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SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of propyl gallate (E 310) as a food additive[1]

EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of propyl gallate (E 310). Propyl gallate is an antioxidant authorised as a food additive in the EU. In 1987, the SCF established a group ADI of 0-0.5 mg/kg bw/day for three gallates (propyl, octyl, dodecyl). JECFA in its last evaluation in 1996 allocated an ADI only to propyl gallate of 0-1.4 mg/kg bw/day and did not establish ADIs for octyl and dodecyl gallate. The Panel considered that no substantial new toxicological data have emerged since this last evaluation. The Panel concluded that the 90-day toxicity study in rats was the key study for the evaluation of propyl gallate considering the uncertainties and lack of a NOAEL in the carcinogenicity database on propyl gallate. Based on the NOAEL of 135 mg propyl gallate/kg bw/day of this study and taking account of the Opinion of the Scientific Committee of EFSA on Default values, the Panel concluded that an uncertainty factor of 300 should be applied for extrapolation from a subchronic to chronic data and due to the limitations in the reproductive toxicity database and derived an ADI of 0.5 mg/kg bw/day for propyl gallate.The Panel also concluded that there was no longer a basis for the present group ADI and that propyl, octyl and dodecyl gallates should be evaluated separately and the present group ADI should be withdrawn. The high level of exposure exceeded the ADI in adults and the elderly. However, given the conservatism of the exposure assessment, the Panel concluded that the use of propyl gallate as food additive at the current uses and use levels is not of safety concern.

[1] On request from European Commission, Question No EFSA-Q-2011-00479, adopted on 1 April 2014.

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† Deceased.

* Minor changes of editorial nature were made. The changes do not affect the contents of this report. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

Amendment note: In section 2.3, the word “propanoyl” was modified to “propanol”. An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request.

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KEY WORDS

propyl gallate, n-propyl 3,4,5-trihydroxybenzoate, gallic acid propyl ester, n-propyl gallate, E 310, CAS no.: 121-79-9, food antioxidant

SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of propyl gallate (E 310) when used as food additive.

Propyl gallate (E 310) is an antioxidant authorised as a food additive in the EU.

Initial evaluations were based on a read- across approach on the basis of presumed toxicokinetic similarities for a group of three gallates (propyl, octyl and dodecyl). In its last evaluation in 1987 (SCF, 1989), the SCF established a group ADI for propyl, octyl and dodecyl gallate of 0-0.5 mg/kg bw/day. JECFA last evaluated the three gallates at the forty-sixth meeting in 1997 and decided that the grouping of propyl, octyl and dodecyl gallates was not scientifically justifiable and evaluated them separately. In its last evaluation JECFA established an ADI of 1.4 mg/kg bw/day for propyl gallate but ADIs could not be established for for octyl and dodecyl gallate (JECFA, 1997). Therefore, new monograph was not prepared in 1997, the last monograph was published in 1993 (JECFA, 1993).

Specifications have been defined in the EU legislation Commission Regulation (EU) No 231/2012 and by JECFA (JECFA 2006). The purity is specified to be not less than 98 %.

Little is known about the occurrence of propyl gallate in food and only fragmentary data exist. These data were published in articles which focussed on development of analytical methods. The data on manufacturing were very limited and provided no details on side reactions, impurities in chemicals and by products originating from different synthesis conditions. Furthermore, there was no information on the specific technological reasons for the selection of a particular gallate (octyl gallate, dodecyl gallate or propyl gallate). It is possible that the choice could be due to differences in fat solubility as reflected by different P_{ow} values. The Panel noted that the use of hydrochloric acid in the manufacture of propyl gallate could result in chlorinated by-products and that there were limits for chlorinated organic compounds in the specification but no information on the identification or quantification of potential chlorinated by-products was available to the Panel.

The SCF considered that biotransformation data were scarce but that propyl gallate was well absorbed and hydrolysed to propyl alcohol and gallic acid (SCF, 1989). Gallic acid was further metabolised to 4-O-methyl gallic acid by O-methylation, glucuronidated and excreted via urine. Propyl alcohol was incorporated into intermediate metabolism of the individual. JECFA (JECFA, 1993) had temporarily concluded that in vitro metabolism studies in different tissues demonstrated some similarities in the metabolism of the different gallates, had however requested in vivo pharmacokinetic and metabolic studies to confirm these in vitro results. As these studies had not been performed, JECFA concluded that there was no longer sufficient evidence to allocate a group ADI for the gallates.

The Panel concluded that no substantial new toxicological data have emerged since the last JECFA monograph (JECFA, 1993) and evaluation (JECFA, 1997).

Acute toxicity studies exist in different species for propyl gallate which had low, if any, acute toxicity.

There were short-term and subchronic toxicity studies on propyl gallate in rats, mice and dogs. In a 13-week study in rats (Speijers et al., 1993) a NOAEL of 135 mg propyl gallate/kg bw/day was

established in the second highest dose group. In the highest dose group, adverse effects were observed on the haematopoietic system. This study was the basis of the ADI established by JECFA (JECFA, 1997). The Panel agreed with this conclusion.

A large number of genotoxicity studies exist on propyl gallate and several have emerged since the latest JECFA evaluation. The literature search revealed new genotoxicity studies (comparative studies, SCE, single strand breaks and other DNA damage, bone marrow micronucleus test).

In mutagenicity tests in bacteria, propyl gallate was weakly mutagenic in *Salmonella* Typhimurium TA102, a strain sensitive to oxidative DNA damage, with and without metabolic activation; negative results were obtained with the other tester strains. In cytogenetic tests in vitro, propyl gallate was clastogenic in rodent cell lines, where it induced micronuclei, chromosomal aberrations and sister chromatid exchanges with and without metabolic activation; conversely, a most uniform negative results were obtained in cytogenetic tests in human cells. A questionable positive response, with no dose-effect relationship, was also observed in a gene mutation assay in mouse lymphoma cells (only performed without metabolic activation). In vivo, propyl gallate was reported to be not genotoxic in limited oral studies in rats (chromosomal aberrations and dominant lethal assays) and negative in two mouse bone marrow micronucleus assays, in which propyl gallate was administered by i.p. injection up to lethal doses. Despite the limited database available, the Panel considered that the weight of the available evidence including the antioxidant action of the compound and the complete hydrolysis of propyl gallate in vivo indicates that the genotoxic activity elicited by propyl gallate in vitro is unlikely to be expressed in vivo.

Based on the above mentioned considerations, the Panel concluded that propyl gallate is not an in vivo genotoxin. No further genotoxicity studies are needed.

There were chronic toxicity and carcinogenicity studies; two studies in mice, three studies in rats and one in guinea pig on propyl gallate (JECFA, 1993). Overall, propyl gallate was not considered to be carcinogenic.

The Panel considered that the reproduction studies were not appropriate for hazard characterisation since they are old, poorly described and lack information about reproductive performance.

Data for developmental toxicity were less limited. Oral studies in mice, rats, rabbits and hamsters were available. Doses around 300 mg/kg bw/day did not appear to be associated with adverse effects and could be regarded as a NOAEL for developmental toxicity.

Propyl gallate was shown to be a modulator of oestrogen receptor activity in vitro (Ter Veld et al., 2006; Amadasi et al., 2009). Whether the in vitro oestrogenic activity of propyl gallate is associated with an adverse effect in an intact organism remains to be investigated. The Panel noted that the significance of these in vitro findings for risk assessment of propyl gallate is difficult to be ascertain in absence of additional studies in vivo. The Panel considers that in light of the limitation of reproductive toxicity database any potential adverse effects due to the oestrogenic activity of propyl gallate in vivo might be further explored to reduce this uncertainty.

The Panel concluded that the 90-day toxicity study in rats was the key study for evaluation of propyl gallate. Based on the NOAEL of 1910 mg/kg feed (equivalent to 135 mg/kg bw/day) in this 90-day study and taking account of the Scientific Committee of EFSA Opinion on Default values (EFSA, 2012), the Panel concluded that an uncertainty factor of 300 instead of 100 should be applied for extrapolation from subchronic to chronic data and due to the limitations in the reproductive toxicity database. The Panel, therefore, derived an ADI of 0.5 mg/kg bw/day for propyl gallate.

The SCF established a group ADI for propyl gallate, together with octyl and dodecyl gallate. This group ADI was based on a read-across approach using presumed toxicokinetic similarities (metabolism to gallic acid and the corresponding alcohol) for a group of the three gallates (propyl,

octyl and dodecyl). However, having reviewed the data on the toxicokinetics (rate and extent of metabolism) of propyl, octyl and dodecyl gallate in this evaluation of propyl gallate and ongoing evaluations of octyl and dodecyl gallate, the Panel considered that the available data were insufficient for the read-across to be valid. Therefore, there was no longer a basis for the present group ADI and the Panel concluded that propyl, octyl and dodecyl gallate should be evaluated separately and the present group ADI should be withdrawn. The Panel noted that JECFA had reached a similar conclusion in 1997.

In this opinion, the dietary exposure to propyl gallate was estimated according to two exposure scenarios: 1) using maximum permitted levels (MPLs) for propyl gallate, and 2) using reported data on analytical levels, supplemented with MPLs for those food categories for which no analytical data were reported (refined exposure assessment). Using MPLs the the high level of exposure was maximally 1.11 mg/kg bw/day in adults. In the refined exposure assessment, this exposure estimate was reduced to 1.04 mg/kg bw/day in adults. The higher exposure in adults (as well as the elderly) as opposed to children was due to the exposure to propyl gallate via the consumption of food supplements : food supplements contributed maximally for 43 % to the total exposure in adults and 48 % in the elderly in three countries. Removal of this source of exposure from the assessment resulted in a reduction in the high level exposure in adults to maximally 0.33 mg/kg bw/day using MPLs and 0.25 mg/kg bw/day using reported data on analytical levels.

The Panel noted that also the refined exposure assessment is still conservative. Of the nine food categories considered in this assessment, six had no or too few reported analytical data. For these categories, MPLs were therefore used. Furthermore, for the remaining three food categories, almost all reported concentrations were below the limit of quantification (LOQ). Of the 1029 data points reported, only two (two pork lard samples) had quantifiable levels of propyl gallate.

Except via food, there is additional exposure to propyl gallate from non-food products. Gallates are permitted in FCMs and may be present in all foodstuffs to a SML of 30 mg/kg expressed as the sum of the three gallates. The Panel noted that exposure to propyl gallate from its use in food contact materials may contribute substantially to the total exposure to propyl gallate and that combined exposure to propyl gallate from its use as food additive in foods and additive to food contact materials does exceed the ADI for most population groups on average and on high level consumption.

Furthermore, the gallates are also permitted in cosmetic products both leave-on products such as skin creams and wash-off products such as hand and bathing soaps. According to the Regulation 2009/1223/EC on cosmetic product there is no limit. Furthermore, gallates may also be bought as part of products available on the Internet, e.g. as capsules, tablets although such sale may not be legal. The exposure via these routes is however unknown, and could therefore not be taken into account in this opinion.

The high level of exposure exceeded the ADI in adults and the elderly. However, given the conservatism of the exposure assessment the Panel concluded that the use of propyl gallate as food additive at the current uses and use levels is not of safety concern. The Panel noted that to refine further the exposure assessment additional analytical or use data on propyl gallate in especially breakfast cereals, soups and broth, processed nuts and food supplements would be needed.

If after conducting these further refined exposure assessments exposure remained greater than the ADI, or if additional uses and use levels were proposed, the Panel considered that given the uncertainties identified, additional toxicological data would be requested.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union (EU) before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[4]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU5 of 2001. The report "Food additives in Europe 20006" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] OJ L 80, 26.03.2010, p19
[5] COM(2001) 542 final.
[6] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. INTRODUCTION

The present opinion deals with the re-evaluation of the safety of propyl gallate (E 310) when used as a food additive.

Propyl gallate (E 310) is an antioxidant authorised as a food additive in the EU. It has previously been evaluated by the EU Scientific Committee for Food (SCF, 1976, 1989), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1962, 1965, 1967, 1972a, 1972b, 1974, 1976, 1980, 1987, 1993, 1997) and Nordic Working group on Food Toxicology and Risk Assessment (TemaNord, 2002).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that became available since then and the data available following a public call for data[7]. Not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

The evaluation of propyl gallate was initially performed by the SCF on a read-across approach on the basis of presumed toxicokinetic similarities for a group of three gallates (propyl, octyl and dodecyl). However, in its last evaluation JECFA (1997) decided that the grouping of propyl, octyl and dodecyl gallates was not scientifically justifiable and evaluated them separately. TemaNord (2002) also recommended that the three permitted gallates should be evaluated individually.

2. TECHNICAL DATA

2.1. Identity of the substance

Propyl gallate is the propyl ester of 3,4,5-trihydroxybenzoic acid. Its molecular formula is $C_{10}H_{12}O_5$ and has a molecular weight of 212.20 g/mol. The CAS Registry Number is 121-79-9 and the EINECS number is 204-498-2. Its chemical name is n-propyl 3,4,5-trihydroxybenzoate and its structural formula is shown in Figure 1.

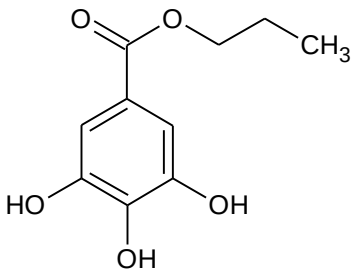

Figure 1: Structural formula of propyl gallate

Propyl gallate is a white or creamy-white, crystalline odourless solid that has a solubility in water of 3.5 g/L at 25 °C and is freely soluble in ethanol, ether and propane-1,2-diol. It has a melting range of 146-150 °C and an octanol/water partition coefficient (P_{ow}) of 63 ($\log P_{ow}$ 1.8) (Boyd and Beveridge, 1979; JECFA, 2006; TemaNord, 2002). Other synonyms used include gallic acid propyl ester and Progallin P.

[7] EFSA call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. November 2009 . http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.pdf

2.2. Specifications

Specifications for propyl gallate according to Commission Regulation (EU) No 231/2012[8] and by JECFA (JECFA 2006) are listed in table 1.

Table 1: Specifications for propyl gallate (E 310) according to Commission Regulation (EU) No 231/2012[8] and to JECFA (2006).

