SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation Tartrazine (E 102)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Tartrazine (E 102). Tartrazine has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1966 and the EU Scientific Committee for Food (SCF) in 1975 and 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-7.5 mg/kg bw/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. New studies included a study by Sasaki *et al*. from 2002 reporting effects on nuclear DNA migration in the mouse *in vivo* Comet assay, a study by McCann *et al*. from 2007 that concluded that exposure to a mixture including Tartrazine resulted in increased hyperactivity in 3-year old and 8- to 9-year old children and studies on neurodevelopment by Tanaka. The Panel notes that Tartrazine was negative in long-term carcinogenicity studies and that the effects on nuclear DNA migration observed in the mouse *in vivo* Comet assay are not expected to result in carcinogenicity. The Panel also concurrs with the conclusion from a previous EFSA opinion on the McCann *et al*. study that the findings of the study cannot be used as a basis for altering the ADI, and additionally considered that the Tanaka study can also not be used as a basis for altering the ADI. The Panel concludes that the present database does not give reason to revise the ADI of 7.5 mg/kg bw/day. The Panel also concludes that at the maximum reported levels of use, refined intake estimates are below the ADI. The Panel concludes that Tartrazine appears to be able to elicit intolerance reactions in a small fraction of the exposed population.The Panel also notes that sensitive individuals may react to Tartrazine at dose levels within the ADI.

KEY WORDS

Tartrazine, FD&C Yellow No. 5, E 102, CAS 1934-21-0, 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazole trisodium salt, food colouring substance.

---

1 On request from the European Commission, Question No EFSA-Q-2008-222, adopted on 23 September 2009.

2 Panel members: F. Aguilar, U.R. Charrondiere, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Guertler, J. Koenig, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R.A. Woutersen. Correspondence: Unit-ANS@efsa.europa.eu.

3 Acknowledgement: The Panel wishes to thank the members of the Working Group B on Food Additives and Nutrient Sources for the preparation of this opinion: D. Boskou, R. Charrondiere, B. Dusemund, D. Gott, T. Hallas-Møller, K.F.A.M. Hulshof, J. König, D. Parent-Massin, I.M.C.M. Rietjens, G.J.A. Speijers, P. Tobback, T. Verguieva, R.A. Woutersen.

For citation purposes: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) ; Scientific Opinion on the re-evaluation Tartrazine (E 102) on request from the European Commission. EFSA Journal 2009; 7(11):1331. [52 pp.]. doi:10.2903/j.efsa.2009.1331. Available online: www.efsa.europa.eu

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of Tartrazine (E 102) when used as a food colouring substance.

Tartrazine (E 102) is an azo dye authorised as a food additive in the EU and previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1966 and the Scientific Committee for Food (SCF) in 1975 and 1984. Both committees established an Acceptable Daily Intake (ADI) of 7.5 mg/kg bw/day. In 2002, TemaNord in its assessment recommended that the evaluation should be updated with inclusion of newly published data including results from studies on genotoxicity, chronic toxicity/carcinogenicity and reproductive and developmental toxicity.

Specifications for Tartrazine have been defined in the EU Commission Directive 2008/128/EC and in the Codex Alimentarius. Tartrazine consists essentially of 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazole trisodium salt and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Tartrazine is described as the sodium salt. The calcium and the potassium salts are also permitted (Directive 2008/128/EC).

The purity is specified as not less than 85 % total colouring matters, calculated as the sodium salt. The remaining 15 % may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly), water insoluble matter not more than 0.2 %, subsidiary colouring matters not more than 1.0 % and organic compounds other than colouring matters with a total not more than 0.5 % (4-hydrazinobenzene sulphonic acid, 4-aminobenzene-1-sulphonic acid, 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid, 4,4′-diazoaminodi(benzene sulphonic acid), tetrahydroxysuccinic acid).

The absorption, distribution, metabolism and excretion of Tartrazine have been extensively studied in animals and humans. Whilst the majority of studies are 40-50 years old the techniques and methods used for the identification of the parent compound and its metabolites were those used to elucidate and identify the metabolic pathways of most xenobiotics. Following oral administration at a range of doses absorption of intact Tartrazine is negligible to low (< 5 %) and this intact Tartrazine is predominantly excreted unchanged in urine. After oral administration there is extensive metabolism of Tartrazine by the gastrointestinal microflora to sulphanilic acid and aminopyrazalone (which may then be subsequently cleaved to sulphanilic acid and α-amino-β-ketobutyric acid fragments with the latter breaking down further via intermediary metabolism with release of carbon dioxide). Both sulphanilic acid and aminopyrazalone can be absorbed to a greater extent than Tartrazine.

The studies included in the JECFA evaluation have been described in very little detail and appear to be inadequate for a proper evaluation of the subchronic toxicity of Tartrazine. The study by Aboel-Zahab and co-workers gives some more detail on the subchronic toxic effects of mixtures containing Tartrazine. This study cannot however be used for a re-assessment of the ADI of Tartrazine, as animals were exposed to a mixture of food colours rather than Tartrazine alone, the dose level of each colour has not been specified and it is not clear what were the amounts/percentage of the colours used in the diet to achieve the cited level of 0.8 g/kg bw/day. Furthermore, only one dose was tested.

In the previous evaluations there were no indications of Tartrazine-related adverse effects on reproduction or development. In the more recent study by Tanaka, adverse effects on reproductive parameters were also not demonstrated up to and including dose levels of 773 and 1225 mg/kg bw/day for males and females, respectively, the highest dose levels tested. The results from behaviour tests conducted during the lactation

period present some indications of differences in the performance of treated animals compared to the controls, most often in a direction of accelerated achievement of coordination (better performance compared to controls). However, these findings are not consistent, no convincing dose-response relationship could be observed and some findings in the high-dose groups are indicative of faster neurological development. While a statistically significant reduction in locomotor activity at weaning appeared to be a consistent finding, the test method did not take into account the possibility of habituation nor the biphasic (inverted V-shaped) pattern of activity from 15 to 30 days of post-natal life.

The Panel concludes that the studies by Tanaka did not demonstrate any adverse effects of Tartrazine on neurobehavioral development.

The Panel concludes that revision of the ADI based on these data is therefore not warranted.

Studies on micronucleus induction *in vitro* and *in vivo* Sister Chromatid Exchange (SCE), micronucleus and chromosome aberration tests were negative. Data from an unscheduled DNA synthesis (UDS) assay conducted *in vitro* and *ex vivo* on mammalian cells were also negative. Tartrazine induced chromosomal aberrations in Chinese hamster fibroblast cell line and showed a significant increase in SCE and chromosomal aberrations in mouse and rat bone marrow cells, following acute and chronic exposure to high doses of Tartrazine via the diet. Using the Comet assay, Sasaki *et al.* showed that Tartrazine induced DNA damage in the colon of mice at doses close to the ADI. In contrast, in a more recent study by Poul *et al*. Tartrazine did not reveal genotoxic effect in the micronucleus gut assay in mice at doses up to 2000 mg/kg bw. The authors comment on the results of the *in vivo* Comet assay by Sasaki *et al.* that the transient DNA damage observed in the colon of mice are unable to be fixed in stable genotoxic lesions and might be partly explained by local cytotoxicity of the dye.

The available carcinogenicity studies include the six carcinogenicity studies reviewed by JECFA, as well as the three more recent ones described by TemaNord, namely the publications of Maekawa *et al*. from 1987, and Borzelleca and Hallagan from 1988, plus the most recent study by Moutinho *et al*. from 2007. These studies have demonstrated that Tartrazine does not have a potential to induce benign or malignant neoplasias.

The Panel considered, in light of the negative carcinogenicity studies and negative results in standard *in vivo* genotoxicity studies, that the biological significance of the positive genotoxicity results in other studies is uncertain. Therefore the Panel concluded that the effects reported in these studies are not expected to result in carcinogenicity.

A study by McCann *et al*. has concluded that exposure to two mixtures of 4 synthetic colours plus a sodium benzoate preservative in the diet, one of them, Mix A, containing Tartrazine, resulted in increased hyperactivity in 8- to 9-year old and 3-year old children in the general population. In an earlier study by the same research team there was some evidence for adverse behavioural effects of a mixture of 4 synthetic colours (including Tartrazine) and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al*,. 2004).

Recently, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) published an opinion on this McCann *et al*. study. In this opinion the AFC Panel also presented an overview of earlier studies that reported effects of food colours in general on child behaviour, the majority of these studies being conducted on children described as hyperactive or with a clinical diagnosis of Attention-Deficit/Hyperactivity Disorder (ADHD).

In its opinion the AFC Panel concluded that the McCann *et al.* study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although

the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The AFC Panel also concluded that the findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular.

However, the AFC Panel, assisted by experts in human behavioural studies in the *ad hoc* Working group preparing the opinion, also concluded that the clinical significance of the observed effects remains unclear, since it is not known whether the small alterations in attention and activity would interfere with schoolwork and other intellectual functioning.

The AFC Panel also concluded that:

- since mixtures and not individual additives were tested in the study by McCann *et al.*, it is not possible to ascribe the observed effects to any of the individual compounds, and

- in the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed, the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

The ANS Panel concurs with these conclusions.

The Panel concludes that the present database does not give reason to revise the ADI of 7.5 mg/kg bw/day established by the SCF.

In humans, adverse reactions such as urticaria and vasculitis after Tartrazine intake have been reported in a number of studies. Data from animal and human studies have not convincingly demonstrated that Tartrazine is able to induce an immune mediated (hypersensitivity) response, and the adverse reactions reported in humans following exposure to Tartrazine appear to be intolerance reactions. The reports of these adverse effects are often characterised by poorly controlled challenge procedures; sometimes Tartrazine is given with a mixture of other colours. In comparison, recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon. However, given the available information, the Panel concludes that Tartrazine may induce intolerance reactions in a small fraction of the population. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The dietary exposure to Tartrazine from the Maximum Permitted Levels (MPLs) of use was estimated by the Panel using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day for adults, and 13.1 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and the Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Tartrazine, as identified by the Panel from the data by the UK Food Standards Agency, the Food Safety Authority of Ireland, the Agence Française de Sécurité Sanitaire des Aliments, the Union of European Beverage Associations, the European Spirits Organisation, the Federation of European Food Additives, Food Enzymes and Food Culture Industries and the Confederation of the Food and Drink Industries of the EU (Tier 3). For children (aged 1-10 years), estimates have been calculated for 9 European countries (Belgium, France, UK, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Tartrazine intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure of European children (aged 1-10 years) ranged from 0.8 mg/kg bw/day to 3.4 mg/kg bw/day and from 0.8 mg/kg bw/day to 9.4 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (50 % at average level and 80 % for high level consumers).

When considering maximum reported use levels (Tier 3), the mean dietary exposure to Tartrazine for European children (aged 1-10 years), ranged from 0.2 mg/kg bw/day to 1.9 mg/kg bw/day and from 0.4 mg/kg bw/day to 7.3 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure of 0.3 mg/kg bw/day and of 0.5 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

The Panel concludes that at the maximum reported levels of use of Tartrazine, refined (Tier 3) intake estimates are below the ADI of 7.5 mg/kg bw/day.

The Panel further notes that the specifications of Tartrazine need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components.

The Panel notes that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel notes that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the framework Directive 89/107/EEC[4] on food additives, the Scientific Committee on Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor the European Food Safety Authority (EFSA).

Directive 89/107/EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives[5] which will apply as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

In accordance with Regulation (EC) No 1333/2008, the Commission should, after consultation with EFSA, set up by 20 January 2010 an evaluation programme for EFSA to re-evaluate the safety of the permitted food additives. That programme will define the needs and the order of priorities according to which the approved food additives are to be examined.

Food colours were among the first additives to be evaluated, therefore, many of the evaluations are old. For some of these colours new studies have become available and the results of these studies should be included in the evaluation. Therefore, food colours should be evaluated with priority. The order of priorities for the re-evaluation of the remaining permitted food additives will be set in the Regulation for the re-evaluation program.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission asks the European Food Safety Authority to start a systematic re-evaluation of all authorised food additives and to issue scientific opinions on these additives, taking into account that colours as a group should be given the highest priority for the reasons outlined above.

[4] OJ L 40, 11.2.1989, p. 27
[5] OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Tartrazine (E 102) when used as a food colouring substance.

Tartrazine (E 102) is an azo dye allowed as a food additive in the EU, previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1966 and the EU Scientific Committee for Food (SCF) in 1975 and 1984.

The Panel has re-evaluated Tartrazine taking into account previous evaluations and additional literature that has become available since these previous evaluations. The Panel did not evaluate this already authorised food additive based on a newly submitted dossier.

2. Technical data

2.1. Identity of the substance

Tartrazine (E 102) is an azo dye with the formula $C_{16}H_9N_4Na_3O_9S_2$. It has a molecular weight of 534.36 g/mol and CAS Registry Number 1934-21-0. Its full chemical name is 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazole trisodium salt. Its structural formula is:

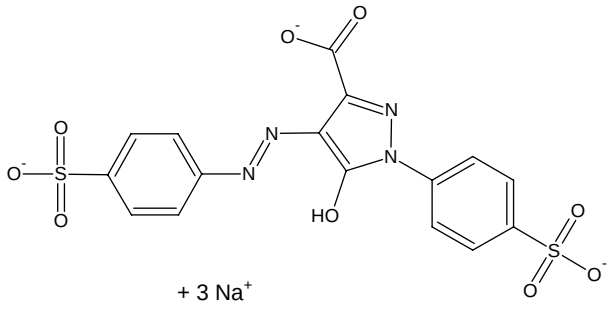

Figure 1. Structural formula of Tartrazine

At least 129 synonyms are in use (ChemIDplus advanced, via internet, 2006). The most commonly used synonyms in published literature are FD & C Yellow No. 5 and CI Food Yellow 4.

Tartrazine is soluble in water and sparingly soluble in ethanol.

2.2. Specifications

Specifications for Tartrazine have been defined in the Directive 2008/128/EC and by JECFA (JECFA, 2006) (Table 1).

Tartrazine consists essentially of 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazole trisodium salt and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Tartrazine is described as the sodium salt but the calcium and the potassium salts are also permitted (EC, 2008).

The purity is specified as not less than 85 % total colouring matters, calculated as the sodium salt. The remaining 15 % may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly), water insoluble matter not more than 0.2 %, subsidiary colouring matters not more than 1.0 % and organic compounds other than colouring matters with a total not more than 0.5 % (4-hydrazinobenzene sulphonic acid, 4-aminobenzene-1-sulphonic acid, 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid, 4,4′-diazoaminodi(benzene sulphonic acid), tetrahydroxysuccinic acid).

Unsulphonated primary aromatic amines may be present at levels of ≤ 0.01 % (calculated as aniline), originating from the manufacturing process.

Thus if the existing specifications could be extended to include ≤ 15.0 % sodium chloride and/or sodium sulphate as the principal uncoloured components, 99.9 % of the material would be accounted for.

Lancaster and Lawrence (1999) determined total benzidine and total aniline in 12 commercial samples of Tartrazine. The results suggested that prior to 1990, samples of Tartrazine contained levels of aniline in the range of 115-182 µg/g and of benzidine in the range of 32 - 38 ng/g. After 1990, the measured amounts were in the range of 0.2-16.6 µg/g for aniline and less than 5 ng/g for benzidine.

