SCIENTIFIC OPINION

Scientific Opinion on the use of Resorcinol as a food additive[1]

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

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ABSTRACT

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the safety of resorcinol when used as an antioxidant in crustaceans. Resorcinol is a specific inhibitor of polyphenol oxidase and therefore it can act as an anti-browning agent in fresh, frozen and deep-frozen crustaceans. Resorcinol is rapidly absorbed from the gastrointestinal tract, metabolised extensively to sulphate and/or glucuronic acid conjugates and excreted primarily in the urine. Resorcinol causes moderate acute toxicity in rats. Subchronic toxicity studies in rats and mice indicate a steep dose-response curve for lethality. Carcinogenicity studies in rats and mice demonstrated the lack of carcinogenic activity of resorcinol. However, clinical signs of toxicity were seen at approximately 100 mg resorcinol/kg bw/day (5 days per week) and above. Based on the available data from *in vitro* and *in vivo* genotoxicity tests the Panel concluded that there is no concern with respect to genotoxicity. Resorcinol has no adverse effects on the developing fetus nor does it cause maternal toxicity in rats and rabbits. The Panel considers the acute neurological signs of toxicity to be the pivotal adverse effect of resorcinol and based on the NOAEL of 36 mg resorcinol/kg bw/day in the carcinogenicity study in rats and using an uncertainty factor of 300 the Panel established an ADI of 0.12 mg/kg bw/day for resorcinol. The conservative estimates of acute consumption of shrimps (the only category for which experimental data were reported) indicate that dietary exposure to resorcinol for adults and for children would exceed the ADI when the residual concentration of resorcinol in whole raw shrimps is above 35 mg/kg. The Panel notes that this value is only applicable if other uses of resorcinol are excluded.

KEY WORDS

Resorcinol, CAS Registry Number 108-46-3

1 On request from the European Commission, Question No EFSA-Q-2006-123, adopted on 26 November 2009.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of resorcinol when used as an antioxidant in crustaceans.

Resorcinol is a specific inhibitor of polyphenol oxidase and therefore it can act as an anti-browning agent in fresh, frozen and deep-frozen crustaceans. Specifications for resorcinol indicated by the petitioner are: the minimum purity of resorcinol is 99 % (stated range of 99.0-100.5 % by iodometry assay), insoluble matter is set at 0.005 % and the residue after ignition 0.01 % maximum. The anti-browning agent intended to be authorised contains resorcinol as a main ingredient (more than 99.5 %) and citric or ascorbic acid at less than 0.3 %.

The petitioner proposes to use resorcinol at a concentration of 5 to 7 g/L in the dipping solution for a dipping time of 4-5 minutes. Under these treatment conditions, the residual resorcinol measured in raw shrimps (whole product) was 120 mg/kg, in edible parts of raw shrimps was 30 mg/kg, and in edible parts of boiled shrimps was 15 mg/kg bw.

Resorcinol is rapidly absorbed from the gastrointestinal tract, metabolised extensively to sulphate and/or glucuronic acid conjugates and excreted primarily in the urine. Studies with ^{14}C-radiolabelled resorcinol administered by gavage indicated no accumulation.

Resorcinol is of moderate acute toxicity. The reported LD_{50} values in rats ranged from 200 mg/kg bw to 980 mg/kg bw depending on the study.

The subchronic toxicity studies in rats and mice indicate a steep dose-response curve for lethality, with lethal effects observed at 520 mg/kg bw/day in rats and at 420 mg/kg bw/day or above in mice. At doses that are not lethal, clinical signs of toxicity manifested by hyperexcitability, tachypnoea, ataxia, prostration, and tremors were observed in mice and rats in subacute, subchronic and chronic toxicity studies.

Other effects reported in subchronic studies at doses that were not lethal were, increased absolute and relative liver weights in rats of both sexes, and increased absolute and relative adrenal weights in male rats, and decreased absolute and relative adrenal weights in male mice. The Panel noted that these changes in organ weights were slight, with no marked dose-response relationship, and were not accompanied by any changes in clinical chemistry parameters indicative of impairment of the liver function, or by any histopathological changes in the liver or adrenals and therefore the Panel did not consider these findings to be biologically significant,

Carcinogenicity studies in rats and mice demonstrated the lack of carcinogenic activity of resorcinol. However, clinical signs of toxicity in treated rats (ataxia, prostration, salivation and tremors) and mice (recumbency and tremors) of both sexes were seen at about 100 mg resorcinol/kg bw/day (5 days per week) and above. These acute signs occurred shortly after dosing and disappeared after approximately 30 to 60 minutes. The no-observed-adverse-effect-level (NOAEL) for these acute neurological effects was 50 mg/kg bw/day, which corresponds to 36 mg/kg bw/day when adjusted from the 5-day dosing week to a 7-day dosing week. The Panel considered these acute neurological effects as critical endpoints in the evaluation of resorcinol toxicity.

Resorcinol was tested in genotoxicity tests covering the three types of genotoxic endpoints, i.e. gene mutations in bacteria and gene mutations and chromosomal aberrations in mammalian cell *in vitro*. Four *in vitro* and one *in vivo* study were conducted according to GLP and in compliance with the relevant OECD test guidelines and additional non-GLP genotoxicity studies were also available. Overall, resorcinol did not induce mutations in bacteria in tests which were performed according to current guidelines; however, resorcinol induced reverse mutations in one study in *Salmonella typhimurium* strain TA100 without metabolic activation and in strain TA1535 with metabolic

activation when tested with a certain bacterial minimal medium. Resorcinol was clastogenic *in vitro* in peripheral human lymphocytes in the absence of metabolic activation. The results from an *in vitro* mouse lymphoma assay indicated that resorcinol could induce gene mutations and/or chromosomal aberrations; however, resorcinol did not induce mutation at the *hprt* locus of L5178Y mouse lymphoma cells when tested in concentrations up to 1101 µg/mL (10 mM) in two independent experiments in the absence or presence of a rat liver metabolic activation system (S9). A valid *in vivo* mouse micronucleus assay was negative. Thus, the clastogenic effects observed in some *in vitro* assays were not confirmed *in vivo*. Therefore, the Panel considered that there was no concern with respect to genotoxicity.

Resorcinol had no adverse effects on the developing fetus when administered to pregnant rats by gavage in doses up to 500 mg/kg bw/day from gestation days 6 to 15, or as a single dose of 1000 mg/kg bw on gestation day 11, or in rabbits in doses up to 100 mg/kg bw/day given by gavage from gestation days 6 to 18. No maternal toxicity was recorded at these dose levels.

Intake of resorcinol in drinking water in a one-generation dose-range finding reproductive toxicity study in rats and in a two-generation reproductive toxicity study in rats in doses amounting up to 233 and 304 mg/kg bw/day for males and females respectively was not associated with reproductive toxicity. In these studies no adverse effects were seen on function and morphology of the thyroid.

The Panel considers the acute clinical signs of toxicity to be the pivotal adverse effect of resorcinol and subsequently derived an ADI from the NOAEL of 50 mg/kg bw/day for these effects in the carcinogenicity study in rats (which corresponds to 36 mg resorcinol/kg bw/day when adjusting the 5-day dosing week to a 7-day dosing week). Because of the steep dose-response curve for resorcinol-induced lethality in acute and chronic animal experiments and the observed species and strain differences the Panel found it appropriate to apply an additional safety factor of 3 to a safety factor of 100 and established an ADI of 0.12 mg/kg bw/day for resorcinol.

The Panel assessed the acute and chronic exposure to resorcinol based on two scenarios resulting in different resorcinol concentrations.

The first scenario corresponds to the use proposed by the petitioner: resorcinol concentration of 5-7 g/L in the dipping solution with a dipping time of 4-5 minutes. The reported residual resorcinol concentrations using these treatment conditions were 120 mg/kg in raw shrimps (whole product), 30 mg/kg in the edible parts of raw shrimps, and 15 mg/kg in the edible parts of boiled shrimps.

In the second scenario, a dipping time of 30 minutes in a solution containing 5-7 g/L resorcinol was considered. The reported residual resorcinol concentrations using these treatment conditions were 470 mg/kg in raw shrimps (whole product), 40 mg/kg in the edible parts of raw shrimps, and 78 mg/kg in the edible parts of boiled shrimps.

For the acute exposure to resorcinol for adults and children, the Panel estimated consumption per eating occasion of 250 g of peeled cooked shrimps or 100 g of whole cooked shrimps as an ingredient of mixed dishes. In the absence of data on resorcinol concentration in cooked dishes prepared with raw shrimps (e.g. paella), it was assumed that the amount of resorcinol originally present in the whole raw shrimps would be present in the whole dish. For the chronic exposure to resorcinol, an estimated level of daily consumption of 50 g of shrimps on a regular basis was considered by the Panel to be sufficiently conservative to cover the food habits of the European population including high consumers. The acute daily exposure ranged from 0.06 to 0.8 mg/kg bw for adults and from 0.12 to 1.6 mg/kg bw for children, depending on the residual concentration of resorcinol. The chronic daily exposure ranged from 0.1 to 0.4 mg/kg bw for adults and from 0.2 to 0.8 mg/kg bw for children consumption, again depending on the residual concentration of resorcinol.

The highest calculated daily exposure to resorcinol corresponds to the consumption of cooked dishes prepared with 100 g of whole raw shrimps as an ingredient. The corresponding figures amount to 0.2-

0.8 mg resorcinol/kg bw for adults and 0.4-1.6 for children. The Panel noted that the exposure would always exceed the ADI for adults and children with the residual resorcinol concentrations considered for whole raw shrimps (120 mg/kg or 470 mg/kg according to dipping time).

In view of exceeding the ADI in these scenarios, the Panel calculated the maximum residual concentration of resorcinol in whole raw shrimps that would not lead to an exposure exceeding the ADI of 0.12 mg/kg bw in the most vulnerable population (children) in any of the exposure scenarios considered. This residual concentration of resorcinol amounts to 35 mg/kg. This defined value is only applicable if other usages of resorcinol are excluded.

The Panel noted that the proposed uses are for all crustaceans but that only experimental data on shrimps have been reported. The current evaluation is therefore related only to the use of resorcinol on raw shrimps and dietary exposure should be re-estimated if new usages are introduced.

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BACKGROUND AS PROVIDED BY THE COMMISSION

The European Commission received an application for the authorisation of resorcinol under Directive 95/2/EC of the European Parliament and Council on food additives other than colours and sweeteners. Resorcinol is proposed to be used as an antioxidant for preventing browning in fresh, frozen and deep-frozen crustaceans. This additive is intended to be used in dipping solutions within the range of concentrations of 5-7 g/L. It is reported to be an anti-browning agent alternative to sulphites with an activity similar to that of 4-hydroxyresorcinol.

The petitioner proposes the authorisation of resorcinol as a food additive other than colours and sweeteners, under the category "Conditionally permitted preservatives and antioxidants", classified as "Other antioxidants".

Resorcinol is authorised as a flavouring substance with FL number 04.047.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29(1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of resorcinol when used as an antioxidant in crustaceans.

ASSESSMENT

1. Introduction

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of resorcinol when used as an antioxidant in crustaceans.

2. Technical data

2.1. Identity of the substance

The chemical name of resorcinol is 1,3-Benzenediol. The synonyms are: resorcin; resorcine; resorcinol; 1,3-dihydroxybenzene; 3-hydroxycyclohexadien-1-one; 3-hydroxyphenol; benzene, 1,3-dihydroxy-; benzene, *m*-dihydroxy-; dihydroxibenzol; *m*-benzenediol; *m*-dihydroxybenzene; *m*-dioxybencene; *m*-hydroxyquinone; *m*-hydroxyphenol; phenol, *m*-hydroxy.

Resorcinol has a molecular weight of 110.11 g/mol. The CAS Registry Number is 108-46-3 and the molecular formula is $C_6H_6O_2$. Its structural formula is presented below:

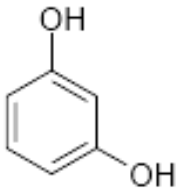

Figure 1: Structural formula of resorcinol

Resorcinol occurs as white needle-like crystals, plates, flakes, rhombic tablets, or powder with a faint, characteristic odour. Solubility in water is 140 g/100 mL. It is also soluble in alcohol, ether, glycerol, acetic acid, chloroform, dimethyl sulfoxide and acetone. The boiling point of resorcinol is 280 ^{o}C. The melting point of resorcinol ranges between 109 ^{o}C and 111 ^{o}C. Its density is 1.2717 g/cm^3.