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Description	White to creamy-white, crystalline, odourless solid	White or creamy-white, crystalline odourless solid
Definition		
Assay	Content not less than 98 % on the anhydrous basis	Not less than 98.0 % and not more than 102.0 % on the dried basis
Identification		
Solubility	Slightly soluble in water, freely soluble in ethanol, ether and propane-1,2-diol	Slightly soluble in water; freely soluble in ethanol, ether and propane-1,2-diol
Melting range	Between 146 °C and 150 °C after drying at 110 °C for four hours	146 – 150 °C after drying
Purity		
Gallic acid	-	Test summary: sample treated to obtain the gallic acid as a precipitate. The melting point of the gallic acid so obtained is about 240°
TLC-separation of gallate esters	-	Test summary: TLC is run with a sample solution and a control solution. The major spot of the sample solution corresponds with that for propyl gallate in the control solution
Loss on drying	Not more than 0.5 % (110 °C, four hours)	Not more than 0.5 % (110°, 4 h)
Sulphated ash	Not more than 0.1 %	Not more than 0.1 %
Free acid	Not more than 0.5 % (as gallic acid)	Not more than 0.5 % as gallic acid
Chlorinated organic compound	Not more than 100 mg/kg (as Cl)	Not more than 100 mg/kg as chlorine
Specific absorption $E_{1cm}^{1\%}$ ethanol	$E_{1cm}^{1\%}$ (275 nm) not less than 485 and not more than 520	-
Arsenic	Not more than 3 mg/kg	-

[8] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1-295.

Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

TemaNord (2002) stated that the toxicological significance of the chlorinated impurities should be considered.

2.3. Manufacturing process

Propyl gallate is produced by the esterification of gallic acid with propanol, at a temperature between 100°C and180°C. The reaction is catalysed by strong acids (e.g. sulphuric acid, hydrochloric acid or p-toluene sulphonic acid). The water produced in the reaction is removed via azeotropic distillation with the unreacted alkyl alcohol. The product is isolated and purified by crystallisation. The crystals can be washed and recrystallised to remove impurities. The final product is dried, sieved and packed (Rebafka and Nickels, 1986, Sas et al, 2001, and additional information provided to EFSA by Inabata in 2010). The Panel noted the presence of chlorinated organic compounds in the final product, however no further information was provided on the nature of these impurities.

2.4. Methods of analysis in food

Different phenolic antioxidants are frequently added simultaneously to the same food product interfering with the analysis of propyl gallate.

Several methods have been developed for the analysis of the food additives, these include paper chromatography, thin layer chromatography (TLC), gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), infrared spectroscopy, fluorometric analyses, ultraviolet spectrophotometry, colorimetric analyses and capillary electrophoresis (CE) (CIR, 2007, Xiang et al., 2007).

Propyl gallate was determined in various food products including safflower oil by GLC after synthesis of the trimethylsilyl derivative (Kline et al., 1978; Page and Kennedy, 1976).

Propyl gallate was determined by microbore HPLC with electrochemical detection in chewing gums and dried potato flakes (Boussenadji et al., 1993).

HPLC (Gertz and Herrmann, 1983) and micellar electroKinetic capillary electrophoretic chromatographic (MEKC) (Hall et al., 1994; Delgado-Zamarreno et al., 2000; Sha et al., 2007) methods are capable of analysing simultaneously different antioxidants containing propyl gallate. The MEKC method has been used to analyse propyl gallate, octyl gallate and dodecyl gallate in cola beverages and low calorie jam (Boyce, 1999).

A method based on a flow injection coupled to a monolithic column for the simultaneous determination of various food additives, including propyl gallate, has also been described. Samples of various foods and beverages were tested. According to the authors, this method was quick, with high repeatability and high reproducibility (Garcia-Jimenez et al., 2007).

A method based upon a biparameter sensor using flow injection with solid-phase spectrometry is described for the simultaneous determination of propyl gallate and BHA in food (e.g. chicken soup, chicken cream and chicken broth). The data correlated with the results obtained by applying an HPLC method (Capitan-Vallvey et al., 2003; Grosset et al., 1990).

An oxidative coupling reaction method between propyl gallate and orcicol in the presence of hydrogen peroxide and horseradish peroxidase followed by a spectrometric quantification has been used for the determination of gallates. The analysis was sensitive and accurate, however, no food samples were analysed (Sekharan et al., 2008).

A capillary electrophoresis method was successfully used for the separation of 6 compounds in food, including propyl gallate (Liu et al., 2008).

2.5. Reaction and fate in food

Propyl gallate is a polyphenolic ester which could be susceptible to ester hydrolysis. According to a review (Anonymous, 2007) *"propyl gallate is stable in neutral or slightly acidic chemical environments but unstable when heated or in mild alkaline environment."* No relevant information on the reaction and fate in foods of gallates was found in the literature.

2.6. Case of need and proposed uses

Maximum Permitted Levels (MPLs) of propyl gallate have been defined in Annex II of Regulation (EC) No 1333/2008[9] as amended on food additives for use in foodstuffs.

Currently, propyl gallate is an authorised antioxidant in the EU with MPLs ranging from 25 to 1000 mg/kg in foods.

Table 2 summarises foods that are permitted to contain propyl gallate, individually, or in combination with octyl and dodecyl gallate, tertiary-butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA) and the corresponding MPLs as set by Annex II of Regulation (EC) No 1333/2008[12].

Table 2: MPLs of propyl gallate (E 310) in foods according to the Annex II of Regulation (EC) No 1333/2008

FCS Category number	Foods	Restrictions/exception	Maximum level (mg/l or mg/kg as appropriate)
1.5	Dehydrated milk as defined by Directive 2001/114/EC	only milk powder for vending machines	200*
2.1	Fats and oils essentially free from water (excluding anhydrous milk fat)	only fats and oils for the professional manufacture of heat-treated foods; frying oil and frying fat (excluding olive pomace oil) and lard, fish oil, beef, poultry and sheep fat	200*[a]
2.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	only frying fat	200*[b]
4.2.5.4	Nut butters and nut spreads	only processed nuts	200*[a]
4.2.6	Processed potato products	only dehydrated potatoes	25*
5.3	Chewing gum		400**
6.3	Breakfast cereal	only pre-cooked cereals	200*[c]
6.7	Pre-cooked or processed cereals	only pre-cooked cereals	200*
7.2	Fine bakery wares	only cake mixes	200*
8.2.1	Non-heat-treated processed meat	only dehydrated meat	200*[c]
12.2.2	Seasonings and condiments		200**[c]
12.5	Soups and broths	only dehydrated soups and broths	200*[c]
12.6	Sauces		200*[c]
15.1	Potato-, cereal-, flour- or starch-based snacks	only cereal-based snack foods	200*
15.2	Processed nuts		200*[c]
17.1	Food supplements supplied in a solid form including capsules and tablets and		400**

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 354, 31.12.2008, p. 16.

FCS Category number	Foods	Restrictions/exception	Maximum level (mg/l or mg/kg as appropriate)
	similar forms, excluding chewable forms		
17.2	Food supplements supplied in a liquid form		400**
17.3	Food supplements supplied in a syrup-type or chewable form		400**

* used singly or in combination with octyl gallate, dodecyl gallate, TBHQ and BHA. When combinations of the antioxidants gallates, TBHQ and BHA are used, the individual levels must be reduced proportionally

** used singly or in combination with octyl gallate, dodecyl gallate, TBHQ, BHA and BHT. When combinations of the antioxidants gallates, TBHQ, BHA and BHT are used, the individual levels must be reduced proportionally

a) expressed on fat basis

b) The maximum level is applicable to the sum and the levels are expressed as the free acid

c) Maximum limit expressed on fat

2.6.1 Reported data on analytical levels of propyl gallate

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call for occurrence data in June 2012 with deadline 1st September 2012[10]. Data on propyl gallate (E 310) including present uses and use patterns (i.e. which food categories and subcategories, proportion of foods within categories/subcategories in which it is used and actual use levels (typical and maximum use levels)) were requested from relevant stakeholders. European food manufacturers, national food authorities, research institutions, academia, food business operators and any other interested stakeholders were invited to submit analytical data on propyl gallate (E 310) in food and beverages. Analytical data on propyl gallate were provided by Denmark (n = 95), Germany (n = 426), Slovakia (n = 500) and Ireland (n=8). Analysed foods were mainly animal and vegetable fats (predominantly pork lard and margarine), but included also fish and potato products. The limit of detection (LOD) and quantification (LOQ) of propyl gallate in these food categories ranged from 0.1 to 18 mg/kg and 0.2 to 58 mg/kg, respectively. Concentrations in all analysed foods were below LOD or LOQ, except for two (out of six) pork lard samples analysed in Denmark, which contained 8.6 and 11 mg propyl gallate/kg. The LOQ of these samples was 5 mg/kg. (NB: the 300 pork lard samples analysed by Slovakia did not contain quantifiable levels).

Apart from the food categories in which propyl gallate is authorised (Table 2), also data were reported in food categories in which propyl gallate is not allowed, including food categories 4.1 (Fruit unprocessed), 6.1 (Whole, broken, or flaked grains), 8.1 (Unprocessed meat), 9.1.1 (Unprocessed fish), 9.1.2. (Unprocessed molluscs and crustaceans), 9.2 (Processed fish and fishery products including molluscs and crustaceans), 9.3. (Fish roe), 12.7 (Salads and savoury based sandwich spreads), and 18 (Processed foods not covered by categories 1 to 17). All reported concentrations were below the LOD or LOQ in these food categories. These food categories were not considered in the exposure assessment (section 2.8).

2.7. Information on existing authorisations and evaluations

In 1976, the SCF established a group ADI of 0-0.2 mg/kg bw/day for three gallates (propyl, octyl, dodecyl). This ADI was the same as that allocated by JECFA in the same year. The ADI was based on a No Observed Effect Level (NOEL) of 50 mg/kg bw/day in long-term studies on rats and mice and the application of a safety factor of 250 due to doubts on their effects on reproduction. The SCF last evaluated the three gallates (propyl, octyl, dodecyl) in 1987 (SCF, 1989) and established that a group

[10] http://www.efsa.europa.eu/en/dataclosed/call/120601.pdf

ADI of 0-0.5 mg/kg bw/day was acceptable. The Committee confirmed the previously established No Effect Level (NEL) of 50 mg/kg bw/day in long-term studies on rats and mice (SCF, 1976) and in the light of new study data applied the usual safety factor of 100.

Propyl gallate and the other gallates (octyl, dodecyl) were first evaluated by JECFA in 1962 (JECFA, 1962) and since then have been evaluated on numerous other occasions (1965, 1967, 1972a, 1972b, 1974, 1976, 1980, 1987, 1993, 1997). For the first evaluation no ADI was established but recommendations were made for more data. JECFA last evaluated the three gallates in 1996 (JECFA, 1997) and allocated propyl gallate an ADI of 0-1.4 mg/kg bw/day based on a No Observed Effect Level (NOEL) of 1910 mg/kg in the feed (equivalent to 135 mg/kg bw/day) in a 90-day study in rats and a safety factor of 100. The temporary ADIs for octyl and dodecyl gallate established by JECFA in 1993 were not extended since the requested data on kinetics and metabolism were not made available.

TemaNord, 2002 recommended that "*The allocation of the ADI for these compounds should be reconsidered individually.*"

Propyl gallate is listed as Generally Recognised As Safe (GRAS) under the US Federal Food and Drug Administration (21CFR 184.1660) at a maximum content of total antioxidants of 0.02 % of the fat or oil content of the food (21CFR 582.3660) (US Federal Register, 1976).

The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) evaluated the results of a project researching the health effects of mixtures of food additives including propyl gallate in in vitro and in vivo experiments (COT, 2008; Stierum, 2008) (see section 3.3).

2.8. Exposure assessment

2.8.1. Food consumption data used for exposure assessment

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with data from national information on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those foods and population groups were the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011b). The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents and adults. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as mentioned in Table 3:

Table 3: Population groups considered for the exposure estimates of propyl gallate

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Bulgaria, Finland, Germany, Netherlands
Children[11]	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[111]	from 65 years and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates.

2.8.2. Exposure to propyl gallate from its use as food additive

Exposure to propyl gallate from its use as a food additive was calculated by using (1) MPLs and (2) reported data on analytical levels if available, supplemented with MPLs (refined exposure assessment), both combined with national consumption data for the five population groups. Table 4 lists the concentrations used in both exposure scenarios. These concentrations were based on the following assumptions which are consistent with the EFSA ANS Panel opinion on BHA (2011):

- Fats and oils for the professional manufacture of heat-treated foodstuffs and frying oils and frying fat (2.1 and 2.2.2) are mainly part of the food categories fine bakery wares (7.2) and potato-, cereal-, flour- or starch based snacks (15.1). These latter two food categories were assumed to have a fat content of 30 %.

- Dehydrated soups and broths (12.5) were assumed to have a fat content of 10 %.

- Sauces (12.6) and herbs, condiments and seasonings (12.2) were assumed to have a fat content of 30 %.

- Pre-cooked cereals are part of the food category breakfast cereals (6.3), which were assumed to have a fat content of 20 %.

- Dehydrated potatoes are part of the food category potato-, cereal-, flour- or starch based snacks (15.1). This food category was assigned the MPL of fats and oils for the professional manufacture of heat-treated foodstuffs corrected for fat content (see first bullet), which is higher than the MPL for dehydrated potatoes (Table 2).

- Consumption of milk powder for vending machines (1.5) and dehydrated meat (8.2.1) were assumed negligible in respect of the amounts consumed and are not included in the assessment.

- In the refined scenario, food categories with at least 10 analytical levels were substituted with the maximum analytical level reported, corrected for fat percentage of the category. When all

[11] The terms “children” and “the elderly” correspond respectively to “other children” and the merge of “elderly” and “very elderly” in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011b).

analytical levels within the food category were below the LOD or LOQ, it was assumed that the additive was not present (0 mg/kg)[12].