Table 1. Specifications for Tartrazine according to Commission Directive 2008/128/EC and by JECFA (JECFA, 2006)

Purity	Commission Directive 2008/128//EC	JECFA (2006)
Water insoluble matter	≤ 0.2 %	≤ 0.2 %
Subsidiary colouring matters	≤ 1.0 %	≤ 1.0 %
- 4-hydrazinobenzene sulphonic acid - 4-aminobenzene-1-sulphonic-acid - 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid - 4,4'-diazoaminodi(benzene sulphonic acid) - Tetrahydrosuccinic acid	≤ 0.5 %	≤ 0.5 %
Unsulphonated primary aromatic amines	≤ 0.01 % (calculated as aniline)	≤ 0.01 % (calculated as aniline)
Ether extractable matter	≤ 0.2 % (under neutral conditions)	≤ 0.2 %
Arsenic	≤ 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-

The Panel notes that the specifications on the purity of Tartrazine would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Tartrazine. Given the maximal allowed concentration of Tartrazine that can be added to food (500 mg/kg food), the concentration of these unidentified unsulphonated primary aromatic amines in food could be up to 50 µg/kg food.

The Panel notes that the limit test for heavy metals as indicated in Table 1 (expressed as lead in the table) is considered obsolete and is being replaced with limits for individual metals of concern.

The Panel noted that the JECFA specification for lead is ≤ 2 mg/kg whereas the EC specification is ≤ 10 mg/kg.

According to Directive 2008/128/EC, the above purity criteria for the pure substance also apply to the raw material from which the aluminium lake is produced. In addition, the aluminium lake should contain not more than 0.5 % HCl-insoluble material and no more than 0.2 % ether-extractable material under neutral conditions. There are no additional specification requirements for the aluminium lake.

JECFA does not give specifications for aluminium lakes of Tartrazine, other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004). The Tartrazine used in the production process should comply with the specifications as given above and the aluminium lake should contain not more than 2 % water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5 % hydrochloric acid-insoluble matter, not more than 0.2 % ether-extractable matter, not more than 3 mg arsenic/kg and not more 5 mg lead/kg. In the aluminium lake, unreacted aluminium oxide may also be present in the final product (not specified).

The Panel notes that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake of 1 mg aluminium/kg bw/week has been established (EFSA, 2008a) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

Tartrazine is prepared from 4-amino benzenesulphonic acid, which is diazotized using hydrochloric acid and sodium nitrite. The diazo compound is then coupled with 4,5-dihydro-5-oxo-1-(4'-sulphophenyl)-1H-pyrazole-3-carboxylic acid or with the methyl ester, the ethyl ester, or a salt of the carboxylic acid. The resulting dye is purified and isolated as the sodium salt.

Tartrazine may be converted to the corresponding aluminium lake under aqueous conditions by reacting aluminium oxide with the colouring matter. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with sodium carbonate or sodium bicarbonate, or aqueous ammonia. Following lake formation, the product is filtered, washed with water and dried (JECFA, 2004).

2.4. Methods of analysis in food

A number of methods for determination of Tartrazine in various foodstuffs have been developed and reported in recent years. Husain *et al.* (2006) and Sawaya *et al.* (2008) determined food colours, including Tartrazine, using High Pressure Liquid Chromatography with Diode Array Detection (HPLC-DAD). For the simultaneous determination of water-soluble synthetic colours in food, including Tartrazine, Ma *et al.* (2006) developed a method where the colours were extracted with dimethylsulphoxide (DMSO) and

quantified using HPLC-DAD electrospray LC/MC. For the determination of selected synthetic food colours, including Tartrazine, in three different kinds of foodstuffs (i.e. solid juice powders, solid jelly powders and soft drinks) Alves *et al.* (2008) used HPLC with UV-DAD detection.

2.5. Reaction and fate in food

No data were available in the published literature specifically related to Tartrazine. However in general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) will affect the colour (Scotter and Castle, 2004).

2.6. Case of need and proposed uses

Permitted use levels have been defined in the EU legislation (Directive 94/36/EC).

Tartrazine is a synthetic food colour authorised in the EU with maximum permitted use levels of 50 to 500 mg/kg food for various foodstuffs. Tartrazine (E 102) is also allowed in alcoholic beverages at levels up to 200 mg/L and non-alcoholic beverages at levels up to 100 mg/L. Table 2 presents those beverages and foodstuffs that may contain Tartrazine up to specified Maximum Permitted Levels (MPLs) of use as set by EC legislation (EC, 1994).

Table 2. Maximum Permitted Levels of use of Tartrazine in beverages and foodstuffs according to the European Parliament and Council Directive 94/36/EC

Beverages	**Maximum Permitted Level** (mg/L)
Non-alcoholic flavoured drinks Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum Permitted Level** (mg/kg)
Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustaceans paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Processed mushy and garden peas (canned)	100

Edible ices Desserts including flavoured milk products	150
Fine bakery wares Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Confectionery Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	*Quantum satis*

2.7. Information on existing authorisations and evaluations

Tartrazine has been evaluated previously by the SCF in 1975 and 1984 and by JECFA in 1966. Both committees established an Acceptable Daily Intake (ADI) of 0-7.5 mg/kg bw/day. Tartrazine was also evaluated by TemaNord in 2002.

2.8. Dietary exposure

2.8.1. Actual levels of use of Tartrazine

More information on current use levels was made available to the Panel for several food categories in finished products.

2.8.1.1. Beverages

For non-alcoholic flavoured drinks, the UK Food Standards Agency (FSA) conducted an *ad hoc* survey in which artificial colours were analytically determined in 201 retail ready-to-drink soft drinks selected for being distinctly coloured (FSA, 2003). Tartrazine was found to be present at a level higher than 0.1 mg/L (Limit of Detection - LOD) in 2 % of the products examined, with levels varying from 3 to 28 mg/L. In another survey, conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Tartrazine was found to be present at a level higher than 1.0 mg/L (Limit of Quantification - LOQ) in approximately 7 % of the 54 soft drinks tested; the concentration in these products ranged from < 1 mg/L to 25 mg/L (unpublished data provided by FSAI). French companies reported maximum use levels of Tartrazine to be below 10 mg/L in soft drinks (unpublished data provided by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA)). A usage survey, conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggests that the highest current use level of Tartrazine in non-alcoholic flavoured drinks is 20 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009 gives a range of use levels from 1 to 20 mg/L (UNESDA, 2009). The Confederation of the Food and Drink Industries of the EU (CIAA) also reported other current use levels of Tartrazine ranging from 1 to 20 mg/L (CIAA, 2009).

The Federation of European Food Additives, Food Enzymes and Food Culture Industries (ELC) has provided from its UK member association, Food Additives and Ingredients Association (FAIA), further data which give a range of typical, low - maximum use levels for Tartrazine from 0.25 to 10 mg/L (ELC, 2009).

For spirituous beverages, including products with less than 15 % alcohol, the survey conducted by the FSAI (2009), gives a range of analytical data from <1 to 3 mg/L for 4 detected samples out of 14 retail samples. The European Spirits Organisation (CEPS) reported a range of use levels of Tartrazine from 0 to 100 mg/L (CEPS, 2009).

For fruit wines (still or sparkling), cider and perry, the CIAA (2009) reported a range of typical maximum use levels below 1 mg/L.

2.8.1.2. Foodstuffs

For confectionery products, the Panel was provided with data from an *ad hoc* survey conducted by the FSA, in which artificial colours were analytically determined in 195 retail samples of brightly coloured packaged sweets selected for being distinctly coloured (FSA, 2002). Tartrazine was found to be present at a level higher than 0.5 mg/kg (LOD) in 3.6 % of the products, with levels varying from 3 to 62 mg/kg. According to the FSAI data, Tartrazine was present at a level higher than 1.0 mg/kg (LOD/LOQ) in 14 % of 183 confectionery products, with levels varying from 1 to 145 mg/kg (unpublished data provided by the FSAI). Data provided by French industries on Tartrazine in sweets showed use levels varying from 0 to 28 mg/kg (unpublished data provided by AFSSA). Data provided by the ELC (2009), give a range of typical low and maximum use levels of Tartrazine from 10 to 220 mg/kg. A range of typical low and maximum use levels from 0 to 180 mg/kg has been reported by the CIAA.

For candied fruit, vegetables, mostarda di frutta, preserves of red fruit, a range of typical low and maximum use levels from 10 to 50 mg/kg has been reported by the CIAA (2009).

For decorations and coatings, the FSAI survey (2009) gave a maximum value below 10 mg/kg for 4 retails samples; the CIAA (2009) reported a range of typical low and maximum use levels of Tartrazine from 10 to 180 mg/kg.

For fine bakery wares, the CIAA (2009) reported a range of typical low and maximum use levels of Tartrazine from 2 to 15 mg/kg, whereas the ELC provided further data from FAIA, which gave a range of typical low and maximum use levels from 14 to 30 mg/kg.

For edible ices, the ELC (2009) gave a range of typical low and maximum use levels of Tartrazine from 0.8 to 16 mg/kg and the CIAA reported a range of typical low and maximum use levels from 0 to 15 mg/kg.

For flavoured processed cheese and edible cheese rind and edible casing, the CIAA reported a typical maximum value of 27 mg/kg.

For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from <1 to 202 mg/kg for 6 detected samples out of 35 retail samples, and the CIAA reported a maximum use level of Tartrazine of 10 mg/kg.

For sauces, seasonings, pickles, relishes, chutney and mustard, the FSAI survey (2009) gave a range of analytical values from < 2 to 20 mg/kg for one detected sample out of 5 retail samples, and the CIAA reported a range of typical low and maximum use levels of 34 to 425 mg/kg.

For extruded or expanded savoury snack products and savoury snack products and savoury coated nuts, the CIAA reported a range of typical low and maximum use levels from 10 to 50 mg/kg.

For foods for Particular Nutritional Purposes (PARNUTS) and food supplements, the CIAA's members provided a range of typical low and maximum use levels from 0 to 50 mg/kg.

For jams, jellies and marmalades, the FSAI survey gave no detected samples (LOD/LOQ from <2 to <5 mg/kg) for 5 retail samples.

For processed mushy and garden peas, the FSAI survey gave a range of analytical values from <2 to 70 mg/kg for 10 detected samples out of 11 retail samples, and the CIAA reported a low and maximum use level from 10 to 50 mg/kg.

In order to refine the exposure assessment for children and adults to food colours, the Panel has defined some rules to identify maximum reported use levels based either on maximum actual usage, maximum analytical data or *quantum satis* rules for Tartrazine. The rules followed in order to deal with *quantum satis* authorisation, with usage data or observed analytical data, for all regulated colours re-evaluated by the Panel, are given in Annex A. Table 3 summarises the maximum reported use levels of Tartrazine in beverages and foodstuffs used for the refined exposure assessment; they have been defined by applying the rules reported in Annex A to the data available to EFSA.

Table 3. Maximum reported use levels of Tartrazine in beverages and foodstuffs used for the refined exposure assessment

Beverages	**Maximum Reported Use Level** (mg/L)
Fruit wines, cider and perry	1
Non-alcoholic flavoured drinks	20
Liquid food supplements/dietary integrators	50
Americano Bitter soda, bitter vino Spirituous beverages	100
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails	200
Foodstuffs	**Maximum Reported Use Level** (mg/kg)
Desserts including flavoured milk products	10
Edible ices	20
Flavoured processed cheese Edible cheese rind and edible casings* Fine bakery wares	30

Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Complete formulae for weight control intended to replace total daily food intake or an individual meal Solid food supplements/dietary integrators Complete formulae and nutritional supplements for use under medical supervision Soups Extruded or expanded savoury snack products Savoury snack products and savoury coated nuts Processed mushy and garden peas (canned)	50
Fish paste and crustaceans paste Smoked fish Meat and fish analogues based on vegetable proteins	100
Decorations and coatings	180
Confectionery	220
Mustard Fish roe	300
Pre-cooked crustaceans	250
Sauces, seasonings, pickles, relishes, chutney and piccalilli	425
Salmon substitutes Surimi	500

* For the Tier 2 approach, the Panel defined some rules in Annex A for identifying the maximum practical use levels to deal with *quantum satis* authorisation. A value of 100 mg/kg was proposed for edible cheese rinds and 25 mg/kg for edible casings.

2.8.2. Exposure assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each successive Tier, this involved a further refinement of intake estimates. The approach goes from the conservative estimates that form the first Tier (Tier 1) of screening, to progressively more realistic estimates that form the Second (Tier 2) and Third Tier (Tier 3).

2.8.2.1. Crude estimates (Budget method)

The dietary exposure to Tartrazine from the maximum permitted use levels was estimated using the Budget method (Tier 1), with the assumptions described in the report of the SCOOP Task 4.2 (EC, 1998).

In the case of Tartrazine, the maximum permitted use level considered for beverages was 200 mg/L. The maximum permitted level considered for solid foods was 500 mg/kg.

The default proportion (25 %) of beverages and solid food that could contain the additive was considered adequate. In effect, even though Tartrazine may be used in a variety of solid foods that could represent more than 25 % of processed foods, it is unlikely that a person would systematically choose all processed solid foods with the same colour added. In the case of beverages, uses are reported for a limited number of beverages; however, some of these may constitute a significant proportion of liquid intake (i.e., non-alcoholic flavoured drinks) with consumer loyalty to a single brand (and therefore to a specific colour)

often being high for this category of product. The 25 % proportion was therefore considered adequate also for beverages (EC, 1998). This assumes that a typical adult, weighing 60 kg, consumes daily 1.5 litres of beverages and 375 g of solid foods, containing Tartrazine. The theoretical maximum daily exposure for adults would therefore be:

(200 x 0.1 x 0.25) + (500 x 0.025 x 0.25) = 5 + 3.12 = 8.1 mg/kg bw/day.

For children, the level of Tartrazine considered in beverages was 100 mg/L (after exclusion of alcoholic drinks), and in solid food was 500 mg/kg. The proportion of 25% used, for beverages, was changed to 100% for children, in order to compensate the fact that the corresponding consumption rate of 375 mL/day could easily be exceeded by young children. This conclusion was derived from UK data on consumption of soft drinks by children aged less than 5 years, where the 97.5th percentile of consumption was between 70 and 80 mL/kg bw/day and a proportion factor of 100 % for beverages was recommended for children in the SCOOP Task 4.2 (EC, 1998). This assumes that a typical 3-year old child, weighing 15 kg, consumes daily 1.5 litres of beverages and 94 g of solid foods, containing Tartrazine.

The overall theoretical maximum daily exposure to Tartrazine in children would therefore be:

(100 x 0.1 x 1) + (500 x 0.025 x 0.25) = 10 + 3.12 = 13.1 mg/kg bw/day.

It was noted that Tartrazine may be used *quantum satis* in edible cheese rind and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, it was excluded from the Budget calculation, since it is not expected to influence the outcome of this exposure calculation to any relevant extent.

2.8.2.2. Refined estimates

Refined exposure estimates have been performed for Tier 2 using maximum permitted use levels presented in Table 2 and maximum practical use levels presented in Table 3 to deal with the specific cases of *quantum satis* authorisation for edible cheese rinds and edible casings, and for Tier 3 using the maximum reported use levels presented in Table 3, for children and adult populations.