2.2. Specifications

Specifications for resorcinol indicated by the petitioner are: the purity of resorcinol is minimum 99 % (stated range of 99.0-100.5 % by iodometry assay), insoluble matter is set at 0.005 % and the residue after ignition 0.01 % maximum.

The anti-browning agent for which the petitioner seeks authorisation contains resorcinol as the main ingredient (more than 99.5 %) and citric and/or ascorbic acid at less than 0.3 %. The anti-browning agent is mixed with water according to the manufacturer's instructions.

2.3. Manufacturing process

No details were provided by the petitioner other than that the manufacturing process follows HACCP principles and resorcinol is fully traceable and therefore compliant with current European legislation relating to the food chain.

2.4. Methods of analysis in foods

The petitioner presented a method developed for the determination of resorcinol in raw or boiled shrimps by High Performance Liquid Chromatography (HPLC), and a method developed for its determination in food simulants.

According to the petitioner, resorcinol can also be analysed by several published methods:

- Bromate titration: AOAC 942.21 (Association of Official Analytical Chemists, 1990),

- Semivolatile organic compounds by isotope dilution Gas Chromatography-Mass Spectrometry (GCMS): EAD Method 1625,

- Gel Permeation Chromatography (GCP) cleanup procedure: OSW Method 3640A,

- Extraction and analysis of organics in biological tissue: SFSAS Method SFSAS_29.

2.5. Stability, reaction and fate in food

According to the petitioner, resorcinol is very stable. Resorcinol is incompatible with strong oxidising agents and may discolour on exposure to air or light. Resorcinol has no known potential for formation of electrophilic reactive intermediates comparable to those derived from other dihydroxybenzenes.

The petitioner provided no information about any possible effect of resorcinol on nutrients.

2.6. Case of need and proposed uses

Black spot formation (melanosis) occurs in the shell of raw, refrigerated and frozen crustaceans within a few hours after catching, by the action of polyphenol oxidase on phenols that are naturally present in foods; this results in the formation of coloured reaction products. These substances subsequently polymerise to form insoluble dark polymeric substances (melanins). Refrigeration alone does not prevent these reactions from occurring, but only slows them down. The enzyme remains active during refrigeration, post-freezing and thawing.

At present, inorganic sulphites (E 220-224, E 226-228) and 4-hexylresorcinol (4HR) (E 586) are used to inhibit melanosis in crustaceans. Resorcinol is also a specific inhibitor of polyphenol oxidase. Data submitted by the petitioner show that resorcinol can be used as an alternative to sulphite and 4HR for preventing browning of crustaceans. According to the petitioner, resorcinol, being highly soluble in water, has a stronger direct antioxidant effect than 4HR.

The petitioner proposed to use resorcinol to prevent melanosis in shrimps. The shrimps are to be dipped into an aqueous solution (either tap water or seawater when treatment is performed on board of the fishing vessel) containing resorcinol (99.53 %), citric acid (0.32 %), ascorbic acid (0.15 %).

The petitioner provided the results of efficacy studies under laboratory conditions and analyses of residual resorcinol in boiled and raw shrimps exposed to different resorcinol concentrations in dipping solutions at temperatures between 5 and 10 °C, and different treatment durations. After dipping the shrimps were allowed to drip dry for 5 to 15 minutes.

No black spots were recorded after 1 to 5 days post-treatment with resorcinol at concentrations of 5, 7, or 10 g/L in seawater and dipping-durations of 5, 15 or 30 minutes. Black spots were recorded at the concentration of 3 g/L from day 3 after a dipping treatment for 5 minutes and from day 4 for dipping treatments of 15 and 30 minutes.

After dipping shrimps for 5 minutes in resorcinol solutions at concentrations of 5, 7 or 10 g/L the concentrations of residual resorcinol were 70, 120, and 320 mg/kg, respectively, in raw shrimps

(whole product), and 30, 30, and 40 mg/kg, respectively, in edible parts of raw shrimps. After dipping shrimps for 30 minutes in resorcinol solutions at concentrations of 5, 7 or 10 g/L the concentrations of residual resorcinol were 190, 470, and 370 mg/kg, respectively, in raw shrimps (whole product) and, 40, and 60 mg/kg respectively, in edible parts of raw shrimps (Table 1).

After dipping shrimps for 5 minutes in resorcinol solutions at concentrations of 5, 7 or 10 g/L the concentrations of residual resorcinol in edible parts of boiled shrimps were 16, 15, and 57 mg/kg, respectively. After dipping shrimps for 30 minutes in resorcinol solutions at concentrations of 5, 7 or 10 g/L, the concentrations of residual resorcinol in edible parts of boiled shrimps were 71, 78, and 159 mg/kg, respectively (Table 1).

Table 1: Analysis of residual resorcinol in the shrimps.

		Residual resorcinol (mg/kg)		
Concentration of resorcinol	**Duration of the dipping** (minutes)	**Raw shrimps (whole product)**	**Raw shrimps (edible parts)**	**Boiled shrimps (edible parts)**
5 g/L	5	70	30	16
	30	190	40	71
7 g/L	5	120	30	15
	30	470	40	78
10 g/L	5	320	40	57
	30	370	60	159

The petitioner proposes to use resorcinol at a concentration of 5-7 g/L in the dipping solution for a dipping duration of 4-5 minutes. The Panel noted that these treatment conditions leave residual resorcinol at concentrations of up to 120 mg/kg in raw shrimps (whole product), up to 30 mg/kg in the edible parts of raw shrimps, and up to 15 mg/kg in the edible parts of boiled shrimps.

The concentrations of 5 to 7 g/L were chosen from efficacy trials with resorcinol investigating the inhibition of the development of melanosis in crustaceans. These concentrations were also considered optimal when focusing on the residual resorcinol found in the edible parts of the shrimps after cooking.

The Panel considered that the dipping time might not always be precisely controlled and therefore also took into account the results for the dipping duration of 30 minutes

2.7. Information on existing authorisations and evaluations

Resorcinol is authorised as a flavouring substance (FL number 04.047; Chemical Status group 25) by Commission Decision 2002/113/EC of 23 January 2002 amending Commission Decision 1999/2177/EC as regards the register of flavouring substances used in or on foodstuffs (EC, 2002).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered resorcinol to be of no safety concern when used as a flavouring agent at an estimated daily intake of 1 µg per person in Europe (JECFA, 2000).

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) evaluated resorcinol in the Flavouring Group Evaluation 58 (FGE.58) (EFSA, 2008) in the context of the consideration of 44 phenol derivatives, evaluated by JECFA (55th meeting), structurally related to ring substituted phenolic substances evaluated by EFSA in FGE.22. The Scientific Panel was requested to consider JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission

Regulation (EC) No 1565/2000. The Panel concluded that the data available on genotoxicity do not preclude evaluation of the 44 JECFA evaluated phenol derivatives including resorcinol through the Procedure and the Panel agreed with the JECFA conclusion that there was *"No safety concern at estimated levels of intake as flavouring substances"* based on the Maximised Survey-derived Daily Intake (MSDI) approach (EFSA, 2008).

The SCF (1986) established a Tolerable Daily Intake (TDI) of 0.04 mg/kg bw for resorcinol.

Resorcinol (1,3-dihidroxybenzene) is included in the list of authorised monomers and other starting substances which may be used in the manufacture of plastic material and articles of the Commission Directive on plastic material and articles intended to come into contact with foodstuffs (EC, 2002), with a Specific Migration Limit (SML) of 2.4 mg/kg.

Resorcinol is included in Annex I as an existing active substance in biocidal products in accordance with the requirements of Article 3(1) of Commission Regulation (EC) 1451/2007 concerning the placing of biocidal products on the market (EC, 2007).

Resorcinol is listed in Annex III of Council Directive on cosmetic products (EC, 1976). Resorcinol is permitted for restricted use in rinse-off hair products (Annex III, part 1, No. 22).

A negative opinion for topical medicinal use of resorcinol on the basis of a risk benefit analysis has been published in Germany (Bundesanzeiger, 1994).

The safety of resorcinol was evaluated by experts of the International Programme on Chemical Safety (IPCS, 2006). The experts established a tolerable intake of 0.4 mg resorcinol/kg bw/day based on a no-observed-adverse-effect-level (NOAEL) of 50 mg/kg bw/day (equivalent to approximately 36 mg/kg bw/day when adjusted from the 5-day dosing week to a 7-day dosing week) from a long-term rat study conducted by the US National Toxicology Program (NTP, 1992).

The Scientific Committee on Consumer Products (SCCP) evaluated the use of resorcinol as an oxidative hair dye. The SCCP noted that there was no evidence of carcinogenic activity of resorcinol in male or female F344/N rats and in male or female B6C3F1 mice. However the Panel noted that the SCCP in 2008 had requested additional mutagenicity data (SCCP, 2008).

The Panel noted that a related substance, 4-hexylresorcinol (E 586), was evaluated by the SCF in 2003. Based on the available data on 4-hexylresorcinol, the SCF concluded "*that the available data do not allow the establishment of an ADI, nevertheless it was considered as toxicologically acceptable for the prevention of melanosis in shrimps under the conditions described provided residues in crustacean meat do not exceed 2 mg/kg*" (SCF, 2003).

2.8. Exposure

The estimated dietary exposure to resorcinol presented by the petitioner was based on concentrations ranging from 15 to 78 mg/kg of residual resorcinol in the edible part of boiled shrimps following dipping durations of 5 or 30 minutes in a solution at the proposed use levels (up to 7g/L of resorcinol). Chronic and acute dietary exposure assessments were performed by the petitioner considering a daily consumption of shrimps of 150 g/day on a regular basis and of 250 g on a single eating occasion respectively.

The Panel performed its own assessment of acute and chronic exposures based on two scenarios of shrimp consumption which correspond to two different ranges of resorcinol concentration levels (Table 1).

In relation to acute consumption, one scenario was that of consumption of shrimps for which the shell is either discarded before cooking or which are cooked without other ingredients. In this case, both the

range of residual resorcinol in the edible part of boiled shrimps (15 to 78 mg/kg) and the quantity per eating occasion (250 g) as suggested by the petitioner were considered adequate to provide a conservative estimate of acute consumption. As shown in Table 2, depending on dipping duration, the potential estimated acute dietary exposure ranged from 0.06 to 0.33 mg/kg bw for adults and from 0.12 to 0.66 mg/kg bw for children.

Another scenario of acute consumption was that of the consumption of shrimps within mixed dishes such as paella (corresponding to 100 g shrimps per eating occasion). In this case, the overall quantity of resorcinol present in the whole shrimp could be ingested through migration to the rest of the mixed dish. In the absence of experimental data provided by the petitioner on the concentration of resorcinol in the whole shrimp product once cooked and on the migration of resorcinol in cooked products, in order to evaluate this scenario the Panel decided to perform a conservative assessment of dietary exposure based on the resorcinol level reported in raw whole shrimps. Resorcinol concentrations of 120 to 470 mg/kg of shrimps were considered, corresponding to the levels found in the whole shrimp (with shell) by the petitioner after dipping in a resorcinol solution at concentrations up to 7 g/L for 5 minutes (120 mg/kg) and 30 minutes (470 mg/kg). In this case, the consumption of shrimps in one eating occasion would be lower than 250g since shrimps are only an ingredient of a mixed dish. A value of 100 g of shrimps per eating occasion was considered, based on the 97.5th percentile of eating occasion of paella in French consumers (700 g, unpublished data from the INCA survey (Volatier, 2000) and on the hypothesis that shrimps represent around 15% of a paella (corresponding approximately to 100 g shrimp for eating occasion). In this scenario, the potential estimated acute dietary exposure to resorcinol ranged from 0.2 to 0.8 mg/kg bw for adults and from 0.4 to 1.6 mg/kg bw for children for dipping durations of 5 or 30 minutes respectively.