Table 4: Summary of levels used in the MPL and refined exposure assessment (mg/kg)

Category number and foods	Exposure scenario		Additional information on analytical level
	MPL	Refined[1]	
5.3 Chewing gum	400	400	No analytical data available
6.3 Breakfast cereals	40	40	No analytical data available
7.2 Fine bakery wares	60	3.3	Based on highest analytical level reported in pork lard (11 mg/kg)
12.2 Herbs, spices, seasonings	60	60	Analytical data not used, n<10
12.5 Soups and broths	20	20	Analytical data not used, n<10
12.6 Sauces	60	0	Data on dressing, curry sauce and mayonnaise; Germany n=104, Denmark n=24, Jensen et al., 2006: n=104
15.1 Potato-, cereal-, flour- or starch-based snacks	60	0	Data on potato crisps and corn chips; Germany n=18, Denmark n=10
15.2 Processed nuts	60	60	No analytical data available
17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	400	400	Analytical data not used, n<10

[1] Refined exposure scenario: use of reported data on analytical levels if available, otherwise MPL

High level exposure (typically 95th percentile of consumers only) was calculated by adding the 95th percentile of exposure from one food group (i.e. the one having the highest exposure value) to the mean exposure resulting from the consumption of all other food groups. This is based on the assumption that an individual might be a high level consumer of one food group and an average consumer of the other food groups. This approach has been tested several times by the Panel in the re-evaluation of food colours and has shown a reasonable correlation with high level total exposures when using the raw food individual consumption data. Therefore, this approach was preferred in order to avoid excessively conservative estimates.

Furthermore, the Panel noted that the exposure estimates should be considered conservative as it is assumed that all processed foods belonging to a food category contain the antioxidant propyl gallate added at the MPLs or the maximum reported data on analytical levels.

Table 5 summarises the estimated exposure to propyl gallate from its use as food additive of all five population groups according to the two exposure scenarios. Exposures were estimated using the Food Additives Intake Model (FAIM), available on the EFSA website (http://www.efsa.europa.eu/en/topics/topic/additives.htm). The exposure estimates per population group and survey are presented in Appendix A.

[12] This was true for the food categories 12.6 and 15.1 (Table 4), for which no concentrations above the LOD or LOQ were reported. It can however be argued that since the additive is allowed in these two categories that propyl gallate may be present. When assuming that propyl gallate is present at the level of the LOD or LOQ (so-called upper bound (UB) estimate), a mean UB concentration of 8.13 and 5 mg/kg could be calculated for these categories, respectively. After correction for the fat content (30 %), a concentration of maximally 2.4 and 1.5 mg/kg could be calculated, respectively. Using these concentrations in the exposure assessment instead of 0 mg/kg (Table 4) did however not influence the exposure estimate.

Table 5: Summary of anticipated exposure to propyl gallate (mg/kg bw/day) from its use as food additive using MPLs or reported analytical data, supplemented with MPLs (refined exposure assessment) in five population groups (mg/kg bw/day)

Exposure	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	0.04-0.22	0.07-0.36	0.05-0.18	0.02-0.11	0.02-0.10
• High level[13]	0.14-0.57	0.16-0.59	0.11-0.37	0.08-1.11	0.05-0.72
Estimated exposure using reported data on analytical levels, supplemented with MPLs*					
• Mean	0.02-0.05	0.02-0.14	0.01-0.07	0.01-0.05	0-0.05
• High level	0.09-0.25	0.04-0.35	0.04-0.20	0.05-1.04	0.02-0.67

* Refined exposure assessment

2.8.3. Main food categories contributing to exposure of propyl gallate using MPLs

Table 6 lists the main food categories contributing most to the total mean to propyl gallate with a contribution of at least 5% using MPL.

Table 6: Main food categories contributing to the dietary exposure to propyl gallate using MPLs (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing.

Category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		range of % contribution to total exposure (number of surveys*)				
5.3	Chewing gum	8 (1)	6 -17 (3)	10 (1)	10-11 (2)	-
6.3	Breakfast cereals	7-46 (3)	6-24 (12)	6-20 (8)	8-25 (6)	6-39 (3)
7.2	Fine bakery wares	20-83 (4)	21-86 (15)	22-78 (12)	30-74 (14)	37-69 (6)
12.2	Herbs, spices and seasonings	9 (1)	-	10 (1)	7-15 (2)	6-15 (2)
12.5	Soups and broths	9 (1)	6-31 (7)	6-29 (5)	6-36 (6)	18-39 (2)
12.6	Sauces	11-15 (3)	7-28 (12)	5-32 (10)	7-28 (14)	7-22 (6)
15.1	Potato-, cereal-, flour- or starch based snacks	7-11 (3)	5-13 (10)	6-17 (9)	6-10 (9)	6 (1)
15.2	Processed nuts	-	-	-	5-19 (3)	12 (1)
17	Food supplements	-	-	-	6-29 (2)	6-41 (2)

* Total number of surveys may be greater than total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific age range.

2.8.4. Main food categories contributing to exposure of propyl gallate using reported data on analytical levels, supplemented with MPLs (refined exposure assessment)

Table 7 lists the main food categories contributing most to the total mean exposure to propyl gallate with a contribution of at least 5% using reported data on analytical levels, supplemented with MPLs (refined exposure assessment, Table 4).

[13] typically 95th percentile of consumers only

Table 7: Main food categories contributing to exposure to propyl gallate using reported data on analytical levels, supplemented with MPLs (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing.

FCS Category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		range of % contribution to total exposure (number of surveys*)				
5.3	Chewing gum	12 (1)	6-40 (5)	5-24 (4)	8-25 (4)	9 (1)
6.3	Breakfast cereals	33-69 (4)	10-67 (15)	12-79 (12)	7-72 (15)	5-55 (6)
7.2	Fine bakery wares	16-44 (3)	5-43 (13)	6-37 (11)	7-30 (12)	10-32 (5)
12.2	Herbs, spices and seasonings	14-43 (2)	6-35 (4)	7-50 (3)	9-51 (5)	27-47 (3)
12.5	Soups and broths	17-43 (2)	13-71 (8)	19-77 (6)	16-82 (7)	9-81 (3)
12.6	Sauces	-	-	-	-	-
15.1	Potato-, cereal-, flour- or starch based snacks	-	-	-	-	-
15.2	Processed nuts	6-10 (2)	5-12 (8)	5-22 (8)	6-58 (12)	6-51 (5)
17	Food supplements	-	9-11 (2)	7 (1)	9-43 (7)	19-48 (2)

* Total number of surveys may be greater than total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific age range.

2.8.5. Exposure via other sources

Propyl gallate, octyl gallate and dodecyl gallate may be added to all feedstuffs to a maximum content of 100 mg/kg feedstuff expressed as the sum of the three gallates (European Commission, 2004). Therefore, theoretically, gallates could be present in food for humans as carry-over from feedstuff. However, no actual measurements of the gallate residual content in meat, milk, poultry and eggs have been found in our literature search.

According to Commission Regulation (EU) No 10/201114 on plastic materials and articles, all three gallates are permitted in Food Contact Materials (FCMs). In the case of food additives permitted also in FCMs the so-called dual-use rule applies. This means that when migration from the FCMs occurs, the limits and permitted food groups in the food additives legislation must be observed. According to Commission Regulation (EU) No 10/2014 on plastic materials the three gallates have a collective specific migration limit (SML) of 30 mg/kg food. Therefore, the exposure resulting from this source is considerably higher than those from its use as food additive. The exposure estimates from food contact materials are based on the assumption that individuals consume 1 kg of food packed in plastics regardless of their age. Using average body weights (EFSA, 2012) and the assumption of 1 kg of packed foods consumed, exposure to propyl gallate would be 2.5, 1.3, 0.6 and 0.4 mg/kg bw/day for toddlers, children, adolescents and adults, respectively.

Furthermore, the gallates are also permitted in cosmetic products both leave-on products such as skin creams and wash-off products such as hand and bathing soaps. According to the Regulation 2009/1223/EC on cosmetic product there is no limit. Furthermore, gallates may also be bought as part of products available on the Internet, e.g. as capsules, tablets although such sale may not be legal. The exposure via these routes is however unknown, and could therefore not be taken into account in this opinion.

[14] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 012, 15.01.2011, p. 89.

2.9. Uncertainty analysis

Some uncertainties in the exposure assessment of propyl gallate have been discussed in the related chapters of this chapter. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties were considered and summarised below:

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate of propyl gallate as food additive

Sources of uncertainties	Direction *
Consumption data: different methodologies / representativeness / under reporting / misreporting	+/-
Estimation chronic exposure based on food consumption data covering a few days	+
Linkage between MPLs or reported analytical levels and food items in the consumption database: uncertainties on the composition of foods	+/-
Assumption that all foods in a category contain additive at the MPL or maximum analytical level.	+
Assumptions on the proportion of fat in different food categories	+
Possible national differences in use levels of food categories, data set not fully representative of foods on the EU market	+

* + = uncertainty with potential to cause over-estimation of exposure; - = uncertainty with potential to cause underestimation of exposure.

The Panel considered that the uncertainties identified would tend to an overestimate of the real exposure to propyl gallate as food additive in European countries.

3. BIOLOGICAL AND TOXICOLOGICAL DATA

The latest evaluations of propyl, octyl and dodecyl gallates by the SCF was in 1987 (SCF, 1989), by JECFA in 1996 (JECFA, 1997) and by TemaNord in 2002 (TemaNord, 2002). The latest JECFA Monographs for propyl, octyl and dodecyl gallates were published in 1996 (and reviewed 2006) (JECFA, 1993). BIBRA evaluated the gallates in 1989 (BIBRA, 1989) The studies on propyl gallate reported in these evaluations are summarised below, together with all other relevant and available information.

3.1. Absorption, distribution, metabolism and excretion

ADME/toxicokinetic studies on the three gallates were reported in the JECFA evaluations which concluded overall that *"Although there are similarities in the metabolism of the different gallates as evidenced by earlier limited data and a newly available* in vitro *metabolism study, the Committee concluded that there was not enough evidence to allocate a group ADI for the gallates when* in vivo *pharmacokinetic and metabolic studies were not available"* (JECFA, 1993). Studies from the JECFA evaluation (1993) are summarised below with the findings from additional literature.

Mice

A mouse study (Vora et al., 1999) compared the toxicokinetics of propyl gallate when administered in 2 different vehicles: an ethanol:saline solution (2:3, 0.9 % w/v), and a solution of the inclusion complex hydroxypropyl-beta-cyclodextrin (concentration not specified) in saline (0.9 % w/v). No dosing volumes were specified. Harlan Sprague-Dawley mice (4-6 animals/group; 20-30 g bw) were administered 100 mg propyl gallate/kg bw p.o. and sacrificed in groups at various post-dosing time-points (0-180 minutes) for blood collection and plasma separation. The results suggested that the rate of absorption of propyl gallate was slower in the ethanol:saline solution. This might be explained by the effects of ethanol on blood flow to the gastrointestinal tract and liver. However, the overall absorption of approximately 5 % was not different between the 2 vehicles (Vora et al., 1999).

No data on the distribution of propyl gallate to organs has been identified.

Rats

Adult albino rats were administered 100 mg propyl gallate per animal by gavage (vehicle not specified). The major metabolite detected in urine was 4-O-methyl-gallic acid. Gallic acid, 2-methoxypyrogallol and glucuronides of the methoxylated products were minor metabolites (CIR, 2007). This indicates hydrolysis of the ester followed by 4-O-methylation of gallic acid (Booth et al., 1959, as reported in JECFA, 1993).

In a study in which rats were given 100 mg gallic acid per animal either by gavage or by intraperitoneal (i.p.) injection (vehicles not specified) or fed a diet containing 0.5 % (equivalent to 600 mg/kg bw) gallic acid, urinary excretion of 4-O-methyl gallic acid as well as gallic acid was reported. In rats given the i.p. injection of gallic acid, an additional metabolite, suspected to be pyrogallol, was also detected along with trace levels of 2-O-methyl-pyrogallol (Booth et al., 1959, as reported in JECFA, 1993).

Metabolism of propyl gallate by intestinal bacteria in the rat was investigated. Several strains were isolated from the faeces of rats which converted propyl gallate to gallic acid which was then decarboxylated to produce pyrogallol (Niimura et al., 1986).

Rabbits

4-O-methyl-gallic acid, and pyrogallol were found in the urine of New Zealand White rabbits (weighing approximately 2 kg) fed 0.5 % (equivalent to approximately 150 mg/kg bw/day) gallic acid in the diet (duration not specified) (Booth et al., 1959).

In rabbits fed 1 g propyl gallate (no further details), the major urinary metabolite was a glucuronide conjugate hypothesised to be 4-O-methyl galloyl-β:D-glucosiduronic acid (72.0 % of the dose administered) and unconjugated phenols: 4-O-methyl gallate, gallic acid and pyrogallol (6.7 % of the dose administered) (Dacre, 1960). In rabbits fed gallic acid for 10 days, most of the dose was excreted in the urine unchanged, although some 4-O-methyl-gallic acid and pyrogallol were also detected (Conning et al., 1986).

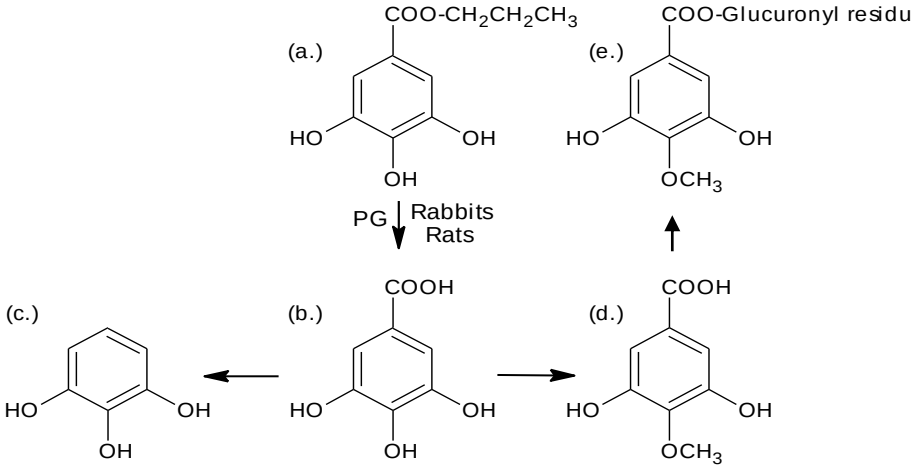

Figure 2: Metabolic pathways and major metabolites of propyl gallate (from Dacre, 1960). a. propyl gallate (PG). b. gallic acid. c. pyrogallol. d. 4-O-methyl-gallic acid. e. 4-O-methyl galloyl-β:D-glucosiduronic acid.