Exposure estimates for children (aged 1-10 years) have been performed by the EXPOCHI consortium, based on detailed individual food consumption data from eight European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany) for Tier 2 and Tier 3. As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC on food colours from Table 2 (Tier 2 approach), and with the maximum reported use levels from Table 3 (Tier 3 approach).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and as estimates were provided on more refined adult food consumption data, in comparison to those available to the Panel (e.g. EFSA Concise European Food Consumption Database, which gives access to aggregate food categories consumed in 15 European countries), the Panel decided to select the UK population as representative of the EU consumers for the Tartrazine intake estimates for adults.

Estimates of Tartrazine exposure from the UK adult population (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 2000-2001) available from the UNESDA report (Tennant, 2006) and with the MPLs as specified in the Directive 94/36/EC (EC,

1994) for Tier 2 approach (Table 2), and with the maximum reported use levels for Tier 3 approach (Table 3).

Table 4 summarises the anticipated exposure of children and adults to Tartrazine.

In the case of Tartrazine, when considering MPLs of use (Tier 2), the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium, ranged from 0.8 mg/kg bw/day to 3.4 mg/kg bw/day and from 0.8 mg/kg bw/day to 9.4 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries) were soft drinks (13 to 41 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (14 to 47 %) and desserts including flavoured milk products (12 to 63 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 10 to 50 % of exposure in three countries. Confectionery accounted for 11 % of exposure in one country.

For UK children aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 3.1 mg/kg bw/day and 7.3 mg/kg bw/day for high level (97.5thpercentile) consumers of beverages. The main contributors to the total anticipated exposure (>10 %) for UK pre-school children were soft drinks (55 %), confectionery (13 %) and desserts, including flavoured milk products (12 %).

Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (50 % at average level and 80% for high level consumers).

Further data suggest that current use levels of Tartrazine in some food categories are lower than the MPLs. Therefore, it was decided that concentration data made available to the Panel by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA surveys, would be used to refine the estimate of dietary exposure to Tartrazine (Tier 3).

When considering the maximum reported use levels from Table 3, the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium, ranged from 0.2 mg/kg bw/day to 1.9 mg/kg bw/day and from 0.4 mg/kg bw/day to 7.3 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries) were soft drinks (11 to 38 %), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (10 to 75 %), confectionery and fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12 to 32 %). Desserts, including flavoured milk products accounted for 14 to 26 % of exposure in 4 countries and surimi accounted for 11 to 17 % of exposure in 3 countries.

For UK children, aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 0.85 mg/kg bw/day and 1.7 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) for UK pre-school children were soft drinks (40 %) and confectionery (35 %).

Estimates reported for the UK adult population give a mean dietary exposure to Tartrazine of 0.3 mg/kg bw/day and of 0.5 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (30 %), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (22 %) and confectionery (16 %).

Table 4 Summary of anticipated exposure to Tartrazine using the tiered approach (EC, 2001) in children and adult population

	Adult UK population (>18 years old)	**Pre-school UK children** (1.5 to 4.5 years old, 15 kg body weight)	**Children EXPOCHI population** (1-10 years old, 25-30 kg body weight
	mg/kg bw/day		
Tier 1. Budget method	8.1	13.1	
Tier 2. Maximum Permitted Level			
• Mean exposure	0.9	3.1	0.8-3.4
• Exposure 95th* or 97.5th percentile**	2.1	7.3	0.8-9.4
Tier 3. Maximum reported use levels			
• Mean exposure	0.3	0.85	0.2-1.9
• Exposure 95th* or 97.5th percentile**	0.5	1.7	0.4-7.3

* For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.
** For UK, estimates are based on the UNESDA report which gives the 97.5th percentile intake from beverages plus *per capita* average from the rest of diet (Tennant, 2006).

3. Biological and toxicological data

Tartrazine (E 102) was previously evaluated by JECFA (1966), the SCF (1975 and 1984) and TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions with a more detailed description of any relevant new data identified in the scientific literature.

3.1. Absorption, distribution, metabolism and excretion

The JECFA evaluation (1966) describes several studies which have focussed on the toxicokinetic aspects of Tartrazine. No further new literature has been published except for some studies describing azoreduction by intestinal bacteria.

Ryan and Wright (1962) demonstrated low biliary excretion of Tartrazine (1 %) following intravenous administration of an unspecified dose. By synthesising the ethyl ester (77 % of the dose excreted in bile) and dichlorotartrazine (25 % of the dose excreted in bile) plus studies on other azo dyes, they demonstrated that this low biliary excretion was due to the carboxyl group. After a dose of 2 mg (not specified but presumably per animal), unchanged Tartrazine could be detected in bile, but there was no evidence of ring fission products. Following intraperitoneal injection, an unidentified and unquantified Tartrazine conjugate was rapidly excreted in bile, but again none of the previously reported reductive ring fission products.

Jones and co-workers (1964) studied the metabolism of carbon-14 Tartrazine randomly labeled in the phenyl azo group in rat, rabbit and human. In both animal species Tartrazine was administered orally and intraperitoneally whilst humans received oral Tartrazine. Urine was collected for 48 hours, except in the oral rabbit study where it was collected for 72 hours; no other samples were taken. After intraperitoneal administration to 6 rats of 2.4 mg/kg bw of Tartrazine, between 64 and 96 % of the dose was recovered unchanged in urine within 24 hours; no other products were reported. In rabbit, at a dose of 2.4 mg/kg bw of Tartrazine administered intraperitoneally, 94 % of the dose was recovered unchanged in urine within 24 hours, with a further 1.4 % recovered as conjugated sulphanilic acid. However, after an intraperitoneal

dose of 1000 mg in the rabbit (dose not specified on a body weight basis, and body weight unreported but based on the other rabbits, around 300-350 mg/kg bw) only 57.3% was recovered unchanged in urine within 24 hours, with a further 25.7 and 6 % recovered as free and conjugated sulphanilic acid, respectively. After oral administration to 3 rats at 5 mg/rat (approximately 20 – 27.5 mg/kg bw, body weight not specified, so based on the range of 180- 250 g, normally used in metabolism studies), no free Tartrazine was measured but means of 28 and 34.6 % were recovered in urine as free and conjugated sulphanilic acid, respectively. In the rabbit dosed 1000 mg (dose not specified on a body weight basis, and body weight unreported but based on the other rabbits, around 300-350 mg/kg bw) 8.2 % was recovered unchanged in urine within 24 hours with a further 27 and 26.8 % as free and conjugated sulphanilic acid respectively within 72 hours. In 4 humans receiving a single capsule containing 89-100 mg of Tartrazine (body weights not specified but approximately 1.2-1.5 mg/kg bw), no free Tartrazine was measured in urine for any subject; in one subject 106 % was recovered as free sulphanilic acid whilst for the other 3 subjects mean recoveries of free and conjugated sulphanilic acid were 40.6 and 49.7 % respectively. The urinary Tartrazine results do not provide clear information whether the Tartrazine is free or conjugated since the methodology does not appear capable of distinguishing these forms.

Ryan and co-workers (1969a) examined the fate of the pyrazole fragment of Tartrazine using sulphur-35 labelled Tartrazine and 1-(4-sulphophenyl)-3-methyl-4-(4-sulphophenylazo)-5-pyrazolone (SPMP an analogue of Tartrazine) and carbon-14 labelled SPMP. Following oral administration, both Tartrazine and SPMP labelled with sulphur-35 were predominantly excreted in faeces (90 and 89 % of the dose respectively after 72 hours) with small amounts in urine (8 and 7.2 % of the dose, respectively, after 72 hours). The urinary radioactivity excreted in 48 hours with sulphanilic acid and 4-sulphophenylhydrazine was 23 and 23 % after Tartrazine administration, and 54 and 22 % after SPMP administration; the remaining radioactivity was not characterised.

The 4-sulphophenylhydrazine metabolite was also labelled with sulphur-35 and administered orally and intraperitoneally. Excretion of this metabolite differed with the route of administration (35 and 49 % in urine and faeces, respectively, 48 hours following oral, and 90 and 5 % in urine and faeces, respectively, 48 hours following intraperitoneal administration). Following oral administration, 69 % of urinary radioactivity excreted in 48 hours was sulphanilic acid and 21 % was 4-sulphophenylhydrazine, whereas following intraperitoneal administration, 9 % of urinary radioactivity excreted in 48 hours was sulphanilic acid and 73% was 4-sulphophenylhydrazine. These data suggest there is a marked conversion of 4-sulphophenylhydrazine to sulphanilic acid presumably in the gut lumen.

Honohan and colleagues (1977) studied the excretion and metabolism of Tartrazine and Sunset Yellow together with polymeric derivatives of these dyes in female Simonson Sprague Dawley rats. Following oral administration of 10 mg per animal (approximately 50-55 mg/kg bw) to bile duct and urethra cannulated or intact rats, samples were collected for 72 or 96 hours respectively (the Panel noted that the duration of cannulation was unlikely to be permitted today). In the cannulated animals no Tartrazine was detected in urine and trace amounts were detected in bile, whereas 21.4 ± 6.9 % of the dose was recovered as sulphanilic acid in urine. In intact animals urinary excretion of radioactivity was 4 ± 0.52 % with a further 0.03 ± 0.01 % in tissues, faecal elimination was 87.3 ± 3.43 % with a further 0.05 ± 0.04 % in gastrointestinal contents. When the aminopyrazalone fragment/metabolite of Tartrazine was dosed at the same dose, urinary excretion of radioactivity was 8.9 ± 2.46 % with a further 0.08 ± 0.01 % in tissues, faecal elimination was 86.3 ± 4.86 % with a further 0.09 ± 0.04 % in gastrointestinal contents. Although traces of intact Tartrazine were detectable by radioactivity they were too low to quantify; in urine 1.6 ± 0.0 % of the dose was excreted as aminopyrazalone and 19.8 ± 1.77 % of the dose as sulphanilic acid (the sulphanilic acid was determined colourimetrically as the radiolabel was not located in this moiety).

Roxon and co-workers (1966, 1967) demonstrated conversion of Tartrazine following anaerobic incubation with *Proteus vulgaris*. Ryan and co-workers (1969b) demonstrated conversion of SPMP and 4-

sulphophenylhydrazine following 48 hours anaerobic incubation with *Proteus vulgaris* or gut contents. Whilst gut contents were more efficient at converting 4-sulphophenylhydrazine to sulphanilic acid, there appeared to be greater metabolism of SPMP by *Proteus vulgaris*. There was evidence of conversion of 4-sulphophenylhydrazine to sulphanilic acid in control anaerobic incubations at a level similar to that with *Proteus vulgaris*.

Whilst the majority of the absorption, distribution, metabolism and excretion studies on Tartrazine are 40-50 years old the techniques and methods used for the identification of the parent compound and its metabolites were those used to elucidate and identify the metabolic pathways of most xenobiotics. Although modern analytical methods might identify some additional minor metabolites or elucidate intermediate metabolites, this would not alter the overall assessment of Tartrazine metabolism. Following oral administration at a range of doses, absorption of intact Tartrazine is negligible to low (< 5 %) and this is predominantly excreted unchanged in urine. After oral administration there is extensive metabolism of Tartrazine by the gastrointestinal microflora to sulphanilic acid and aminopyrazalone (which may then be subsequently cleaved to sulphanilic acid and α-amino-β-ketobutyric acid fragments with the latter breaking down further via intermediary metabolism with release of carbon dioxide). Both sulphanilic acid and aminopyrazalone can be absorbed to a greater extent than Tartrazine with urinary excretion of up to 20-25 % of the dose as sulphanilic acid in rats at around 50-55 mg/kg bw and 55 % at 20-27.5 mg/kg bw and 40-49 % of the dose as sulphanilic acid in humans at around 1-1.5 mg/kg bw. These data suggest that the rat is a suitable metabolic model for man and given the extensive metabolism in rats there would be extensive exposure to the microfloral metabolites in the toxicity studies and no need for toxicity data on the metabolites.

3.2. Toxicological data

3.2.1. Acute oral toxicity

Acute oral toxicity has been assessed in mice and rats. In mice, the LD_{50} value was determined to be 12750 mg/kg bw (NIHS, 1964), and in rats the LD_{50} was defined as > 2000 mg/kg bw (Sasaki *et al*., 2002).

3.2.2. Short-term and subchronic toxicity

The JECFA (1966) evaluation describes several short-term and subchronic studies in rats, cats and dogs.

Three groups of dogs (2/sex) were given 0, 1 or 2 % Tartrazine (corresponding to 0, 250 and 500 mg/kg bw/day) in their diets for two years. No Tartrazine-related effects were reported (Davis *et al*., 1964).

In the TemaNord evaluation (2002) one additional study is described.

Male rats received in the diet two mixtures containing Tartrazine, Brilliant Blue FCF, Sunset Yellow and Carmoisine (mixtures A and B) for 30- and 60-day periods at a dose level of 800 mg mixture/kg bw (Aboel-Zahab *et al*., 1997). A third mixture, C, did not contain Tartrazine. The compositions of mixtures A and B were not specified as they were stated to be company secrets and so the concentration of each colour is not reported. The effects on body weight, blood picture, liver and kidney functions, blood glucose, serum and liver lipids, liver nucleic acids (DNA and RNA), thyroid hormones (T3 and T4) and growth hormone, and histopathological examinations of liver, kidney and stomach sections were evaluated. These parameters were also investigated 30 days after the end of exposure.

Rats fed diets supplemented with both mixture A and B showed significant increases in serum total lipids, cholesterol, triglycerides, total protein, globulin and serum transaminases. Haematological investigations demonstrated selective neutropenia and lymphocytosis (no significant alterations of total white blood cell counts), and significantly decreased haemoglobin concentrations and red blood cell counts. Eosinophilia was noted only in rats receiving mixture A. Histopathological studies showed brown pigment deposition in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. Congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in rats receiving mixture B. No histopathological effects were recorded in the stomach tissue.

The Panel concludes that the results of this study (Aboel-Zahab *et al*. 1997) cannot be used as the basis for a re-assessment of the ADI for Tartrazine, as the exposure of the experimental animals has been to a mixture of food colours in which the dose level of each colour has not been specified and it is not clear what were the amounts/percentage of the individual colours added in the diet to achieve the cited level of 0.8 g of mixture/kg bw/day.

3.2.3. Genotoxicity

3.2.3.1. *In vitro* studies

The JECFA (1966) evaluation describes a single study in which Tartrazine was tested for mutagenic effects in cultures of *Escherichia coli* and no mutagenic effect was found (Lück and Rickerl, 1960).

No evidence of mutagenic activity was found in *in vitro* studies in *Salmonella typhimurium* (Izbirak *et al.,* 1990) and *Escherichia coli* (Henschler and Wild, 1985; Karpliuk *et al*., 1984; Pollastrini *et al*., 1990).

The mutagenicity of four azo dyes (Ponceau 4R, Amaranth, Sunset Yellow FCF and Tartrazine) that are widely used to colour food has been evaluated. These were tested for mutagenicity in *Salmonella typhimurium* TA98 and TA100 in both plate-incorporation and pre-incubation assays in the absence and presence of rat-liver S9. No mutagenic activity was seen for any of the azo dyes tested in these assays (Izbirak *et al*., 1990).

The Panel noted that Prival and Mitchell (1982) demonstrated that the metabolic conditions of the standard Ames test protocol were not appropriate for testing azo dyes for mutagenic activity in *Salmonella typhimurium* and developed a specific protocol including use of flavin mononucleotide (FMN) rather than riboflavin to reduce the azo compounds to free amines, and hamster liver S9 rather than rat liver S9 for metabolic activation. The Panel therefore noted that a final conclusion from negative Ames test results obtained under standard conditions cannot be drawn.