In relation to chronic consumption, the Panel considered that the estimate used by the petitioner (150 g/day) was unrealistically high. In an individual food consumption survey conducted in 2005 in France among high consumers of sea foods, the 97.5th percentile of daily shrimp consumption for consumers (including shrimps as ingredients of recipes) was around 25 g (Bemrah *et al.*, 2008). This survey was conducted with a food frequency questionnaire, which tend to overestimate consumption. High levels of daily consumption for the whole category of crustaceans reported in the Scoop Task 3.2.11 (EC, 2004) for 5 European countries varied from 2.6 g in Ireland to 37 g in Norway (EC, 2004). Since the study contained data for only 5 European countries and shrimp consumption might be higher in other EU countries, the Panel considered a level of shrimp consumption of 50 g/person/day. This is intended to be a conservative estimate of chronic consumption of shrimps (consumed as such or as an ingredient in recipes) covering dietary habits in all EU countries. Based on French INCA (Individuelle et Nationale sur les Consommations Alimentaires) data (Volatier, 2000), the Panel observed that the consumption of shrimps could be as high in children as in adults. Potential exposure in children was therefore estimated based on the levels of consumption reported for the adult population. Exposure levels referred to a standard body weight of 30 kg for children and 60 kg for adults.

The residual resorcinol concentrations of 120 and 470 mg/kg of shrimps were considered in order to cover the case of consumers that would always consume shrimps within mixed dishes such as paella. In this conservative scenario the estimated potential chronic exposure to resorcinol ranged from 0.10 to 0.4 mg/kg bw/day for adults and from 0.2 to 0.8 mg/kg bw/day for children.

Table 2: Scenarios for acute and chronic exposure to resorcinol exposure

	Duration of dipping for a resorcinol solution at 7g/L	Estimated residual resorcinol concentration (mg/kg) in shrimp after dipping	Consumption (g/day or g/eating occasion)	Dietary exposure to resorcinol (mg/kg bw/day)	
				Adults (60 kg body weight)	Children (30 kg body weight)

Acute consumption, shrimps as such (edible part of boiled shrimps)	5 minutes	15	250 g/eating occasion	0.06	0.12
	30 minutes	78	250 g/eating occasion	0.33	0.66
Acute consumption, raw shrimps (with shells) as an ingredient of mixed dishes	5 minutes	120	100 g/eating occasion	0.2	0.4
	30 minutes	470	100 g/eating occasion	0.8	1.6
Chronic consumption, raw shrimps (with shells) as such or as an ingredient of recipes	5 minutes	120	50 g/day	0.10	0.20
	30 minutes	470	50 g/day	0.4	0.8

The Panel noted that exposure to resorcinol may also arise from its use in cosmetic products.

3. Biological and toxicological data

3.1. Absorption, distribution, metabolism and excretion

3.1.1. Animal studies

3.1.1.1. Oral administration

A study of metabolism and excretion of resorcinol in F344 rats (N=3/sex) following oral administration (Kim and Matthews, 1987) was submitted by the petitioner. Following a single oral administration of 112 or 225 mg/kg bw, the radiolabelled compound (^{14}C-resorcinol) was rapidly absorbed from the gastrointestinal tract, metabolised, and 90 % or 80 % of the administered dose, respectively was excreted in the urine within 24 hours. Only 1 to 2 % of the dose was excreted in faeces and less than 0.1 % was eliminated as CO_2. Approximately 70 % of the total radioactivity in the urine of both sexes was in the form of a glucuronide. No sex-related difference was observed in the ability of the rats to excrete resorcinol. However, in females, a higher proportion was excreted as the sulphate conjugate, whereas males excreted a higher proportion of a diconjugate (both sulphate and glucuronide groups). From these data, the authors concluded that male rats have a higher capacity for glucuronidation of resorcinol than female rats. Repeated exposures to doses up to 225 mg resorcinol/kg bw/day for 5 consecutive days did not result in a significant change in the rate of excretion. Comparison of the concentrations of ^{14}C-resorcinol in the samples from the major tissues indicated that no specific organ or tissue, including the thyroid gland, accumulated the compound to appreciably higher concentrations than any other (Kim and Matthews, 1987).

Three rabbits (sex not stated) received single doses of 100 mg resorcinol/kg bw by stomach tube and 24-hours urine was collected. A total of 11.4 % of the administered resorcinol was recovered unconjugated from the urine, 52 % of the dose was excreted as the monoglucuronide and 13.5 % as monosulphate. Trihydroxybenzenes were not detected (Garton and Williams, 1949).

3.1.1.2. Subcutaneous administration

The petitioner presented a study investigating the toxicokinetics of resorcinol in male Sprague Dawley rats following subcutaneous (s.c.) administration (Merker *et al.*, 1982). After single doses of 10, 50 or 100 mg resorcinol/kg bw (in an aqueous solution containing trace amounts of ^{14}C-resorcinol) the rats (N= 2 or 3) were sacrificed at 1, 3, 6 and 24 hours after administration. Urine, faeces, blood, kidney, liver, brain, intestines, spleen and muscle samples were collected. Blood samples were also collected at 15 minutes intervals for the first hour after the injection. Following a single dose administration of

50 or 100 mg resorcinol/kg bw the highest concentrations of resorcinol in blood were seen after 15 minutes. About 90 % of the dose was eliminated during the first 2 hours. Initial half-lives (plasmatic $T_{1/2}$ (α)) values were 21 and 18 minutes for 50 and 100 mg resorcinol/kg bw, respectively, and terminal phase half-life ($T_{1/2}$ (ß)) values were 8.6 and 10.5 hours for these doses, respectively.

In the liver and kidneys the concentration of resorcinol was highest one hour after a single s.c. administration of 10 mg/kg bw, but only 0.2 % and 0.3 % of the administered compound, respectively, was present. One hour after dosing, resorcinol levels in the brain and the thyroid were negligible, which was also the case for other analysed tissues. Within 24 hours after dosing with 10 mg resorcinol/kg bw s.c. 98 % of the applied dose was excreted via urine and 1 % via faeces, mainly as the glucuronic acid conjugate (84 %) (Merker *et al.*, 1982).

Resorcinol was also administered to male Sprague Dawley rats for 14 or 30 days at a total daily dose of 100 mg/kg bw s.c. (administered as two doses of 50 mg/kg bw 6 hours apart). After 14 or 30 days of treatment, the rats were injected with a single dose of 50 mg resorcinol/kg bw containing trace amounts of ^{14}C-resorcinol. Three animals were sacrificed 1, 3, 6 and 24 hours after injection and the biological samples referred to above were collected for analysis. Plasmatic $T_{1/2}$ (α) values were 31 and 32 minutes, and $T_{1/2}$ (ß) values were 5.0 and 7.3 hours for the 14 and 30-day assays, respectively. After repeated administration the distribution profile of resorcinol after 24 hours was similar to that after single administration (Merker *et al.*, 1982). The latter indicates absence of accumulation.

3.1.2. Human studies

No data on absorption, metabolism and excretion of resorcinol in humans following oral exposure were available.

3.1.2.1. Topical application

Resorcinol was applied topically to 3 healthy men, twice a day, 6 days per week over 4 weeks, in a hydroalcoholic vehicle at a 2 % concentration, in a total of 20 mL of formulation per application (800 mg resorcinol per day corresponding to a daily dose 12 mg/kg bw). No detectable levels of free resorcinol or its conjugates were found in blood (detection limit of the method applied was 0.1 µg/mL). In 24-hour urine samples after 14 days of continuous product treatment, about 0.5 - 2.9 % (maximum 23 mg resorcinol) of the applied daily dose was excreted and detected as the glucuronide and sulphate conjugates of resorcinol (Yeung *et al.*, 1983).

3.2. Toxicological data

3.2.1. Acute oral toxicity

3.2.1.1. Oral studies

Various LD_{50} values have been reported for resorcinol. Acute oral LD_{50} in male rats (strain not given, n=5/group) was reported to be 980 mg/kg bw (Flickinger, 1976). In another study with CFY rats (n=5/sex/group), an acute oral LD_{50} of 370 mg/kg bw was reported (Lloyd *et al.*, 1977). Other sources reported an acute oral LD_{50} value of 301 mg/kg bw in rats (strain and sex not given) (Koppers Company, 1970) or 300 mg/kg bw in rats (CIR, 1986). For female Wistar rats, an acute oral LD_{50} of 202 mg/kg bw was reported by Hoechst (1979).

In rabbits (giant chinchilla), doses of ≤500 mg/kg bw caused no apparent toxic effects, whereas after dosing with 600 mg/kg bw, temporary muscular twitching and increased respiration rate were noted (Garton and Williams, 1949).

The oral maximal non-lethal dose of resorcinol in Sprague-Dawley Rj:SD (IOPS Han) female rats was 200 mg/kg bw (Sire, 2004a).

In humans an oral lethal dose low (LDLo) of 29 mg/kg bw was reported (Deichmann, 1969).

In a case of poisoning in a pregnant woman accidentally given orally 50 g resorcinol instead of glucose at 30 weeks of pregnancy, the major systemic effects were unconsciousness, drowsiness, respiratory failure, tonic-clonic seizures and hypothermia. Laboratory findings were leukocytosis, high bilirubin levels, severe metabolic acidosis, and green coloured urine. The fetus died but the mother recovered (Duran *et al*., 2004)

3.2.2. Short-term and subchronic toxicity

The petitioner provided reports from two 17-day studies in rats and mice performed under the direction of the US National Institute of Environmental Health Sciences (NIEHS) and conducted in compliance with the US National Toxicology Program (NTP). The aim of these studies was to determine appropriate doses of resorcinol for 13-week studies (NTP, 1992).

Groups of F344 rats (N=5/sex) were administered 0, 27.5, 55, 110, 225, or 450 mg/kg bw/day resorcinol by gavage, 5 days per week for 17 days. The animals were observed daily for mortality and clinical signs. Body weights were recorded at the start and end of the study and at weekly intervals. All animals were subjected to macroscopic examination. Weights of the brain, heart, right kidney, liver, lung, and thymus were recorded and these organs from the control and the high-dose group were examined microscopically. No rats died during the study. Final body weights of the dosed rats were similar to those of the controls. Clinical signs of toxicity appeared within one-half hour after dosing and lasted 1 to 2 hours. Hyperexcitability and tachypnoea were observed in males from the 225 and 450 mg/kg bw/day groups. Females receiving doses of 55 mg/kg bw/day and above showed hyperexcitability and those receiving 110 and 450 mg/kg bw/day showed tachypnoea. Thymus weights, both absolute [344mg ± 17 (SE) *vs.* control, 412 mg ± 16, $p < 0.01$] and relative [2.33 ± 0.1 *vs.* control, 2.71 ± 0.1, $p < 0.01$] were statistically significantly decreased in high-dose females in comparison to the control. No gross and microscopic lesions attributable to resorcinol administration were observed (NTP, 1992). Based on the clinical signs reported, the Panel concluded that the NOAEL was 27.5 mg resorcinol/kg bw/day.

The protocol for the 17-day mouse study with resorcinol was the same as for the 17-day rat study described above. Groups of B6C3F_1 mice (N=5/sex) received resorcinol by gavage at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg bw/day, 5 days per week for 17 days. All females and four males receiving 600 mg/kg bw/day and one male receiving 300 mg/kg bw/day died as a result of the resorcinol administration. The final body weights of surviving dosed mice were similar to those of the controls. Clinical findings including prostration and tremors were recorded among males from the 150, 300, and 600 mg/kg bw/day groups, and among females from the 300 and 600 mg/kg bw/day groups. These clinical signs appeared usually within one-half hour after dosing and lasted 1 to 2 hours in surviving animals. No biologically significant changes in organ weights were recorded. No gross and microscopic lesions attributable to resorcinol administration were observed (NTP, 1992). Based on the clinical effects reported the Panel concluded that the NOAEL was 75 mg/kg bw/day.

The petitioner provided reports from two 13-week studies in rats and mice performed under the direction of the NIEHS and conducted in compliance with the NTP (NTP, 1992). These studies were performed in order to evaluate the cumulative toxic effects of repeated exposure to resorcinol and to determine appropriate dose levels for 2-year studies of toxicity and carcinogenicity.