Dogs

No propyl gallate was detected in the urine of dogs fed 0.0117 % propyl gallate in the diet for 14 months (Orten et al., 1948 as reported in CIR, 2007).

Pigs

Madhavi (1996) reported that the metabolism of gallic esters in the pig is similar to that in rats.

Rat and human comparison.

In a project for the UK Food Standards Agency, Tullberg and colleagues (2004) compared the kinetics of four food additives (BHT, curcumin, propyl gallate and thiabendazole) in vivo in rats and humans and in hepatocytes from human and rat to examine the adequacy of the kinetic uncertainty factors.

The plasma concentration-time curves for propyl gallate in rats showed rapid absorption and elimination, with significantly higher concentrations in males than in females. The plasma concentrations in humans dosed at the current group ADI (0.5 mg/kg bw/day) were close to the limit of quantification, whereas more reliable data were obtained at a dose of 10 times the ADI. The kinetics were essentially linear in both rats and humans at the doses studied. The data indicated that the default uncertainty factors for interspecies differences and human variability would be adequate for propyl gallate. Studies with octyl gallate and dodecyl gallate showed the presence of extremely low plasma concentrations, which combined with the low ADI values for these food additives, meant that further in vivo studies were impracticable.

In vitro studies on propyl gallate using rat and human liver preparations showed that there were minor species differences in the rates of metabolism and in the estimated Vmax and Km values for the BHT, propyl gallate and TBZ metabolising enzyme systems. The estimated inter-species adjustment factors based on Vmax/Km were 2.4 for propyl gallate compared with in vivo values of 2.0 for propyl gallate (at dose-equivalence). The in vitro values do not take into account differences in organ blood flow, and such analyses would require the development of a full PBPK model. Interestingly the Km values for propyl gallate were considerably higher than the in vivo plasma levels, indicating little likelihood of saturation of metabolism (Tullberg et al, 2004).

3.1.1. Other studies

The JECFA (JECFA, 1993), evaluation reported the following, based on a TNO report from De Bie and Van Ommen (1992, unpublished report), *"In vitro incubations with propyl, octyl and dodecyl gallate were performed using homogenates of liver, mucosa of the small intestine, and contents of caecum/colon as a source of intestinal microflora. The various homogenates were incubated at 37° C with the individual gallate esters. At various time points up to 24 hours, samples were taken and analysed by HPLC in order to determine the concentration of gallic acid and residual ester. From the time-course of gallic acid formation, as well as the disappearance of the specific esters, the rate of hydrolysis of the three esters was calculated. All test substances were extensively metabolized by the homogenate of the intestinal mucosa, which was demonstrated by the appearance of peaks in the chromatograms. Furthermore, the caecum and colon contents also showed a high metabolic capacity, especially towards propyl gallate. The amount of gallic acid detected in the incubations was always much smaller than the total decrease of the amount of ester. It seems likely that apart from hydrolysis of the ester bond, other biotransformation routes (oxidation and/or conjugation) are of major importance for all three gallate esters. The three homogenates show quantitatively different structure-activity relationships for the three esters. Homogenates of liver and of contents of caecum and colon metabolize propyl gallate most extensively, followed by octyl or dodecyl gallate. Homogenate of the mucosa of the small intestine shows the highest rates with octyl gallate, lower rates with dodecyl gallate and propyl gallate. For this homogenate, the rate of formation of gallic acid is inversely related to the chain length of the ester (de Bie and van Ommen, 1992)."* The Panel noted that the species from which the samples were taken are not stated. However, the Panel noted also that the rate and extent of metabolism of propyl, octyl and dodecyl gallates would differ. Therefore, the Panel concluded that the available data were insufficient for the read across to be valid.

Bianchi et al., (1997) described a sensitive HPLC method with electrochemical detection for the measurement of propyl gallate in mammalian tissues. Tissue specimens of omentum were collected from 50 patients (20 females, mean age 69±4 years and body weight 60±3 kg; 30 males, mean age 59±3 years and body weight 73±3 kg). Forty-two of the 50 omentum samples and 45 plasma samples from a hospital blood bank were analysed for propyl gallate. Propyl gallate was not detected in the plasma samples but was detected in 30 % (13 out of 42) of the omentum samples.

3.2. Acute oral toxicity

The BIBRA toxicity profile for propyl gallate (1989a) reports the following oral LD_{50} values: 2.6-3.8 g/kg bw for rats, 1.7-35 g/kg bw for mice, 2.48 g/kg bw for hamsters, 2.75 g/kg bw for rabbits, and in pigs no overt toxicity was observed at doses up to 6 g/kg bw. The i.p. LD_{50} value in the rat was reported to be 380 mg/kg bw (BIBRA, 1989a). The Cosmetic Ingredient Review (2007) safety assessment on propyl gallate reported acute oral LD_{50} values of 2100-7000 mg/kg bw for rats.

In an NTP study (1982), groups of F344/N rats (5/sex/group) and B6C3F1 mice (5/sex/group) were administered by gavage a single dose of propyl gallate at 125, 250, 500, 1000 or 2000 mg/kg bw in 20 % ethanol in water. No controls were used. In rats, the only death was of a single male receiving 1000 mg/kg propyl gallate on day 5; no other compound-related effects were observed (NTP, 1982). In mice, 1 male and 3 females receiving the top dose died within 2 hours of dosing, whilst the survivors were slightly inactive up to 1 day after dosing. No other compound related effects were observed at the lower doses (NTP, 1982).

Dose levels of 100, 500, 1000, 2000, 3000 and 4000 mg propyl gallate/kg bw were administered by intubation to male rats (5 animals/group; average 250 g bw). The LD_{50} for this study was determined as 2100 mg/kg bw (Litton Bionetics, 1974). Another study performed by the same author showed that on one occasion a single dose of 5000 mg/kg bw propyl gallate, administered by intubation to 10 male rats resulted in the death of all animals within 24 hours, but on another occasion the same dose induced no deaths and no signs of toxicity were observed up to 7 days after the treatment period. No explanation is given for the divergent results. The LD_{50} for the latter trial was considered by the authors to be greater than 5000 mg propyl gallate/kg bw.

The Panel noted that the available studies in different species on acute toxicity of propyl gallate indicate low acute toxicity.

3.3. Short-term and subchronic toxicity

Short-term and subchronic toxicity studies as reported in the JECFA evaluation (JECFA, 1993) are summarised below. No new relevant studies have been identified, with the exception of the 28 day studies reported by Stierum et al. (2008). These studies involved administration of propyl gallate alone or in combination with other additives (BHT, curcumin and thiabendazole) and measurement of various end-points, including effects on body weight, liver weight and the various biomarkers measured (e.g. enzyme activities, mRNA levels No overt toxicity was observed with the four additives (25 to 1000 mg/kg bw/day for BHT and curcumin, 20 to 600 mg/kg bw/day for propyl gallate and 10 to 500 mg/kg bw/day for thiabendazole) either individually or in combination.

Mice

In a NTP study in B6C3F1 mice, groups of mice (5 animals/sex/group; 5 weeks old) were fed diets containing 1200, 2500, 5000, 10000 or 20000 mg propyl gallate /kg bw/day for 14 days (NTP, 1982). All mice receiving the top dose and all but one male at 10000 mg/kg bw/day died. Mean body weight gains for males and females were inversely proportional to dose. Feed consumption was variable in the males but showed a slight trend to increase with dose in the females (NTP, 1982).

In NTP subchronic toxicity study, groups of B6C3F1 mice (10 animals/sex/group; 5 weeks old) were fed diets containing 0, 133, 250, 500, 1000, 2083 mg/kg bw/day propyl gallate for 13 weeks. No mice died in this study. Weight gains could not be interpreted due to control animals being dehydrated due

to a technical problem. No compound-related gross or microscopic pathological effects were reported (NTP, 1982).

Rats

Various subchronic studies have been briefly reported in the BIBRA toxicity profile for propyl gallate (BIBRA, 1989a). At doses of 1-5 % propyl gallate (equivalent to 500- 2500 mg/kg bw/day) in the diet for 13 weeks or longer, some fatalities and damage to the stomach, forestomach, kidneys and prostate were reported in various studies (BIBRA, 1989a). Doses of 100-250 mg/kg bw/day given over 6 and 26 week periods were reported not to have effects on growth, the liver or adrenal glands (BIBRA, 1989a). Other studies have reported that at 100 mg/kg bw/day for various durations, altered enzyme activities in the lung, liver and intestines and caused slight fatty changes in the liver and increased liver weight. One study which was considered to be inadequately reported, indicated that 4 propyl gallate mg/kg bw/day for 6 months followed by 8 mg propyl gallate/kg bw/day for another 6 months caused reduced liver weight and degenerative changes in the testes (BIBRA, 1989a). The administration of propyl gallate at doses around 1-2 mg/kg bw/day alone or in an antioxidant mixture over a 13/52 weeks period induced no effects on the growth or survival of rats (approximately 30 animals/group). The microscopic examination of the major tissues revealed no treatment-related effects (Den Tonkelaar et al., 1968, as cited in BIBRA, 1989a).

Rats (7-8 animals/group) were administered five doses of 0 to 500 mg propyl gallate/kg bw/day by gavage for 1 week (Feuer et al., 1965 as reported in CIR, 2007). Animals were killed 24 hours after the last dose. Four additional groups (6 animals/group) that had been kept on the high dose were killed 14 or 28 days after the last dose. Histopathology and biochemical analyses were performed on the liver. Extensive fatty change observed at 24 hours after 500 mg/kg bw/day was markedly reduced after 14 days recovery and almost returned to normal at 28 days recovery. An increase in abnormal mitotic figures in hepatocytes was observed in all doses which was still present at 14 days post-treatment but not at 28 days post-treatment (Feuer et al., 1965 as reported in CIR, 2007).

In a NTP dose ranging study, groups of rats (5 animals/sex/group; 5 weeks old) were fed diets containing 600, 1250, 2500, 5000, or 10000 mg propyl gallate/kg bw/day for 14 days. All animals fed 10000 propyl gallate mg/kg bw/day died, as did 1 male fed 5000 mg propyl gallate/kg bw/day diet. Feed consumption was higher for all dosed groups compared to laboratory controls (no controls specific to the study) and feed consumption of male rats on 5000 mg propyl gallate/kg bw/day was comparable to that of rats fed on lower doses. However, male rats on 5000 mg propyl gallate/kg bw/day lost weight and females on this dose had weight gain less than 25 % of that for female rats on lower doses (NTP, 1982).

Concentrations of 0, 1000, 5000 and 25000 mg propyl gallate/kg bw/day were fed to rats (6 animals/group/sex) for 4 weeks. Parameters studied were growth, food and water intake, biochemistry, haematology, organ weights and histopathology. Both females and males from the high-dose group gained less weight compared to those in the control group. Haemoglobin concentration, packed cell volume, red blood cell concentration, mean corpuscular volume and mean corpuscular haemoglobin were lowered in the high-dose group. An increased extramedullary haematopoiesis and slightly decreased haemosiderosis were noted in the spleen, which are consistent with the anaemia. In the kidneys, hyperplastic tubuli in the outer medulla were detected In the liver of the animals of the 500 and 2500 mg propyl gallate/kg bw/day groups, increases in the activity of aminopyrine-N-demethylase, glucuronyl-transferase and glutathione-S-transferase and an increase in cytochrome P-450 content were detected (Strik, 1986, as reported in JECFA, 1993). It is noted that the high concentration in the feed might compromise eating habits and the nutritional value of the feed and may also cause damage to the gastrointestinal tract.

Weanling rats (12 animals/group) were fed diets for 6 weeks that contained 20 % lard providing dose of 0, 120, 240, 360, 480, 600 mg propyl gallate/kg bw/day. There was no effect on growth rate, relative liver weight, relative left adrenal weight or any consistent effect on the clinical biochemical parameters as measured in serum: cholesterol, esterified to total cholesterol ratio or sodium. In the

liver, the concentrations of total lipid, cholesterol, polyunsaturated fatty acids and the esterified to total cholesterol ratio were unaffected, as were the adrenal concentration of cholesterol and the esterified to total cholesterol ratio (Johnson and Hewgill, 1961 as reported in JECFA, 1993, CIR, 2007).

In another NTP study, groups of F344/N rats (10 animals/sex/group; 5 weeks old) were fed diets providing doses of 0, 75, 150, 300, 625 or 1250 mg propyl gallate /kg bw/day for 13 weeks (NTP, 1982). All animals survived except for 1 female on 625 mg/kg bw/day and one control female. Feed consumption tended to increase with dose. However, males receiving 625 or 1250 mg/kg bw/day and females receiving 1250 mg/kg bw/day weight gain depressions of 10 % or more. At 1250 mg/kg bw/day, all rats had dirty tails, indicative of digestive tract disturbances. At necropsy, male and female animals on 1250 mg/kg bw/day, had reddish duodenal mucosa, thickened stomach wall, necrosis and ulceration of the mucosal surface of the stomach, and a moderate to severe granulomatous inflammatory response in the submucosa and muscular wall of stomach (NTP, 1982). At 300 and 625 mg/kg bw/day, no stomach or duodenal lesions were observed (NTP, 1982).

Propyl gallate at 18 mg/kg bw/day was added to the fat content of feed of weanling rats for 13 weeks. The fat content provided 30 % of the caloric value of the diet. There was a very slight inhibition of growth. The same rats were subsequently placed on a partial starvation diet and kept until death. The survival time of the animals which had received the propyl gallate was considerably reduced and the reduction in their total body protein was greater than that for control rats (Bukhman, 1962 as reported in JECFA, 1993).

