applicant also bases its estimation of a 750 mg/kg bw per day NOAEL on the fact that the weight reductions of various organs observed with 300 or 750 mg/kg bw per day are due to palatability issues. Accordingly, we would ask for clarification and possible evidence to support the assertion that this effect is attributable to such palatability issues. One Member State commented that the NOAEL in the subchronic toxicity rat study should be rather 700 mg/kg per day considering the reduced feed intake.
- The subchronic study submitted by the applicant showed effects which require further investigation in a long-term study. Toxicological studies of *trans*-resveratrol are currently being conducted as part of the National Toxicology Program (NTP) of the USA's National Institute of Environmental Health Sciences. The substance was included in the NTP because of doubts about whether it had been sufficiently tested for potential toxicological effects.
- If a child were to consume this supplement (either intentionally or by misadventure), the 450 mg daily dose in a 15-kg child would equate to 30 mg/kg bodyweight which would result in a margin of safety of only 25.
- Potential interactions between resveratrol food supplements and medicines should be examined further, especially for those medicines that are metabolised by sulfation in the human body, as resveratrol has the potential to inhibit sulfation. Resveratrol given to human volunteers (1,000 mg/day for 4 weeks) had a significant effect on several key cytochrome P450s. Resveratrol has the potential to modify both Phase 1 and Phase 2 drug metabolism and to interfere with the effectiveness of pharmaceuticals taken concomitantly with the resveratrol supplement. This may be a significant concern for high-dose consumers or consumers taking this supplement over an extended period.
- Resveratrol binds to oestrogen receptors and acts as an oestrogen antagonist (Bhatt et al. 2001). Some intervention studies found that resveratrol in doses greater than 1 g/day can trigger post-menopausal symptoms in women, such as hot flushes (Crandall et al. 2012, Chow et al. 2010).
- The applicant should provide reassurance that all the available clinical studies have been taken into account. The applicant should also address the uncertainties expressed in the review of Cottart et al. (2014) on 25 studies published between 2009 and 2012, of which 13 studies were in humans.
- Timmers et al. (2011) showed, in a 4-week intervention study with 11 healthy obese men (BMI > 30) that 150 mg of resveratrol per day significantly decreases metabolic rate at rest. A lower metabolic rate at rest will lead over the long term to weight gain unless calorie intake is reduced correspondingly.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to provide a scientific opinion by carrying out the additional assessment for resveratrol as a novel food in the context of Regulation (EC) No. 258/97.

2. Data and Methodologies

2.1. Data

The assessment of the safety of this novel food is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States, the responses of the applicant and additional information submitted by the applicant following EFSA requests for supplementary information (see 'Documentation provided to EFSA').

In accordance with Commission Recommendation 97/618/EC, resveratrol (resVida[®]) was allocated to Class 6 (foods produced using a novel process) by the competent authority of Ireland (FSAI) in its initial assessment report. The assessment of the safety of this novel food is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States, and the responses of the applicant. The data are required to comply with the information required for the novel foods of Class 6, i.e. structured schemes I, II, III, IX, XI, XII and XIII of the Commission Recommendation 97/618/EC. In the text, these structured schemes are listed in nine sections. This assessment only concerns risk that might be associated with consumption within the proposed conditions of use and it does not address risk beyond the proposed conditions of use. The Opinion does not address efficacy of resveratrol (resVida[®]) with regard to any claimed benefit.

2.2. Methodologies

The assessment follows the methodology set in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Assessment

3.1. Specification of the novel food

The novel food (resVida[®]) is described as an off-white to beige crystalline substance, with a *trans*-resveratrol content of > 99% (w/w). *trans*-Resveratrol (IUPAC nomenclature: 5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) has a molecular weight of 228.25 Da (Fig. 1). Synonym for the compound is 3,5,4'-trihydroxy-*trans*-stilbene. Its empirical formula is C₁₄H₁₂O₃. Its CAS number is 501-36-0.

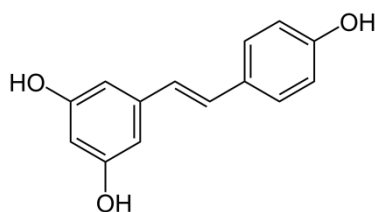


Figure 1: Structural formula of *trans*-resveratrol

The production process is based on a Wittig reaction followed by demethylation. The applicant has identified possible process impurities related to resveratrol including pinostilbene (3-[(*E*)-2-(4-hydroxyphenyl)ethenyl]-5-methoxyphenol, 3-methoxyresveratrol), pinostilbene isomer (4'-methoxyresveratrol, pterostilbene (4-[(*E*)-2-(3,5-dimethoxyphenyl)ethenyl]phenol), 2-methylresveratrol, 3-methylresveratrol and homo-resveratrol. *Cis*-resveratrol, resveratrol trimethyl ether and piceatannol are mentioned as possible other process impurities. In response to EFSA's request, the applicant stated that *cis*-resveratrol is consistently not detected and is below the 0.1% specification limit. According to the proposed specifications, total impurities are ≤ 0.5% with no impurity exceeding 0.1%.

The specifications are provided in Table 1. Test results for three batches show compliance with the specification limits apart for the values for mercury (Table 2).

No certificate has been provided regarding accreditation of the applicants' laboratory. However, the analyses of resveratrol and its by-products have been carried out using a validated HPLC method.

Table 1: Specifications of the Novel Food

Parameter	Target	Method of detection
Appearance	Off-white to beige crystals	
<i>trans</i> -Resveratrol	≥ 99%	HPLC-MS, HPLC-UV
Total by-products (related substances)	≤ 0.5%	HPLC-UV
Pinostilbene	≤ 0.1%	
Pinostilbene isomers	≤ 0.1%	
Pterostilbene	≤ 0.1%	
2-Methylresveratrol	≤ 0.1%	
3-Methylresveratrol	≤ 0.1%	
'Homo'-resveratrol ^(a)	≤ 0.1%	
Unknown impurities	≤ 0.1%	
Heavy metals	≤ 10 ppm	AAS (Ph.Eur)
Lead	≤ 1 ppm	
Mercury	≤ 0.1 ppm	
Cadmium	≤ 0.5 ppm	
Arsenic	≤ 1 ppm	AAS (Ph.Eur)
Loss on drying	≤ 0.5%	
Sulfated ash	≤ 0.1%	
Diisopropylamine	≤ 50 mg/kg	GC (Ph. Eur)
Microbiological purity	Compliant	Ph.Eur

^(a): Trivial DSM internal name for 5-[(1*E*,3*E*)-4-(4-hydroxyphenyl)-1,3-butadien-1-yl]-1,3-Benzenediol, its CAS number is 945484-65-1

Table 2: Analytical results for testing of three batches of the Novel Food

Parameter	Specification	Batch C10030512	Batch C10050612	Batch C10080612
Description	Off-white to beige colour powder	Beige powder colour	Off-white to beige colour powder	Off-white to beige colour powder
Identification	IR	Complies	Complies	Complies
Assay HPLC on dried basis (%w/w)	Not less than 99.0	99.9	100.5	99.4
Related substances (by HPLC, % area)	0.05	0.04	0.1	0.07
Pterostilbene	≤ 0.1%	nd ^(a)	nd	nd
Pinostilbene	≤ 0.1-%	0.02	0.02	0.01
Pinostilbene isomer	≤ 0.1%	nd	nd	nd
2-Methyl resveratrol	≤ 0.1%	nd	nd	nd
3-Methyl resveratrol	≤ 0.1%	0.01	0.01	0.01
Resveratrol trimethylether	≤ 0.1%	nd	nd	nd
'Homo'-resveratrol ^(b)	≤ 0.1%	nd	0.01	nd
Unknown impurities	≤ 0.1%	0.01	0.01	0.01
Total heavy metals expressed as lead	< 10 mg/kg	< 10	< 10	< 10