Ishidate *et al*. (1984) did find a small increase in the incidence of polyploid cells after a 48-hour Tartrazine treatment in Chinese hamster fibroblast cell line.

Pollastrini *et al*. (1990) studied the genotoxic character of 21 food dyes containing Tartrazine at concentrations of 8.5 to 35 % with salt and either wheat flour or corn starch as the other ingredients. *In vitro* studies performed with *S. typhimurium* (TA98, TA100, TA1535 and TA1538) and *E. coli* (Wp2, Wp2uvrA and Wp2uvrApkM101) with metabolic activation at concentrations up to 1000 or 5000 µg/plate Tartrazine. No mutagenic activity was observed.

Das and Mukherejee (2004) tested the mutagenic and genotoxic effects of Tartrazine in the Ames assay in *Salmonella typhimurium* strains without metabolic activation and in an *in vivo* mouse bone marrow assay. In the Ames test, Tartrazine at concentrations of 10, 100, 250, 500 and 1000 µg/plate failed to induce

mutations in TA97a and TA100. However, in TA 98 there was an increase in the number of revertant colonies at the lower doses, but not at the two highest doses.

3.2.3.2. In vivo studies

Giri *et al*. (1990) showed a significant increase in Sister Chromatid Exchange (SCE) and chromosomal aberrations in mouse and rat bone marrow cells, following acute and chronic exposure to high doses of Tartrazine via the diet.

Durnev *et al.* (1995) studied *in vivo* mutagenic activity of six food colours including Tartrazine. The colour was given *per os* to C57BL/6 mice at an age of 8-12 weeks and body weight of 18-20 g (5 animals per group) for a period of 5 days in a daily doses of 0.5 and 5 mg/kg bw. Bone marrow smears were taken and stained according to the Dean, 1969 method. From every treated and control animal, 100 cells in metaphasis on bone marrow smears were examined. The cytogenetic analysis of altogether 500 metaphases from the control group cells with anomalies were seen in 1.4 ± 0.5 % of cells. After the 5-day treatment with Tartrazine, the percentage of the metaphases with anomalies did not differ statistically from those in controls. The authors concluded that Tartrazine did not increase the spontaneous incidence of chromosome aberrations in mice bone marrow cells.

The Panel notes that types of chromosome aberrations were not described in detail nor using the internationally used terminology.

Farag *et al*. (2001) treated pregnant mice orally with 68 mg Tartrazine/kg bw/day from gestational days 0-7, and evaluated cytogenetic changes in mothers and embryos. In dams the frequency of chromosome aberrations was increased and mitotic indices were depressed.

Sasaki *et al*. (2002) studied the genotoxicity of 39 chemicals currently in use as food additives. They treated groups of four male ddY mice once orally with each additive at up to half its LD_{50} or the limit dose (2000 mg/kg bw) and performed Comet assays on glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow, 3 and 24 hours after treatment. Tartrazine induced dose-related DNA damage in the glandular stomach, colon, and/or urinary bladder. All 7 food dyes tested induced DNA damage in the gastrointestinal organs at low doses (10 or 100 mg/kg). Among them, Amaranth, Allura Red, New Coccine, and Tartrazine induced DNA damage in the colon. Tartrazine also induced DNA damage in the stomach at doses of 10 and 2000 mg/kg bw without a dose-effect relationship.

In the *in vivo* genotoxicity test, Swiss albino mice per (4 animals/dose) were administered Tartrazine (50, 100 and 200 mg/kg bw) intraperitoneally. The frequency of aberrant cells and the number of breaks per cell was not significantly higher than controls in the groups treated with Tartrazine. The authors concluded that Tartrazine was not genotoxic (Das and Mukherejee, 2004).

Recently Poul *et al.* (2009) demonstrated a lack of genotoxicity of Tartrazine in the gut micronucleus assay in mice after administration by oral gavage of 20, 200 or 1000 mg/kg bw twice at 24-hour intervals, and examination 24 hours later. The authors assessed the genotoxic effects by recording the frequency of micronucleated cells and cell toxicity by identification of the apoptotic and mitotic cells (Poul *et al.*, 2009). The concentrations of parent compound and its main metabolites (sulphanilic acid) were measured in faeces during a 24-hour period after single oral administrations of the food dye. Parent dye compounds and their main aromatic amine metabolites were detected in significant amounts in the environment of colonic cells. Acute oral exposure to Tartrazine did not induce genotoxic effect in the micronucleus gut assay at doses up to 2000 mg/kg bw. Food dye administration increased the mitotic cells at all dose levels

when compared to controls. According to the authors, these results suggest that the transient DNA damages previously observed in the colon of mice treated with Tartrazine in the *in vivo* Comet assay (Sasaki *et al.,* 2002), are unable to be fixed in stable genotoxic lesions, and might be partly explained by local cytotoxicity of the dyes.

The TemaNord (2002) evaluation mentions nine additional studies but gives no details. These studies include among others an *in vitro*/*in vivo* rat hepatocyte assay, two *Salmonella typhimurium* assays, and a somatic assay in *Drosophila melanogaster* and conclude that Tartrazine is not genotoxic (Brown and Dietrich, 1983; Chung, 1983; Combes, 1986; Kornbrust and Barfknecht, 1985; Münzner and Wever, 1987; Prival *et al*., 1988; Rosenkranz and Klopman, 1989; Roychoudhury and Giri, 1989; and Tripathy *et al*., 1989).

3.2.3.3. Tartrazine metabolites genotoxicity testing

After dosing rats by gavage, unidentified urinary metabolites of Tartrazine had dose-dependent mutagenic activities in the Ames test with *Salmonella typhimurium* TA98 but not TA100, in the presence of rat liver S9 (Henschler and Wild, 1985). After oral administration of Tartrazine in rats, bile and faeces of treated rats were investigated for mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation. In the presence of S9-mix, faecal extracts developed a weak but reproducible dose-related response in strain TA100. In bile, no metabolites exerting mutagenic activity were found (Münzner and Wever, 1987). The mutagenic component in faecal extracts was not identified and it is not possible to draw conclusions on Tartrazine genotoxicity from this study. The Panel considers that these studies are not relevant to the assessment of the mutagenic potential of Tartrazine and its metabolites.

Azo reduction of Tartrazine may produce sulphonated aromatic amines. Jung *et al*. (1992) have reviewed the genotoxicity data of a range of sulphonated aromatic amines. To study the effect of sulphonation on the genotoxic potential of phenyl- and naphtylamines, the genotoxicity of sulphonated aromatic amines was compared with their unsulphonated analogues. It was found that, in general, sulphonated phenyl- and naphtylamines, including the azo reduction products of Tartrazine, are non-mutagenic to *Salmonella typhimurium* in the Ames test. For some other sulphonated aromatic amines the absence of genotoxicity was also demonstrated with a variety of other test systems *in vitro* and *in vivo*. Based on the available data, the authors concluded that sulphonated aromatic amines, in contrast with their unsulphonated analogues, have no or very low genotoxic potential. Hence, the authors concluded that exposure to sulphonated aromatic amines derived from metabolic cleavage or present as contaminants in colourings, is unlikely to induce any significant genotoxic risk.

The Panel concludes that despite some studies reporting possible mutagenic effects, based on the available data no genotoxic effects of Tartrazine have been convincingly demonstrated.

3.2.4. Chronic toxicity and carcinogenicity

The JECFA (1966) evaluation describes seven long-term toxicity studies, one conducted in mice and six conducted in rats. The Panel noted that these studies were all performed before OECD guidelines and Good Laboratory Practice (GLP) were established.

A total of 117 male and female mice received a daily diet containing 1 mg Tartrazine per animal (equivalent to approximately 2.5 mg/kg bw/day) (duration of exposure not stated). After 500 and 700 days

tumour incidence was not significantly increased compared to controls (Waterman and Lignac, 1958). In rats fed 4 % Tartrazine in their diets (equivalent to 2000 mg/kg bw/day) for approximately 18 months, discolouration of the stomach, small intestine and colon was observed; tumours were not observed (Willheim and Ivy, 1953).

Ten rats were exposed to diets containing 0.2 % Tartrazine (equivalent to 100 mg/kg bw/day) for 417 days. After observation periods of up to 922 days no tumours were observed (DFG, 1957).

Four groups of rats (15 of each sex) were given diets with 0, 0.03, 0.3, or 1.5 % Tartrazine (equivalent to 0, 15, 150, or 750 mg/kg bw/day) for 64 weeks. The dye had no effects on mortality, food intake, growth, organ weights, histopathology, blood picture or tumour incidence (Mannell *et al.*, 1958). This study was used by JECFA to establish the ADI based on the No-Observed-Adverse-Effect Level (NOAEL) of 750 mg/kg bw/day (being the highest dose level tested) and a safety factor of 100.

Five groups of 24 Osborne-Mendel weanling rats, evenly divided by sex, were fed 0.0, 0.5, 1.0, 2.0, and 5.0 % Tartrazine (equivalent to 0, 250, 500, 1000 and 2000 mg/kg bw/day) mixed in their chow diet for two years. Growth effect was negligible, and there was no effect on survival, haematology, or organ weights. The rats showed diarrhoea at the 5 % dosage level, slight diarrhea at the 2 % level, and no diarrhoea at the 1 % level. The incidence of tumors was unaffected. No organ pathology attributable to treatment was noted except at the highest dosage level, where the feeding of Tartrazine may have influenced the deposition of small amounts of gritty material in the renal pelvis of some of the surviving male rats (Davis *et al.,* 1964)

Three groups of two male and two female beagle dogs each were fed diets containing 0, 1 and 2 % (equivalent to approximately 0, 1.125-2.225 mg/kg bw/day and 2.25-4.45 mg/kg bw/day) Tartrazine for 2 years. No clinical symptoms of toxicity or hematologic abnormalities were noted during the study. No gross lesions were noted at autopsy. Histopathology demonstrated only incidental lesions which, with the possible exception of pyloric gastritis in one high-dose level dog, were not attributable to Tartrazine toxicity (Davis *et al*., 1964).

The TemaNord (2002) evaluation describes three additional chronic toxicity/carcinogenicity studies.

Groups of ICR derived mice (60 per sex/group) at an age of 42 days at the start of the study were fed diets containing 0, 0.5, 1.5, or 5 % Tartrazine (equivalent to approximately 0, 714 and 870, 2173 and 2662 and 8103 and 9735 mg/kg bw/day, in males and females respectively) for 104 weeks (Borzelleca and Hallagan, 1988b). Deaths, morbidity and gross signs of toxicity were recorded twice daily and individual body weight and food consumption were determined weekly for the first 14 weeks, bi-weekly for weeks 16-26 and monthly thereafter. Ten animals of each sex and group were randomly selected for haematology tests at 3, 6, 12, 18, and 24 months. The haematological parameters evaluated were haemoglobin, haematocrit, erythrocytes count and morphology, total and differential leucocytes count.

Necropsies were conducted on all animals (including those dying spontaneously or killed in a moribund condition). A complete histological examination was conducted on all animals from the two control groups and from the high-dose group and on any low-dose and middle-dose group animals with gross lesions or masses. The survival of the animals in control and treated groups was similar; mean body weights of male and female mice in the 5 % group and male mice in the 1.5 % group were slightly but statistically significantly lower at several sampling intervals and at study termination in the 5 % group only. The authors did not consider this decrease as toxicologically significant and suggested that this was due to decreased caloric intake due to the Tartrazine component of the diet. Yellow-brown coloured urine was noted in all treatment groups within one week after the start of the study. The faeces of mice fed

Tartrazine at dietary levels of 1.5 and 5 % were purple and yellow-brown, respectively. Yellow hair and skin were noted in all treatment groups (Borzelleca and Hallagan, 1988b).

The authors stated that there were no consistent or dose-related effects on behaviour, morbidity, mortality, haematology (figures not presented for the different haematological parameters), or the general physical observations.

A variety of neoplasms was noted in all groups. Neither the incidence of neoplasias nor their primary location and histological characteristics differed significantly between the control and treated animals. The authors stated that all of the neoplasias observed were of types commonly found in ageing mice and that there were no statistically significant differences in the time of tumour appearance between treated and control animals.

The authors derived a NOAEL of 5 % in the diet equivalent (the highest dose tested) to 8103 and 9735 mg Tartrazine/kg bw/day for males and females, respectively. The Panel agrees with this conclusion.

Two separate chronic toxicity/carcinogenicity studies on rats were conducted in the same laboratory and in the same time period by Borzelleca and Hallagan (1988a). The studies were initiated with an *in utero* phase in which Tartrazine was administered to the F0 generation (63-70 day-old rats) for two months prior to mating. In the "original" study 60 animals/sex/group received Tartrazine in the diet at levels 0.1, 1 or 2 % (48 and 58, 491 and 589, 984 and 1225 mg/kg bw for males and females, respectively). Two concurrent control groups (60 animals/sex/group) of the same size received no Tartrazine in the diet. In the "high dose" study 60 animals/sex/group were used; one group was fed a diet containing 5 % Tartrazine (2641 and 3348 mg/kg bw) and a control group fed a diet without Tartrazine. Female rats were weighed on gestation days 0, 4, 14, 21. A maximum of two rats/sex/litter were randomly selected for the chronic study phase following the completion of the *in uterus* phase. After random selection of the F1 animals (70 animals/sex/group) the chronic phase was initiated and these offspring were exposed to the same dietary levels as their parents. Three control groups were used. F1 males and females received Tartrazine for 113 and 114 weeks respectively, in the original study and for 122 and 125 weeks in the "high" dose study. Deaths, morbidity, gross signs of toxicity, individual body weights and detailed physical examinations and palpation of masses and ophtalmoscopic examinations were recorded regularly. At 3, 6, 12, 18, 24 months and at termination of the experiment ten animals per sex and group were selected for blood haematological and biochemical examination and urine analysis. A complete histopathological study was conducted on all animals from the three control groups and the highest dose group from each study (2 and 5 %) and also on ten rats of each sex randomly selected from each group for an interim sacrifice at 12 months.

A yellow tint of the fur was noted in all treated animals and in the faeces of the 1.0, 2.0 and 5.0 % groups. The authors stated that there were no differences between control and treated groups in the incidence of palpable masses, opthtalmoscopic findings, and that few of the haematological, clinical chemistry and urine analysis parameters differ significantly but none of the differences appeared to be treatment-related. The results from these examinations were not presented. There were no Tartrazine-related effects on the number of rats surviving, as well as on gross changes, including organ weights. Decreases in group mean body weights at termination of the studies were detected for male (-8.3 %, non-significant) and female rats (-14.4 %, $p < 0.01$) on the 1% diet, and for male (-12.2, $p < 0.01$) and female (-16.9, $p < 0.01$) rats on the 5 % diet.

A variety of lesions, including neoplasms detected by histological examinations, were present in similar incidence in control and treated groups. The lesions appeared to be spontaneous and were of the types commonly found in ageing rats. No differences in overall incidence of both benign and malignant neoplasias were found between groups (Borzelleca and Hallagan, 1988a).

The authors stated that the NOAEL was 5.0 % in the diet (the highest dose tested), equivalent to 2641 and 3348 mg/kg bw/day for male and female rats, respectively. The Panel considers that the body weight changes observed were not adverse and therefore agrees with this NOAEL.