SPF-derived Wistar RIVM:Tox rats (10 animals/group/sex) were fed a semisynthetic diet containing 0, 490, 1910 or 7455 mg propyl gallate/kg feed (equivalent to 0, 35, 135, 527 mg/kg bw/day) for 13 weeks. The Panel noted that the study of Speijers et al was designed to investigate specific adverse effects seen in other toxicity studies and as such only relevant parameters were measured, Body-weight gain was recorded weekly and food-intake was recorded twice weekly. Other parameters included haematology, biochemical analyses in urine, serum and liver and complete histopathological examinations (Speijers et al., 1993). Adverse effects noted in the male high-dose group were reflected as decreased haematological parameters (Hb, Hct and RBC) and decrease in extramedullary haematopoiesis in the spleen. In the high-dose group, a decreased incidence of nephrocalcinosis (which is normally seen in female rats on semisynthetic diet) and a decreased liver microsomal activity of ethoxy-resorufin-O-deethylase (EROD) were observed. In the mid-dose and high-dose groups an increased activity of the conjugating enzymes glucuronyl-transferase and glutathione-S-transferase was observed. The effects on the liver enzymes suggest that other biotransformation routes additional to hydrolysis of the gallate seem to be involved. The effects on nephrocalcinosis and on the conjugating enzymes were not considered adverse. Therefore the NOAEL was 1910 mg propyl gallate/kg feed, equivalent to 135 mg propyl gallate/kg bw/day (Speijers et al., 1993). .

The Panel observed that the 90-day toxicity study in rats by Speijers et al., 1993, identifying a NOAEL of 135 mg propyl gallate/kg bw/day, was the key study for the evaluation of propyl gallate by JECFA.

In the last SCF evaluation on gallates it was concluded, based on a number of short-term studies, that propyl gallate did not cause hyperplasia in the rat forestomach during short-term studies (no further details provided) (SCF, 1989).

Pigs

No toxic effects or blood abnormalities were observed in pigs given a diet containing 0.2 % propyl gallate (approximately equivalent to a dose of 80 mg/kg bw/day) for 3 months (van Esch, 1955 as reported in BIBRA, 1989a).

Dogs

Young female mongrel dogs were divided into a control group (5 animals) and a group (7 animals) administered 2 925 mg/kg bw/day propyl gallate for 14 months (Orten et al., 1948). Throughout the study, there was no evidence of toxic effects, including appearance, activity and other gross characteristics, haemoglobin concentration, urine analyses (glucose, acetone-bodies, blood, bile, protein, specific gravity). The authors concluded *"There is thus no suggestion of any abnormality due to propyl gallate feeding, in renal or hepatic function or of any detectable metabolic aberration"*(Orten et al., 1948).

3.4. Genotoxicity

Several genotoxicity studies on propyl gallate have been described in JECFA's latest evaluation on gallates (JECFA, 1993). The findings from existing evaluations and more recent literature are summarised below.

3.4.1. In vitro assays

A number of studies reporting propyl gallate as negative for mutagenicity in the Ames assay are briefly described below. Propyl gallate was not mutagenic in *Salmonella* Typhimurium strains TA98 and TA100 in the presence or absence of Aroclor-induced rat liver S9 mix (Rosin and Stich, 1980 as reported in JECFA, 1993; Shelef and Chin, 1980, as reported in CIR, 2007); no information about the range of doses and the purity of the compound was provided. Propyl gallate (>98 % pure) was not mutagenic in *Salmonella* Typhimurium strains, TA98, TA100, TA1535 and TA1537 at doses up to 10 mg/plate, with or without metabolic activation (Mortelmans et al., 1986). Propyl gallate was not mutagenic in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 and in *Escherichia coli* strain WP2, with or without metabolic activation from 1 to 333 µg/plate (Prival et al., 1991). In another study, propyl gallate was tested in *Salmonella* Typhimurium strains TA98 and TA100 at 0, 125, 250, 500 and 1000 µg per plate with and without metabolic activation (Chen and Chung, 2000). Propyl gallate was negative in the Ames test using strains TA98, TA100, TA1537 with and without metabolic activation, using a 20 minutes preincubation method before plating (up to 40 µg/plate)) (Ishidate et al., 1980). No mutagenic effect on *S.*Typhimurium strains TA1530 and G46 and no recombination induction in *Saccharomyces cerevisiae* D3 strain were demonstrated without metabolic activation using the spot test method (Litton Bionetics, 1974).

Weak mutagenic activity was reported for propyl gallate tested in *S.*Typhimurium strain TA102 in the presence and absence of an S9 mix in the preincubation Ames assay at the top dose of 100 µg/plate. It was negative in TA97 strain with and without S9mix. (Fujita et al., 1988).

Von der Hude et al (1988) tested propyl gallate, among 124 other chemicals in SOS chromotest in *E.coli* PQ37, with and without metabolic activation, and found no genotoxic potential of propyl gallate. The Panel notes that the chromotest is not currently validated.

Propyl gallate was tested in the Ames test with strains TA100 and TA98, in a *rec* assay with *Bacillus subtilis* and chromosomal aberration (CA)/sister chromatid exchange (SCE) assays in hamster lung cells and in human fibroblasts (Kawachi et al, 1980). Propyl gallate was genotoxic in the *rec* assay and clastogenic in the CA assay without metabolic activation using hamster lung fibroblasts but it was negative in human embryo fibroblast cells for CA and SCE without metabolic activation (Kawachi et al., 1980). The Panel noted the limited protocol and inadequate reporting of the results of this study, and the lack of validation of the *rec* assay.

Propyl gallate was tested in an in vitro cytogenetic study using human embryonic lung cultures (WI-38). Doses tested were 0.5, 5 and 50 µg/mL. The cells were examined at 24, 48 and 72 hours. The low and high doses caused 2 % and 3 % CAs respectively, compared to 1 % in the negative control, these variations were not considered as significant. Anaphase preparations were examined in this test. There was no demonstration of a clastogenic effect with 0, 3 and 2 % cells with aberrations at 0.5, 5 and 50 µg/mL respectively vs. 2 % in the negative control). (Litton Bionetic, 1974 unpublished non GLP study report as reported in CIR, 2007). The Panel noted that a limited experimental protocol was

applied in this study, which was based on the examination of only 100 metaphases or 100 anaphases per concentration.

Propyl gallate was added to cultures of Chinese hamster fibroblasts (CHL) at concentrations up to 0.04 mg/mL in saline and chromosomal preparations were made 24 hours later. At 0.023 mg/mL propyl gallate, with metabolic activation (3-hours with S9 mix from liver homogenate of Wistar rats pretreated with PCB, followed by 21-hour recovery period) and without metabolic activation (24 and 48h continuous treatment), chromosomal gaps, breaks, exchanges and fragments were induced in 20 % of the cells. The dose at which chromosome aberrations were detected in 20 % of metaphases (D_{20}) was calculated as about 10 µg/mL (Ishidate et al., 1980).

Gulati et al. (1989) reported that propyl gallate (unknown purity) at dose levels of 5-50 µg/mL significantly induced CAs in CHO cells, only in the absence of metabolic activation and with extended harvest times; however, no information about cytotoxicity level was provided..

Propyl gallate, dissolved in DMSO, was tested at final concentrations of 0, 0.0021 and 0.0212 mg/mL in a CA and SCE tests using a diploid human embryo fibroblast cell line HE 2144; exposures were 40- 48 hours; for detection of SCE, 20-30 metaphase spread cells were observed, and the frequency of CAs was calculated scoring 100 metaphase cells per dose (Sasaki et al., 1980 as reported in CIR, 2007). In this study propyl gallate was toxic at the highest concentration used; at the next lower concentration, propyl gallate did not induce significant CAs or SCEs. The Panel noted that for CA, a limited experimental protocol was applied in this study, which was limited to the examination of only 100 metaphases at only one dose without information on the cytotoxicity.

Induction of SCEs, CAs and endoreduplications (ERDs) were reported in CHO-K1 cells treated with 0.25-1.5 mM propyl gallate with S9 activation for 3 hours (Tayama and Nakagawa, 2001, as reported in CIR, 2007). In the absence of exogenous metabolic activation, propyl gallate at concentrations higher than 1.0 mM caused cell-cycle delay in over 50 % of cells, and at 2.0 mM it arrested cell division. At 0.25–1.25 mM, propyl gallate caused statistically significant and dose-dependent increase in the SCEs. In the same study, propyl gallate induced a significant increase of CAs, excluding gaps, at ≥0.5mM with dose dependency up to 0.75 mM. Endoreduplicated cells increased with increasing doses of propyl gallate, and their frequency at 1.5 mM was remarkably high (23.5 %). The authors concluded that in the presence or absence of S9, propyl gallate can induce SCEs, CAs, and endoreduplication which may be partly due to the generation of oxygen-free radicals during the treatment. The authors also noted that *"during the 3 hours incubation, the medium changed from clear red to dark brown, and propyl gallate dimer and EA (ellagic acid) were detected along with decreasing propyl gallate concentration"*, indicating the production of degradation products and their reaction with medium components, and that *"the pH of the medium increased from 7.5 to 8.0 during treatment, which might have facilitated the autoxidation of propyl gallate"*. These observations call for caution in the interpretation of these results. As to the induction of endoreduplication by propyl gallate, the Panel considered that endoreduplication, differently from polyploidy, is a hallmark of cell cycle perturbation which is not by itself related to genotoxicity.

Gulati et al. (1989) reported that propyl gallate at dose levels of 5-50 µg/mL is a potent inducer of SCEs in CHO cells, with or without metabolic activation; the increase of SCE was much less significant in the presence of metabolic activation.

Propyl gallate was tested in an in vitro micronucleus assay without metabolic activation in a 3-hour treatment + 21-hour recovery period protocol, in which the response of rodent (V79, CHO and CHL) and human cells (peripheral lymphocytes, TK6 and HepG2) were compared (Fowler et al., 2012). In this study propyl gallate induced high levels of toxicity in all cell types, and a significant increase of micronuclei in V79, CHO, CHL and TK6. Conversely, primary human lymphocytes and HepG2 cell were negative for micronucleus induction, despite treatments induced similar levels of cytotoxicity. Propyl gallate was reported to be positive in a L5178Y tk+/tk- Mouse Lymphoma Assay (MLA). Cultures were exposed to propyl gallate at concentrations ranging from 0 to 1000 µg/mL for 4 hours

only without metabolic activation (McGregor et al., 1988). Significant mutagenic responses were observed in the absence of metabolic activation in 6 experiments at all doses tested. The lowest positive concentrations were 50 µg/mL in five experiments and 5 µg/mL in one experiment. No information on size of induced colonies was provided. Overall, propyl gallate was evaluated as positive for mutagenicity because of the reproducibility of the effect. However, the authors noted that the dose-related response was inverted in the high dose range, and that the mechanism causing this type of inverse dose effect relationship response is unknown.

Propyl gallate at concentrations up to 100 µM did not induce single strand breaks in the DNA of bacteriophage PM2 and did not demonstrated DNA strand breaks in human fibroblasts using the alkaline elution method at concentrations of 0.15 to 0.5 mM propyl gallate that reduced weakly the cell viability, as measured by MTT assay (Jacobi et al., 1998). However, propyl gallate in the presence of Cu(II) induced DNA strand breaks in PM2 phage DNA and in human fibroblasts with an inhibition of cell growth of in a concentration dependant manner, while neither propyl gallate nor Cu(II) alone induced any DNA damage. DNA strand breakage in PM2 phage was inhibited by addition of catalase or the Cu(I) chelator neocuproine, indicating the involvement of H_2O_2 and a Cu(II)/Cu(I) redox cycling in the DNA damage.

The mechanism of in vitro genotoxicity of propyl gallate was investigated by Hendriks et al (2013), in mouse embryonic stem cells with a reporter gene under transcriptional regulation of Bscl2 (responsive to the inhibition of DNA replication subsequent to genotoxic damage), and Srxn1 (responsive to oxidative stress). In this novel genotoxicity assay (ToxTracker), propyl gallate tested positive, but was classified equivocal as to the mode of action, as treatments induced the expression of reporter genes under control of both Bscl2 and Srxn1. The authors noted that PG selectively induced expression of the Srxn1-GFP reporter, suggesting that the primary toxic properties of this compound are associated with the induction of oxidative stress. The Panel noted that this test is not currently validated.

3.4.2. In vivo assays

The mutagenic effects of propyl gallate were investigated in host-mediated assays in mice with *Salmonella* Typhimurium or *Saccharomyces cerevisiae* as indicator cells, and in chromosomal aberration and dominant lethal assays in rats (Litton Bionetics 1974, unpublished non-GLP report as cited in JECFA, 1993 and CIR, 2007). In the host mediated, propyl gallate induced no significant increases in mutants or recombinants frequencies with *Salmonella* Typhimurium or *Saccharomyces cerevisiae*, respectively. However, the Panel noted the lack of validation of this test method.

As part of the same investigation, Sprague-Dawley CD strain male rats (10 animals/group) were dosed with 5, 50 and 500 mg/kg bw propyl gallate in a dominant lethal test. In the acute study, a single dose was administered by oral intubation and the males mated with 2 females per week for 8 weeks. In the subacute study, 5 daily doses were administered by oral intubation to males (test one, 0, 5, 50, 500 mg/kg; test two, 0, 5000 mg/kg), which were mated with 2 females per week for 7 weeks. Females were sacrificed 2 weeks after mating and the fertility index, preimplantation loss and lethal effects were determined. The authors overall concluded that propyl gallate does not induce dominant lethal mutations.

In the in vivo CA test, 10-12 week old male albino rats (3 animals/control, 5 animals/ treated groups) were dosed by gastric intubation with propyl gallate at single doses of 5, 50 and 500 mg/kg; animals were sacrificed at 6, 24 or 48 hours after one treatment. In the first trial, propyl gallate given as single doses produced a weak but significantly higher percentage of aberrations in bone marrow cells at the intermediate dose at 48 hours and at the high dose at 24 and 48 hours. In a subacute study using the same dose levels given for five days as above (with 24 hours intervals), there was a slight increase in the chromosomal aberrations at all dose levels compared to the negative control (4, 4 and 6 % cells with aberrations at 5, 50 and 500 mg/kg respectively vs. 2 % in the negative control). In a second assay propyl gallate was administered to rats (3 animals/control, 5 animals/ treated groups) at 5000 mg/kg both as single dose and in five repeated administrations with 24 hour intervals. In this second

trial neither the variety nor the number of aberrations in treated rats differed significantly from the negative controls. The authors concluded that propyl gallate was non-genotoxic in this test. The Panel noted the limited protocol of this study, with only 3 negative control animals and the examination of only 50 metaphases per animal.