Lead	≤ 1 mg/kg	≤ 1	≤ 1	≤ 1
Arsenic	≤ 1 mg/kg	< 1	< 1	< 1
Cadmium	≤ 0.5 mg/kg	0.007	0.005	0.005
Mercury	≤ 0.1 mg/kg	≤ 1.0	≤ 1.0	≤ 1.0
Diisopropylamine	≤ 50 mg/kg	2	4	4
Total aerobic count	≤ 1000 cfu/g	70	10	< 10
Total combined yeast and mould	≤ 100 cfu/g	< 10	< 10	< 10
Enterobacteria	≤ 10 cfu/g	< 10	< 10	< 10
<i>Escherichia coli</i>	neg in 10 g	neg	neg	neg
<i>Staphylococcus aureus</i>	neg in 10 g	neg	neg	neg
<i>Pseudomonas aeruginosa</i>	neg in 10 g	neg	neg	neg
<i>Salmonella subsp.</i>	neg in 25 g	neg	neg	neg
Isopropyl alcohol	≤ 150 mg/kg	nd	nd	nd
Tetrahydrofuran	≤ 50 mg/kg	1	nd	nd
Ethyl acetate	≤ 50 mg/kg	nd	nd	nd
Methanol	≤ 50 mg/kg	nd	3	3
Toluene	≤ 50 mg/kg	1	6	4
Ethanol	≤ 1500 mg/kg	322	164	223
Diisopropylamine	≤ 50 mg/kg	2	4	4

^(a) not detected

^(b) 5-[(1*E*,3*E*)-4-(4-hydroxyphenyl)-1,3-butadien-1-yl]-1,3-benzenediol, its CAS number is 945484-65-1

Testing for potential residual aluminium for three batches (other than those in Table 2) with induced couple plasma mass spectrometry (ICP/MS) resulted to levels of below the limit of quantification (< 1 ppm).

Additional impurities that can be present at very low concentrations are residual solvents including diisopropylamine. The applicant proposes a limit of 50 mg/kg for diisopropylamine by taking into consideration that according to the threshold of toxicological concern (TTC) scheme diisopropylamine is assigned to Cramer Class III with TTC of 90 µg/day. A supplemental dose of the maximum proposed dose of 150 mg/day would correspond to 7.5 µg/day of diisopropylamine which is below the TTC.

The Panel considers that the information provided on the composition and specifications of the Novel Food is sufficient. The Panel notes that the results of batch testing did not meet the specifications for mercury.

3.2. Effect of the production process applied to the novel food

trans-Resveratrol subject to this assessment is produced by a chemical process, consisting of a Wittig reaction between 3,5-dimethoxybenzylphosphonate ester and 4-methoxy-benzaldehyde in tetrahydrofuran in the presence of sodium hydride followed by purification to yield trimethoxyresveratrol. This intermediate is dealkylated with aluminium trichloride and diisopropylamine and crystallised from isopropanol/water to yield resveratrol. Final step is recrystallisation from ethanol/water.

In response to a Member State's concerns, the applicant clarified that an old production method used a styrene derivative (5-ethenyl-1,3-benzenediol diacetate) and 4-bromo-phenyl acetate as starting materials. In that old method, the impurities included *p*-bromophenol (≤ 140 mg/kg), *N*-methyl-2-pyrrolidone (≤ 280 mg/kg) in addition to resveratrol-related impurities (≤ 0.4%).

The applicant provided a summary and a flow chart on the production process. The applicant claims that the production site is ICHQ7 and ISO 9001:2008 certified, although no certification has been

provided. In response to MS concerns, the applicant clarified that the purity of *trans*-resveratrol obtained with the old and the new production methods is the same, $\geq 99\%$. The analysis was performed using HPLC-MS. Only one peak, corresponding to *trans*-resveratrol, was visible.

In response to MS concern, the applicant states that the tests conducted meet the HACCP standard concept.

Stability

The final product is packed as a dry powder into polyethylene-laminated aluminium bags in order to be protected from light and humidity. Regarding stability under different pH condition, the applicant stated that resveratrol like other polyphenols is not stable in a solution with $\text{pH} > 8$. The Novel Food has a low solubility in water.

In response to MS concerns, the applicant provided additional information which indicated that the Novel Food as an ingredient at various concentrations and in different formulations (soft gels, tablets) produced with the old production process, was stable in different storage conditions at least up to 48 months at 25°C and 60% relative humidity (RH).

In response to EFSA's request to provide the results from stability testing of resveratrol produced with the new production process, the applicant provided data which demonstrated stability of the Novel Food when packed in low-density polyethylene bags inserted in high-density polyethylene containers for at least 36 months at 25°C with 60% RH and at least 6 months at accelerated conditions with a temperature of 40°C with 75% RH.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.3. History of the organism used as a source of the novel food

Not applicable.

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

The original application **suggested the applicant's intention** to market the Novel Food as a food supplement in capsule or tablet form. In response to EFSA's request, the applicant Novel Food indicated a maximum intake level of 150 mg/day. The applicant indicated that the Novel Food is to be marketed to supplement manufacturers. The target population is adults.

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

Resveratrol occurs naturally in grapes, grape juice and wine with low amounts found in peanuts, pistachios, and in blueberries (Zamora-Ros et al. 2008). *trans*-Resveratrol in red wines ranging from 0.05 to 10.9 mg/L, *cis*-resveratrol from 0.04 to 8.7 mg/L as well as the resveratrol glycosides *trans*- and *cis*-piceid (0.7–4.0 mg/L and 0.4–1.2 mg/L, respectively) have been reported (Goldberg et al., 1995; Lamuela-Raventós et al., 1995; Paulo et al., 2011). *trans*-Piceid up to 7.3 mg/kg fresh weight (fw) was found in freshly harvested table grapes (Cantos et al., 2002). In red grape juice, *trans*-resveratrol (up to 1.1 mg/L), *cis*-resveratrol (up to 0.2 mg/L), *trans*-piceid (up to 7.3 mg/L) and *cis*-piceid (up to 5.7 mg/L) have been reported (Romero-Pérez et al., 1999).

According to Zamora-Ros et al. (2008), the most important source of resveratrol and piceid were wines (98.4%), and grape and grape juices (1.6%), whereas peanuts, pistachios and berries contribute to less than 0.01% to the dietary intake in the Spanish adult cohort of the European Prospective Investigation Cancer (EPIC) study.

The applicant has estimated that the highest average per capita intake of naturally occurring resveratrol among countries is 0.46 mg/day using the European Data Food Networking Project (DAFNE), UN Food and Agriculture Organization (FAO) food balance sheets (FBS), the EFSA Comprehensive European Food Consumption Database for the entire population and the UK National Dietary and Nutrition Survey (NDNS) (Tennant, 2012). The highest 95th percentile intake of the EU Member States covered by the EFSA Database was 2.93 mg/day consumed by very elderly subjects.

In 2012, resveratrol (*trans*-resveratrol > 98%) produced with genetically modified *S. cerevisiae* was authorised in the EU as a novel food with a maximum permitted dose of 500 mg/day based on substantial equivalence to resveratrol derived from Japanese knotweed (*Polygonum cuspidatum*) which has a history of consumption within the EU prior to 1997. The applicant also indicates that on the European markets there are grape and grape seed food supplements containing 202 µg–500 mg of resveratrol per serving.

The Novel Food has been on the US market as a food supplement since June 2008 and as a food ingredient since June 2012. The Novel Food is also marketed in Russia since July 2008 as a food supplement. According to the applicant, the doses are up to 150 mg/day.

The Panel notes that the intended maximum supplemental intake of 150 mg/day value is about 50 times higher than the high percentile background intake from foods.