In a 13-week study, groups of F344 rats (N=10/sex) were administered 0, 32, 65, 130, 260, or 520 mg resorcinol/kg bw/day by gavage, 5 days per week. The rats were observed twice daily for mortality, and weekly for clinical signs of toxicity. Body weights were recorded at the start and end of the study, and at weekly intervals. Blood samples for haematology and clinical chemistry were collected at

termination. In addition to standard clinical parameters, measurements of triiodothyronine (T_3) and thyroxine (T_4) were performed in the control and 130 mg resorcinol/kg bw/day 5 days per week groups. All animals were subjected to macroscopic examination. Organ weights were recorded for adrenal glands, brain, heart, right kidney, liver, lungs, and thymus for all animals, and the right testis of all males. Histopathological examination was performed on animals from the control, 260 and 520 mg/kg bw/day groups.

All female and 8 male rats from the high-dose group died from resorcinol-related toxicity during the first four weeks of the study. On day 2 of the study, rats from the 260 mg/kg bw/day group were given 520 mg/kg bw by mistake. Within 5 days, two males and four females in this group died. These deaths were attributed to incorrect dosing because no further deaths occurred among rats receiving the correct dose during the study. The final mean body weights and changes in mean body weights of rats receiving resorcinol were similar to those of the controls. Tremors were observed in high-dose rats of both sexes. No differences were observed in haematology or clinical chemistry parameters that could be attributed to the resorcinol administration. The few significant differences in various parameters were scattered among the groups, but none were considered biologically significant. The levels of T_3 and T_4 in the 130 mg resorcinol/kg bw/day 5 days per week group were comparable to the control values [T_3: control 107 µg/dL ± 7 (SE) *vs.* 109 ± 6; T_4: control 7 µg/dL ± 0 (SE) *vs.* 7 ± 0]. There were no gross or microscopical lesions attributable to resorcinol administration. Changes in organ weights were recorded in the liver of both sexes and in the adrenal glands of males. Absolute and relative liver weights of males dosed with 130 mg/kg bw/day [11.75 g ± 0.24 (SE) and 34.4 mg/g bw ± 0.5, respectively] or 260 mg/kg bw/day [11.74 g ± 0.18 and 34.9 mg/g bw ± 0.5, respectively] were statistically significantly ($p<0.01$) increased compared to controls [10.84 g ± 0.3 and 32.0 mg/g bw ± 0.7, respectively]. For females, statistically significant increased absolute liver weights were recorded after doses higher than 32 mg/kg bw/day [4.77 g ± 0.16 (control); 5.15 g ±0.18 (NS); 5.43 g ± 0.15 ($p<0.05$); 5.41 g ± 0.32 ($p<0.05$); 5.49 g ± 0.16 ($p<0.05$), for doses 0, 32, 65, 130 and 260 mg resorcinol/kg bw/day respectively] but relative liver weights were increased in all these dose groups [26.0 mg/g bw ± 0.9 (control); 28.03 mg/g bw ± 0.8 ($p<0.05$); 29.7 mg/g bw ± 0.05 ($p<0.01$); 28.8 mg/g bw ± 0.8 ($p<0.01$); 30.2 mg/g bw ± 0.7 ($p<0.01$)]. The Panel noticed that the increases in liver weights in the treated groups were slight, with no marked dose-response relationships, and not accompanied by any changes in clinical chemistry parameters indicative of impaired liver function, or by any histopathological changes. The Panel considered therefore that the effect on the liver weight was not biologically significant. The weights of the adrenal glands in males from all dosed groups were statistically significantly increased ($p<0.01$) compared to the controls [absolute: 4.73 mg ±0.24 (SE) (control), 5.42 mg ± 012, 5.48 mg ± 0.09, 5.21 mg ± 0.12, 5.74 mg ± 0.24; relative: 0.14 mg/g bw x 10 ± 0.01, 0.16 mg/g bw x 10 ± 0.00, 0.16 mg/g bw x 10 ± 0.00, 0.15 mg/g bw x 10 ± 0.00, 0.17 mg/g bw x 10 ± 0.00, in the control, 32, 65, 130 or 260 mg/kg bw/day dose-groups, respectively]. The Panel noted that the absolute adrenal weights were low in the male controls, that no dose-response relationship was apparent, and that the changes in adrenal weights were not accompanied by histopathological findings (NTP, 1992). Due to the incorrect dosing of the animals in the 260 mg resorcinol/kg bw/day dose-group the Panel concluded that this dose-group should not be used to define the NOAEL. The Panel therefore considered 130 mg resorcinol/kg bw/day as the NOAEL.

In a 13-week study, groups of $B6C3F_1$ mice (N=10/sex) were administered 0, 28, 56, 112, 225, or 420 mg/kg bw/day resorcinol by gavage, 5 days per week (NTP, 1992). Clinical observations, body weight records, blood sampling, macroscopic examination, and organ weight records followed the protocol as described above in the 13-week rat study. Histopathological examinations were performed on all animals from the control, 225 or 420 mg/kg bw/day groups.

In the high-dose group seven mice of each sex died during the first week of the study, another male died during week 4 and another female died during week 12. The authors of the study attributed these deaths to resorcinol-related toxicity. Furthermore, one male died in the 112 mg/kg bw group due to improper gavage technique. The final body weights of the 2 surviving high-dose male mice were statistically significantly less than controls. The final body weights and changes in body weights of all other mice receiving resorcinol were similar to those of the controls. Clinical signs of toxicity recorded

for the high-dose animals were dyspnoea, prostration, and tremors. These signs appeared within 30 minutes of dosing. No resorcinol-related, biologically significant changes in haematology or clinical chemistry parameters were seen. Statistically significant decreases ($p<0.01$) were noted in absolute and relative adrenal gland weights for males receiving 28, 56, 112, and 225 mg/kg [absolute: 8.30 mg ±. 0.52 (SE) (control), 6.60 mg ± 0.34, 5.90 mg ± 0.18, 5.89 mg ± 0.20, 5.70 mg ± 0.26; relative: 0.31 mg/g bw ± 0.02 (control), 0.25 mg/g bw ± 0.01, 0.22 mg/g bw ± 0.01, 0.23 mg/g bw ± 0.01, 0.23 mg/g bw ±0.01]. The Panel noticed that there was no dose-response relationship for the decreased adrenal weights and that the changes were not accompanied by microscopical findings. A few other differences in various organ weights were scattered among the study groups, and none were considered biologically significant. There were no gross or microscopic lesions attributable to resorcinol administration (NTP, 1992) Based on the clinical effects reported the Panel concluded that the NOAEL was 225 mg resorcinol/kg bw/day. The Panel noticed that the dose causing mortality was less than two-fold greater than this NOAEL.

In a 13-week study, groups of Sprague-Dawley rats (N=10/sex) were administered daily 0, 40, 80 or 250 mg resorcinol/kg bw/day by gavage. Six rats of each sex from the control and the high-dose groups were treated for 13 weeks and then kept for a 4-week treatment-free period. Six animals of each sex in the resorcinol treated groups were used for toxicokinetic investigations. The rats were observed twice daily for mortality/morbidity and daily for clinical signs. Body weights were recorded once a week. Detailed clinical observations were performed on each rat in a standard arena once before the beginning of the treatment period and then once a week until the end of the study. Any animal found dead was subjected to a macroscopic *post-mortem* examination.

Two males in the mid-and one female in the high-dose group died due to incidental gavage errors. In the high-dose group all males and females (including animals in the satellite groups) showed intermittent convulsive movements, starting between weeks 6 and 8 and lasting until the end of the treatment period. Also excessive salivation (majority of animals) and loud breathing (2 males) were reported in the high-dose group. With the exception of the 2 males which had convulsions and died due to gavage error, no clinical observations were recorded for the mid-dose group. No treatment related effects on body weight, feed intake, blood and urine parameters were noted. The female high-dose group gained less weight (86 % of the body weight gained by the controls) from week 4 to 8. Examination of the animals during the functional observation battery did not reveal any treatment-related effects. No abnormalities were noted at necropsy. The only findings in organ weights were slightly decreased absolute and relative thyroid gland weights in the high-dose group compared to the controls. The authors of the study considered the decreased thyroid weights of no toxicological importance considering the lack of a dose response and of relevant histopathological changes (Foulon, 2005). The Panel considered 80 mg resorcinol/kg bw/day as the NOAEL.

3.2.2.1. Effects in humans exposed repeatedly to resorcinol by dermal route.

Several case reports of poisoning especially in infants, with sometimes fatal outcomes were reported in the literature (Graham and Tisdall, 1922; Becker 1933; Cunningham, 1956). In most cases ointments or pastes containing up to 50% resorcinol were applied dermally over varying time intervals, but additional oral uptake cannot be excluded. The reported symptoms included burning sensation or convulsions, central nervous system (CNS) disturbances such as dizziness, vertigo, confusion, disorientation, amnesia, or tremors, or red blood changes, such as methaemoglobinaemia, haemolytic anaemia, haemoglubinuria, or cyanosis (Graham and Tisdall, 1922; Wüthrich *et al*., 1970; Bontemps *et al*., 1995; Tush and Kuhn, 1966; Hernández-Pérez, 2002; Duran, 2004).

In the three case reports where ointments containing up to 12% resorcinol were applied onto the skin of patients suffering from leg ulcers over long periods, enlargement of thyroid glands and hypoactivity were reported (Bull and Fraiser, 1950).

A female patient with leg ulcers treated dermally for 13 years with an ointment containing 12.5% resorcinol had an enlarged thyroid gland (Thomas and Gisburn, 1961).

One case of hypothyroidism in a 70-year-old male patient was reported after about 3 months of dermal application of large amounts of a paste containing 2% resorcinol x (Katin *et al*., 1977). After cessation of treatment, free T4 and thyroid-stimulating hormone (TSH) returned to physiological levels within 2 weeks.

About 10 cases of hypothyroidism linked to resorcinol used in treating persistent skin ulcers dermally with doses of about 34 – 122 mg/kg bw/day for many days or years have been reported (Gans, 1980).

3.2.3. Genotoxicity

The petitioner presented several studies including an original report from studies performed under the direction of the US NIEHS and conducted in compliance with the NTP (NTP, 1992), reviews of genotoxicity studies by scientific bodies (IARC, 1999; JECFA, 2000; NTP, 1992, Lynch *et al.,* 2002), and published papers on *in vitro* bacterial and mammalian assays and *in vivo* studies. Furthermore, five unpublished study reports were provided by the secretariat of the Scientific Committee on Consumer Products (SCCP). These studies were performed under GLP conditions and were in compliance with current OECD test guidelines.

3.2.3.1. *In vitro* testing

Resorcinol was tested in a GLP study performed according to OECD test guideline 471 (1997) in strains TA98, TA100, TA1535, TA1537 and TA102 of *Salmonella typhimurium* at 6 concentrations from 1.6 to 5000 µg/plate, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9), in two separate experiments using the standard plate incorporation procedure as well as a pre-incubation step. Resorcinol did not induce gene mutations in bacteria (Williams, 2005).

Resorcinol did not induce gene mutations in *Salmonella typhimurium* strains TA98, TA100, and TA1535, TA1537 at concentrations ranging from 33 to 3333 µg/plate when tested with a pre-incubation protocol in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Haworth *et al.*, 1983, NTP, 1992).

Negative results were reported in the standard assay for reverse mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 incubated with resorcinol with and without metabolic activation. However, in an assay with a modified minimal ZLM medium for *Escherichia coli* (in place of Vogel-Bonner medium), resorcinol was mutagenic in TA1535 with metabolic activation and in TA100 without metabolic activation (Gocke *et al.*, 1981).

Furthermore, the petitioner provided other publications, which reported that resorcinol did not induce gene mutations in any of several strains of *Salmonella typhimurium* with or without metabolic activation (McCann *et al*., 1975; Florin *et al.,* 1980; Shahin *et al*., 1980; Probst *et al*., 1981; Haworth *et al*., 1983; Crebelli *et al*., 1981, 1984, 1985) and did not induce the SOS response in the genetically engineered *Salmonella* strain TA1535/pS-K1002, with or without S9 (Nakamura *et al*., 1987).

Resorcinol induced gene mutations at low pH in *Salmonella typhimurium* TA98 (SD 510) and *Escherichia coli* B/r WP2 $trp^{-}hcr^{-}$ (Hosono *et al.*, 1991). The mutagenicity was highest at pH 3 while it decreased with increase in pH. No mutagenicity was observed above pH 6. The Panel noted that the low pH could not be considered as standard condition.