In a study by Maekawa *et al.* (1987) groups of 50 male and 50 female F344 rats were given *ad libitum* drinking water with 0, 1 or 2 % of Tartrazine (equivalent to approximately 0, 500 and 1000 mg/kg bw/day) for up to two years.. No toxic lesions caused by Tartrazine were detected in any treated group of either sex. Except for mesotheliomas in males and endometrial stromal polyps in females, there were no significant increases in the incidences of any tumours compared to those in the corresponding control group. The incidence of these tumours was statistically significantly greater in the group given 1 % Tartrazine only. However, no positive trend was noted in the occurrence of these two tumours using an age-adjusted statistical analysis. Mesotheliomas and endometrial stromal polyps are frequently observed spontaneous tumours in this rat strain, and their incidences in authors historical controls were 4.1 and 21.9 %, respectively. However, in the concurrent controls no mesotheliomas occurred in any of the male control rats and the incidence of endometrial stromal polyps was only 10.6 % in the female control group. In addition there were no significant differences between the control and treated groups in hyperplastic or pre-neoplastic changes in the mesothelium or endometrium. The authors concluded that the significant increases in the incidences of mesothelioma and endometrial stromal polyp in the groups given 1 % Tartrazine, but not at the higher exposure level (2 %) were not attributable to Tartrazine administration and that Tartrazine was not carcinogenic in F344 rats. The Panel concurs with this conclusion.

In the Moutinho *et al*. (2007) study, 22 male Wistar rats at weaning were assigned to a control group to receive a mineral water for ten months (46 weeks) and 23 to a treatment group. The treatment group received 7.5 mg/kg bw Tartrazine daily in mineral water offered *ad libitum*. In the 46th week the animals were killed and necropsied, removing the oesophagus-gastro-duodenal segment, subsequently opened through the greater curvature and processed according to a standardised procedure for a histological examination. For each of four regions: squamous gastric fundus, glandular fundus, body and atrum, the number of mitosis, the presence of atypia and atrophy, the number of eosinophils and lymphocytes, and the number of argentaffin granular cells were observed. One animal from each group died before the 40th week. The cause of death for the control animal was not identified; for the treated group animal, the cause was undifferentiated round cell malignancy, involving the intestinal wall and pancreas. The statistically significant differences between the treatment and control group were in the higher number and extent of lymphocytes infiltrates ($p<0.01$) and number of eosinophils ($p<0.05$) in the antral mucosa of treated animals. Mild atypia predominated in most of the animals from both groups, but in 3 control rats there was no atypia whatsoever whereas 3 treated rats showed foci of moderate to severe atypia ($p<0.05$). The conclusion of the authors was that no carcinogenic changes in any gastric area were observed during this study.

The available carcinogenicity studies have demonstrated that Tartrazine does not induce benign or malignant neoplasias.

3.2.5. Reproductive and developmental toxicity

The JECFA evaluation (1966) stated that “additional investigations are particularly needed into the effects on reproduction and foetus”. The SCF (1984) concluded on the basis of available studies that reproductive function was not affected and no teratogenic potential was noted in rats and rabbits. No details were given on which studies gave rise to this conclusion.

The TemaNord (2002) evaluation describes two reproductive and developmental toxicity studies on Tartrazine (Collins *et al*. 1990, 1992).

In the first study, Tartrazine was given to Osborne-Mendel rats (no detail on number) by gavage at dose levels of 0, 60, 100, 200, 400, 600, or 1000 mg/kg bw/day on days 0-19 of gestation. No dose-related effects were observed on the number and type of implantations, fetal viability or external fetal development. Fetal skeletal and visceral development was not different from controls (Collins *et al*., 1990).

In the second study, Tartrazine was given to Osborne-Mendel rats (no detail on number) in the drinking water at levels of 0.05, 0.1, 0.2, 0.4, or 0.7 % (based on typical fluid consumption equivalent to 0, 67, 132, 292, 568, and 1064 mg/kg bw/day, respectively) throughout gestation. No dose-related changes were seen in maternal clinical findings, number and type of implantations, fetal viability or fetal size (weight and length). No dose-related fetal terata or visceral and skeletal aberrations were noted (Collins *et al.,* 1992).

In addition, TemaNord (2002) indicates that reproductive parameters were also examined in studies on chronic toxicity/carcinogenicity (see below).

Two separate chronic toxicity/carcinogenicity studies on rats were conducted in the same laboratory and in the same time period by Borzelleca and Hallagan (1988a). The studies were initiated with an *in utero* phase in which the compound was administered to the F0 generation (63-70 day-old rats) two months before mating. In the "original" study 60 animals/sex/group received Tartrazine in the diet at levels of 0.1, 1 or 2 % (48/58, 491/589, 984/1225 mg/kg/bw for males and females, respectively) Two concurrent groups of the same size received no Tartrazine in the diet (control groups). In the "high dose" study the same number of rats per group were used; one group receiving a diet containing 5 % Tartrazine (2641-3348 mg/kg/bw), and the control group receiving a Tartrazine-free diet. Female rats were weighed on gestation days (GD) 0, 4, 14, 21. The only statistically significant effects considered to be Tartrazine-related were decreased (mean) body weights in the high-dose groups, and discolouration of fur and faeces. The authors state that "there were no compound–related effects on fertility, gestation, parturition, lactation, pups survival through weaning and lactation or number of live and still-born pups", but no actual data were provided in the text nor in the tables presented in the publication. A slight decrease in body weights and a slight increase in food consumption were noted in F0 rats treated at a dietary level of 5 %. The mean body weights of the pups in the 5 % group at lactation day 21 were slightly lower than those of controls, although the differences were not statistically significant. There were no compound-related effects on the survival of pups. The Panel derives a NOAEL of 5 % Tartrazine in the diet (equivalent to 2641-3348 mg/kg bw/day, for males and females, respectively).

Tanaka (2006) investigated several reproductive and neurobehavioral parameters in the mouse. Groups of 20 mice (10 per sex) were given 0, 0.05, 0.15, or 0.45 % Tartrazine in their diets (equivalent to approximately 83, 259, and 773 mg/kg bw/day, respectively) from five weeks of age in the F0 generation to nine weeks of age in the F1 generation.

It was found that F0 animals were not affected. In the F1 generation no effects were observed regarding litter size, litter weight, or sex ratio at birth. Average body weight and survival indices were slightly affected in the lactation period (significantly lower survival index in the middle-dose group and higher average body weight of male offspring in the highest dose group on postnatal day (PND) 0 and 21), but this could not be attributed to Tartrazine treatment.

Concerning neurobehavioral development, the following effects were observed in males: surface righting at PND 4 was significantly accelerated in the high-dose group, cliff avoidance was accelerated in the middle-dose group, and exploratory behaviour tended to be lower at all dose levels (not statistically significant). Females in the high-dose group displayed delayed negative geotaxis.

In another study by Tanaka *et al.* (2008), Tartrazine was given to mice in the diet at levels of 0 (control), 0.05, 0.15 or 0.45 % (equivalent to, approximately, 83, 259, and 773 mg/kg bw/day, respectively) from five weeks of age of the F0 generation to nine weeks of age of the F2 generation, and selected reproductive and neurobehavioral parameters were measured. The reproductive parameters including survival index were not affected for F1 and F2 generations. Significantly higher average body weight of progeny was recorded for the low-dose and middle–dose groups during the lactation period. In the F1 generation, the development of swimming direction at PND 7 was accelerated significantly in male offspring in a dose-related manner. Surface righting at PND 7 was delayed significantly in female offspring of the F1 generation at a dose level of 0.15 % in the diet but not at 0.5 %. Several variables in exploratory behaviour showed significant tendencies to be affected in the treatment groups in male offspring at three weeks of age.

In the F2 generation, the development of swimming direction at PND 7 was accelerated significantly in the high-dosed group in male offsprings. Time taken for olfactory orientation at PND 14 was accelerated significantly in male offspring in a dose-related manner. Several variables in exploratory behaviour showed significant tendencies to be affected in the treatment groups in male offspring at three weeks of age, and in males at eight weeks of age.

3.2.6. Hypersensitivity and intolerance

The JECFA (1966) evaluation only describes one study regarding intolerance and allergenicity. In this study Tartrazine revealed no sensitisation activity in guinea pigs (no further details given) (Bär and Griepentrog, 1960).

In The TemaNord (2002) evaluation a number of additional publications are mentioned, but the results of only three publications are very briefly described. In two studies "pseudo"allergic reactions (some as severe as an anaphylactic reaction) were reported not to be mediated by antibodies (no further details) (Safford and Goodwin, 1985; Schaubschlager *et al.,* 1988). In another study it was found that in a human lymphocyte model Tartrazine showed some immunosuppressive effects *in vitro* (Koutsogeorgopoulou *et al.,* 1998).

In tests on laboratory animals with Tartrazine and its metabolites by methods which had the potential to detect induction of antibody formation, no antibodies were detected except by methods which are not relevant to human exposure. Similarly, laboratory methods have shown that Tartrazine metabolites, and in some cases Tartrazine itself, can induce contact sensitisation in guinea pigs, although there is little evidence that Tartrazine can induce similar changes in man (Safford and Goodwin, 1985).

Koutsogeorgopoulou *et al.* (1998) describe a sensitive and reproducible microassay model using human peripheral blood lymphocytes (PBL) for discrimination between the cytotoxic and immunosuppressive effects of food colours such as Amaranth and Tartrazine. The cytotoxic effects of a wide range of concentrations of these substances were studied on human PBL by the colorimetric *in vitro* cytotoxicity assays, Neutral Red uptake (NRU) and thiazolyl blue tetrazolium bromide (methylthiazolyldiphenyl-tetrazolium bromide, MTT). The immunotoxic properties of these two substances were determined by a [^{3}H]-thymidine DNA incorporation assay on phytohaemagglutinin stimulated or non-stimulated lymphocytes, as well as by a Cr51 release Natural Killer assay. The results showed clear immunosuppressive effects of the two substances tested, at concentrations chosen, proved for this study, to be non-cytotoxic by NRU and MTT assays.

Kalender (2000) studied the effects of Tartrazine on dermal mast cell degranulation in the mouse 1, 6, 12 and 24 hours after a single intradermal injection. After 1 and 12 hours the dye caused partial

degranulation; at the end of the 6-hour period an internal degranulation was predominant. The effects were no longer visible after 24 hours.

3.3.6.1. Human studies

Reactions to food colourings, including those triggered by immune (hypersensitivity) and non-immune (intolerance) mechanisms, are assumed to be infrequent in the population, and prevalence of 0.14 to around 2 % have been reported (Young *et al.*, 1987; Hannuksela and Haahtela, 1987; Fuglsang *et al.*, 1993, 1994). Reports are often characterised by poorly controlled challenge procedures. Recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon (Supramaniam and Warner, 1986; Simon, 2003)

Novembre *et al.* (1992) reported two cases of unusual reactions to food additives (Tartrazine and benzoates) involving mainly the central nervous system (headache, migraine, overactivity, concentration and learning difficulties, depression) and joints (arthralgias), confirmed with diet and double-blind challenge.

The studies merely mentioned in the TemaNord (2002) evaluation consist of:

- Case reports (Baumgartner, 1989; Bhatia, 1996; Michel *et al*., 1984; Orchard and Varigos, 1997; Pohl *et al*., 1987; Prieto *et al*., 1986; Thuvander, 1995).
- Challenge studies (Corder and Buckley, 1995; David, 1987; Devlin and David, 1992; Grzelewska-Rzymowska *et al*., 1986; Hong *et al*., 1989; Jimenez-Aranda *et al*., 1996; Kemp and Schembri, 1985; Marques *et al*.,1995; Montano and Orea, 1989; Morales *et al*., 1995; Rowe, 1988,1994; Schulte-Korne *et al*., 1996; Timberlake *et al*., 1992; van Bever *et al*., 1989; Virchow *et al*., 1988; Wilson and Scott,1989)
- Reviews (Collins-Williams, 1985; Simon, 1986; Wuthrich, 1993).

The TemaNord (2002) evaluation concluded that whilst these studies added further data, they did not identify new elements, and therefore would not give rise to additional concern.

Bhatia (2000) studied consecutive outpatients (May 1996 to April 1998) who developed allergic reactions or intolerance to Tartrazine in psychotropic drugs. The subjects showing allergic reactions to Tartrazine were then exposed to non-Tartrazine-containing brands of psychotropic drugs. Of 2210 patients exposed to Tartrazine-containing drugs, 83 (3.8 %) developed allergic reactions. The symptoms subsided within 24 to 48 hours of stopping the drug. None of the patients showed allergy to non-Tartrazine-containing brands. History of allergy to Tartrazine was present in 13.2 % of patients, and 15.7 % of patients had a history of aspirin sensitivity.

Nettis *et al*. (2003) determined the incidence of intolerance to Tartrazine among subjects with a history of food-induced urticaria/angioedema. They found that out of 102 subjects only one had reactions after ingestion of 5 mg Tartrazine.

Worm *et al*. (2001) found that Tartrazine (among other tested food additives) increased sulphidoleukotriene production by peripheral leucocytes in patients with a proven food intolerance towards food additives (in the form of atopic dermatitis). The authors state that this process may be a pathophysiological mechanism involved in food additive mediated aggravation of atopic dermatitis.

Inomata *et al.* (2006) present a paediatric case of Multiple Chemical Sensitivities (MCS). This was the case of a 5-year-old girl who suffered from recurrent reactions accompanied by urticaria, angioedema, headaches, dyspnoea, loss of consciousness, and abdominal pain that were not eradicated, but were instead exacerbated, by various treatments with antihistamines and intravenous corticosteroids. Open challenge tests with Tartrazine, aspirin and acetaminophen were positive, whereas skin prick tests using additives and NSAIDs and prick-prick tests using candies and jellybeans were all negative. Consequently, intolerance to azo dyes and non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, was diagnosed.

Caldas *et al.* (2007) challenged 166 atopic adult volunteers with previous medical histories of asthma, rhinitis, urticaria (chronic or acute) and with hypersensitivity of non-steroidal anti-inflammatory agents, with Tartrazine (E 102) in order to verify whether Tartrazine can evoke hypersensitive reactions. The results from the 99 volunteers who fulfilled the clinical, laboratorial and protocol requirements revealed that Tartrazine is capable of provoking IgE and non-IgE dependent reactions in 6 % of the volunteers.

A total of 54 patients with an allergic disease randomly recruited in five university hospitals in Korea were enrolled in a double-blind, placebo-controlled (DBPC) oral challenge with crossover design. (Park *et al.*, 2008) After seven days on a low–food additive diet, oral challenges were performed with a mixture of seven food additives or placebo. Enrolled patients completed a questionnaire designed to screen for the presence of a food-related hypersensitivity and underwent skin prick and patch testing using seven common food additives: Amaranth, Erythrosine, Tartrazine, Sunset Yellow FCF, sodium sulphite, sodium benzoate, and monosodium glutamate. The amount of each food additive in the mixture was 1/10 of the ADI determined for the Korean population. Test substances were administered in three divided doses (i.e., 1/6, 2/6, and 3/6 of the total amount in order), with 30-minute intervals between doses.

Five patients (9.3 %) had a positive reaction to food additives but no reactions to the placebo, two patients (3.7 %) reacted to both food additives and to the placebo, three patients (5.5 %) reacted to the placebo only, and the remaining 44 patients (81.5 %) reacted to neither. Urticaria, periorbital oedema, and facial flushing accompanied by itching were the major symptoms. Statistically significant differences between those who reacted positively to food additives and those who reacted positively to the placebo were not observed.