3.6. Nutritional information on the novel food

The Panel considers that resveratrol does not have a nutritionally relevant role in the human diet and that the consumption of the novel food ingredient is not nutritionally disadvantageous.

3.7. Microbiological information on the novel food

The Panel considers that the data provided do not raise safety concerns with regard to the microbiological quality of the Novel Food (see Section 1).

3.8. Toxicological information on the novel food

The applicant provided the toxicological study reports of a subacute (28-day), subchronic and developmental study conducted with synthetic *trans*-resveratrol of equally high purity (99.1%), produced with the previous ('old') production method of the applicant. *trans*-Resveratrol produced with the new process was used only in an Ames test. In addition, the applicant provided published articles on toxicological studies performed with high purity *trans*-resveratrol produced by other manufacturers.

The Panel considers that the new production process does not introduce any impurities at levels which significantly change the toxicity profile of the NF. Considering the high-purity of the material used in the previous toxicological studies, these data can be used for the safety assessment of the NF.

3.8.1. Absorption, distribution, metabolism and excretion (ADME)

In *in vitro* studies using Caco-2 cells and rat hepatocytes, synthetic *trans*-resveratrol produced by the applicant was rapidly converted to glucuronide and sulfate conjugates (Beck et al., 2006).

Such conjugations of this substance were also shown in rat studies (Mair et al., 2006; Beck et al., 2007). The first used ¹⁴C-*trans*-resveratrol administered as single gavage dose of 1 mg/kg bw. The mono-glucuronides of resveratrol and of dihydro-resveratrol were identified in urine, each accounting for around 30% of the administered radioactivity.

Other metabolites identified in the rat were conjugates of piceatannol (hydroxylated resveratrol), of dihydro-piceatannol, and of methylated dihydropiceatannol. In the study by Beck et al. (2007) which used both ¹⁴C-labelled (1 and 10) and unlabelled (1 000 mg/kg bw), *trans*-resveratrol the plasma elimination half-life was between 7 and 10 h for the high dose and was between 8 and 12.5 h for the labelled material. A study of tissue distribution and excretion (Beck and Bruchlen, 2007) used a dose of 1 mg/kg bw synthetic ¹⁴C-*trans*-resveratrol. After 72 h, 36.6% of activity was found in urine and 45.9% in faeces, whereas only 1.05% and 0.15% were remaining in the body after 48 and 72 h, respectively. The distribution in tissues was wide with slightly higher levels in those tissues associated with absorption and elimination. The time course of plasma levels showed two peaks, indicating enterohepatic circulation.

A study of 40 healthy adults given a single oral dose of 0.5, 1, 2.5 or 5 g synthetic *trans*-resveratrol not produced by the applicant, reported peak plasma levels of resveratrol and metabolites between 0.83 and 1.5 h after administration (Boocock et al., 2007). Urinary excretion was rapid with 77% of resveratrol-derived molecules within 4 h. The three most abundant metabolites were two resveratrol

monoglucuronides and resveratrol-3-*O*-sulfate with a maximum concentration of 1135–4294 ng/mL. The plasma half-lives of the three resveratrol conjugates, 3.2–11.5 h for the sulfate and 2.9–10.6 h for the glucuronides, were similar to those of parent resveratrol (2.9–8.9 h). The major human metabolite appears to be resveratrol-3-sulfate. Also in humans, a second, smaller peak was observed which occurred 5–6 h after intake. In faeces, traces of resveratrol metabolites were detected, consistent with enterohepatic recirculation.

According to the pharmacokinetic data from 15 human studies with high purity (> 98%), *trans*-resveratrol (synthetic, from yeast or from Japanese knotweed) included in two reviews on published human studies (Cottart et al., 2010; 2014), at least 70% of ingested resveratrol is rapidly absorbed followed by conjugation and excretion, mostly via the kidneys with no evidence of tissue accumulation. Small amounts of resveratrol and its metabolites in the faeces indicate an enterohepatic cycle.

In an open-labelled, single dose, cross-over bioequivalence study in 12 healthy adult subjects, total *trans*-resveratrol plasma levels over 8 h were compared following oral supplementation with of 30 mg of the Novel Food or with a supplement already in the EU market containing 32 mg resveratrol from Japanese knotweed (Norkus et al., 2007). The area under the time–concentration curve in the 8 h-period (AUC 0-480min) for the Novel Food and the comparator were 32.07 µg*min/mL and 29.95 µg*min/mL, respectively. The Novel Food produced a significant greater C_{max} (273 ± 17 vs. 199 ± 21 ng/mL; p < 0.05 by t-test) and a shorter T_{max} (71 ± 17 vs. 132 ± 15 min; p < 0.05 by t-test). According to the applicant, the smaller plasma peak following ingestion of the natural product maybe due to slower absorption of resveratrol from *Polygonum cuspidatum* due to specific matrix effects or interactions with other components or due to absorption delays because of the necessity to cleave glycosylated resveratrol in the comparator product. The Panel considers that it is unlikely that the small difference of the AUC and the observed differences in the C_{max} and T_{max} translate to relevant different safety profiles of resveratrol obtained from the two products.

3.8.2. Genotoxicity and carcinogenicity

A *Salmonella* typhimurium reverse mutation assay using strains TA1535, TA1537, TA98, TA100 and TA102 compliant with GLP and OECD guideline 471, was carried out with three samples of resveratrol at concentrations up to 5,000 µg/plate (Sokolowski, 2012). There was no indication of a mutagenic response in any of the strains tested, neither in the presence nor in the absence of S9 mix. This is in conformity with earlier results in a reverse mutation assay using strains TA1535, TA1537, TA98, TA100 and *E. coli* strain WP2 *uvrA* (pKM101) compliant with GLP and OECD guideline 471 and at concentrations up to 5,000 µg/plate with *trans*-resveratrol (resVida[®]) produced by a different process (Edwards and May, 2007). In addition, a photo mutagenicity study in *Salmonella* Typhimurium strains TA1537, TA98, TA100 and TA102 conducted with *trans*-resveratrol (resVida) (Edwards and Sokolowski, 2009) at doses up to 5,000 µg/plate and in compliance with GLP was also negative.

In addition, *trans*-resveratrol from a different source (*Yucca schidigera* bark), purity not indicated, was also reported negative in the Ames test at concentrations up to 500 µg/plate using four strains of *S. typhimurium* (Czeczot et al., 2003).

An *in vitro* chromosome aberration study with *trans*-resveratrol (99.1%) using human lymphocytes (Dunn, 2007) was conducted according to OECD guideline 473 and in compliance with GLP. Doses of 100 µg/ml and above were toxic to the cells, thus tested doses were 5, 10, 20 and 30 µg/ml without activation and 5, 30, 40 and 50 with metabolic activation. There were significant increases in structural chromosome aberrations at doses of 10, 20 and 30 µg/ml without activation and at 40 and 50 µg/ml with activation.

Schmitt et al. (2002) investigated potential genotoxicity in an *in vitro* assay utilising murine L5178Y lymphoma cells treated with 1, 10, 20, 40 or 60 µM resveratrol (from a different manufacturer) for 4 h. Thereafter, cells were washed, spun onto microscope slides and stained with fluorescent dyes. The number of micronuclei per 1,000 L5178Y cells, distorted spindles per 100 L5178Y cells in metaphase, dislocated chromosomes per 100 cells in metaphase and kinetochore-positive micronuclei per 100 micronuclei were determined. Treatment with resveratrol increased all of these parameters in a dose-dependent manner in murine L5178Y lymphoma cells. The authors noted that the determination of kinetochore signals in micronuclei and cell cycle analysis suggested that resveratrol

would not directly disturb mitosis and that also a cell-free tubulin polymerisation experiment indicated no direct effect of resveratrol on microtubule assembly. In this experiment, even at 200 mM concentration, resveratrol was unable to elicit any effect on the assembly of microtubule proteins, in contrast to diethylstilbestrol, which markedly inhibited this assembly at 20 mM. The author considered that the observed *in vitro* genotoxicity of resveratrol is not due to interference with the assembly of the mitotic spindle, despite the close structural similarity of resveratrol and diethylstilbestrol.