Resorcinol was tested in an *in vitro* micronucleus assay performed in compliance with GLP and according to the draft OECD test guideline 487 (2004) using duplicate human lymphocyte cultures prepared from the pooled blood of 2 female donors in two independent experiments up to a concentration of 1100 µg/mL (equivalent to 10 mM). Treatments were performed both in the absence and presence of metabolic activation (Aroclor 1254 induced rat liver S9). Dose-related and statistically significant increases in the frequencies of micronuclei in cultured human peripheral blood

lymphocytes (up to 2-fold compared to control in experiment 1 and up to 5.2-fold compared to control in experiment 2) were observed following a 20 hours treatment period + a 28 hours recovery period in the absence of a metabolic activation system, where treatment commenced either 24 or 48 hours following mitogen stimulation. Dose-related and statistically significantly increased frequencies of micronucleated cells were also observed following a 3 hours treatment period + a 45 hours recovery period in the presence of S9 (up to 3.7-fold compared to control in experiment 1) where treatment commenced 24 hours post mitogen stimulation. No such increases in micronucleated cells were apparent following 3 hours treatment period + a 45 hours recovery period in the presence of S9 where treatment commenced 48 hours post mitogen stimulation at concentrations up to its limit of cytotoxicity (Whitwell, 2004). Accordingly, the Panel considers the result positive in the absence of metabolic activation and equivocal in the presence of metabolic activation, since the positive result in the first experiment could not be reproduced in the second experiment.

Resorcinol was tested for mutation at the *hprt* locus in mouse lymphoma L5178Y cells in the absence and presence of a rat liver metabolising system (S9) (Lloyd, 2009). The study was performed in compliance with GLP and according to the OECD test guideline 476 (1997). After a preliminary toxicity test, resorcinol was tested in two independent experiments, in the absence and the presence of S9. In the first experiment the highest resorcinol concentration of 1101 µg/mL gave 29 % and 19 % relative survival (RS) in the absence and the presence of S9, respectively. In the second experiment the highest concentration of 1101 µg/mL gave 35 % and 16 % RS in the absence and the presence of S9, respectively. No statistically significant increases in mutant frequency were observed following treatment with resorcinol at any concentration tested in the absence and presence of S-9 in both experiments. The author concluded that resorcinol under conditions of the study did not induce mutation at the *hprt* locus of L5178Y mouse lymphoma cells in the absence or presence of a rat liver metabolic activation system (S9) (Lloyd, 2009).

Resorcinol was tested in a mouse lymphoma ($TK^{+/-}$) assay in L5178Y cells (Sire, 2004b). The study was performed in compliance with GLP and according to the OECD test guideline 476 (1997). After a preliminary toxicity test, resorcinol was tested in two independent experiments, with and without a metabolic activation system prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254, at concentrations up to 10 mM (corresponding to 1100 µg/mL). Without metabolic activation, in both experiments, a moderate to marked toxicity was noted at all dose-levels as shown by 54-76 % decrease in Adjusted Relative Suspension Growth (Adj. RSG) and 46-83 % decrease in Adj. RTG. Increases in the mutation frequency (up to 3.2-fold accompanied by 28 % Adjusted Relative Total Growth (Adj. RTG) compared to the vehicle control value in experiment 1 and up to 4.8-fold accompanied by 17 % Adj. RTG in experiment 2) were observed following the 3-hour treatment in both experiments. In both experiments, the frequencies of large and small colonies were increased, indicating mutagenic as well as clastogenic potential. In the presence of metabolic activation, a slight to marked toxicity was observed at dose-levels ≥ 5 mM, as shown by 30-78 % decrease in Adj. RSG and 28-65 % decrease in Adj. RTG. A weak increase in the mutation frequency was observed at the dose-level of 10 mM in the first experiment (up to 1.9-fold accompanied by 72 % Adj. RTG compared to the vehicle control value). This increase could not be reproduced in the second experiment (Sire, 2004b). Accordingly, the Panel considers that the positive results in this study were obtained at concentrations that result in moderate to marked toxicity.

In a mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells, performed under the direction of the NIEHS and conducted in compliance with the NTP, resorcinol gave a dose-related and reproducible positive response in the absence of S9 at concentrations ranging from 156.25 to 2500 µg/mL. Resorcinol was not tested with S9 in this study (McGregor *et al*., 1988; NTP, 1992).

In the NTP (1992) study resorcinol was tested in cultured Chinese Hamster Ovary (CHO) cells for induction of sister chromatid exchanges and chromosomal aberrations both in the presence and in the absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Each test consisted of concurrent solvent and positive controls and of at least 3concentrations of resorcinol. The high concentration was limited by toxicity or solubility, but did not exceed 5 mg/mL resorcinol.

Resorcinol induced sister chromatid exchanges at doses of 167 and 500 µg/mL in the absence of S9 and at 1670 and 5000 µg/mL in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (NTP, 1992).

With S9, a significant increase in chromosomal aberrations in CHO cells was observed at all three reported concentrations (4000, 4500, and 5000 µg/mL). Without S9, the response in this test was equivocal, with a significant increase in chromosomal aberrations observed only at 1000 µg/mL (Galloway, 1985, 1987; NTP, 1992).

In vitro exposure to resorcinol did not induce sister chromatid exchanges either in CHO cells (without or with exogenous metabolic activation) or in human lymphocytes (tested only without exogenous metabolic activation) (Darroudi and Natarajan, 1983).

Chromosomal aberrations were induced *in vitro* in human lymphocytes (Schulz *et al*., 1982; Darroudi and Natarajan, 1983) and amniotic cells (tested only without exogenous metabolic activation) (Schulz *et al*., 1982) but not in CHO cells (without or with exogenous metabolic activation) or human fibroblasts (tested only without exogenous metabolic activation) (Darroudi and Natarajan, 1983).

Sister chromatid exchange was not induced in human lymphocytes by resorcinol at concentrations up to 28 µg/mL (Jansson *et al.,* 1986, 1988), and no evidence of sister chromatic exchange was found in CHO cells exposed to resorcinol at concentrations of 0.6-2 µg/mL (Wild *et al*., 1981); however, the validity of these studies cannot be evaluated since relevant details were not reported.

Furthermore, resorcinol was reported to be positive, with and without S9, in tests for induction of chromosomal aberrations in Chinese hamster lung fibroblasts (Sakano *et al.*, 1985) and CHO cells (Stich *et al*., 1981); however, the study by Sakano *et al.* (1985) was reported only as an abstract and the validity of the study by Stich *et al.* (1981) is limited.

Resorcinol did not induce strand breaks in isolated DNA (Yamada *et al.,* 1985; Kawanishi *et al*., 1989; Miura *et al.,* 2000).

3.2.3.2. *In vivo* testing

Resorcinol was tested in a rat micronucleus assay which was performed in compliance with GLP and according to the OECD test guideline 474 (1997). After a dose range finding assay, resorcinol was tested at doses of 125, 250 and 500 mg/kg bw in groups of 5 male and 5 female Crl:CD (SD)BR rats. Resorcinol was formulated in water and administered once by oral gavage. Bone marrow was sampled at 24 hours after treatment and the bone marrow of the high dose group was also sampled at 48 hours. Resorcinol induced mortality in 1 female and signs of clinical toxicity in the animals treated at 500 mg/kg bw, which included tremors, rapid respiration, salivation, and/or squinted eyes. Resorcinol did not induce statistically significant increases in micronucleated polychromatic erythrocytes (PCEs) at any dose examined. It induced a statistically significant decrease in the PCE:NCE (normochromatic erythrocytes) ratio in females at 500 mg/kg bw at the 48-hour harvest time point, indicating that the bone marrow was exposed to the test compound (Erexson, 2005). The Panel considers that resorcinol was negative in this rat bone marrow assay.

In a mouse micronucleus assay, male CBA mice (N=4/group) received single doses of resorcinol at doses of 37.5, 75, 150 or 300 mg/kg bw intraperitoneally. The bone marrow was sampled after 24 and 48 hours and 1000 PCEs were analysed per mouse. No increase in the frequency of micronuclei could be detected (Darroudi and Natarajan, 1983). However, the study did not fully meet the criteria of the current OECD test guideline 474 (1997) with respect to the number of animals used and the number of PCEs counted.

The frequency of micronucleated polychromatic erythrocytes was not increased in groups of four (two male and two female) mice after two intraperitoneal injections of resorcinol at doses of 55-220 mg/kg

bw (at 0 and 24 hours, and bone marrow was sampled at 30 hours) (Gocke *et al.*, 1981; Wild *et al.*, 1981); however, this study did not meet the criteria of the current OECD test guideline 474 (1997) with respect to the sampling time, the number of animals used and the number of PCEs counted.

Treatment of mice with resorcinol did not induce micronuclei in bone marrow cells at a dose of 75 mg/kg bw (Paschin *et al.,* 1986) and at 2 equal doses of 250 mg/kg bw separated by an interval of 24 hours (the bone marrow was sampled 6 hours after the second dose) (Hossack and Richardson, 1977); however, the validity of these studies is insufficient due to the sampling times used.

In the NTP study, resorcinol (11 000 mg/kg feed) was negative for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered to adult flies by feeding. Administration of resorcinol by injection (concentration 1194 mg resorcinol/L, injected volume 0.2-0.3 µl) yielded an increase in mutations, which was equivocal (P=0.06 and mutation frequency of 0.12 % in the treated group) (Zimmering *et al.*, 1985, NTP, 1992).

Resorcinol, at doses of up to 100 mg/kg bw either orally or intraperitoneally, or up to 200 mg/kg bw topically, did not induce sister chromatid exchanges in rat bone marrow cells *in vivo* (Bracher *et al.,* 1981).

Resorcinol was reported not to induce inhibition of DNA synthesis in testicular cells in mice at a dose of 100 mg/kg bw (Seiler, 1977), or sperm abnormalities (Wild *et al.*, 1981); however, the validity of these studies cannot be evaluated since relevant details were not reported.

3.2.4. Chronic toxicity and carcinogenicity

The studies summarised below were performed under the direction of the US NIEHS and were conducted in compliance with the NTP (NTP, 1992).

3.2.4.1. Rats

F344 rats (N=50/sex/group) received orally by gavage resorcinol in doses of 0, 112, or 225 mg/kg bw/day, 5 days per week, for 103 weeks. As 16 females from the high-dose group had died after 22 weeks, the 2-year female rat study was restarted using lower dose levels of 0, 50, 100, or 150 mg/kg bw/day resorcinol, 5 days per week. Additionally, 10 rats of each sex per dose group were designated for interim evaluations of haematology, clinical chemistry, organ weights and histopathology after 15 months (66 weeks) of treatment. Because substantial early mortalities occurred among the high-dose male rats, animals from this group were not killed at 15 months. Instead, 10 high-dose male rats that either died or were killed in a moribund condition between weeks 62 and 67 of the study were used for the high-dose 15-month interim histopathological evaluation; but organ weights, haematology, and clinical chemistry data were not collected from this group.

At the 15-month interim evaluation, no treatment-related differences were seen in haematology or clinical chemistry parameters. No treatment-related histopathological changes were recorded. The changes in organ weights were limited to a statistically significantly increased relative brain weight of males receiving 112 mg/kg bw/day (5.19 ± 0.09 *vs.* 4.75 ± 0.07 control, $p < 0.01$), and to a statistically significantly increased relative liver weight of females receiving 150 mg/kg bw/day (3.19 ± 0.09 *vs.* 2.95 ± 0.06 control, $p < 0.05$). These differences in relative weights were considered by the authors of the report to be associated with the decreased body weights in these groups (NTP, 1992).

In the main study, the mean body weights of the males receiving 225 mg resorcinol/kg bw/day were 10 % to 15 % lower than those of the control from week 87 to study termination. The mean body weights of male rats receiving 112 mg resorcinol/kg bw/day were similar to those of control male rats. The mean body weights of the females receiving 150 mg resorcinol/kg bw/day were 11 % to 14 % lower than those of the controls from week 95 to study termination. The mean body weights of the females from the 100 mg resorcinol/kg bw/day group were slightly lower than the controls during the

second half of the study, while the mean body weights of the females from the 50 mg resorcinol/kg bw/day group were similar to the controls throughout the study.