The results of this study suggest that a mixture of seven common food additives, each at 10 % of the ADI, do not cause dermatologic adverse reactions or aggravated atopic dermatitis symptoms in patients with allergic diseases.

The author commented that the clinical relevance of skin prick and patch testing with food additives for the diagnosis of adverse reactions to food has not yet been confirmed in scientific literature and their observations indicate that skin prick and patch testing are limited in their ability to determine whether food additives are likely to cause adverse skin reactions furthermore. Moreover, positivity showed no significant association with a history of an adverse reaction related to food on the screening questionnaire or DBPC oral challenge outcome.

Reus *et al*. (2000) conducted a literature study on whether a causal connection exists between food additives and various medical complaints. For azo dyes including Tartrazine, no link with medical symptoms was demonstrable.

The objective of the Ardern and Ram (2001) literature search was to assess the overall effect of Tartrazine (exclusion or challenge) in the management of asthma. The selection criteria were Randomized Control trials (RCTs) of oral administration of Tartrazine (as a challenge) *versus* placebo or dietary avoidance of Tartrazine *versus* normal diet were considered. Studies which focused upon allergic asthma were also

included. Studies of Tartrazine exclusion for other allergic conditions such as hay fever, allergic rhinitis and eczema were only considered if the results for subjects with asthma were separately identified. Study quality was assessed and data abstracted by two reviewers independently. Ninety abstracts were found, of which 18 were potentially relevant. Six met the inclusion criteria, but only three presented results in a format that permitted analysis and none could be combined in a meta-analysis. In none of the studies did Tartrazine challenge or avoidance in diet significantly alter asthma outcomes. The reviewer's conclusions were that due to the paucity of available evidence, it was not possible to provide firm conclusions as to the effects of Tartrazine on asthma control. However, the six RCTs included in this review all arrived at the same conclusion.

On the basis of a literature search from 1959 and including articles published up to 2003. Elkhim *et al.* (2007) stated that most studies have been conducted on selected patients with intolerance reactions in a hospital setting. The authors came to the conclusion that "The risk of intolerance reactions attributed to Tartrazine has still to be considered as probable, even if it seems to be low in prevalence. The level of Tartrazine supposed to cause intolerance reactions in specific people is less than milligram quantities and can be reached through consumption of the food additives under normal conditions of use. For this aim, the presence of this dye must be clearly mentioned on the labelling of products destined for human food and pharmaceuticals, in order to provide clear and essential information to intolerant people."

The Panel concludes that Tartrazine does appear to be able to trigger intolerance reactions in a small fraction of the exposed population.

3.2.7. Other studies

A study by McCann *et al*. (2007) has concluded that exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet resulted in increased hyperactivity in 3-year old and 8- to 9-year old children in the general population. In an earlier study by the same research team there was some evidence for adverse behavioural effects of a mixture of 4 synthetic colours and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al.,* 2004). In the McCann *et al*. (2007) study, the effects of two combinations of Tartrazine (E 102), Quinoline Yellow (E 104), Sunset Yellow FCF (E 110), Ponceau 4R (E 124), Allura Red AC (E 129), Carmoisine (E 122) and sodium benzoate (E211) on children's behaviour were studied.

The study involved 153 3-year old and one 144 8- to 9-year old children, selected to represent a broad range of behaviour in the general population including children with normal to high level behavioural activity. Children who were medicated for Attention-Deficit Hyperactivity Disorder (ADHD) were not included. A Global Hyperactivity Aggregate (GHA) score was the main outcome of the study, and this parameter was based on aggregated z-scores of observed behaviours and ratings by teachers, class room observers and parents, plus, for 8- to 9-year old children, a computerised test of attention.

Mix A in this study contained Tartrazine and in addition, Ponceau 4R, Sunset Yellow, Carmoisine and sodium benzoate. Mix B in this study contained Allura Red AC, and in addition Sunset Yellow FCF, Carmoisine, Quinoline Yellow and 45 mg of sodium benzoate.

Mix A significantly increased the GHA scores for all 3-year old children compared to the placebo control GHA scores (effect size 0.20 [CI 0.01 to 0.39], $p < 0.05$). This result persisted when analysis was restricted to 3-year old children who consumed more than 85 % of juice and had no missing data (complete case group); in this analysis the effect of Mix A in the 3-year old children was still significantly increased compared to placebo control (effect size 0.32 [CI 0.05 to 0.60, $p < 0.05$).

For the 8- to 9-year old children, a significant effect of Mix A (effect size 0.12 [CI 0.02 to 0.23], p <0.05) and Mix B (effect size 0.17 [0.07 – 0.28], p< 0.001 was seen when analysis was restricted to those children consuming at least 85 % of drinks with no missing data (complete case group). When all 8- to 9-year old children that completed the study were taken into account, Mix A had no effect on the GHA scores compared to the placebo control (effect size 0.08 [CI -0.02 to 0.17]). The clinical significance of the observed effects for normal functioning of the exposed children remains unclear.

Pollock and Warner (1990) conducted a study on Tartrazine-related aberrant behaviour in children. The behavior of 39 children was observed, by their parents, to improve on an artificial food additive-free diet and to deteriorate with dietary lapses. Nineteen children completed a double-blind placebo controlled challenge study with a combination of synthetic food colours (Azorubine (25 mg), Tartrazine (50 mg), Sunset Yellow (25 mg) and Amaranth (25 mg)) and these colours were shown to have an adverse effect on a daily Conners' rating of behaviour, although most parents could not detect these changes.

4. Discussion

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

Tartrazine (E 102) is an azo dye authorised as a food additive in the EU and previously evaluated by JECFA in 1966 and the SCF in 1975 and 1984.

Specifications for Tartrazine have been defined in the EU Commission Directive 2008/128/EC and in the Codex Alimentarius. Tartrazine consists essentially of 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazole trisodium salt and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Tartrazine is described as the sodium salt. The calcium and the potassium salts are also permitted (Directive 2008/128/EC).

The purity is specified as not less than 85 % total colouring matters, calculated as the sodium salt. The remaining 15 % may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly), water insoluble matter not more than 0.2 %, subsidiary colouring matters not more than 1.0 % and organic compounds other than colouring matters with a total not more than 0.5 % (4-hydrazinobenzene sulphonic acid, 4-aminobenzene-1-sulphonic acid, 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid, 4,4′-diazoaminodi(benzene sulphonic acid), tetrahydroxysuccinic acid).

Whilst the majority of the absorption, distribution, metabolism and excretion studies are 40-50 years old the techniques and methods used for the identification of the parent compound and its metabolites were those used to elucidate and identify the metabolic pathways of most xenobiotics. Although modern analytical methods might identify some additional minor metabolites or elucidate intermediate metabolites, this would not alter the overall assessment of Tartrazine metabolism. Following oral administration at a range of doses absorption of intact Tartrazine is negligible to low (< 5 %) and this is predominantly excreted unchanged in urine. After oral administration there is extensive metabolism of Tartrazine by the gastrointestinal microflora to sulphanilic acid and aminopyrazalone (which may then be subsequently cleaved to sulphanilic acid and α-amino-β-ketobutyric acid fragments with the latter breaking down further via intermediary metabolism with release of carbon dioxide). Both sulphanilic acid and aminopyrazalone can be absorbed to a greater extent than Tartrazine, with urinary excretion of up to 20-25 % of the dose as sulphanilic acid in rats at around 50-55 mg/kg bw,and 55 % of the dose at 20-27.5 mg/kg bw, and 40-49 % of the dose as sulphanilic acid in humans at around 1-1.5 mg/kg bw. These data

suggest that the rat is a suitable metabolic model for man and given the extensive metabolism in rats there would be extensive exposure to the microfloral metabolites in the toxicity studies and no need for toxicity data on the metabolites.

The studies included in the JECFA (1966) evaluation have been described in very little detail and appear to be inadequate for a proper evaluation of the subchronic toxicity of Tartrazine. The study by Aboel-Zahab *et al*. (1997) gives some more detail on the subchronic toxic effects of mixtures containing Tartrazine but no discrimination between the different constituents is possible. The Aboel-Zahab *et al*. (1997) study cannot be used for a re-assessment of the ADI of Tartrazine, as animals were exposed to a mixture of food colours rather than Tartrazine alone, the dose levels of each colour has not been specified and it is even not clear what were the amounts/percentage of the colours used in the diet to achieve the cited level of 0.8 g/kg bw. Furthermore, only one dose was tested.

In the previous evaluations there were no indications of Tartrazine-related adverse effects on reproduction or development. In the more recent study by Tanaka (2006; 2008), deleterious effects on reproductive parameters were also not demonstrated up to and including dose levels of 773 and 1225 mg Tartrazine/kg bw/day for males and females, respectively, the highest dose levels tested. The results from behaviour tests conducted during the lactation period present some indications of differences in the performance of treated animals compared to the controls, most often in a direction of accelerated achievement of coordination (better performance compared to controls). However, these can be considered to be of little importance as these findings are non-consistent and no convincing dose-response relationship could be observed. It should be made clear that this type of test is used in developmental toxicity studies with the aim to reveal a delay of acquisition of coordination abilities, orientation and muscle strength.

The Tanaka studies (2006; 2008) are generally well-designed developmental neurobehavioural studies in which the methods are generally fully described. However they did not standardise litter sizes by culling to a uniform number of pups/litter on the day of birth. Since differences in litter size can have a significant impact on nutrition and hence rates of development, with respect to physical landmarks, reflex, locomotor and cognitive development, the lack of standardisation may have influenced the results in a way that could have confounded treatment-related findings. The authors do not explain in their statistical analysis whether litters or individual pups were used as the independent variable for any of the endpoints investigated. As presented, the results appear to suggest individual pups have been used, however for neurobehavioral parameters, siblings within a litter should not be treated as independent variables; instead, litter averages should be the scores used in statistical analyses. Otherwise, statistical significance is erroneously inflated. Interpretation of the studies is made difficult by the loss of litters, for various reasons, reducing the number of litters per dose to as low as 7 in some instances.

It is important in the interpretation of neurobehavioral outcomes to consider the consistency of findings within dose groups at closely-related time points and also across the two studies, whilst accepting that male pups may behave differently from females. The design of the two studies was identical except for continuation to a second generation in the 2008 study.

The results show (with one exception) that the statistically significant differences found were:

- Not dose-related (Tanaka 2006 cliff avoidance, Tanaka 2008 swimming behaviour)
- Observed only on PND 4 and not PND 7 (Tanaka 2006 surface righting, negative geotaxis)
- Only seen in one of the two studies (surface righting, negative geotaxis, cliff avoidance, swimming behaviour, olfactory orientation, T-water maze)

- Only seen in one of the two generations within Tanaka 2008 (surface righting, swimming behaviour, olfactory orientation).

This suggests that either the numbers of pups/litters evaluated per dose group were too low to consistently detect minor changes, or that the statistically significant differences found were chance findings.

It should also be noted that some of the statistically significant findings in the high-dose groups from both studies are indicative of faster neurological development, some possibly related to slightly higher pup body weight, and should not be interpreted as adverse effects. On the contrary, they could be interpreted as reassurance of the absence of such effects of Tartrazine.

The one exception to the inconsistencies described above is the statistically significant reductions in locomotor activity when analysed by a trend test. Reductions were observed at three weeks of age in the 2006 study and at three weeks of age but not eight weeks of age in the F1 generation of the 2008 study. The F2 generation of the 2008 study showed reduced locomotor activity at both three and eight weeks. While these may appear to be consistent findings, the method of testing employed did not take account of the possibility of habituation over time within each test period, which if it was the cause of the reduced activity at the high dose would be regarded as improved cognition rather than adverse. More importantly, testing at only single time points at three and eight weeks of age does not take account of the fact that normal rat locomotor development in the first three weeks of life follows a biphasic (inverted V-shape) pattern, reaching a peak at 15 days of age and declining thereafter to adult levels by around 30 days of age (Annau, 1985; Henck, 2002). This pattern is attributable to the fact that inhibitory neural circuits develop later than activation circuits. Thus, it is better to test for spontaneous activity away from the home cage several days before, during and after the peak period so that the biphasic response can be followed and compared. If, for example, the high-dose animals were slightly more advanced in their development than the other groups (as some of the other tests suggest), then they may have been further down the declining part of the activity curve at the time of testing at 21 days than the other groups, which would account for the findings, but would not be interpreted as adverse. The Panel considers that the results from these two studies with respect to locomotor activity are uninterpretable in the absence of repeated testing.

The Panel concludes that studies by Tanaka (2006; 2008) did not demonstrate any adverse effects of Tartrazine on neurobehavioral development.

The Panel concludes that revision of the ADI based on these data is therefore not warranted.

Studies on micronucleus induction *in vitro* and *in vivo* SCE, micronucleus and chromosome aberration tests were negative. Data from an UDS assay conducted *in vitro* and *ex vivo* on mammalian cells were also negative. Tartrazine induced chromosomal aberrations in Chinese hamster fibroblast cell line and showed a significant increase in SCE and chromosomal aberrations in mouse and rat bone marrow cells, following acute and chronic exposure to high doses of Tartrazine via the diet. Using the Comet assay, Sasaki *et al.* showed that Tartrazine induced DNA damage in the colon of mice at doses close to the ADI. In contrast, in a more recent study by Poul *et al*. Tartrazine did not reveal genotoxic effect in the micronucleus gut assay in mice at doses up to 2000 mg/kg bw. The authors comment on the results of the *in vivo* Comet assay by Sasaki *et al.* that the transient DNA damage observed in the colon of mice are unable to be fixed in stable genotoxic lesions and might be partly explained by local cytotoxicity of the dye.

The available carcinogenicity studies, including the six carcinogenicity studies reviewed by JECFA, as well as the three more recent ones described by TemaNord, namely the publications of Maekawa *et al*., (1987) and Borzelleca and Hallagan (1988a, 1988b) plus the most recent study by Moutinho *et al*. (2007). These have demonstrated that Tartrazine does not have a potential to induce benign or malignant neoplasias.

The Panel considered, in light of the negative carcinogenicity studies and negative results in standard *in vivo* genotoxicity studies, that the biological significance of the positive genotoxicity results is uncertain. Therefore the Panel concluded that the effects reported in these studies are not expected to result in carcinogenicity.

The conversion of Tartrazine by azo reduction *in vivo* results in the formation of sulphonated naphtylamines that may not be formed in the standard *in vitro* genotoxicity tests. In a review by Jung *et al.* (1992), a range of sulphonated aromatic amines was shown, in general, not to be associated with genotoxicity *in vitro* and *in vivo*. Since all the sulphonated aromatic amine metabolites that could in theory be formed by azo reduction of Tartrazine were included in the study, the Panel concluded that the data reviewed by Jung *et al.* (1992) are sufficiently re-assuring to support the conclusion that the sulphonated aromatic amines formed from Tartrazine by azo reduction do not give reason for concern with respect to genotoxicity.

A study by McCann *et al*. (2007) has concluded that exposure to two mixtures of four synthetic colours plus a sodium benzoate preservative in the diet, one of them, Mix A, (containing Tartrazine) resulted in increased hyperactivity in 3-year old, but not in 8- to 9-year old children in the general population. In an earlier study by the same research team there was some evidence for adverse behavioural effects of a mixture of 4 synthetic colours (including Tartrazine) and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al,*. 2004).