Kawachi et al. (1980) tested propyl gallate in rat bone marrow chromosomal aberration test. Details on the protocol and the results are poorly reported (doses, timing for treatment /sampling, number and sex of animals were not reported), but authors concluded that it was negative in this test.

Propyl gallate was tested in a mouse bone marrow micronucleus test employing 3 daily administrations by i.p. injection. Bone marrow samples were obtained 24 hours after the final exposure. Propyl gallate was negative when administered at doses of 0, 75, 150, and 300 mg/kg bw; lethality was noted at the 2 high doses, but the percentage of PCE was not significantly affected by treatments (Shelby et al., 1993). In another study, i.p administration of 217 mg propyl gallate/kg bw produced no increases in micronuclei in a B6C3F1 mouse bone marrow assay (Raj and Katz, 1984 as cited in Gulati et al., 1989 and by Shelby et al., 1993).

In conclusion, propyl gallate was weakly mutagenic in a bacterial strain (TA 102) sensitive to oxidative damage and clastogenic in rodent cell lines in vitro, with and without metabolic activation. An equivocal positive response was also elicited by propyl gallate in a gene mutation assay in cultured mouse lymphoma cells, only performed without metabolic activation. Conversely, results in human cells were almost uniformly negative. In vivo, no genotoxic effects were detected in limited oral studies in rats, and in cytogenetic studies in mice treated by i.p. injection up to - and above - the maximum tolerated dose. Despite the limited database available, the Panel considered that the weight of the available evidence including the antioxidant action of the compound and the complete hydrolysis of propyl gallate in vivo indicates that the genotoxic activity elicited by propyl gallate in vitro is unlikely to be expressed in vivo.

3.5. Chronic toxicity and carcinogenicity

In its last evaluation the SCF stated *"at least six long-term toxicity/carcinogenicity studies in rats and mice have been carried out with propyl gallate. About half of these studies were old and/or inadequate. Nevertheless, there was no evidence of any increase in tumor formation. The NEL was 1170 mg/kg feed in one study and 5000 mg/kg feed in at least two other studies"* (no further details) (SCF, 1989).

Mice

In a two-year mouse chronic toxicity study, propyl gallate at a concentration of 7 450 mg propyl gallate/kg bw/day in the diet gave rise to patchy hyperplasia in the stomach. At a concentration of 1 490 mg/kg bw/day no difference was observed between treated and control animals (Lehman et al., 1951 as reported in JECFA, 1993).

Albino mice (University Animal Breeding Station closed strain colony) (25 animals/sex/group), were maintained on diets containing 745, or 1490 mg propyl gallate /kg bw/day for 21 months (Dacre, 1974). Water intake, food consumption and growth of treated test animals were comparable to controls. Treated male mice showed a greater percentage survival than control mice at termination. Haematologic measurements (haemoglobin, packed cell volume, differential white cell count) were similar for test and control animals. At autopsy, a comparison of relative organ weight/body weight (liver, spleen, kidneys, adrenals) only showed a reduction in the relative spleen weight of males at 1 490 mg/kg bw/day but no compound-related histopathological changes were observed in any of the organs including the spleen from male mice (Dacre, 1974). The authors concluded a NEL of 1 % equivalent to an intake of 1 490 mg/kg bw/day.

As an integrated part of an NTP study (NTP, 1982), *"Abdo and coworkers maintained groups of B6C3F1 mice of each sex on diets containing 0, 6000 or 12000 mg propyl gallate/kg feed for 105 -*

107 weeks. Lower body weights compared to controls were observed throughout most of the duration of the study in both sexes and both dose groups. At week 104, mean body weights of the male mice were 6 % and 8 % lower than controls in the high- and low-dose groups respectively. In the females, both dose groups had about a 12 % lower body weight than the controls at week 104. Feed consumption in low and high-dose males was 91 and 100 %, respectively, of that of controls while the corresponding figures for females were 109 and 106 %, respectively. No other compound-related clinical signs were observed. . There was no significant effect of treatment on survival. The survival rate averaged 80 % in males and 75 % in females. Tumour incidences of the haematopoietic system and liver in the treated groups showed significant increases. Tumours at other sites were not significantly different from controls. There was a significant positive trend in the incidence of histiocytic lymphoma in male mice (8 %) relative to controls. The historical control rate for histiocytic lymphomas was 3.3 % (21/640). There was a significant positive trend in the incidence of all malignant lymphomas in male mice (1/3/8), and significantly increased incidence by a direct comparison between high-dose and control (p<0.028). However the high dose incidence was not statistically significant when compared to the historical control rate at the performing laboratory for all malignant lymphomas of 9.4 (60/640).). In females the highest incidence was noted in the control group. The number of male rats in which hepatic adenomas or carcinomas occurred showed a significant negative trend. Hepatocellullar adenomas in female mice occurred with a positive trend (p < 0.022) and the incidence of adenoma in the high-dose females is significantly greater than in controls (p < 0.039). However the incidence in the high-dose group was not different from the historical incidence of this tumour (94/3127; 3 %). Further, the combined incidence of hepatocellular adenomas or carcinomas was similar in dosed and control groups (Abdo et al., 1983 as reported in JECFA, 1993).

The NTP concluded:*" Propyl gallate was not considered to be carcinogenic for B6C3F1 mice of either sex, although the increased incidence of malignant lymphoma in male mice may have been related to the dietary administration of propyl gallate*".

The Panel noted that although a trend was reported in lymphomas compared to concurrent controls, the reported values were within the historical control ranges and as such considered these changes were unlikely to be a compound related effect.

Rats

Weanling albino male and female rats (Connecticut Agricultural Experimental Station strain) (21-23 days old) were fed 0, 0.5 5, 60, 600 and 1 150mg propyl gallate /kg bw/day in the diet for 2 years (Orten et al., 1948 as reported in JECFA, 1993 and in CIR). Groups consisted of 10 male and 10 female rats, except the highest dosed group that consisted of 10 males and 6 females. The groups receiving 600 and 1 150 mg propyl gallate/kg bw/day propyl gallate showed stunted growth and macroscopic evidence of renal damage. The stunted growth could be caused by the fact that these levels of propyl gallate imparted a distinctly bitter taste to the diet. In the other groups, no detectable effect on growth was found. Neither gross appearance, haemoglobin, erythrocyte, or leukocyte counts in the blood, nor the histopathological appearance of the organs (heart, lung, liver, spleen, gonads) examined were affected at any dose (Orten et al., 1948).

In rats (16 animals/group) fed diets containing 0, 52, 130, 260, 520, or 2600 mg propyl gallate/kg bw/day no effect on mortality was seen after 2 years of feeding with 0, 52, 130, 260, 520, or 2600 mg/kg bw/day (no further details) (Lehman et al., 1951, unpublished study as cited in JECFA, 1993, van der Heijden et al., 1986).

In another study, five-week-old male and female F344 rats (50 females and 50 males per group) were fed 312, or 624 mg/kg bw/day propyl gallate for 105-107 weeks "*Throughout the study, there was a dose-related depression in body weights at both dose levels and in both sexes. Mean feed consumption was 94 % and 98 % of the controls in the low and high-dose males, while the corresponding values for females were 95 and 115 % respectively. In males 78 % of the controls, 76 % of the low- dose and 88 % of the high-dose group lived to the end of the study. In females the corresponding values were*

78 %, 76 % and 84 % respectively. No treatment-related clinical signs were observed. There were no significant differences in survival between the groups. For males the survival was 78 %, 76 % and

88 %, for control, low, and high-dose groups, respectively, and the corresponding values for female rats were 78 %, 76 % and 84 %. ... in male rats the incidence of three types of neoplasms was increased in the low-dose treatment compared to the control group, namely, phaeochromocytoma of the adrenal medulla, islet cell neoplasms of the pancreas and neoplasm of preputial gland origin. Equal or greater increases were not observed in the high-dose male groups. The occurrence of these tumours was not considered to be treatment related. The combined incidence of male rats with follicular cell adenomas or carcinomas of the thyroid was significant ($p < 0.05$) by the trend test, but the high-dose incidence was not statistically different in any tests in direct comparison with the control. In the high-dose females there were 3 mammary adenomas while there were none in the other two groups. The trend test was statistically significant but the incidence in the high-dose group was not significantly higher than control. There was an increase in the incidence of females with endometrial stromal polyps of the uterus with a marginally significant trend. The high-dose incidence falls within the overall historical control range (4-36 %). Tumours of the brain (an astrocytoma and a glioma) were found in two low-dose female rats. None of the high-dose female rats showed this tumour. The incidence of these tumours in the brain of the low-dose females was not considered to be related to propyl gallate, since none of the high-dose females had this tumour....under the conditions of this bioassay, propyl gallate was not considered to be clearly carcinogenic for F344 rats, although the increased incidence of preputial gland tumors, islet cell tumors of the pancreas and phaeochromocytoma of the adrenal glands in the low-dose male rats have been related to compound administration " (Abdo et al., 1983, as cited in JECFA, 1993).

The NTP concluded: *"Under the conditions of this bioassay, propyl gallate was not considered to be carcinogenic for F344/N rats, although there was evidence of an increased proportion of low-dose male rats with preputial gland tumors, islet-cell tumors of the pancreas, and pheochromocytomas of the adrenal glands; rare tumors of the brain occurred in two low-dose females."*

Rats of both sexes (26 animals/group)) were fed diets containing bread made with different concentrations of antioxidants, resulting in concentrations of 0, 0.405, and 20.25 mg propyl gallate/kg diet, for 1 year. Some animals were necropsied at weeks 13 and 26 and at study termination. It was concluded that propyl gallate had no significant effects on growth rates or organ weights. In treated females, a low incidence of renal tubular degeneration and glomerulonephritis was observed (Graham et al., 1954 as reported in CIR, 1974). In a second study, Graham and Grice (1955) added the unbaked bread ingredients (same as used in previous study) to the diet of 14 groups of rats (15 animals/group) for 32 weeks. No significant differences in body weight, organ weight, haematological parameters, organ lesions, appearance, behaviour, mortality were atttibuted at concentrations up to 20.25 mg propyl gallate/kg diet (Graham and Grice, 1955 as reported in CIR, 2007).

Guinea pigs

Guinea pigs, (4 weeks old; 200-300 g bw), were fed 0 or 4.68 mg propyl gallate /kg bw/day (14 males and 6 females/group) for 52 weeks (Orten et al., 1948). There was no effect on gross appearance or growth rate. Histology of liver, kidney, spleen, testes and ovary, adrenals heart and lungs were not affected.

Overall the Panel concluded that propyl gallate do not induce increased incidence of tumour and consequently is not considered as carcinogenic for mice of either sex. Furthermore the Panel concluded that propyl gallate was not carcinogenic in rats, the increased preputial gland tumours, islet-cell tumours of the pancreas, and pheochromocytomas of the adrenal glands in low-dose male rats and rare tumours of the brain occurred in two low-dose females were not dose related and were considered to be chance findings. The Panel noted that a trend was reported in lymphomas in male mice compared to concurrent controls, the reported values were within the historical control ranges. The Panel therefore noted that these changes were unlikely to be a compound related effect.

3.6. Reproductive and developmental toxicity

3.6.1. *Reproductive toxicity*

Rats

Rats were fed propyl gallate in the diet at concentrations equivalent to doses of 18.2, 104 or 260 mg/kg bw/day for 2 successive generations. No effects on reproduction performance nor on indices of reproduction were reported, and at autopsy no abnormalities were observed in the organs or tissues of the rats. (van Esch, 1955, as reported in JECFA, 1993).

In a review, it was reported that rats were fed propyl gallate in the diet at concentrations equivalent to 31.5, 180 or 450 mg/kg bw/day for more than 3 months until a few litters had been produced (van Esch, 1955 as reported in CIR, 2007). Propyl gallate was reported not to have produced any significant changes in growth or reproduction and no significant abnormalities were attributed to treatment at necropsy. A 31.5 mg/kg bw/day, organ weights and haematological values did not differ significantly from control (van Esch, 1955 as reported in CIR, 2007).

At doses of 4 mg propyl gallate /kg bw/day in rats for 6 months followed by 8 mg propyl gallate /kg bw/day for a further 6 months, degenerative changes in the testes were observed (Karplyuk, 1960 as reported by BIBRA, 1989a). However this study was judged to be inadequately reported by BIBRA (1989a).

Guinea pigs

Male and female guinea pigs (14 males and 6 females/group; 3-4 weeks old) were dosed in the diet with 0 or 4.68 mg propyl gallate/kg bw/day (Orten et al., 1948). After 12 months, the 6 females in each group were mated with males from the same group. Only one litter with 3 offspring was obtained from the 6 control females while 3 litters of 3 offsprings were obtained from the 6 dosed females. There was no effect on gross appearance or rate of growth and no gross abnormalities were found at autopsy. Histological examination of liver, kidney, spleen, testes ovaries, adrenals, heart and lungs did not disclose any pathological effects. It was not specified if mothers and/or offspring were examined. There was no compound effect on the growth of 1 litter of the dosed females when compared to 1 litter of the control animals. The rationale for this 1 litter study design was not specified

Pigs

In a review, it was reported that pigs were fed propyl gallate in the diet at concentrations of 0.035, 0.2 or 200 mg/kg diet for more than 3 months until a few litters had been produced (van Esch, 1955, as reported in CIR, 2007). Propyl gallate was reported not to have produced significant changes in growth or reproduction and no significant abnormalities were attributed to treatment at necropsy. At 14 mg/kg bw/day 0.035 % diet, organ weights and haematological values did not differ significantly from control (van Esch, 1955, as reported in CIR, 2007).

3.6.2. *Developmental toxicity*

Mice

Propyl gallate was tested in a prenatal developmental toxicity study in mice given orally 3-300 mg propyl gallate /kg/bw on gestation days (GD) 6-15 (22-25 animals/group) (FDRL, 1972, as reported in CIR, 2007). Dams were killed and fetuses removed on GD 17. Propyl gallate up to 300 mg/kg bw for 10 consecutive days had no effect on implantation or on maternal or fetal survival (CIR, 2007). The number of visceral, skeletal and external abnormalities in the test group fetuses did not differ to control animals (fed corn oil) (FDRL, 1972, as reported in CIR, 2007).