Clinical signs of toxicity that included ataxia, prostration, salivation and tremors, were observed in males and females treated with 100 and 150 mg resorcinol/kg bw/day. These clinical signs began shortly after dosing and disappeared after approximately 30 to 60 minutes, and became more pronounced at the end of the 5-day dose periods.

The survival of high-dose males and females was statistically significantly lower than that of the controls (males: 15 % *vs.* 57 % in the control group; females: 50 % *vs.* 68 % in the control group). The remaining dose groups had survival rates similar to those of the controls.

There were no treatment-related increases in the incidences of neoplasms or non-neoplastic lesions (NTP, 1992). The Panel noticed that the NOAEL was 50 mg/kg bw/day with regard to acute clinical signs of toxicity, which were considered a resorcinol-related effect on the CNS. This NOAEL corresponds to a daily dose of 36 mg/kg bw/day when adjusted from the 5-day dosing week to a 7-day dosing week.

3.2.4.2. Mice

Groups of B6C3F$_1$ mice (N=60/sex) received orally by gavage resorcinol in doses of 0, 112, or 225 mg/kg bw/day, 5 days per week. An interim sacrifice of 10 mice per sex was performed after 15 months (66 weeks) for evaluations of haematology, clinical chemistry, organ weights and histopathology. The study with the remaining animals continued for 104 weeks. At the 15-month interim evaluation, there were no statistically significant differences in absolute or relative organ weights. No chemical-related changes in haematology or clinical chemistry parameters were seen. No chemical-related histopathological changes were recorded.

In the main study, the mean body weights of the females receiving 225 mg/kg bw/day were 10 to 15 % lower than those of the controls from week 85 until study termination. The mean body weights of the males receiving 225 mg/kg/day and of the male and female mice receiving 112 mg/kg/day were similar to those of the controls throughout the study period. Clinical symptoms recorded included recumbency and tremors occurring for a short period after dosing in mice of both sexes in both treatment groups. The terminal survival of males and females receiving resorcinol was similar to that of the controls although several males from the high-dose group died during the early part of the study (e.g. by week 45 of the study 8 high-dose male mice had died but the survival of control and low-dose males was 100 % at that time point). There was no treatment-related increase in the incidence of neoplasms or non-neoplastic lesions (NTP, 1992). The Panel considered the clinical symptoms a resorcinol-related effect and concluded that a NOAEL could not be established from this study because clinical symptoms of toxicity were reported for both dose groups.

3.2.5. Other studies related to carcinogenicity

Feeding studies with resorcinol in transgenic mice CB6F1-Tg rasH2 carrying the human prototype c-Ha-ras gene, were part of an inter-laboratory program for evaluating new models for short-term (26 weeks) carcinogenicity testing (Gulezian *et al.*, 2000). Administration of resorcinol to CB6F1-Tg rasH2 mice by gavage at a dose of 225 mg/kg bw/day, 5 days per week, for 26 weeks was reported by 2 independent laboratories not to be associated with tumour development (Yamamoto *et al.*, 1998; Maronpot *et al.*, 2000; Morton *et al.*, 2002).

The petitioner also submitted summaries of animal studies on administration of resorcinol together with known genotoxic carcinogens. In these studies resorcinol was administered in the diet (0.2 or 0.8 %) to rats (Miyata *et al.*, 1985; Hirose *et al.*, 1986, Yamaguchi *et al.*, 1989; Kurata *et al.*, 1990; Okazaki *et al.*, 1993) or to hamsters (0.25 or 1.5%) (Hirose *et al.*, 1986; Maruyama *et al.*, 1991).

Under the conditions of these bioassays resorcinol did not enhance the incidence of tumours in the bladder (Miyata *et al*., 1985; Kurata *et al.*, 1990), forestomach (Hirose *et al*., 1989), liver or kidneys (Okazaki *et al.*, 1993) in the rat, or tumours of the urinary bladder, forestomach (Hirose *et al.*, 1986), or pancreas (Maruyama *et al.,* 1991) in the hamster. However, treatment of rats initiated with 3 i.p. injections of 25 mg/kg bw of *N*-nitrosomethyl-*n*-amylamine within the initial 2-week period, and administered commencing 1 week after 0.8 % resorcinol in a powdered basal diet for 49 weeks was associated with an increased incidence of tongue papilloma (6/12 in the group treated with resorcinol after initiation *vs.* 1/11 in the group treated only with initiator, $p < 0.05$) and of oesophageal carcinoma (7/12 *vs.* 0/11 the group treated only with initiator $p < 0.01$) The authors of the study noted that the mechanism behind the observed increased incidence was unclear and concluded that resorcinol might contribute to the development of human upper gastrointestinal cancer (Yamaguchi *et al.*, 1989).

In a study of the potential inhibitory effects on carcinogenesis of several antioxidants in rats with initiated lung tumorigenesis, resorcinol was reported to have an inhibitory effect (Hasegawa *et al.* 1990).

Oral administration of resorcinol (0.8 % in the diet) to rats did not induce hyperplasia or DNA synthesis, as measured by bromodeoxyuridine-labeling index in the forestomach epithelium. No cell proliferation, increased DNA synthesis or increase in pepsinogen-altered neoplastic foci was observed in the pyloric mucosa (Shibata *et al.*, 1990).

Male rats (N=9-11/group) given a diet containing 0 or 0.01 % resorcinol for 6 weeks beginning 1 week after partial hepatectomy and intraperitoneal injection of 300 mg/kg bw N-nitrosodiethylamine to initiate liver carcinogenesis demonstrated that resorcinol after initiation did not increase the multiplicity of enzyme-altered (gamma-glutamyltranspeptidase) foci (Stenius *et al.*, 1989).

3.2.6. Reproductive and developmental toxicity

The petitioner presented a study assessing the potential teratogenic effect of resorcinol. Resorcinol in doses of 0, 125, 250, or 500 mg/kg bw/day was administered by gavage to pregnant Sprague-Dawley rats (N=10-13/group) on gestation days 6 to 15. The clinical condition of the dams was observed daily throughout gestation and body weights were recorded on gestation days 0, 6, 16 and 20. Dams were killed 24 hours before parturition and viability of each fetus was determined. Resorcinol in doses up to 500 mg/kg bw/day caused no embryotoxicity and no adverse effects on the mean number of corpora lutea, total implantations, viable fetuses, or mean fetal body weights. There was no increase in fetal anomalies or malformations. In dams only a slight (not statistically significant) decrease in maternal weight gain was seen on gestation days 6-16 at 500 mg/kg bw/day (Di Nardo *et al.,* 1985). The Panel noticed that the NOAEL was 500 mg/kg bw/day, the highest dose tested.

For further studies addressing the potential teratogenicity of resorcinol the petitioner referred to a toxicology review by Lynch *et al.* (2002) in which other studies were mentioned. These studies were also reviewed either by the International Agency for Research on Cancer (IARC, 1999), the NTP (1992), or JECFA (2000) and summaries are presented below.

Administration of resorcinol by gavage in doses of 40, 80, or 250 mg/kg bw/day to pregnant rats (strain and number/group not given) on gestation days 6 to 15 or to pregnant rabbits in doses 25, 50, or 100 mg/kg bw on gestation days 6 through 18 failed to cause any signs of maternal toxicity or embryotoxicity, teratogenicity in either species (Spengler *et al.*, 1986). The Panel noted that this limited report indicated a NOAEL of 250 mg/kg bw/day in rats and 100 mg/kg bw/day in rabbits, the highest doses tested.

In another study, resorcinol was administered by gavage to a group of Sprague-Dawley rats (N=15-20) on gestation day 11 at doses of 0, 333, 667, or 1000 mg/kg bw/day. No effects of treatment were recorded on any of the developmental parameters measured including litter size, rate of perinatal loss, average pup weight, and total litter weight. Slight maternal weight loss was reported on the first day

after dosing at all dose levels. However, no effect on maternal weights was found 72 hours post-dosing (Kavlock, 1990).

No multigeneration reproduction study or other data addressing potential effects on postnatal development and function, including neurological function and behaviour, physical, functional and behavioural development of animals exposed from at least the beginning of embryogenesis throughout weaning were submitted by the petitioner. The studies presented below were located by the Panel in a publication from the International Programme on Chemical Safety (IPCS, 2006).

In a dose-range finding one-generation reproductive toxicity study Sprague-Dawley rats (F_0, N=14/sex/group, age ca. 8 weeks) received resorcinol in drinking water at concentrations of 0, 10, 40, 120, or 360 mg/L (corresponding to doses of 0, 4, 13, and 37 mg/kg bw/day for males and 0, 1, 5, 16, and 47 mg/kg bw/day for females) for a minimum of 28 consecutive days prior to mating, during mating and then throughout gestation and lactation (the latter only for F_0 females). One F_1 pup per sex per litter was selected for resorcinol exposure from weaning on postnatal day (PND) 21 to PND 28. Three F_1 pups per sex per litter were selected for behavioural testing, while the rest of the F_1 were necropsied on PND 21. F_0 parental and F_1 (following selection) rats underwent complete gross necropsy following the breeding period (N=7 F_0 males/group), following completion of weaning of F_1 (F_0 females and 7 remaining F_0 males/group), on PND 28 (F_1 exposed pups) or on PND 30 or 70 (F_1 pups selected for behaviour tests). Several organs were weighed, and hormone analyses (TSH/T_3/T_4) were performed at scheduled necropsy on all F_0 parents, on PND 28 for exposed F_1 pups, and on PND 4 for all culled F_1 pups. Thyroid gland from F_0 parents were subjected to microscopy and brain measurements were performed on all exposed F_1 pups selected to behavioural testing. In controls and the high-dose groups a qualitative histopathological analysis of the brain (forebrain, midbrain, and hindbrain) was also performed for the aforementioned animals. No histopathological changes were reported.

Locomotor activity for F_1 males and females was unaffected on PND 21. On PND 61 statistically significantly increases in cumulative total (34-41 %) and/or ambulatory counts (37-54 %) were noted for F_1 males in the 40, 120, and 360 mg/L groups. However owing to the absence of a dose-response relationship, other indicators of developmental delay, or other changes in CNS function, the Panel considered that these effects did not provide conclusive evidence of change in CNS function.

For the thyroid, a non-statistically significant increase in TSH levels was reported for interim males but not at scheduled necropsy. In high-dose females, T_3 levels were increased, while there was no effect on T_3 or T_4 levels in males. The microscopic examination of the thyroid revealed minimal changes (follicular hyperplasia), which were not statistically significant between the control and individual dose groups.

There were no adverse effects on reproductive performance, mortality, and body or organ weights in all treated groups (RTF, 2003). The Panel notes that the NOAEL was 360 mg/L (equal to 37 mg/kg bw/day for males and to 47 mg/kg bw/day for females), the highest doses tested.

Based on these data, a 2-generation reproductive toxicity study with doses of resorcinol of 0, 120, 360, 1000, or 3000 mg/L in drinking water for the F_0 and F_1 was performed. Groups of Sprague-Dawley rats (F_0, N=30/sex/group, aged approx. 6 weeks at the start) were treated for a minimum of 70 days prior to mating. The F_0 and F_1 males continued to receive resorcinol through mating until termination. The F_0 and F_1 females were treated with resorcinol throughout mating, gestation, and lactation until termination after weaning of F_1 and F_2 respectively.

There were no resorcinol-related deaths or clinical findings in the F_0 and F_1 parental animals during the weekly detailed physical examination. Reproductive performance (oestrus cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) and parturition in the F_0 and F_1 animals were unaffected by administration of resorcinol. No effect of resorcinol was found for spermatogenic end-points (mean testicular and epididymal sperm numbers and sperm

production rate, motility, progressive motility and morphology) in the F_0 and F_1 males. No resorcinol-related effects on F_0 and F_1 pup survival or the general physical condition of the pups during the pre-weaning period were observed. In F_0 animals dosed with 3000 mg resorcinol/L decreased cumulative body weight gains without clear trends were noted during the premating period (females) or the entire generation (males). In F_0 females dosed with 3000 mg/resorcinol/L mean body weights were decreased by up to 6.3 % from study days 56 to 70 and during the first week of gestation (up to 5.5 %) throughout lactation (up to 8.4 %) and after the lactation period (6.3 %).