An *in vivo* mammalian erythrocyte micronucleus test (Hynes, 2007) was conducted in compliance with GLP and according to OECD guideline 474 (1997) using Sprague–Dawley rats given 0, 500, 1,000 or 2,000 mg/kg bw per day *trans*-resveratrol for 2 consecutive days by gavage. There was no change in the proportion of immature erythrocytes. There was no increase in micronucleated erythrocytes at any dose of resveratrol, thus the test indicates the absence of clastogenic activity *in vivo*. Exposure of the target tissue (bone marrow) was demonstrated by concomitant analysis of the plasma of three rats at the highest dose level in which the presence of free and conjugated resveratrol was confirmed.

An *in vivo* micronucleus test of *trans*-resveratrol (98.2%) from a different source (*Polygonum cuspidatum*) at doses up to 1,200 mg/kg bw per day (Chetelat et al., 2003) gave also a negative result.

In accordance with the EFSA Scientific opinion on genotoxicity testing strategies (EFSA, 2011), the Panel considers that the negative genotoxicity assay *in vivo* (Hynes, 2007) supported by Chetelat et al. (2003), is sufficient to rule out the concern based on the positive *in vitro* chromosomal aberration findings by Dunn (2007) and Schmitt et al. (2002) considering the endpoints structural and numerical chromosome aberrations (clastogenicity and aneugenicity).

3.8.3. Subacute, subchronic and chronic toxicity

A 90-day study has been conducted on synthetic *trans*-resveratrol (Edwards et al., 2007a) produced with the previous process by the applicant. Groups of 10 Wistar rats of each sex were given diets containing *trans*-resveratrol for 90 days at concentrations designed to provide an intake of 0, 120, 300 or 750 mg/kg bw per day. Additional groups of five rats of each sex were given control or high-dose (750 mg) diets for 90 days followed by at least 4 weeks on control diet (recovery animals). The study was performed in compliance with GLP and was based on OECD guideline 408. Dietary concentrations were adjusted weekly. Achieved doses were lower than targeted (male 0,118, 285, 668; female 0, 123, 300, 729 mg/kg bw per day). The exposure level of the animals was assessed from blood samples taken from 5 rats of each sex per group at weeks 4, 8, 13 and recovery weeks 1 and 5.

Animals were housed in groups of 5 and examined for clinical signs at least once daily. Observations included a functional observation battery, ophthalmoscopy, body weight, food consumption, water consumption and oestrous cycle determination. Blood was collected from all animals prior to necropsy and examined for a full range of haematological parameters (WBC, RBC, red blood cell distribution width (RDW), reticulocytes, Hb, haematocrit, MCV, MCH, MCHC, platelets, prothrombin time and activated partial thromboplastin time). Serum was examined for clinical biochemistry parameters (ALT, AST, ALP, total protein, albumin, globulin, albumin/globulin ratio, bilirubin, urea, creatinine, glucose, cholesterol, triglycerides, phospholipids, Na, K, Cl, Ca and inorganic phosphate). Urine was collected from all animals overnight and examined for a range of endpoints (volume, specific gravity, clarity, colour, pH, blood, WBC, bilirubin, urobilinogen, protein, ketones, glucose and nitrite) and the sediment examined.

A necropsy including macroscopic examination of tissues and weighing of organs (adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, spleen, seminal vesicles, testes, thymus, thyroid and uterus) was conducted on all main study animals at week 14 and the recovery animals at week 18. A full range of tissues was preserved for histological examination. Samples of liver and kidney were taken from all animals and frozen in liquid nitrogen for possible analysis. Histopathological examination was confined to control and high-dose animals together with any abnormal tissues from intermediate groups.

Measurement of total and free serum resveratrol confirmed a dose-related level of both which remained constant from 4 to 13 weeks of treatment.

One high-dose male died at day 30 but the cause of death could not be established. There were no treatment-related differences in clinical signs, functional observations, ophthalmoscopy, urinalysis, sperm analysis or oestrous cycle.

Body weight and body weight gain of high-dose animals were lower than that of controls throughout the study for males and from week 4 for females, and remained lower during the recovery period. The reductions were approximately 10% but were not always statistically significant. Body weight gain of the intermediate group (300 mg/kg bw per day) was slightly lower (approximately 3%), although statistically not significant, than controls for both sexes and body weight of females from this group was lower from week 4. The reduced body weight at the mid and highest dose was associated with (approximately 7%) reduced food consumption, however, due to group caging statistical analysis of these data is rather crude. Food consumption returned to control levels during the recovery period. No effect was observed on the body weight and food consumption of the 120 mg/kg bw per day treatment group.

Haematological results showed statistically significantly increased platelet counts, APTT and RDW and reduced Hb in the 300 mg/kg bw per day males but not at the higher dose. There were no comparable differences in females and no dose-related differences in the haematological results. Clinical biochemistry measurements showed a statistically significant dose-related increase in levels of plasma albumin in treated males but no difference in albumin/globulin ratio. Females showed a similar trend but the difference was not statistically significant.

At necropsy, there was no observation of any macroscopic changes associated with treatment. Organ weights and relative organ weights were generally similar in all groups with no evidence of dose-related differences. Microscopic examination of tissues showed no treatment-related differences in the incidence of most histopathological findings. A slight increase in tubular basophilia was seen in kidneys from high-dose females (3 vs 1). This would normally not be considered relevant, however in the light of previous findings (Crowell et al., 2004) with resveratrol, further investigation was conducted by the applicant. A review of the renal pathology in all treatment groups was conducted by an expert who concluded that there was no evidence of a treatment-related renal effect in this study and this view was accepted by the Panel.

The effects on body weight and food intake at the highest dose indicate that the dose tested is close to the maximum tolerated by this route. The authors of the study argued that the reduced weight gain seen at the highest dose represents lack of palatability. However, reduced weight gain was also reported at the highest dose for both sexes and for females at 1,000 mg/kg bw per day in a 28-day study in 20 Charles River CD rats of each sex 0, 300, 1,000 and 3,000 mg/kg bw per day *trans*-resveratrol (99.7% purity) by gavage (Crowell et al., 2004). In the 3,000 mg/kg bw dose group, there was also significantly increased incidence and severity of nephropathy (renal tubule dilatation, papillary necrosis, ulceration of pelvic epithelium, acute inflammation of the pelvis, acute inflammation of pelvic adventitia, glomerular necrosis, papillary fibrosis, hyperplasia of pelvic epithelium). Feed consumption was significantly reduced only in the 3,000 mg group. Although the dose at which reduced body weight was seen is higher than in the recent 90-day study, it confirms this as an effect of *trans*-resveratrol, even when administered by gavage; thus the reduced weight gain observed in the subchronic rat study cannot be fully explained by lack of palatability. The Panel, therefore, considers that the intermediate dose in this subchronic study (300 mg/kg bw per day) causing slight reductions in body weight and weight gain is to be the NOAEL for the Novel Food in this study.

In order to obtain a more refined estimate, the applicant was asked to perform a benchmark dose (BMD) analysis on the observed body weight effects **separately for either sex**. The US EPA's BMDS software was used in version 2.6.

For body weight gain data of male rats, a homogenous variance model was considered appropriate (Test 3 p-value > 0.05). The goodness-of-fit p-value, however was < 0.05 for all models. Therefore, all models had to be rejected as not appropriate. For body weight gain data of female rats, neither a homogenous nor a non-homogenous variance model was considered appropriate (Test 3 p-value < 0.05). Therefore, all models were rejected as not appropriate and the applicant concluded that the endpoint body weight gain in the subchronic toxicity study with resveratrol in rats cannot be modelled with the current version of BMDS.