Recently, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) published an opinion on this McCann *et al*. study (EFSA, 2008a). In this opinion the AFC Panel also presented an overview of earlier studies that reported effects of food colours in general on child behaviour, the majority of these studies being conducted on children described as hyperactive or with a clinical diagnosis of ADHD.

In its opinion, the AFC Panel concluded that the McCann *et al.* (2007) study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The AFC Panel also concluded that the findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular.

However, the AFC Panel, assisted by experts in human behavioural studies in the *ad hoc* Working group preparing the opinion, also concluded that the clinical significance of the observed effects remains unclear, since it is not known whether the small alterations in attention and activity would interfere with schoolwork and other intellectual functioning.

The AFC Panel also concluded that:

- since mixtures and not individual additives were tested in the study by McCann *et al.*, it is not possible to ascribe the observed effects to any of the individual compounds, and

- in the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed, the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

The ANS Panel concurs with these conclusions.

Overall, the Panel concludes that the present database on genotoxicity, semi-chronic, reproductive, developmental and long-term toxicity, and carcinogenicity as well as the McCann *et al.* study (2007) does not give reason to revise the ADI of 7.5 mg/kg bw/day.

In humans, adverse reactions such as urticaria and vasculitis after Tartrazine intake have been reported in a number of studies. Data from animal and human studies have not convincingly demonstrated that Tartrazine is able to induce an immune mediated (hypersensitivity) response, and the adverse reactions reported in humans following exposure to Tartrazine appear to be intolerance reactions. The reports of these adverse effects are often characterised by poorly controlled challenge procedures; sometimes Tartrazine is given with a mixture of other colours. In comparison, recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon. However, given the available information, the Panel concludes that Tartrazine may induce intolerance reactions in a small fraction of the population. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tier. The dietary exposure to Tartrazine from the MPLs of use was estimated by the Panel using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day for adults, and 13.1 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and the Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Tartrazine, as identified by the Panel from the data by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA (Tier 3).

For children (aged 1-10 years), estimates have been calculated for nine European countries (Belgium, France, UK, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Tartrazine intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure of European children (aged 1-10 years) ranged from 0.8 mg/kg bw/day to 3.4 mg/kg bw/day and from 0.8 mg/kg bw/day to 9.4 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries) were soft drinks (13 to 55 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (14 to 47 %) and desserts, including flavoured milk products (12 to 63 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 10 to 50 % of exposure in three countries. Confectionery accounted for 11-13 % of exposure in two countries.

Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (50 % at average level and 80 % for high level consumers).

When considering maximum reported use levels (Tier 3), the mean dietary exposure to Tartrazine for European children (aged 1-10 years), ranged from 0.2 mg/kg bw/day to 1.9 mg/kg bw/day and from 0.4 mg/kg bw/day to 7.3 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries) were soft drinks (11 to 40 %) sauces, seasonings (e.g. curry powder,

tandoori), pickles, relishes, chutney, piccalilli (10 to 75 %), confectionery (12 to 35 %) and fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12 to 32 %). Desserts, including flavoured milk products accounted for 14 to 26 % of exposure in 4 countries and surimi accounted for to 11 to 17 % of exposure in 3 countries.

Estimates reported for the UK adult population give a mean dietary exposure of 0.3 mg/kg bw/day and of 0.5 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (30 %), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (22 %) and confectionery (16 %).

The Panel further notes that the specifications of Tartrazine need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components.

The Panel notes that the JECFA specification for lead is $\leq$ 2 mg whereas the EC specification is $\leq$10 mg/kg.

The Panel notes that the aluminium lake of the colour could add to the daily intake of aluminium for which a TWI of 1 mg aluminium/kg bw/week has been established (EFSA, 2008b) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

CONCLUSIONS

Tartrazine (E 102) is an azo dye allowed as a food additive in the EU, and has been previously evaluated by the Joint WHO/FAO Expert Committee on Food Additives (JECFA) in 1966 and the EU Scientific Committee for Food (SCF) in 1975 and 1984. Both committees established an ADI of 0-7.5 mg/kg bw/day.

The Panel concludes that the present dataset does not give reason to revise the ADI of 7.5 mg/kg bw/day.

The Panel concludes that at the maximum reported levels of use of Tartrazine, refined (Tier 3) intake estimates are below the ADI of 7.5 mg/kg bw/day.

The Panel concludes that Tartrazine appears to be able to elicit intolerance reactions in a small fraction of the exposed population. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The Panel further notes that the specifications for Tartrazine need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components. The Panel notes that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel notes that the aluminium lake of the colour could add to the daily intake of aluminium for which a TWI of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document on Tartrazine (E 102) prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
2. CEPS (European Spirits Organisation), 2009. Letter sent to DG SANCO, dated 17 September 2009/GP.TS-006-2009.
3. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009).
4. ELC (Federation of European Food Additives, Food Enzymes and Food Culture Industries), 2009. ELC comments to EFSA in response to a written request from DG Sanco: "EFSA re-evaluation of food colours" – DG Sanco's additional call for data dated 8 April 2009, letter to EFSA on 20 May 2009).
5. UNESDA (Union of European Beverage Associations), 2009. Comments to the CIAA/DG Sanco in response to a written request from DG Sanco to the CIAA, dated April 8 2009: 'Use of certain colour additives in non-alcoholic beverages' (May 26, 2009).

REFERENCES

Aboel-Zahab H, el-Khyat Z, Sidhom G, Awadallah R, Abdel-al W and Mahdy K, 1997. Physiological effects of some synthetic food colouring additives on rats. Boll.Chim. Farm. 136, 615-627 (as referred to by TemaNord, 2002).

Alves SP, Brum DM, de Andrade ECB and Netto ADP, 2008. Determination of synthetic dyes in selected foodstuffs by high performance liquid chromatography with UV-DAD detection. Food Chemistry, volume 107, Issue 1, 489-496.

Annau Z, 1985. Chapter 30. Behavioural Toxicology: Animal Experimental Models. In: Neurotoxicology. Eds Blum, K and Manzo, L. Marcel Dekker, New York and Boston.

Ardern KD and Ram FS, 2001. Tartrazine exclusion for allergic asthma. Cochrane Database Syst. Rev. 4: CD000460.

Bär F and Griepentrog F, 1960. Die Allergenwirkung von Fremden Stoffen in der Lebensmitteln. Med. u. Ernähr. 1, 99-104 (as referred to by JECFA 1966).

Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C, Stevenson J, 2004. The effects of a double blind, placebo-controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch. Dis. Child. 89, 506-511.

Baumgardner DJ, 1989. Persistent urticaria caused by a common coloring agent. Postgrad. Med. 85, 265-266 (as referred to by TemaNord, 2002).

Bhatia MS, 1996. Allergy to Tartrazine in alprazolam. Indian J. Med. Sci. 50, 285-286 (as referred to by TemaNord, 2002).

Bhatia MS, 2000. Allergy to Tartrazine in psychotropic drugs. J. Clin. Psychiatry 61, 473-476.

Borzelleca JF and Hallagan JB, 1988a. Chronic toxicity/carcinogenicity studies of FD& C Yellow No.5 (Tartrazine) in rats. Food Chem. Toxicol. 26, 179-187 (as referred Toby TemaNord, 2002).

Borzelleca JF and Hallagan JB, 1988b. A chronic toxicity/carcinogenicity study of FD & C Yellow No.5 (Tartrazine) in mice. Food Chem. Toxicol. 26, 189-194 (as referred to by TemaNord, 2002).

Brown JP and Dietrich PS, 1983. Mutagenicity of selected sulfonated azo dyes in the *Salmonella*/microsome assay: use of aerobic and anaerobic activation procedures. Mutat. Res. 116, 305-315 (as referred to by TemaNord, 2002).

Caldas, LQA, Moreira MD, Olej B, Pestana S, Morais L, Piumbini R, 2007. Double-blind and crossed-over randomized placebo controlled study in atopic patients challenged with a yellow colorant Tartrazine. In: IV Congreso International da Sociedade Cubana de Toxicologia, 2007, Havana. Computer Disk of the Conference. Havana Cuba: Universidad de Havana, v.1. p. 49.

ChemIDplus Advanced (via internet, 2006). Accessible via: http://chem.sis.nlm.nih.gov/chemidplus/

Chung KT, 1983. The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes. Mutat. Res. 114, 269-281 (as referred to by TemaNord, 2002).

Codex Alimentarius (via internet, 2006). Accessible via: http://www.codexalimentarius.net/gsfaonline/index.html

Collins TF, Black TN, Brown LH and Bulhack P, 1990. Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. Food Chem. Toxicol. 28(12), 821-827

Collins TF, Black TN, O'Donnell MW Jr and Bulhack P, 1992. Study of the teratogenic potential of FD & C yellow No. 5 when given in drinking-water. Food Chem.Toxicol. 30(4), 263-268.

Collins-Williams C, 1985. Clinical spectrum of adverse reactions to Tartrazine. J. Asthma 22, 139-143 (as referred to by TemaNord, 2002).

Combes RD, 1986. On the mutagenicity of Tartrazine (FD&C Yellow No5) [letter]. Arch.Toxicol. 59, 68-70 (as referred to by TemaNord, 2002).

Corder EH and Buckley CE, 1995. Aspirin, salicylate, sulfite and tartrazine induced bronchoconstriction. Safe doses and case definition in epidemiological studies. J. Clin.Epidemiol. 48, 1269-1275 (as referred to by TemaNord, 2002).

Das A and Mukherjee A, 2004. Genotoxicity testing of the food colours amaranth and Tartrazine. Int. J. Hum. Genetics, 4, 277-280.

David TJ, 1987. Reactions to dietary Tartrazine. Arch. Dis. Child 62, 119-122 (as referred to by TemaNord, 2002).

Davis KJ, Fitzhugh OG and Nelson AA, 1964. Chronic rat and dog toxicity studies on Tartrazine. Toxicol. Appl. Pharmacol. 6, 621-626.

Devlin J and David TJ, 1992. Tartrazine in atopic eczema. Arch. Dis. Child 67, 709-711(as referred to by TemaNord, 2002).

DFG, 1957. Deutsche Forschungsgemeinschaft. Farbstoff Kommission, Mitteilung 6, 2, Auflage toxikologische Daten von Farbstoffen und ihre zulassung fur Lebensmittel inverschiedenen Landern, Franz Steiner Verlag GmbH, Wiesbaden (as referred to by JECFA, 1966).

Durnev AD, Oreshchenko AV, Kulakova AV and Beresten NF, 1995. [Analysis of cytogenetic activity of food dyes]. Vopr. Med. Khim. 41, 50-53 (as referred to by TemaNord, 2002).

EC (European Community), 1994. European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs.

EC (European Commission), 1998. Report on Methodologies for the Monitoring of Food Additive Intake Across the European Union. Final Report Submitted by the Task Co-ordinator 16 January 1998, Reports of a Working Group on Scientific Cooperation on Questions Relating to Food. Task 4.2. SCOOP/INT/REPORT/2 (Brussels: European Commission Directorate General I11 Industry).

EC (European Commission), 2001. Commission of the European Communities (COM). 542 final. Report from the commission on dietary food additive intake in the European Union. Brussels, 01.10.2001.

EC (European Commission), 2008. Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs.

EFSA (European Food Safety Authority), 2008a. Assessment of the results of the study by McCann *et al.* (2007) on the effect of some colours and sodium benzoate on children's behaviour. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). Adopted on 7 March 2008. The EFSA Journal (2008) 660, 1-54.

EFSA (European Food Safety Authority), 2008b. Safety of aluminium from dietary intake Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). Adopted on 22 May 2008. The EFSA Journal (2008) 754, 2-4.

Elhkim MO, Fanny Héraud F, Bemrah N, Gauchard F, Lorino T, Lambré C, Frémy JM, Jean- Poul J-M, 2007. New considerations regarding the risk assessment on Tartrazine. An update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regulatory Toxicology and Pharmacology 47, 308–316.

Farag IM, Abdel Aziz KB, El-Nahass E, Zaher M, Hegazy MA and Roshdy HM, 2001. Cytogenetic studies on the effects of Tartrazine, beta-carotene and a mixture of both dyes on pregnant mice and their embryos. Bulletin of the National Research Centre (Egypt) 26, 93-109.

FSA (Food Standards Agency), 2003. Survey of colours in soft drinks. Food Survey Information Sheets. No.37/03 May 2003. Available at: http://www.food.gov.uk/multimedia/pdfs/fsiscoloursinfoods032003.pdf.

Fuglsang G, Madsen C, Saval P, Osterballe O, 1993. Prevalence of intolerance to food additives among Danish school children. Pediatr Allergy Immunol. 4(3), 123-9.

Fuglsang G, Madsen G, Halken S, Jørgensen S, Ostergaard PA, Osterballe O, 1994. Adverse reactions to food additives in children with atopic symptoms. Allergy. 49(1), 31-7.

Giri AK, Das SK, Talukder G and Sharma A, 1990. Sister chromatid exchange and chromosome aberrations induced by Curcumin and Tartrazine on mammalian cells *in vivo*. Cytobios. 62 (249), 111-7.

Grzelewska-Rzymowska I, Szmidt M, Kowalski ML and Roznіecki J, 1986. Sensitivity and tolerance to Tartrazine in aspirin-sensitive asthmatics. Allergol. Immunopathol. (Madr.) 14, 31-36 (as referred to by TemaNord, 2002).

Hannuksela M and Haahtela T, 1987. Hypersensitivity reactions to food additives. Allergy 42(8), 561-75.

Henck JW, 2002. Chapter 1. Developmental Neurotoxicology. In: Handbook of Neurotoxicology, Volume II. Ed. Massaro, EJ. Humana Press inc. Ottawa

Henschler D and Wild D, 1985. Mutagenic activity in rat urine after feeding with the azodye Tartrazine. Arch. Toxicol. 57, 214-215 (as referred to by TemaNord, 2002).

Hong SP, Park HS, Lee MK and Hong CS, 1989. Oral provocation tests with aspirin and food additives in asthmatic patients. Yonsei. Med. J. 30, 339-345 (as referred to by TemaNord, 2002).

Honohan T, Enderlin FE, Ryerson BA, Parkinson TM, 1977. Intestinal absorption of polymeric derivatives of the food dyes sunset yellow and tartrazine in rats. Xenobiotica 7(12), 765-74.

Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N, Al-Sinan M, 2006. Estimates of dietary exposure of children to artificial food colours in Kuwait. Food Additives and Contaminants: Part A, volume 23, Issue 3 March 2006, 245 – 251.

Inomata N, Osuna H, Fujita H, Ogawa1 T and Ikezawa1 Z, 2006. Multiple Chemical Sensitivities following intolerance to azo dye in sweets in a 5-year-old girl. Allergology International. 55, 203-205.

Ishidate MJ, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22, 623-636.

Izbirak A, Sumer S and Diril N, 1990. [Mutagenicity testing of some azo dyes used as food additives]. Mikrobiyol. Bul. 24, 48-56 (as referred to by TemaNord, 2002).

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1966. 8th Report of the Joint FAO/WHO Expert Committee on Food Additives. Specifications for identity and purity and toxicological evaluation of food colours. WHO Food additives series, 66.25, 88-92 (out of print).

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. 63rd meeting of the Joint FAO/WHO Expert Committee on Food Additives. Aluminium Lakes of Colouring Matters, General Specifications (via internet, 2006). Accessible via: http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-013.pdf

Jimenez-Aranda GS, Flores-Sandoval G, Gomez-Vera J and Orea-Solano M, 1996. [Prevalence of chronic urticaria following the ingestion of food additives in a third tier hospital]. Rev. Alerg. Mex., 43, 152-156 (as referred to by TemaNord, 2002).