In F_1 males from the 3000 mg resorcinol/L group, mean cumulative body weight gains were decreased without clear trends. Mean body weights were decreased by up to 7.1 %. In F_1 females from the 3000 mg resorcinol/L group, decreased mean body weights were recorded during lactation (up to 6.1 %) and after (up to 7 %).

The mean water intake was decreased in F_0 and F_1 rats from 3000 mg/L groups during the premating period (females) or the entire generation (males) and also in F_1 pups gang-housed by litter from PND 21 to 28. A decreased water intake was also recorded on several occasions in the group dosed with 1000 mg/L but the decrease was less severe and the onset was seen later than in the high-dosed rats. The authors of the study considered the decreased water intake to be related to poor palatability of resorcinol in the water as no effects were observed on both feed intake and feed utilisation.

No resorcinol-related macroscopic findings, organ weight changes, or adverse microscopic target organ effects were observed in F_0 and F_1 parental animals.

No statistically significant resorcinol-related changes in the mean concentrations of T_3, T_4, or TSH were observed in the F_0 and F_1 parental animals or in the F_1 and F_2 pups selected for analysis (PND 4 or 21). Higher TSH values were recorded in the F_0 males at scheduled necropsy, but these were not considered treatment-related in the absence of effects on T_3 or T_4, organ weights or adverse macro- or microscopic findings. Decreased colloid (not statistically significant) in the thyroid gland was observed the high-dose males. As the finding was not associated with functional effects it was not considered an adverse effect (RTF, 2005). The Panel noted that the NOAEL was the highest dose tested, 3000 mg/L, with regard to reproductive toxicity and thyroid effects. When expressed on a body weight basis (average of F_0 and F_1 animals) this concentration corresponds to approximately 233 mg resorcinol/kg bw/day for males and 304 mg resorcinol/kg bw/day for females.

3.2.7. Other studies

3.2.7.1. Allergenicity /skin sensitisation

Concentrated solutions of resorcinol are irritating to the skin. Resorcinol is also a rare cause of contact dermatitis, and may also induce generalised eczema, urticaria and angioneurotic oedema (IARC, 1999). By using the Local Lymph Node Assay, it has been suggested that the skin sensitising potency of resorcinol is approximately two orders of magnitude lower than that of p-phenylenediamine and similar to that of hexyl cinnamic aldehyde (Basketter *et al.*, 2007).

3.2.7.2. Goitrogenic activity of resorcinol in animals and humans

The petitioner submitted a review on the thyroid effects of resorcinol (Lynch *et al.*, 2002). The animal toxicology and human data were reviewed. The paper is summarised below.

Resorcinol administered at high doses to rodents can disrupt thyroid hormone synthesis and produce goitrogenic effects. According to Lynch *et al.*, (2002), the effects of resorcinol on the thyroid are ascribed to the inhibition of thyroid peroxidase enzymes and subsequent interruption of the synthesis of thyroid hormone. There are species-specific differences in synthesis, binding, and transport of thyroid hormone. Rodents, especially rats, have been reported to be particularly susceptible to

goitrogens, due primarily to the lack of thyroid-binding protein, which is the primary thyroid hormone binding and transport protein. The effects on the thyroid in animals, especially rats, must be interpreted with caution as thyroid function studies have shown that thyroid active chemicals exert significant effects in some animal species, including rats, but do not produce clinically significant effects in humans. Given the relative insensitivity of humans and other primates to changes in the thyroid gland, high doses of substances that cause hormonally-induced changes of the thyroid in rodents, may have little relevance to humans. According to the authors of the review, antithyroid activity of resorcinol in animals occurs only after administration in a manner that allows for continued systemic exposure e.g., via diet, drinking water, subcutaneous administration in hydrophobic vehicles, or by twice daily dermal application and then only at high doses. This conclusion was based on the review of selected animal studies published between 1950 and 1995.

The human data reviewed by Lynch *et al.* (2002) included clinical case reports referring to medical use of resorcinol in ointments to treat persistent skin ulcers, epidemiological studies focusing on the effects of resorcinol on occupationally exposed persons and in populations living in geographical regions where drinking water contained resorcinol and other phenolic derivates from natural sources. Based on the reviewed studies, Lynch *et al.* (2002) reported that thyroid effects (reversible hypothyroidism) may occur as a result of integrity-compromised skin exposed to resorcinol at dose levels in the range of 34 to 122 mg/kg bw/day over a prolonged period (months to years). From these data, the authors of the review established an effect threshold value of 10 mg resorcinol/kg/day for dermal exposure based on the application of a 3-fold safety factor. They also noted that there was no evidence that intermittent or low-dose exposure to resorcinol caused hypothyroidism or any other adverse health effects.

According to the authors of the review, with high use conditions, the potential human exposure to resorcinol from its topical use in ointments to treat acne is estimated to be up to 1.2 mg/kg bw/day. From more realistic use conditions an exposure of about 0.2 mg/kg bw/day would result. These exposure estimates are well below the levels that could be associated with adverse thyroid effects (i.e., higher than 10 mg/kg bw/day) and therefore could not be expected to be associated with thyroid effects (Lynch *et al.*, 2002).

According to Lynch *et al.* (2002) the available epidemiological studies indicated that exposure via inhalation and dermal contact with concentrations of resorcinol found in occupational settings did not indicate thyroid abnormalities. The few cases of hypothyroidism reported in cross-sectional studies of exposed workers may simply reflect the background occurrence of this disorder. The prevalence of hypothyroidism in exposed workers did not appear to be unusual when compared with general population values. There was no convincing evidence of causal relationship between resorcinol in drinking water and the occurrence of goiter or hypothyroidism. Overall the epidemiology studies provided no clear evidence to indicate that resorcinol was an etiological agent for goitrogenic effects in the human population (Lynch *et al.*, 2002).

During topical application of 2 % resorcinol in a hydroalcoholic vehicle, in a total of 20 mL of formulation per application (daily dose 12 mg resorcinol/kg bw) to 3 healthy men, twice a day, 6 days per week, thyroid function remained normal throughout the study (Yeung *et al.*, 1983).

Apart from the publications reviewed above, the petitioner submitted publications concerning evaluation of certain biological effects of resorcinol (and other phenolic compounds) in *in vitro* assays (Passi *et al.*, 1987; Mutoh *et al.*, 2000). The Panel considered these publications, not directly applicable for the evaluation of the safety of resorcinol. Therefore the studies are not further discussed in this opinion.

4. Discussion

The presented studies indicate that resorcinol is rapidly absorbed from the gastrointestinal tract, metabolised extensively to sulphate and/or glucuronic acid conjugates which are excreted primarily in

the urine. Studies using ^{14}C-radiolabelled resorcinol administered orally by gavage or subcutaneously indicated no accumulation.

The data on acute toxicity after oral exposure indicate that resorcinol is of moderate acute toxicity as the median lethal dose after oral exposure in rats ranged from 202 to 980 mg/kg bw depending on the study. However, the subacute and subchronic studies in F344 rats and $B6C3F_1$ mice indicate a steep dose-response curve for lethality with lethal effects at 520 mg resorcinol/kg bw/day in rats and at 420 mg resorcinol/kg bw/day or above in mice. At doses that were not lethal clinical signs of toxicity manifested by hyperexcitability, tachypnea, ataxia, prostration and tremors were observed in mice and rats in subacute, subchronic and chronic toxicity studies. The NOAELs for clinical signs of toxicity were: 27.5 mg/kg bw/day in rats and 75 mg/kg bw/day in mice in the subacute studies, and 225 mg/kg bw in mice in the 90-day studies. In the subchronic study in F344 rats, 260 mg resorcinol/kg bw/day was the highest dose with no clinical signs of toxicity in the animals surviving to termination. The Panel noted that NOAELs for the clinical signs of toxicity in subchronic studies in rodents were 2 fold or less than the doses causing lethality. The Panel noted that the clinical signs of toxicity manifested as intermittent convulsive movements, starting between week 6 and 8 in the subchronic (13-week) study were recorded in Sprague Dawley rats at 250 mg resorcinol/kg bw/day. The Panel also noted that the neurological effects were not reported in the reproductive toxicity studies in Sprague Dawley rats.

The other effects recorded in subchronic studies in F344 rats and $B6C3F_1$ mice at doses that were not lethal were changes in organ weights: (I) statistically significantly increased absolute and relative liver weights of rats of both sexes (for males at doses of 130 and 260 mg/kg bw, for females absolute weights at doses of 65, 130, 260 mg/kg bw, relative weights at doses of 32, 65, 130 and 260 mg/kg bw/day (II) statistically significantly increased absolute and relative adrenal weights in all male rats treated with resorcinol doses ranging from 32 to 260 mg/kg bw/day, and (III) decreases in absolute and relative adrenal weights recorded for male mice receiving resorcinol doses ranging from 28 to 225 mg/kg bw/day.

The Panel noted that the reported increases in liver weights in the treated groups were slight, with no apparent dose-response relationship, and not accompanied by any changes in clinical chemistry parameters indicative of impaired liver function, or by any histopathological changes, and therefore the Panel did not consider this finding biologically significant.

The Panel also noted that the changes in adrenal weights in rats and mice were limited to the males and that no dose-response relationship was apparent; the effects on the adrenal weights were opposite in male rats and mice, and were not accompanied by any microscopical changes. Furthermore, the Panel noted that no treatment-related non-neoplastic and neoplastic lesions were seen in the adrenal glands of both sexes in the carcinogenicity studies in rats and mice. Therefore, the Panel considered that the changes in adrenal weights in male rats and mice were not attributable to resorcinol toxicity.

The Panel noted that the reported decreases in the absolute and relative thyroid weights of Sprague Dawley rats in the 13-week study were slight, with no apparent dose-response relationship and not accompanied by any histopathological changes, and therefore the Panel considers these effects not to be biologically significant.

Carcinogenicity studies in rats and mice demonstrated the lack of carcinogenic activity of resorcinol. However, in these studies acute clinical signs of toxicity like ataxia, prostration, salivation and tremors were observed in treated male and female rats and recumbency and tremors in treated mice at about 100 mg/kg bw/day and above. These acute clinical signs occurred shortly after dosing and disappeared after approximately 30 to 60 minutes. It was noted by the authors of the report that the severity of these acute signs became more pronounced at the end of the 5-day dose periods, indicating a possible cumulative effect with repeated dosing. The Panel noted that the NOAEL for these acute neurological effects was 50 mg/kg bw/day in rats. This dose corresponds to a daily dose of 36 mg/kg bw/day when adjusted for the 5-day dosing week to a 7-day week. The Panel considered the acute neurological

effects indicative of a resorcinol-related effect and given the steep dose-response relationship for lethality, a critical endpoint in the evaluation of resorcinol-induced toxicity.

Treatment of rats initiated with 3 i.p. injections of 25 mg/kg bw of *N*-nitrosomethyl-*n*-amylamine within the initial 2-week period, and administered commencing 1 week after 0.8 % resorcinol in a powdered basal diet for 49 weeks, was associated with an increased incidence of tongue papilloma and of oesophageal carcinoma. However, since this result was not reproduced in another study and in the light of the negative results seen in several other initiation-promotion studies in which resorcinol was administered together with a known genotoxic carcinogen to rats and hamsters, the Panel considered the tumour-promoting effect observed with resorcinol in this single study not significant.