For modelling the body weight of male rats, a homogenous variance model was considered appropriate. Acceptance criteria were met only by the 2nd degree polynomial model. The fit of the 2nd degree polynomial model resulted in a BMDL₀₅ of 367 mg/kg bw per day.

Modelling body weight data of female rats, neither a homogenous nor a non-homogenous variance model were considered appropriate (Test 3 p-value < 0.05). Test 3 p-value for the non-homogenous variance model, however, was > 0.04, closely to, but below, the acceptance criterion (p > 0.05). Applying the other acceptance criteria led to five accepted models. Of those accepted models, the 2nd degree exponential model had the most conservative BMDL₀₅ of 344 mg/kg bw per day.

Despite test 3 p-value of > 0.04 for the non-homogenous variance model, the Panel considers the 344 mg/kg bw per day to be the BMDL₀₅ for this study derived from body weights of female rats.

A 28-day preliminary study performed in the same laboratory and with the same test substance, using doses of 0, 50, 150 or 500 mg/kg bw per day by dietary administration and including a range of observations similar to the 90-day rat study showed no effects of treatment at any dose (Wolz and Scase, 2005).

Elliot et al. (2009) reviewed the available data on synthetic *trans*-resveratrol toxicity and summarised a number of studies using doses up to 3,000 mg/kg bw per day (28-day studies in rats, dogs and rabbits) but gave very limited information on experimental design. The authors claimed that this resveratrol formulation had an increased bioavailability due to its higher stability and micronisation. The only finding reported in rats was a slight haemolytic anaemia without associated pathology at a dose of 1,000 mg/kg bw per day and a NOAEL was concluded at 300 mg/kg bw per day. In dogs, the highest dose tested appears to be 300 mg/kg bw per day and this was concluded to be the NOAEL. Rabbits showed severe weight loss at a dose of 750 mg/kg bw per day; renal effects were seen similar to those reported by Crowell et al. (2004) in rats and the NOAEL was concluded to be 500 mg/kg bw per day for males and 250 mg/kg bw per day for females. The same review also reports the results of a 6-month rat study which showed increased bilirubin levels at a dose of 1,000 mg/kg bw but without any associated histological changes. The NOAEL was concluded to be 300 mg/kg bw per day. A 6-month study in rabbits at doses of 0, 100, 300 and 500 mg/kg bw per day showed a range of minor haematological and clinical biochemistry effects at the highest dose which were all reversible. The NOAEL was concluded to be at the highest dose of 500 mg/kg bw per day.

Johnson et al. (2011) reported a 90-day gavage study using groups of 20 CrI:CD[®][SD]IGS rats of each sex to which synthetic resveratrol of high purity was given at doses of 0, 200, 400 or 1,000 mg/kg bw per day. The study is described in sufficient detail and appears to be consistent with OECD 408. The only effects reported are significant reduction in body weight gain at the intermediate and high dose and elevated but reversible bilirubin levels at the highest dose. The NOAEL was concluded to be 200 mg/kg bw per day by the authors. Measurement of plasma levels of free resveratrol after 13 weeks of exposure gave values of 0.430, 0.912, and 1.87 for males and 185, 308, and 547 ng/ml for female rats. Values for total resveratrol were 18,400, 25,300, and 36,700 ng/ml for males and 19,000, 36,200 and 50,300 ng/ml for female rats. In a second study covered by Johnson et al. (2011), groups of dogs given resveratrol in a capsule form at doses of 0, 200, 600 or 1,200 mg/kg bw per day for 90 days showed evidence of renal neutrophil infiltrate in both sexes (male 2/4 and 1/4; female 2/4 and 3/4) but apart from a reduction in body weight in the high-dose group no other adverse effects were reported. The NOAEL was concluded to be at 600 mg/kg bw per day.

3.8.4. Chronic toxicity/carcinogenicity studies

Chronic toxicity/carcinogenicity studies performed in accordance with OECD Guidance 451 and 452 or 453 were not carried out on *trans*-resveratrol. In response to a Member State's comment to assess the risk of carcinogenicity, the applicant referred to a 6-month study with p53-knockout mice (Horn et al., 2007). In this study, synthetic *trans*-resveratrol with a purity of 99.5% produced by another manufacturer, was administered by gavage to groups of 10 male and 10 female animals per dose group (i.e. 1,000, 2,000 and 4,000 mg/kg bw per day). Histopathology identified the kidney (hydronephrosis in all treatment groups, i.e. 1,000, 2,000 and 4,000 mg/kg bw per day) and urinary bladder (epithelial hyperplasia in the mid-dose group) as target tissues for resveratrol toxicity. Effects on bodyweight were not observed this study in neither dose group. Despite the limitations of the p53-knockout mouse model for carcinogenicity testing (i.e. short duration, not generally accepted as an

alternative to carcinogenicity testing in accordance with OECD Guidance), the Panel notes that this study does not indicate a carcinogenic effect.

The Panel notes that toxicological studies on *trans*-resveratrol are currently being conducted as part of the US National Toxicology Program (NTP) which includes also a 2-year chronic toxicity/carcinogenicity study, along with a study on subchronic toxicity and a modified one-generation study in rats. Although the live phase of these studies is complete, the pathology investigations are yet to be completed and the date for publication of the results is unknown.

3.8.5. Developmental toxicity

In a GLP-compliant embryo–fetal toxicity study conducted in accordance with OECD guideline 414 (Edwards et al., 2007b), *trans*-resveratrol was administered in the diet to groups of 22 pregnant CrI:CD® (SD) IGS BR rats between days 5 and 15 of gestation at concentrations designed to provide a dose of 0, 120, 300 or 750 mg/kg bw per day. The mean achieved dose was 0, 124, 309 and 783 for each group, respectively. Body weight was measured daily and the mean weight was significantly lower than that of controls for the highest dose from day 6 onwards, for the intermediate group from day 7 onwards and for the lowest dose group from day 11 onwards. Differences remained until the end of the study at day 20 with the final body weight of all treated groups being very similar and approximately 4% lower than the controls. Necropsy at day 20 showed no evidence of treatment-related abnormalities in the maternal animals and no differences between the groups in the numbers of resorptions or live young or in pre- or post-implantation losses. Litter size and fetus weight were similar in all groups. A difference in anogenital distance in female pups at the highest dose was considered to be a chance finding as the difference was extremely small, although it was statistically significant (mean 2.7 mm vs control 2.8 mm; $p < 0.05$). Fetal examination for soft tissue and skeletal abnormalities showed no treatment-related effects thus the NOAEL for fetal development was the highest dose tested. The only maternal effect observed was reduction in body weight, however this occurred at all doses. The Panel considers 120 mg/kg bw for maternal toxicity as a LOEL.

A similar study using synthetic *trans*-resveratrol from another manufacturer claimed to have an increased bioavailability due to its higher stability and micronisation, reported by Elliot et al. (2009) used gavage administration of doses of 0, 300, 1,000 and 3,000 mg/kg bw per day between days 7 and 17 of gestation. The authors concluded a development NOAEL of 1,000 mg/kg bw per day and a maternal NOAEL of 300 mg/kg bw per day.

The above reported 90-day rat study with target dietary exposures up to 750 mg/kg bw per day of *trans*-resveratrol did not identify any adverse effects on the oestrous cycle length or sperm count, motility and morphology (Edwards et al., 2007a).

The Panel considers that the studies provided do not raise concerns regarding developmental toxicity.