Rats

In Walter Reed-Carworth Farms rats (9 animals; average body weight 200 g) fed propyl gallate at a dose of 0.5 g in diet (possibly equivalent to 100 mg/kg bw/day) during pregnancy, increased fetal resorption rates were observed (Telford et al., 1962, as reported in BIBRA, 1989a, CIR, 2007). In this study only one dose was tested and the number of animals per group was low. This study was therefore considered as a very limited study.

Propyl gallate was tested in a prenatal developmental toxicity study in rats given orally 3-300 mg propyl gallate/kg bw/day on gestation days (GD) 6-15 (22-25 animals/group). Dams were killed and fetuses removed on GD 20. Propyl gallate up to 300 mg/kg bw/day for 10 consecutive days had no effect on implantation or on maternal or fetal survival. The number of visceral, skeletal and external abnormalities in the test group fetuses did not differ to control animals (fed corn oil) (FDRL, 1972, as reported in CIR, 2007).

Female Wistar rats (20 animals/group, except 18 animals/highest dose-group) were given propyl gallate in the diet at doses of 0, 0.4, 1 and 2.5 % (approximately equivalent to 0, 350, 880 and 2000 mg/kg bw/day) throughout pregnancy (Tanaka et al., 1979, BIBRA, 1989a). On GD 20, at least 13 dams per group were sacrificed for fetal examination (Tanaka et al.,1979); the remaining animals were allowed to deliver their litters which were autopsied at week 8 of age. The highest dose (2000 mg/kg bw/day) caused a decrease in the total number of offspring and induced a slight retardation in fetal development; a marked suppression of maternal body weight gain and food consumption was also noted (Tanaka et al., 1979). There was no evidence of increases in fetal deaths or malformations due to propyl gallate at any concentration. Also, there was no evidence of developmental toxicity for the groups which received 350 or 880 mg/kg bw/day. Five females per group were allowed to deliver normally and appearance, behaviour and organ weights were normal in the dams and offspring (Tanaka et al., 1979 as reported in BIBRA, 1989a).

Rabbits

No increase in fetal abnormalities or evidence of embryotoxicity were observed in rabbits (20-50/group) given daily doses of up to 250 mg propyl gallate /kg bw by stomach tube on GD days 6-18 (FDRL, 1973 as reported in BIBRA, 1989a, CIR, 2007).

Hamsters

Propyl gallate was tested in a prenatal developmental toxicity study in hamsters given orally 2.5-250 mg propyl gallate/kg bw/day on gestation GD 6-10 (22-25 animals/group) (FDRL, 1972, as reported in CIR, 2007). Dams were killed and fetuses removed on GD 14. Propyl gallate up to 250 mg/kg bw/day for 5 consecutive days had no effect on implantation or on maternal or fetal survival (CIR, 2007). The number of visceral, skeletal and external abnormalities in the test group fetuses did not differ to control animals (fed corn oil) (FDRL, 1972, as reported in CIR, 2007).

The Panel considered that the reproduction studies were not appropriate for hazard characterisation since they are old, poorly described and lack information about reproductive performance.

Data for developmental toxicity were less limited. Oral studies in mice, rats, rabbits and hamsters were available. Doses around 300 mg/kg bw/day did not appear to be associated with adverse effects and could be regarded as a NOAEL for developmental toxicity.

3.7. Other studies

3.7.1. Sensitisation

In its latest evaluation in 1987, without identifying any particular study, the SCF stated that *"Gallates may cause skin sensitisation and subsequent exacerbation of the resulting contact dermatitis occurs in some such sensitized individuals after ingestion of gallates."* (SCF, 1989).

Propyl gallate has a high sensitising potential and cases of allergic contact dermatitis to propyl gallate have been reported (Kraus et al., 1990; Garcia-Melgares et al., 2007; Perez et al., 2008). They are usually cheilitis and dermatitis of the hands. The most common sensitising agent in the case of cosmetics is lipstick and, in an occupational setting, are bakery products. Due to this potential, it has been recommended to limit its concentration in cosmetics (Cosmetic Ingredient Review Expert Panel, 2007). However, the frequency of allergic contact dermatitis appears to be low. Previous exposure and orally induced tolerance, may explained the low rates of allergic contact dermatitis to propyl gallate (Kahn, 1974). Only very rare reactions have been reported after oral intake of gallates (Pemberton et al., 1993; Van der Meeren, 1987). Therefore, the available data do not indicate that the use of propyl gallate as a food additive raise concern as regards allergenicity, hypersensitivity and intolerance.

Propyl gallate (dose not specified) tested positive in a murine local lymph node assay (Kimber et al., 1994).

3.7.2. Dermal irritation

The Cosmetic Ingredient Review on propyl gallate reports numerous animal studies on dermal irritation (CIR, 2007). In summary, studies in rabbits and guinea pigs using doses up to 10 % propyl gallate (in cosmetic formulations) were regarded to be practially non-irritating (CIR, 2007).

3.7.3. Oestrogenic activity

Ter Veld et al. (2006) found that propyl gallate was oestrogenic in human osteoblastic cell lines stably transfected with plasmid constructs encoding a luciferase reporter gene under control of a concatemer of 3 oestrogen response elements (EREs) and a minimal "TATA" promoter and expression constructs encoding the human oestrogen receptor α, ERα, or oestrogen receptor β, ERβ, genes. The oestradiol equivalency factor (EEF_{10}= EC_{10}estradiol/EC_{10} propylgallate) for ERα or ERβ cell lines was 6.5 x 10^{-7} and 3.9 x 10^{-6}, respectively.

In a search for potential xenoestrogens among food additives, 1500 compounds were analysed using in silico molecular modelling studies and in vitro binding studies and assays in MCF-7 cells stably transfected with a construct encoding a luciferase reporter gene under control of an oestrogen-responsive promoter (Amadasi et al., 2009). In silico analysis predicted that propyl gallate should bind the ERs in the nM range and this was confirmed in receptor-ligand binding studies in whole cells, in which propyl gallate competed with radiolabelled oestradiol for binding to the ERα with a "binding constant" [Ki] of 54nM. Lack of induction of luciferase expression in response to propyl gallate in the MCF-7 B17 cells was suggested by the authors to indicate that propyl gallate was acting as an ERα antagonist.

The Panel noted that the test methods used in the study, although widely used by the scientific community, are not OECD validated methods, in contrast to the BG1Luc oestrogen receptor transcriptional activation (TA) test method for identifying ER agonists and antagonists. This test method utilises an ER responsive (ERα and weakly expressing ERβ) human ovarian adenocarcinoma BG1Luc cell line stably transfected with a luciferase reporter gene under the control of four estrogen response elements upstream of the mouse mammary tumor virus promoter (OECD TG 457 or OECD TG 455).

3.7.4. Omics

In a study of transcriptomics (Stierum et al., 2008) that was performed to gain insight into mechanisms, propyl gallate was administered to Sprague-Dawley rats in the diet for 28 days in doses of 20.4, 49.9, 160.0, 359.6 or 658.4 mg/kg bw/day. The expression of eight genes in the liver was changed upon propyl gallate administration: CD74 antigen, sub maxillary gland alpha-2U globulin, EST, ID2 protein, cdc25A, ATP citrate lyase, Sth2 sulfotransferase hydroxysteroid gene 2, electron transfer flavoprotein alpha-subunit. No overt toxicity was observed with the four additives (25 to 1000 mg/kg bw/day for BHT and curcumin, 20 to 600 mg/kg bw/day for propyl gallate and 10 to 500 mg/kg bw/day for thiabendazole) either individually or in combination. Without using dose levels that

demonstrated relevant toxicity or being able to extrapolate to such levels it was not possible to interpret the results of the transcriptomics studies with respect to implications for risk assessment. The Committee on Toxicity of chemicals in food, consumer products and the environment (COT concluded that the new research does not raise concerns that combined exposure to the four compounds tested would pose a risk to health at doses below their individual ADIs.

3.7.5. Rat forestomach

Propyl gallate incorporated into the diet (0.52 and 2 %) and fed to male F-344 rats for 9 days neither affected the morphological appearance of the forestomach squamous epithelium or induced changes in the (^{3}H-methyl)thymidine labelling index in the fundic region of the forestomach (Nera et al., 1984).

The FDA reported that a Lowest-No-Effect-Level (LEL) of 500 mg/kg bw/day for mild hyperplasia in rat forestomach following treatment for 364 days. In another study included in the FDA report, a Highest-No-Effect-Level (HNEL) of 500 mg/kg bw/day in rat forestomach following treatment for 252 days was reported (PAFA Propyl Gallate, Feb 2011).

3.7.6. Observations in humans

Boehm and Williams (1943) reported that the examination of the urine of a man who had ingested propyl gallate at a dose of 7-10 mg/kg bw/day for 6 consecutive days revealed no evidence of kidney damage, with urine being negative for albumin, abnormal sedimentation contents, red blood cells, and casts (CIR, 2007).

In a study, a small outbreak of toxic methemoglobinemia occurred among infants in a pediatric ward. The most likely source of toxicity was an approved fat preservative (containing butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate) which had been added to a soybean infant formula by the manufacturer (Nitzan, 1979).

4. DISCUSSION

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following EFSA public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for this re-evaluation.

Propyl gallate (E 310) is an antioxidant authorised as a food additive in the EU.

Initial evaluations were based on a read-across approach on the basis of presumed toxicokinetic similarities for a group of three gallates (propyl, octyl and dodecyl). In its last evaluation in 1987 (SCF, 1989), the SCF established a group ADI for propyl, octyl and dodecyl gallate of 0-0.5 mg/kg bw/day. JECFA last evaluated the three gallates at the forty-sixth meeting in 1997 and decided that the grouping of propyl, octyl and dodecyl gallates was not scientifically justifiable and evaluated them separately. In its last evaluation JECFA established an ADI of 1.4 mg/kg bw/day for propyl gallate based on the 90 day study but ADIs could not be established for octyl and dodecyl gallate since relevant data on kinetics and metabolism were not made available (JECFA, 1997). Therefore, new monograph was not prepared in 1997, the last monograph was published in 1993 (JECFA, 1993).

Specifications have been defined in the EU legislation Commission Regulation (EU) No 231/2012 and by JECFA (JECFA 2006). The purity is specified to be not less than 98 %.

Little is known about the occurrence of propyl gallate in food and only fragmentary data exist. These data were published in articles which focussed on development of analytical methods. The data on manufacturing were very limited and provided no details on side reactions, impurities in chemicals and by products originating from different synthesis conditions. Furthermore, there was no information on the specific technological reasons for the selection of a particular gallate (octyl

gallate, dodecyl gallate or propyl gallate). It is possible that the choice could be due to differences in fat solubility as reflected by different P_{ow} values. The Panel noted that the use of hydrochloric acid in the manufacture of propyl gallate could result in chlorinated by-products and that there were limits for chlorinated organic compounds in the specification but no information on the identification or quantification of potential chlorinated by-products was available to the Panel.

The SCF considered that biotransformation data were scarce but that propyl gallate was well absorbed and hydrolysed to propyl alcohol and gallic acid (SCF, 1989). Gallic acid was further metabolised to 4-O-methyl gallic acid by O-methylation, glucuronidated and excreted via urine. Propyl alcohol was incorporated into intermediate metabolism of the individual. JECFA (JECFA, 1993) had temporarily concluded that in vitro metabolism studies in different tissues demonstrated some similarities in the metabolism of the different gallates, had however requested in vivo pharmacokinetic and metabolic studies to confirm these in vitro results. As these studies had not been performed JECFA concluded that there was no longer sufficient evidence to allocate a group ADI for the gallates.

The Panel concluded that no substantial new toxicological data have emerged since the last JECFA monograph (JECFA, 1993) and evaluation (JECFA, 1997).

Acute toxicity studies exist in different species for propyl gallate which had low if any acute toxicity.

There were short-term and subchronic toxicity studies on propyl gallate in rats ,mice and dogs. In a 13-week study in rats (Speijers et al., 1993) a NOAEL of 135 mg propyl gallate/kg bw/day was established in the second highest dose group. In the highest dose group, adverse effects were observed on the haematopoietic system. This study was the basis of the ADI established by JECFA (JECFA, 1997). The Panel agreed with this conclusion.

A large number of genotoxicity studies exist on propyl gallate and several have emerged since the latest JECFA evaluation. The literature search revealed new genotoxicity studies (comparative studies, SCE, single strand breaks and other DNA damage, bone marrow micronucleus test).

In mutagenicity tests in bacteria, propyl gallate was weakly mutagenic in *S.*Typhimurium TA102, a strain sensitive to oxidative DNA damage, with and without metabolic activation; negative results were obtained with the other tester strains. In cytogenetic tests in vitro, propyl gallate was clastogenic in rodent cell lines, where it induced micronuclei, chromosomal aberrations and sister chromatid exchanges with and without metabolic activation; conversely, a most uniform negative results were obtained in cytogenetic tests in human cells. A questionable positive response, with no dose-effect relationship, was also observed in a gene mutation assay in mouse lymphoma cells (only performed without metabolic activation). In vivo, propyl gallate was reported to be not genotoxic in limited oral studies in rats (chromosomal aberrations and dominant lethal assays) and negative in two mouse bone marrow micronucleus assays, in which propyl gallate was administered by i.p. injection up to lethal doses. Despite the limited database available, the Panel considered that the weight of the available evidence including the antioxidant action of the compound and the complete hydrolysis of propyl gallate in vivo indicates that the genotoxic activity elicited by propyl gallate in vitro is unlikely to be expressed in vivo.

Based on the above mentioned considerations, the Panel concluded that propyl gallate is not an in vivo genotoxin. No further genotoxicity studies are needed.

There were chronic toxicity and carcinogenicity studies; two studies in mice, three studies in rats and one in guinea pig on propyl gallate (JECFA, 1993). Overall, propyl gallate was not considered to be carcinogenic.

The Panel considered that the reproduction studies were not appropriate for hazard characterisation since they are old, poorly described and lack information about reproductive performance.