Resorcinol was tested in genotoxicity tests covering the three types of genotoxic endpoints, i.e. gene mutations in bacteria and gene mutations and chromosomal aberrations in mammalian cell *in vitro*. Four *in vitro* studies and one *in vivo* study were conducted according to GLP and in compliance with the relevant OECD test guidelines and additional non-GLP genotoxicity studies were also available. Overall, resorcinol did not induce mutations in bacteria in tests which were performed according to current guidelines; however, resorcinol induced reverse mutations in one study in *Salmonella typhimurium* strain TA100 without metabolic activation and in strain TA1535 with metabolic activation when tested with a certain bacterial minimal medium. Resorcinol was clastogenic *in vitro* in peripheral human lymphocytes in the absence of metabolic activation. The results from an *in vitro* mouse lymphoma assay indicated that resorcinol could induce gene mutations and/or chromosomal aberrations; however, resorcinol did not induce mutation at the *hprt* locus of L5178Y mouse lymphoma cells when tested in concentrations up to 1101 µg/mL (10 mM) in two independent experiments in the absence or presence of a rat liver metabolic activation system (S9). The Panel notes that *in vivo* phenolic compounds are generally efficiently conjugated in phase II reactions like glucuronidation and sulfation, and excreted; ADME studies confirm that this also occurs for resorcinol. Since the *in vitro* models used to investigate the genotoxicity of resorcinol lack these phase II enzymes, the Panel notes that results obtained in these *in vitro* genotoxicity studies may not reflect what could happen in the *in vivo* situation. A valid *in vivo* mouse micronucleus assay with resorcinol at dose levels up to 300 mg/kg bw was negative. Thus, the clastogenic effects observed in some *in vitro* assays were not confirmed *in vivo*. Altogether, the Panel considered resorcinol was not of concern with respect to genotoxicity.

Resorcinol had no adverse effects on the developing fetus when administered to pregnant rats by gavage in doses up to 500 mg/kg bw/day on gestation days 6 to 15, or as a single dose of 1000 mg/kg bw on gestation day 11, or in rabbits in doses up to 100 mg/kg bw/day given by gavage on gestation days 6 to 18.

Intake of resorcinol in drinking water in a one-generation dose-range finding reproductive toxicity study in rats in doses amounting to 37 and 47 mg/kg bw/day for males and females respectively and in a two-generation reproductive toxicity study in rats in doses amounting to 233 mg/kg bw/day for males and 304 mg/kg bw/day for females was not associated with reproductive toxicity.

No signs of maternal toxicity were recorded for rats receiving resorcinol orally in the diet at doses up to 500 mg/kg bw and in rabbits receiving by gavage up to 100 mg/kg bw in the developmental toxicity studies and in rats receiving up to approximately 304 mg resorcinol/kg bw in the drinking water in reproductive toxicity studies.

The literature on possible thyroid effects of resorcinol has been reviewed by Lynch *et al.* (2002). Based on the data from animal studies, Lynch *et al.* (2002), postulated that the antithyroid effect of resorcinol in animals occurs only after administration that allows for continued systemic exposure, and then only at high doses. According to Lynch *et al.* (2002), the reviewed case reports documented that recurrent exposure to very high daily doses of resorcinol applied dermally to patients with integrity compromised skin may induce reversible hypothyroidism, but that there was no evidence that intermittent or low-dose exposure to resorcinol caused hypothyroidism or any other adverse health

effects in humans. The reviewed epidemiological studies indicated, according to Lynch *et al.* (2002) that occupational exposure to resorcinol had not been sufficient to cause thyroid effects and there was no convincing evidence of a causal relationship between resorcinol in drinking water and the occurrence of goiter and hypothyroidism.

The literature indicates interspecies differences in susceptibility to goitrogens. Given the relative insensitivity of humans (and other primates) to changes in the thyroid gland, high doses of substances that cause hormonally-induced changes of the thyroid in rodents, have little relevance to humans. The Panel noticed that the toxicological studies in rodents did not indicate any thyroid effects of resorcinol. The Panel also noted that while the subacute, subchronic and chronic studies in rats and mice were not designed to investigate the endpoints for thyroid effects, a dose-range finding one-generation reproductive toxicity and a two-generation reproductive toxicity study in rats specifically investigated the potential effect of resorcinol on function and morphology of the thyroid gland. No adverse effects were noted. Furthermore, the Panel noted that the route of administration in these studies allowed continuous systemic exposure, which has been postulated to be essential for demonstration of the goitrogenic activity of resorcinol in rats and that the rat was a species reported to be particularly susceptible to goitrogens.

The Panel considered the recurring clinical signs of acute toxicity observed in long-term toxicity study in rats to be the pivotal adverse effect of resorcinol. The NOAEL of 50 mg/kg bw/day for these effects in the long term study in rat was adjusted from a 5-day dosing week to a 7-day dosing week, to a NOAEL of 36 mg resorcinol/kg bw/day. Because of the steep dose-response curve for resorcinol-induced lethality in acute and chronic animal experiments, and the observed species and strain differences, the Panel found it appropriate to apply an additional uncertainty factor of 3 to a safety factor of 100 and established an ADI of 0.12 mg/kg bw/day for resorcinol.

The Panel assessed the acute and chronic exposure based on two scenarios resulting in different resorcinol concentrations.

The first scenario corresponds to the use proposed by the petitioner to prevent the development of melanosis in shrimps: resorcinol concentration of 5 to 7 g/L in the dipping solution with a dipping time of 4-5 minutes. The Panel noted that the efficacy trials using these treatment conditions resulted in residual resorcinol at concentrations of 120 mg/kg in raw shrimps (whole product), 30 mg/kg in the edible parts of raw shrimps, and 15 mg/kg in the edible parts of boiled shrimps.

As the Panel considered that in real life situations it was unlikely that the dipping time could be this precisely controlled, a second scenario was established: dipping time of 30 minutes in a solution containing 5 to 7 g/L resorcinol. The Panel noted that the efficacy trials using these treatment conditions resulted in residual resorcinol concentrations of 470 mg/kg in raw shrimps (whole product), 40 mg/kg in the edible parts of raw shrimps, and 78 mg/kg in the edible parts of boiled shrimps.

For the acute exposure to resorcinol in adults and children, the Panel estimated consumption per eating occasion of 250 g of peeled cooked shrimps or 100 g of whole cooked shrimps as an ingredient of mixed dishes. In the absence of data on resorcinol concentration in cooked dishes prepared with raw shrimps (e.g. paella), it was assumed that the amount of resorcinol originally present in the whole raw shrimps would be present in the whole dish.

For the chronic exposure to resorcinol, an estimated level of daily consumption of 50 g of shrimps on a regular basis was considered by the Panel to be sufficiently conservative to cover the food habits of the European population including high consumers.

The acute daily exposure ranged from 0.06 to 0.8 mg/kg bw for adults and from 0.12 to 1.6 mg/kg bw for children, depending on the residual concentration of resorcinol.

The chronic daily exposure ranged from 0.1 to 0.4 mg/kg bw for adults and from 0.2 to 0.8 mg/kg bw for children, again depending on the residual concentration of resorcinol.

The highest calculated daily exposure to resorcinol corresponds to the consumption of cooked dishes prepared with 100 g of whole raw shrimps as an ingredient. The corresponding figures amount to 0.2-0.8 mg resorcinol/kg bw/day for adults and 0.4-1.6 mg resorcinol/kg bw/day for children. The Panel noted that the exposure would always exceed the ADI for adults and children with the residual resorcinol concentrations considered for whole raw shrimps (120 mg/kg or 470 mg/kg according to dipping time).

In view of these scenarios in which the ADI is exceeded, the Panel calculated the maximum residual concentration of resorcinol in whole raw shrimps that would not lead to an exposure exceeding the ADI of 0.12 mg/kg bw in the most vulnerable population (children) in any of the exposure scenarios considered. This residual concentration of resorcinol amounts to 35 mg/kg. This defined value is only applicable if other usages of resorcinol are excluded.

CONCLUSIONS

The Panel established an ADI of 0.12 mg resorcinol/kg bw/day based on the application of an uncertainty factor of 300 to the adjusted NOAEL of 36 mg/kg bw/day for acute neurological effects in a carcinogenicity study in rats.

The conservative estimates of acute consumption of shrimps would indicate that dietary exposure to resorcinol for adults and for children would exceed of the ADI when the residual concentration of resorcinol in whole raw shrimps is above 35 mg/kg. The Panel notes that this value is only applicable if other uses of resorcinol are excluded.

The Panel noted that the proposed uses for resorcinol are for all crustaceans but that only experimental data on shrimps have been reported. The current evaluation is therefore related only to the use of resorcinol on raw shrimps and dietary exposure should be re-estimated if new usages are introduced

DOCUMENTATION PROVIDED TO EFSA

1. Application for the Authorisation of Resorcinol as a Food Additive (Antioxidant). July 2006. Submitted by Harinas y Sémolas del Noroeste, S.A. (HASENOSA).
2. Unpublished study reports on genotoxicity were provided by the secretariat of the Scientific Committee on Consumer Products (Erexson, 2005; Lloyd, 2009; Sire, 2004; Williams, 2005; Whitwell, 2004).

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APPENDIX

List of acute, short-term, subchronic, chronic, developmental and reproductive toxicity studies with resorcinol and the no-observable adverse effect levels (NOAEL) or effect levels (LOAEL).

Study	Reference	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	Effects
Acute oral toxicity (oral exposure)				
Rat (♂)	Flickinger, 1976	-	-	LD_{50}=980 mg/kg bw
Rat (♂,♀)	Lloyd *et al.*, 1977	-	-	LD_{50}=370 mg/kg bw
Rat (strain, sex?)	Koppers Company, 1970	-	-	LD_{50}=301 mg/kg bw
Rat (strain, sex?)	CIR,1986	-	-	LD_{50}=300 mg/kg bw
Rat (♀ Wistar)	Hoechst AG, 1979	-	-	LD_{50}= 202 mg/kg bw
17-day studies in rats	NTP, 1992	27.5	55	hyper-excitability in females
17-day studies in mice	NTP, 1992	75	150	clinical findings including prostration and tremors
13-week studies in rats	NTP, 1992	130		given the uncertainty in the dosing and the cause of deaths in the two highest dose group
13-week studies in mice	NTP, 1992	225		Clinical signs of toxicity (dyspnoea, prostration, tremors)
Carcinogenicity study in rats (104 weeks)	NTP, 1992	50 (adjusted to 36 mg/kg bw/ day from the 5–day dosing week to a 7-day week)	100 (♀), 112 (♂)	Clinical signs (ataxia, tremors) (appearing shortly after dosing and disappearing after approximately 30 to 60 minutes)
Carcinogenicity study in mice (104 weeks)	NTP, 1992	112 mg/kg be/day (adjusted to 80 mg/kg bw/day from the 5-day dosing week to a 7-day week)		Recumbency and tremors occurring for a short period after dosing in mice of both sexes
Developmental toxicity in rats (oral gavage)	Di Nardo *et al.*, 1985	500 (the highest dose tested)		
Developmental toxicity in rats (oral gavage)	Spengler *et al.*, 1986	250 (the highest dose tested)		
Developmental toxicity in rabbits (oral gavage)	Spengler *et al.*, 1986	100 (the highest dose tested)		
Developmental toxicity in rats (oral gavage, single dosing on day 11 of gestation)	Kavlock 1990	1000		Maternal weight loss was reported on the first day after dosing at all dose levels
Dose range-finding one generation reproductive toxicity in rats (via drinking water)	RTF, 2003	360 mg/L (=37 mg/kg ♂, or 47 mg/kg bw ♀) (the highest dose tested)		

Two generation reproductive toxicity study in rats (via drinking water)	RTF, 2003	3000 mg/L (= 233mg/kg bw/day for F_0 and F_1 ♂ over the entire generation, and 304 mg/kg bw/day for F_0 and F_1 females during premating and gestation)		This NOAEL also applies to thyroid effects. 3000mg/L: ↓ mean cumulative body weight gains in F_0 and F1 animals, ↓ mean water consumption in parental F_0 and F_1 animals

GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
Adj RSG	Adjusted Relative Suspension Growth
Adj RTG	Adjusted Relative Total Growth
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
bw	body weight
CAS	Chemical Abstract Service
CHO	Chinese Hamster Ovary
CNS	Central Nervous System
EC	European Commission
EFSA	European Food Safety Authority
GCMS	Gas Chromatography-Mass Spectrometry
GCP	Gel Permeation Chromatography
GLP	Good Laboratory Practice
4HR	4-Hexylresorcinol
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
INCA	Individuelle et Nationale sur les Consommations Alimentaires
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
NCE	Normochromatic Rrythrocytes
NOAEL	No-Observed-Adverse-Effect-Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCEs	Polychromatic erythrocytes

PND	Postnatal Day
SCCP	Scientific Committee on Consumer Products
SCF	Scientific Committee on Food
SML	Specific Migration Limit
T_3	triiodothyronine
T_4	thyroxine
TDI	Tolerable Daily Intake
TSH	Thyroid-Stimulating Hormone