3.8.6. Oestrogenic activity

Member States expressed concern that *trans*-resveratrol has potential for oestrogenic activity, due to its stilbene structure and structural similarity to the potent oestrogen diethylstilbestrol (Edwards et al., 2011).

According to Bhat et al. (2001), resveratrol binds to oestrogen receptors and acts as a weak oestrogen antagonist in human breast cancer cell lines. In this study with oestrogen receptor and reporter gene (luciferase) transfected human breast cancer cell lines MCF-7 and S40, resveratrol showed a weak oestrogen activity, but when resveratrol was combined with 17- β -estradiol (E2) (1 nM), a dose-dependent antagonism was observed. In contrast, resveratrol functioned as a pure oestrogen antagonist with T47D and LY2 cells. In another experiment described in this article, resveratrol induced the expression of progesterone receptor in a mouse mammary organ culture model, when administered alone, but expression was suppressed in the presence of E2 (1 nM). Resveratrol also inhibited the formation of oestrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz(a)anthracene in mammary glands and reduced *N*-methyl-*N*-nitrosourea-induced mammary tumorigenesis when administered to female Sprague–Dawley rats by gavage. The authors of this study concluded that in the absence of E2, resveratrol exerts mixed oestrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E2, resveratrol functions as an anti-oestrogen.

Also Schmitt et al. (2002) showed in *in vitro* studies that resveratrol binds to human oestrogen receptor and stimulate MCF-7 cell proliferation in a dose-dependent manner. MCF-7 in a dose-dependent manner at concentrations ranging from 10 nM to 10 mM, whereas higher concentrations lead to decreased relative proliferation.

No signs for an oestrogenic effect were found in the histological examination of ovaries and in the organ weight measurements of ovaries, testes and uterus in rats receiving resveratrol at doses up to 1,000 mg/kg/day for 3 months (Johnson et al. 2011).

When administered to female weanling rats, ***trans*-resveratrol at dose levels up to 100 µg did not** exhibit oestrogenic effects as indicated by uterine weight and epithelial cell height of the uterus (Turner et al. 1999). **In a second experiment of this study, 1000 µg *trans*-resveratrol** administered for 6 days antagonised the E2-induced cholesterol lowering effect in weaning rats.

trans-Resveratrol has also been shown to be non-oestrogenic in a uterotrophic assay studying several relevant endpoints (uterine weight, histopathology, immunohistochemical expression of nuclear oestrogen receptor- α (ER α), progesterone receptor protein, gene expression of ER α and PR and peroxidase induction) in Wistar rats receiving up to 575 mg/kg bw on each of 3 consecutive days (Freyberger et al., 2000). A second rat uterotrophic assay (Ashby et al., 1999) in which up to 120 mg/kg bw *trans*-resveratrol was administered by oral gavage and/or the subcutaneous route for 3 consecutive days, showed no effect on wet and dry uterine weight.

The Panel considers that these data do not indicate concerns with respect to oestrogenic activity of resveratrol *in vivo*.

3.8.7. Human studies

The applicant provided 23 articles on human studies and one review on resveratrol (Cottart et al., 2014) which covered additional 14 human studies.

Fourteen of a total of 38 studies investigated pharmacokinetic, acute tolerability or acute effects of single or multiple doses up to 5 g on metabolic and vascular endpoints such as endothelial function or blood pressure, inflammatory markers and oxidative stress with a maximum duration of 96 hours (Almeida et al., 2009; Boocock et al., 2007; Elliot and Jirousek, 2008; Ghanim et al., 2011; Goldberg et al., 2003; Kennedy et al., 2010; Meng et al., 2004; Norkus et al., 2007; Nunes et al., 2009; Ortuno et al., 2010; Vaz-da-Silva et al., 2008; Vitaglione et al., 2005; Walle et al., 2004; Wong et al., 2010).

The Panel considers that no conclusions with regards to the safety of resveratrol at the intended dose levels can be drawn on the pharmacokinetic studies and short-term clinical studies.

Eight trials studied resveratrol at dose levels (< 1–40 mg/day) significantly below the use level intended by the applicant (Brasnyo et al., 2011; Ghanim et al., 2010; Magyar et al., 2011; Nguyen et al., 2009; Ortuno et al., 2010; Tome-Carneiro et al., 2012a,b; Zamora-Ros et al., 2012). The Panel considers that no conclusions with on these studies due to the relatively low doses studied.

Two studies were with patients suffering from advanced colorectal cancer with study duration of 8 days and 14 days, respectively (Patel et al., 2010; Howells et al., 2011). In an uncontrolled study, 24 patients with multiple myeloma received daily 5 g resveratrol for 21 days (Popat et al., 2013). Those patients with progressive disease after the 21 days received then also bortezomib (proteasome inhibitor). The study was terminated because of acute adverse kidney effects (cast and crystal nephropathy, acute tubular damage, renal failure). The Panel notes the uncontrolled study design, the comedication and the high occurrence of renal impairment in such patients. The Panel considers that no conclusions can be drawn on the three studies with cancer patients.

Four studies investigated supposed beneficial effects of resveratrol on markers of oxidative stress in obese subjects (4 weeks, 150 mg resveratrol) (De Groote et al., 2012), on methylation of cancer-related genes in mammary ductoscopy specimen of 39 adult women with an increased breast cancer risk (12 weeks, 100 mg resveratrol) (Zhu et al., 2012), on endothelial function in adults with metabolic syndrome (3 months, 100 mg resveratrol) (Fujitaka et al., 2011), or on glucose homeostasis, inflammatory markers, resting energy rate, oxidation rate of lipids (4 weeks 1.5 g resveratrol/day) (Poulsen et al., 2013), without or with very limited parameters relevant for the safety assessment

such as blood lipids, blood pressure or ALT which were not altered by the treatment. The Panel notes that these four studies provide no evidence for the safety of the NF.

In addition to the study by Wong et al. (2010), two other studies were conducted with the Novel Food (resVida[®]) produced by the applicant: A 4-week randomized controlled trial (RCT) with a daily dose of 150 mg *trans*-resveratrol by Timmers et al. (2011) and the 12-week RCT with a daily dose of 75 mg by Yoshino et al. (2012). In the RCT by Timmers et al. (2011) with cross-over design, 11 obese but otherwise healthy men (BMI >30) consumed 150 mg *trans*-resveratrol per day. The authors reported a significantly decreased sleeping metabolic rate for the first, but not for the second night after the 28-day treatment. The article also reports a statistically significant higher daytime respiratory quotient for the verum group, whereas the 24-h respiratory quotient, the 24-h energy expenditure and body mass at the end of the intervention did not differ significantly. The Panel also notes the exploratory character of this study with multiple metabolic and haematological endpoints measured. The Panel considers that this reference does not provide evidence that resveratrol has an effect on metabolic rates. In the other RCT with (resVida[®]) intake of 75 mg *trans*-resveratrol of the Novel Food per day over 12 weeks was studied in 30 healthy non-obese postmenopausal women with normal glucose tolerance. No effects were observed on the resting metabolic rate, body composition, plasma lipids, inflammatory markers and insulin sensitivity (Yoshino et al., 2012). Both studies included clinical chemistry and haematological testing of blood samples and reported that no adverse effects have been observed. The Panel notes the short study duration (Timmers et al., 2011) and the low dose studied by Yoshino et al. (2012) compared to the use level intended by the applicant.

No effect on resting energy rate and oxidation rate of lipids was seen in an RCT with 24 obese but otherwise healthy men after 4 weeks of daily intake of thrice 500 mg *trans*-resveratrol derived from yeasts (Poulsen et al., 2013).