Data for developmental toxicity were less limited. Oral studies in mice, rats, rabbits and hamsters were available. Doses around 300 mg/kg bw/day did not appear to be associated with adverse effects and could be regarded as a NOAEL for developmental toxicity.

Propyl gallate was shown to be a modulator of oestrogen receptor activity in vitro (Ter Veld, 2006; Amadasi et al., 2009). According to EFSA's Scientific Opinion on the hazard assessment of endocrine disruptors (EFSA, 2013) *"the fact that a substance in an in vitro assay is binding to an endocrine receptor, then interfering with the intracellular messenger system connecting receptor to target or resulting in an endocrine related response in a target cell must be taken as strong indication for endocrine activity. If a suitable animal model provides further indication for an endocrine-related adverse effect, this substance should be considered an endocrine disruptor"*. Whether the in vitro oestrogenic activity of propyl gallate is associated with an adverse effect in an intact organism remains to be investigated. The Panel considered that the significance of these in vitro findings for risk assessment of propyl gallate is difficult to be ascertained in the absence of additional in vivo studies. The Panel considers that in light of the limitation of reproductive toxicity database any potential adverse effects due to the oestrogenic activity of propyl gallate in vivo might be further explored to reduce this uncertainty.

The Panel concluded that the 90-day toxicity study in rats was the key study for evaluation of propyl gallate. Based on the NOAEL of 1910 mg/kg feed (equivalent to 135 mg/kg bw/day) in this 90-day study and taking account of the Scientific Committee of EFSA Opinion on Default values the Panel concluded that an uncertainty factor of 300 instead of 100 should be applied for extrapolation from a subchronic to chronic data and due to the limitations in the reproductive toxicity database. The Panel therefore derived an ADI of 0.5 mg/kg bw/day for propyl gallate.

The SCF established a group ADI for propyl gallate, together with octyl and dodecyl gallate. This group ADI was based on a read-across approach using presumed toxicokinetic similarities (metabolism to gallic acid and the corresponding alcohol) for a group of the three gallates (propyl, octyl and dodecyl). However, having reviewed the data on the toxicokinetics (rate and extent of metabolism) of propyl, octyl and dodecyl gallate in this evaluation of propyl gallate and ongoing evaluations of octyl and dodecyl gallate, the Panel considered that the available data were insufficient for the read-across to be valid. Therefore, there was no longer a basis for the present group ADI and the Panel concluded that propyl, octyl and dodecyl gallate should be evaluated separately and the present group ADI should be withdrawn. The Panel noted that JECFA had reached a similar conclusion in 1997.

In this opinion, the dietary exposure to propyl gallate was estimated according to two exposure scenarios: 1) using maximum permitted levels (MPLs) for propyl gallate, and 2) using reported data on analytical levels, supplemented with MPLs for those food categories for which no analytical data were reported (refined exposure assessment). Using MPLs the estimated mean exposure to propyl gallate was highest in children: maximum of 0.36 mg/kg bw/day (Table 5). The high level of exposure was maximally 1.11 mg/kg bw/day in adults. In the refined exposure assessment, these exposure estimates were reduced to maximally 0.14 mg/kg bw/day in children and 1.04 mg/kg bw/day in adults, respectively (Table 5). The higher exposure in adults (as well as the elderly) as opposed to children was due to the exposure to propyl gallate via the consumption of food supplements (Table 6 and 7): food supplements contributed maximally 43 % to the total exposure in adults and 48 % in the elderly in three countries (appendix A). Removal of this source of exposure from the assessment resulted in a reduction in the high level exposure in adults to maximally 0.33 mg/kg bw/day using MPLs and 0.25 mg/kg bw/day using reported data on analytical levels. The high level of exposure in the elderly was reduced to 0.24 and 0.19 mg/kg bw/day, respectively.

The Panel noted that also the refined exposure assessment is still conservative. Of the nine food categories considered in this assessment, six had no or too few reported analytical data. For these categories, MPLs were therefore used. Furthermore, for the remaining three food categories, almost all reported concentrations were below the limit of quantification (LOQ). Of the 1029 data points

reported, only two (two pork lard samples) had quantifiable levels of propyl gallate. To refine the assessment further, analytical levels of propyl gallate would be needed in the other main food categories contributing to exposure, especially in breakfast cereals, soups and broths, and processed nuts.

Except via food, there is additional exposure to propyl gallate from non-food products. Gallates are permitted in FCMs and may be present in all foodstuffs to a SML of 30 mg/kg expressed as the sum of the three gallates. The Panel noted that exposure to propyl gallate from its use in food contact materials may contribute substantially to the total exposure to propyl gallate and that combined exposure to propyl gallate from its use as food additive in foods and additive to food contact materials does exceed the ADI for most population groups on average and on high level consumption.

Furthermore, the gallates are also permitted in cosmetic products both leave-on products such as skin creams and wash-off products such as hand and bathing soaps. According to the Regulation 2009/1223/EC on cosmetic product there is no limit. Furthermore, gallates may also be bought as part of products available on the Internet, e.g. as capsules, tablets although such sale may not be legal. The exposure via these routes is however unknown, and could therefore not be taken into account in this opinion.

CONCLUSIONS

The Panel concluded that the 90-day toxicity study in rats was the key study for evaluation of propyl gallate. Based on the NOAEL of 1910 mg/kg feed (equivalent to 135 mg/kg bw/day) in this 90-day study and taking account of the Scientific Committee of EFSA Opinion on Default values the Panel concluded that an uncertainty factor of 300 instead of 100 should be applied for extrapolation from a subchronic to chronic data and due to the limitations in the reproductive toxicity database. The Panel therefore derived an ADI of 0.5 mg/kg bw/day for propyl gallate.

The Panel also concluded that there was no longer a basis for the present group ADI and that propyl, octyl and dodecyl gallates should be evaluated separately and the present group ADI should be withdrawn. The Panel noted that JECFA had reached a similar conclusion in 1997.

The high level of exposure exceeded the ADI in adults and the elderly. However, given the conservatism of the exposure assessment the Panel concluded that the use of propyl gallate as food additive at the current uses and use levels is not of safety concern. The Panel noted that to refine further the exposure assessment additional analytical or use data on propyl gallate in especially breakfast cereals, soups and broth, processed nuts and food supplements would be needed.

If after conducting these further refined exposure assessments exposure remained greater than the ADI, or if additional uses and use levels were proposed, the Panel considered that given the uncertainties identified, additional toxicological data would be requested.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the DHI, Denmark, November 2011.

2. FDA, PAFA report, Propyl Gallate, Nov 2011

3. Food and Drug Research Labs (FDRL), 1972. Teratological evaluation of FDA 71-39 (propyl gallate) in rats. Laboratory No 0902v Contract No. FDA 71-260

4. Food and Drug Research Labs (FDRL), 1972. Teratological evaluation of FDA 71-39 (propyl gallate) in rats. Laboratory No 0902v Contract No. FDA 71-260

5. Food and Drug Research Labs (FDRL), 1972. Teratological evaluation of FDA 71-39 (propyl gallate) in hamsters. Laboratory No 0903v Contract No. FDA 71-260

6. Food and Drug Research Labs (FDRL), 1973. Teratological evaluation of FDA 71-39 (propyl gallate) in rabbits. Laboratory No 0904v Contract No. FDA 71-260

7. Inabata, submission of physical data, Feb 2010

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APPENDIX

Appendix A. Summary of total estimated exposure (using maximum permitted levels (MPLs) and reported data on analytical levels, supplemented with MPLs) per age class and survey[(a)]: mean and high level (mg/kg bw/day)

	Number of subjects	MPL		Reported data on analytical levels, supplemented with MPLs	
		Mean	High level	Mean	High level
Toddlers					
Bulgaria (Nutrichild)	428	0.2	0.6	0.0	0.1
Finland (DIPP)	497	0.0	0.1	0.0	0.1
Germany (Donald 2006_2008)	261	0.1	0.3	0.0	0.1
The Netherlands (VCP_Kids)	322	0.2	0.5	0.0	0.3
Children					
Belgium (Regional_Flanders)	625	0.3	0.5	0.1	0.3
Bulgaria (Nutrichild)	433	0.2	0.6	0.0	0.1
Czech Republic (SISP04)	389	0.2	0.4	0.0	0.3
Denmark (Danish Dietary Survey)	490	0.1	0.2	0.0	0.1
Finland (DIPP)	933	0.1	0.2	0.0	0.1
Finland (STRIP)	250	0.4	0.6	0.1	0.3
France (INCA 2)	482	0.3	0.5	0.0	0.1
Germany (Donald 2006_2008)	660	0.1	0.3	0.0	0.1
Greece (Regional_Crete)	839	0.2	0.4	0.0	0.3
Italy (INRAN_SCAI_2005_06)	193	0.1	0.3	0.0	0.0
Latvia (EFSA_TEST)	189	0.3	0.5	0.1	0.3
The Netherlands (VCP_Kids)	957	0.2	0.5	0.0	0.2
Spain (enKid)	156	0.2	0.4	0.0	0.1
Spain (Nut_Ink05)	399	0.2	0.4	0.0	0.2
Sweden (NFA)	1473	0.2	0.5	0.1	0.2
Adolescents					
Belgium (Diet_National_2004)	584	0.1	0.3	0.0	0.2
Cyprus (Childhealth)	303	0.1	0.2	0.0	0.0
Czech Republic (SISP04)	298	0.1	0.3	0.0	0.0
Denmark (Danish Dietary Survey)	479	0.1	0.1	0.0	0.1
France (INCA 2)	973	0.1	0.3	0.0	0.1
Germany (National_Nutrition_Survey_II)	1011	0.1	0.3	0.0	0.2
Italy (INRAN_SCAI_2005_06)	247	0.1	0.2	0.0	0.1
Latvia (EFSA_TEST)	470	0.2	0.4	0.1	0.2
Spain (AESAN_FIAB)	86	0.1	0.2	0.0	0.1
Spain (enKid)	209	0.1	0.3	0.0	0.1
Spain (Nut_Ink05)	651	0.1	0.3	0.0	0.1
Sweden (NFA)	1018	0.1	0.3	0.0	0.1

	Number of subjects	MPL		Reported data on analytical levels, supplemented with MPLs	
		Mean	High level	Mean	High level
Adults					
Belgium (Diet_National_2004)	1304	0.1	0.3	0.0	0.3
Czech Republic (SISP04)	1666	0.1	0.8	0.0	0.8
Denmark (Danish_Dietary_Survey)	2822	0.0	0.1	0.0	0.1
Finland (FINDIET_2007)	1575	0.0	0.2	0.0	0.1
France (INCA2)	2276	0.1	0.2	0.0	0.1
Germany (National_Nutrition_Survey_II)	10419	0.1	1.1	0.0	1.0
Hungary (National_Repr_Surv)	1074	0.0	0.1	0.0	0.0
Ireland (NSIFCS)	958	0.1	0.1	0.0	0.1
Italy (INRAN_SCAI_2005_06)	2313	0.0	0.2	0.0	0.2
Latvia (EFSA_TEST)	1306	0.1	0.2	0.0	0.1
The Netherlands (DNFCS_2003)	750	0.1	0.2	0.0	0.1
Spain (AESAN)	410	0.1	0.2	0.0	0.2
Spain (AESAN_FIAB)	981	0.1	0.5	0.0	0.4
Sweden (Riksmaten_1997_98)	1210	0.1	0.2	0.0	0.1
United Kingdom (NDNS)	1724	0.1	0.2	0.0	0.1
The elderly					
Belgium (Diet_National_2004)	1230	0.1	0.7	0.1	0.7
Denmark (Danish_Dietary_Survey)	329	0.0	0.0	0.0	0.0
Finland (FINDIET_2007)	463	0.0	0.1	0.0	0.1
France (INCA2)	348	0.1	0.2	0.0	0.0
Germany (National_Nutrition_Survey_II)	2496	0.1	0.4	0.0	0.3
Hungary (National_Repr_Surv)	286	0.0	0.1	0.0	0.0
Italy (INRAN_SCAI_2005_06)	518	0.0	0.1	0.0	0.0

(a): The different methodologies used within the European dietary surveys included in the EFSA Comprehensive Database are fully described in the Guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a). A summary is available on p.11, Table 1 of the Guidance.

ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
BHA	Butylated HydroxyAnisole
BIBRA	British Industrial Biological Research Association
bw	Body weight
CA	Chromosome Aberration
CAS	Chemical Abstract Services
CHO	Chinese Hamster Ovary
CIR	Cosmetic ingredients review
COT	Committee On Toxicity of chemicals in food
DMBA	7,12-DiMetylBenz(a)Antracene
EC	European Commission
EFSA	The European Food Safety Authority
EINECS	European Inventory of Existing Commercial chemical Substances
ERD	Endoreduplication
EROD	Ethoxy-Resorufin-O-Deethylase
EU	The European Union
FAO	The Food and Agriculture Organisation of the UN
FCS	Food Categorisation System (food nomenclature) presented in the Annex II of Regulation (EC) No 1333/2008
FCM	Food Contact Material
FDRL	Food and Drug Research Labs
GLC	Gas Liquid Chromatography
GRAS	Generally Recognised As Safe
Hb	Hemoglobin
HcT	Hematocrit
HNEL	Highest-No-Effect-Level

HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD50	Lethal Dose for 50% animals
LEL	Lowest-no-Effect-Level
MEKC	Micellar ElectroKinetic Capillary electrophoresis
MLA	Mouse Lymphoma Assay
MPL	Maximum Permitted Level
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NEL	No-Effect-Level
NNK	Nitrosamine-4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
MPL	Maximum Permitted Level
NTP	National Toxicology Program
PAFA	Priority based Assessment of Food Additives
PCB	Polychlorinated Biphenyl
PG	Propyl Gallate
Pow	Octanol/water partition coefficient
RBC	Red Blood Cell count
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SCOOP	Scientific CO-OPeration
SML	Specific Migration Limit
TBHQ	Tert-Butylated HydroQuinone
TemaNord	Nordic Working Group on Food Toxicology and Risk Assessment
TLC	Thin-Layer Chromatography
WHO	The World Health Organisation

w/v	Weight/volume ratio