In a 4-week open-label study, Crandall et al. (2012) enrolled ten (seven female) overweight to obese subjects with a mean age of 72 years with impaired glucose tolerance to study effects of resveratrol (daily dose 1, 1.5 or 2 g) on several metabolic parameters. The article reports that no serious adverse events or changes in the clinical chemical and haematological parameters (including serum creatinine, liver enzymes, blood cell counts) and urine analysis. Among adverse events considered 'possibly' related to study treatment, one subject reported mild diarrhoea (dose not given for this subject) and two women reported increased 'hot flushes'.

Hot flushes were also reported by one post-menopausal women enrolled in an uncontrolled 4-week study by Chow et al. (2010) with 42 healthy non-smoking adults (31 women, 11 men) received 1 g resveratrol/day to determine the effect of resveratrol on drug metabolising enzymes. Other adverse events reported possibly or probably related to resveratrol were diarrhoea (n=4), heartburn (n=3), increased appetite, mood alteration, menstrual changes (each 2 subjects), flatulence, nausea, abdominal pain, vivid dreams and urine odour.

Another open-label, single-arm, within-subject control study in eight healthy subjects investigating the steady-state 12-hour pharmacokinetics of 2 g *trans*-resveratrol twice daily consumed with the breakfast and with a snack in the evening (in total 4 g/day) for 8 days reported the occurrence of diarrhoea in six out of eight subjects (la Porte et al., 2010).

No adverse effects were observed in an open-label randomised controlled trial with 62 patients suffering from type 2 diabetes mellitus receiving 250 mg resveratrol over 3 months on biochemical and clinical parameters including blood pressure, blood lipids, serum creatinine among others (Bhatt et al., 2012). Also no differences were observed for the body weight and BMI between the groups at the end of the intervention.

In an uncontrolled intervention study, 44 healthy subjects received a daily dose of 0.5, 1, 2.5 or 5 g synthetic resveratrol of another manufacturer for 29 days (Brown et al., 2010). Mild to moderate gastrointestinal symptoms such as nausea, flatulence, abdominal discomfort and diarrhoea reported only for subjects receiving 2.5 or 5 g/day, were considered to be treatment related. These effects occurred most often at 30–60 min after resveratrol ingestion and commenced 2–4 days after the treatment stopped. The article reports that weight loss or adverse effects on clinical chemistry and haematological parameters were not observed.

Elliot et al. (2009) briefly summarised two RCTs with in total 240 male type 2 diabetes patients receiving with either once or twice 2.5 g synthetic *trans*-resveratrol per day for 28 days. According to

this article, there were no dose-related or dose-limiting effects on physiological, clinical chemistry, haematological, cardiovascular, liver and renal parameters in any treatment group and adverse events were generally mild in nature and reversible. The Panel notes that the article did not specify the type adverse events.

The Panel notes that diarrhoea or other gastrointestinal symptoms were reported in four uncontrolled intervention studies (Brown et al., 2010; Chow et al., 2010; Crandall et al., 2012; la Porte et al., 2010) comprising in total 104 subject given doses of 1 g resveratrol/day or higher. No effects were reported on clinical chemistry, haematology parameters, serum creatinine, liver enzymes and urinalyses in two RCTs with duration of 1 month (Elliot et al., 2009) with in total 240 subjects receiving 2.5 or 5 g resveratrol/day, respectively, and in one study with 250 mg/day with study duration of 3 months. The Panel notes the limited number of subjects and/or duration of the provided studies.

The Panel considers that the human studies do not indicate adverse effects below 1 g.

3.8.8. Interaction with drugs

Referring to the initial assessment report by the Irish FSAI that some *in vitro* evidence suggested that resveratrol (especially resveratrol-3-*O*-sulfate) may result in weak interference with the metabolism of pharmaceuticals, one MS expressed concerns that resveratrol may interfere with the efficacy of certain medication, especially with those which are metabolised by sulfation.

In *in vitro* studies, CYP inhibition by *trans*-resveratrol was observed in low micromolar range, *trans*-resveratrol sulfate caused inhibition at higher concentrations whereas *trans*-resveratrol glucuronide (up to 500 µM) did not cause CYP inhibition (Chun et al., 1999; Piver et al., 2001; Yu et al., 2003; Regev-Shoshani et al., 2004; Elliott et al., 2009). *In vitro* data for resveratrol-3-*O*-sulfate showed an IC₅₀ for CYP2C9 inhibition (9 µM) similar to the maximum plasma concentration reached after the 1000 mg dose in the study by Boocock et al. (2007).

In a 4-week study with 42 healthy non-smoking adults, all adults received 1 g resveratrol/day to determine the effect of resveratrol on drug- and carcinogen-metabolising CYP (CYP1A2, CYP2D6, CYP2C9 and CYP3A4) and phase II enzymes. As compared to baseline values resveratrol intervention was found to inhibit the phenotypic indices of CYP3A4, CYP2D6, CYP2C9 and to induce (by about 16%) the phenotypic index of 1A2 (Chow et al., 2010).

The Panel considers that the metabolite *trans*-resveratrol sulfate could inhibit CYP enzymes in human and may interact with medicines which are mainly metabolised by CYP2C9.

3.9. Allergenicity

Considering the chemical nature and the purity of the NF, the Panel has no concerns regarding allergenicity.

4. Discussion

Reduced body weight gain is seen consistently in animal studies. Although it has been considered by some authors to be an effect of palatability, the effect is also seen after gavage administration indicating a possible toxic effect. Effects on the kidney have been reported at high doses in several animal studies. In the subchronic toxicity study (Edwards et al., 2007a), the Panel notes that body weight and body weight gain were the critical endpoints. The Panel considers the 344 mg/kg bw per day derived from body weights of female rats to be the BMDL₀₅ for this study. This BMDL₀₅ of 344 mg/kg bw per day is close to the NOAELs derived in other studies on the same endpoint.

In a developmental toxicity study with rats, reduced body weight was seen already at 124 mg/kg bw per day for dams, which was the lowest dose tested in this study. This indicates that pregnant rats may be especially susceptible.

On the basis of the BMDL₀₅ of 344 mg/kg bw per day and the intended intake of 150 mg/day (about 2 mg/kg bw for an adult), the margin of exposure is 172. For pregnant rats, it is below 62.

The Panel notes that diarrhoea or other gastrointestinal symptoms were reported in four uncontrolled intervention studies at doses of 1 g resveratrol/day or higher, corresponding to about 14 mg/kg bw per day, which is by a factor of 10 lower than the LOEL of the animal studies. No treatment related

effects were reported for doses of below 1 g/day in studies up to 3 months of duration. Taking into account the limitations of the available human studies, i.e. low number of subjects, short duration, limited safety-relevant endpoints studied, no study reports available, the Panel considers that these studies per se do not provide sufficient evidence of safety. Considering the weight of evidence, the Panel concludes that the intended intake level of 150 mg/day for adults does not raise safety concerns.

The Panel considers that the metabolite *trans*-resveratrol sulfate could inhibit CYP enzymes in human and may interact with medicines which are mainly metabolised by CYP2C9.

5. Conclusions

The Panel concludes that the novel food, synthetic *trans*-resveratrol is safe under the proposed conditions of use.

Documentation provided to EFSA

1. Dossier 'resVida[®] DSM1437-001, October 2012; submitted to FSAI by DSM' received on 3 April 2014. Additional data were provided on 9 and 29 October 2014 and 12 January 2015.
2. Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on resveratrol as a novel food ingredient. SANCO/E6/SH/ks D (2014) 1054798; Ref. Ares(2014)1042397, dated 3 April 2014.
3. Initial assessment report carried out by the Food Safety Authority of Ireland (FSAI): 'Safety Assessment of Resveratrol (resVida[®])'.
4. Member States' comments and objections.
5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMD	Bench mark dose
BMDL	Benchmark Dose Lower confidence limit
bw	body weight
EPA	Environmental Protection Agency
fw	fresh weight
GLP	good laboratory practice
Hb	haemoglobin
LOEL	lowest-observed-effect level
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentrations
MCV	mean corpuscular volume
MCV	mean corpuscular haemoglobin concentrations
NOAEL	No observed adverse effect level
RCT	Randomized controlled trial

Appendix A – Benchmark Analysis

A.1. Data

Results on body weight from the 90-day rat study by Edwards et al. (2007a)

Endpoint	Sex	Dose levels (mg/kg bw/d)	No of animals	Mean	Standard deviation
Body weight Week 13 (g)	m	0	15	482	47.3
		120	10	518	46.9
		300	10	480	36.1
		750	14	435**	30.9
Body weight Week 13 (g)	f	0	15	284	42.4
		120	10	289	17.0
		300	10	264	21.6
		750	15	268	17.9

*/** Dun nett-test based on pooled variance significant at 5% (*) or 1% (**) level

A.2. BMR:

Default value (per cent change = 5%)

A.3. Software:

US EPA's BMDS version 2.6 (<http://www.epa.gov/bmnds>)

A.4. Additional assumptions:

None

A.5. Table of BMD results from analysis of body weight data

Results of the BMD analysis of body weight of male rats in week 13 of the 90-day repeated dose study with resveratrol (Edwards et al. 2007a).

Model	Variance model ^(a)	p-value Test 3 ^(b)	Goodness-of-fit p-value	Scaled residual	BMD/BMDL ratio	Model accepted ^(c)	Akaike Information Criterion	BMD ₀₅	BMDL ₀₅
Exponential2	H	0.3555	0.0148	0.454	1.44	No	422.94	273.96	190.53
Exponential3	H	0.3555	0.0203	-0.473	1.76	No	421.89	453.55	257.05
Exponential4	H	0.3555	0.0148	0.454	1.59	No	422.94	273.96	172.38
Exponential5	H	0.3555	N/A	0.000	1.25	No	423.33	293.25	233.93
Hill	H	0.3555	0.0282	0.000	1.29	No	421.33	295.01	228.17
Linear	H	0.3555	0.0166	0.412	1.40	No	422.70	280.37	200.88
Polynomial2	H	0.3555	0.0650	-0.336	1.17	Yes	419.97	427.66	366.61
Polynomial3	H	0.3555	0.0196	-0.464	1.25	No	421.95	457.32	366.81
Power	H	0.3555	0.0201	-0.484	1.77	No	421.91	458.35	259.05

^(a): H: homogenous (constant) variance model

non-H: non-homogenous (power) variance model

^(b): Is the chosen variance model acceptable?

^(c): Acceptance criteria are listed in Section 3.6: Plausible dose-response curve, Test 3 p-value > 0.05, goodness-of-fit p-value > 0.05, scaled residual at nearest dose level between -2 and +2.

Results of the BMD analysis of body weight of female rats in week 13 of the 90-day repeated dose study with resveratrol (Edwards et al. 2007a).

Model	Variance model ^(a)	p-value Test 3 ^(b)	Goodness-of-fit p-value	Scaled residual	BMD/BMDL ratio	Model accepted ^(c)	AIC	BMD ₀₅	BMDL ₀₅
Exponential2	H	0.0008	0.2216	0.347	1.89	No	388.60	558.49	294.99
Exponential3	H	0.0008	0.2216	0.347	1.89	No	388.60	558.49	294.99
Exponential4	H	0.0008	0.1158	-0.987	257.25	No	390.06	321.77	1.25
Exponential5	H	0.0008	N/A	-0.280	2.12	No	389.93	261.19	123.35
Hill	H	0.0008	0.5614	0.351	1.65	No	387.93	204.23	123.56
Linear	H	0.0008	0.2185	0.339	1.84	No	388.63	567.87	308.48
Polynomial2	H	0.0008	0.2185	0.339	1.84	No	388.63	567.87	308.48
Polynomial3	H	0.0008	0.2185	0.339	1.84	No	388.63	567.87	308.48
Power	H	0.0008	0.2185	0.339	1.84	No	388.63	567.87	308.48
Exponential2	H	0.0404	0.0992	0.573	1.54	Yes ^(d)	381.94	527.49	343.52
Exponential3	H	0.0404	0.0992	0.573	1.56	Yes ^(d)	381.94	527.49	343.52
Exponential4	H	0.0404	0.5749	1.443	2.93	No	379.63	127.63	43.58
Exponential5	H	0.0404	N/A	1.239	2.99	No	381.31	138.82	46.36
Hill	H	0.0406	0.9617	1.240	3.82	No	379.31	125.87	32.94
Linear	H	0.0406	0.1926	0.565	1.50	Yes ^(d)	380.04	536.96	357.29
Polynomial2	H	0.0406	0.1926	0.565	1.50	Yes ^(d)	380.04	536.96	357.29
Polynomial3	H	0.0406	0.0008	1.050	-	No	392.07	-9999.00	-
Power	H	0.0404	0.1933	0.565	1.50	Yes ^(d)	380.04	536.96	357.29

^(a): H: homogenous (constant) variance model
non-H: non-homogenous variance model (model variance model in BMDS)
^(b): Is the chosen variance model acceptable?
^(c): Acceptance criteria are listed in Section 3.6: Plausible dose-response curve, Test 3 p-value > 0.05, goodness-of-fit p-value > 0.05, scaled residual at nearest dose level between -2 and +2, BMD/BMDL ratio < 2.
^(d): Models accepted as Test 3 p-value of 0.04 is considered sufficiently close to the threshold of 0.05.

A.6. Figure

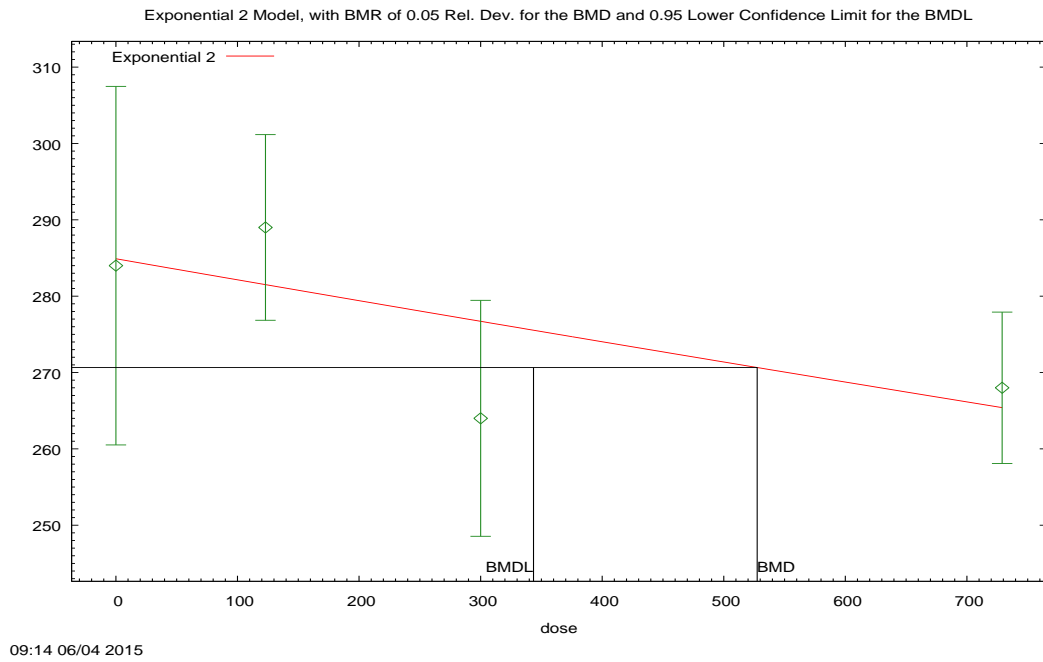


Figure: Fitted curve for female body weight data