Jones R, Ryan AJ and Wright SE, 1964. The metabolism and excretion of Tartrazine in the rat, rabbit and man. Food Cosmet. Toxicol. 2(4), 447-452 (as referred to by JECFA, 1966).

Jung R, Steinle D and Anliker R, 1992. A compilation of genotoxicity and carcinogenicity data on aromatic aminosulphonic acids. Food Chem. Toxicol. 30 (7), 635-660.

Kalender S, 2000. Degranulation effect of Tartrazine on the dermal mast cells of the mouse. Turk Hijven Ve Deneysel Biyoloji Dergisi, 57, 65-70.

Karpliuk IA, Volkova NA, Okuneva LA, Gogol AT and Rybakova KD, 1984. [Mutagenic effect of the food-coloring agents Tartrazine and indigo carmine]. Vopr. Pitan. 58-61 (as referred to by TemaNord, 2002).

Kemp AS and Schembri G, 1985. An elimination diet for chronic urticaria of childhood. Med. J. Aust., 143, 234-235 (as referred to by TemaNord, 2002).

Kornbrust D and Barfknecht T, 1985. Testing of 24 food, drug, cosmetic, and fabric dyes in the *in vitro* and the *in vivo*/*in vitro* rat hepatocyte primary culture/DNA repair assays. Environ. Mutagen. 7, 101-120 (as referred to by TemaNord, 2002).

Koutsogeorgopoulou L, Maravelias C, Methenitou G and Koutselinis A, 1998. Immunological aspects of the common food colorants, amaranth and tartrazine. Vet.Hum. Toxicol. 40, 1-4 (as referred to by TemaNord, 2002).

Lancaster FE and Lawrence JF, 1999. Determination of benzidine in the food colours Tartrazine and sunset yellow FCF, by reduction and derivatization followed by high-performance liquid chromatography. Food Additives and Contaminants: Part A, Volume 16, Issue 9, pages 381 – 390.

Lück H and Rickerl E, 1960. Food additives and mutative effects. VI Report, Z. Lebesmitt.-Untersuch. 112, 157-174 (as referred to by JECFA, 1966).

Ma M, Luoa X, Chenb B, Sub S and Yao S, 2006. Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high-performance liquid chromatography–diode array detection–electrospray mass spectrometry. Journal of Chromatography A, volume 1103, Issue 1, 170-176.

McCann D, Barrett A, Cooper C, Crumpler D, Dalen L, Grimshaw K, Kitchin E, Lok K, Porteous L, Prince E, Sonuga-Barke E, O'Warner J, Stevenson J, 2007. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community a randomized, double-blinded, placebo-controlled trial. The Lancet, Vol. 370, Issue 9598, 1560-1567.

Maekawa A, Matsuoka C, Onodera H, Tanigawa H, Furuta K, Kanno J, Jang JJ, Hayashi Y and Ogiu T, 1987. Lack of carcinogenicity of Tartrazine (FD & C Yellow No. 5) in the F344 rat. Food Chem. Toxicol. 25, 891-896 (as referred to by TemaNord, 2002).

Mannell WA, Grice HC, Lu FC and Allmark MGJ, 1958. Chronic toxicity studies on food colours. IV. Observations on the toxicity of Tartrazine, amaranth and Tartrazine in rats. Pharm Pharmacol. 10, 625-34 (as referred to by JECFA, 1966).

Marques L, Dordal MT and Marti E, 1995. [Tartrazine-induced rhinitis? (letter)]. Arch. Bronconeumol. 31, 255 (as referred to by TemaNord, 2002).

Michel O, Naeije N, Bracamonte M, Sergysels R and Duchateau J, 1984. [Indications for the aspirin provocation test in the asthmatic]. Rev. Pneumol. Clin. 40, 369-371 (as referred to by TemaNord, 2002).

Montano GM and Orea M, 1989. [Frequency of urticaria and angioedema induced by food additives]. Rev. Alerg. Mex. 36, 15-18 (as referred to by TemaNord, 2002).

Morales C, Penarrocha M, Bagan JV, Burches E and Pelaez A, 1995. Immunological study of Melkersson-Rosenthal syndrome. Lack of response to food additive challenge. Clin. Exp. Allergy, 25, 260-264 (as referred to by TemaNord, 2002).

Moutinho IL, Bertges LC, Assis RV, 2007. Prolonged use of the food dye Tartrazine (FD&C yellow no 5) and its effects on the gastric mucosa of Wistar rats. Braz. J. Biol. 67(1), 141-145.

Münzner R and Wever J, 1987. Mutagenic activity of the feces of rats following oral administration of Tartrazine. Arch. Toxicol. 60, 328-330 (as referred to by TemaNord, 2002).

Nettis E, Colanardi MC, Ferrannini A and Tursi A, 2003. Suspected tartrazine-induced acute urticaria/angioedema is only rarely reproducible by oral challenge. Clin. Exp.Allergy 33, 1725-1729.

NIHS, 1964. National Institute of Hygienic sciences, Department of Pharmacology, Japan. Unpublished data submitted to WHO, September 1964.

Novembre E, Dini L, Bernardini R, Resti M and Vierucci A, 1992. [Unusual reactions to food additives]. Pediatr. Med. Chir., 14, 39-42 (as referred to by TemaNord, 2002).

Orchard DC and Varigos GA, 1997. Fixed drug eruption to Tartrazine. Australas. J.Dermatol. 38, 212-214 (as referred to by TemaNord, 2002).

Park H-W, Park C-H, Park S-H, Park JY, Park HS, Yang J, Ahn K-M, Kim K-H, Oh J-W, Kim K-E, Pyun B-Y, Lee H-B and Min K-U, 2008. Dermatologic adverse reactions to 7 common food additives in patients with allergic diseases: A double-blind, placebo-controlled study. Letter to the Editor. Journal of Allergy and Clinical Immunology volume 121, Issue 4, 1059-1061.

Pohl R, Balon R, Berchou R and Yeragani VK, 1987. Allergy to Tartrazine in antidepressants. Am. J. Psychiatry 144, 237-238 (as referred to by TemaNord, 2002).

Pollastrini MT, Barea M and Salas J, 1990. [Genotoxic study of commercial dyes with Tartrazine base in *S. typhimurium* his- and *E. coli* trp-]. Rev. Sanid. Hig. Publica. (Madr.) 64, 203-209 (as referred to by TemaNord, 2002).

Pollock I and Warner JO, 1990. Effect of artificial food colours on childhood behaviour. Arch. Dis. Child 65, 74-77 (as referred to by TemaNord, 2002).

Poul M, Jarry G, Elhkim MO, Poul J-M, 2009. Lack of genotoxic effect of food dyes amaranth, sunset yellow and Tartrazine and their metabolites in the gut micronucleus assay in mice. Food and Chemical Toxicology 47, 443–448.

Prieto L, Pastor A, Palop A, Castro J, Paricio A. and Piquer A, 1986. [Rhinitis with intolerance to non-steroidal anti-inflammatory agents. Report of 3 cases]. Allergol. Immunopathol. (Madr.) 14, 147-153 (as referred to by TemaNord, 2002).

Prival MJ, Davis VM, Peiperl MD and Bell SJ, 1988. Evaluation of azo food dyes for mutagenicity and inhibition of mutagenicity by methods using *Salmonella typhimurium*. Mutat. Res. 206, 247-259 (as referred to by TemaNord, 2002).

Prival MJ, Mitchell VD 1982. Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. Mutat. Res. 97, 103-116.

Reus KE, Houben GF, Stam M, Dubois AE, 2000. [Food additives as a cause of medical symptoms: relationship shown between sulfites and asthma and anaphylaxis; results of a literature review][Article in Dutch]. Ned Tijdschr Geneeskd 144(38), 1836-9.

Rosenkranz, HS and Klopman G, 1989. Structural basis of the mutagenicity of phenylazoaniline dyes. Mutat. Res. 221, 217-234 (as referred to by TemaNord, 2002).

Roxon JJ, Ryan AJ, Wright SE, 1967. Enzymatic reduction of tartrazine by *Proteus vulgaris* from rats. Food Cosmet Toxicol. 5(5), 645-56.

Roxon JJ, Ryan AJ, Wright SE, 1966. Reduction of tartrazine by a *Proteus* species isolated from rats.Food Cosmet Toxicol. 4(4), 419-26.

Rowe KS, 1988. Synthetic food colourings and 'hyperactivity': a double-blind crossover study. Aust. Paediatr. J. 24, 143-147 (as referred to by TemaNord, 2002).

Rowe KS and Rowe KJ, 1994. Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. J. Pediatr. 125,691-698 (as referred to by TemaNord, 2002).

Roychoudhury A and Giri AK 1989. Effects of certain food dyes on chromosomes of *Allium cepa*. Mutat. Res. 223, 313-319 (as referred to by TemaNord, 2002).

Ryan AJ, Welling PG and Wright SE, 1969a. Further studies on the metabolism of tartrazine and related compounds in the intact rat. Food Cosmet Toxicol. 7(4), 287-95.

Ryan AJ, Welling PG, Roxon JJ, 1969b. Metabolism of a tartrazine analogue by intestinal bacteria. Food Cosmet Toxicol. 7(4):297-9.

Ryan AJ and Wright SE, 1962. Biliary excretion of some azo dyes related to Tartrazine. Nature 195, 1009 (as referred to by JECFA, 1966).

Safford RJ and Goodwin BF, 1985. Immunological studies on Tartrazine and its metabolites. I. Animal studies. Int. Arch. Allergy Appl. Immunol. 77, 331-336 (as referred to by TemaNord, 2002).

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K and Tsuda S, 2002. The Comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res. 519, 103-119.

Sawaya W, Husain A, Al-Otaibi J, Al-Foudari M, Hajji A, 2008. Colour additive levels in foodstuffs commonly consumed by children in Kuwait. Food Control, volume 19, Issue 1, 98-105.

SCF (Scientific Committee for Food), 1975. Reports of the Scientific Committee for Food (1st series), opinion expressed 1974, 17.

SCF (Scientific Committee for Food), 1984. Reports of the Scientific Committee for Food (14th series), opinion expressed 1983, 61.

Schaubschlager W, Ruschmeyer J, Zabel P and Schlaak M, 1988. [Intragastric provocation and antigen-induced *in vitro* histamine liberation by the food additive E 102]. Immun. Infekt. 16, 118-119 (as referred to by TemaNord, 2002).

Schulte-Korne G, Deimel W, Gutenbrunner C, Hennighausen K, Blank R, Rieger C ,and Remschmidt H, 1996. [Effect of an oligo-antigen diet on the behavior of hyperkinetic children]. Z. Kinder. Jugendpsychiatr. Psychother. 24, 176-183 (as referred to by TemaNord, 2002).

Scotter MJ, Castle L, 2004. Chemical interactions between additives in foodstuffs: a review. Food Addit Contam 2, 93-124.

Simon RA, 2003. Adverse reactions to food additives. Curr Allergy Asthma Rep 3(1), 62-6.

Simon RA, 1986. Adverse reactions to food additives. N. Engl. Reg. Allergy Proc. 7, 533-542 (as referred to by TemaNord, 2002).

Supramaniam G, Warner JO, 1986. Artificial food additive intolerance in patients with angio-oedema and urticaria. Lancet 2 (8512), 907-9.

Tanaka T, 2006. Reproductive and neurobehavioural toxicity study of Tartrazine administered to mice in the diet. Food Chem. Toxicol. 44, 179-187.

Tanaka T, Takahashi O, Oishi S, Ogata A, 2008. Effects of Tartrazine on exploratory behavior in a three-generation toxicity study in mice. Reproductive Toxicology 26, 156–163.

TemaNord, 2002. Food additives in Europe 2000; Status of safety assessments of food additives presently permitted in the EU. TemaNord 560, 53-60.

Tennant D, 2006. Screening of Colour Intakes from Non-Alcoholic Beverages. Report prepared for the Union of European Beverages Associations (UNESDA), December 2006.

Thuvander A, 1995. [Hypersensitivity to azo coloring agents. Tartrazine in food may cause rash and asthma]. Lakartidningen. 92, 296-298 (as referred to by TemaNord, 2002).

Timberlake CM, Toun AK and Hudson BJ, 1992. Precipitation of asthma attacks in Melanesian adults by sodium metabisulphite. P.N.G. Med. J., 35, 186-190 (as referred to by TemaNord, 2002).

Tripathy NK, Patnaik KK and Nabi MJ, 1989. Genotoxicity of Tartrazine studied into somatic assays of Drosophila melanogaster. Mutat. Res. 224, 479-483 (as referred to by TemaNord, 2002).

Van Bever HP, Docx M and Stevens WJ, 1989. Food and food additives in severe atopic dermatitis. Allergy 44, 588-594 (as referred to by TemaNord, 2002).

Virchow C, Szczeklik A, Bianco S, Schmitz-Schumann M, Juhl E, Robuschi M, Damonte C, Menz G and Serwonska M, 1988. Intolerance to Tartrazine in aspirin1101induced asthma: results of a multicenter study. Respiration 53, 20-23 (as referred to byTemaNord, 2002).

Waterman N and Lignac GO, 1958. The influence of the feeding of a number of food colours on the occurrence of tumours in mice. Acta. Physiol. Pharmacol. Neerl. 7, 35-55 (as referred to by JECFA, 1966).

Willheim R and Ivy AC, 1953. A preliminary study concerning the possibility of dietary carcinogenesis. Gastroenterology 23, 1-19 (as referred to by JECFA, 1966).

Wilson N and Scott A, 1989. A double-blind assessment of additive intolerance in children using a 12-day challenge period at home. Clin. Exp. Allergy 19, 267-272 (as referred to by TemaNord, 2002).

Worm M, Vieth W, Ehlers I, Sterry W and Zuberbier T, 2001. Increased leukotriene production by food additives in patients with atopic dermatitis and proven food intolerance. Clin. Exp. Allergy, 31, 265-273.

Wuthrich B, 1993. Adverse reactions to food additives. Ann. Allergy 71, 379-384 (as referred to by TemaNord, 2002).

Yoshioka and Ichihashi, 2008. Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection. Talanta 74(5), 1408-13.

Young E, Patel S, Stoneham M, Rona R, Wilkinson JD, 1987. The prevalence of reaction to food additives in a survey population. J R Coll Physicians Lond 21(4), 241-7.

ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated colours to be re-evaluated (30 July 09) and intake estimates

1. Decision rules taken to deal with QS authorisations:

a. In the category 'All other foodstuffs, the value of 500 mg/kg (the highest MPL) is used

b. At the food category level: if a colour is authorised QS in a food category for one or more colours

   i. If a value is available for only one colour, this value is used for all the colours (except if this value is available only for annatto-cf point c)

   ii. If many values are available for more than one colour, the highest value is used

c. At the colour level: if there is no available value or if there is just a single value for annatto, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

Particular cases:

- **Edible casings**: if available use the pork-based products use level; if not available, the highest MPL of 500 mg/kg is used.

- **Edible cheese rinds:** 100 mg/kg (as the flavoured processed cheese category) is used, except for the E 120 (Cochineal) colour whose level is 125 mg/kg for red marbled cheese.

2. Rules defined to identify maximum reported use levels from maximum current usages or maximum observed analytical values:

a. If the identified maximum reported use level, adjusted for the highest current usage data or the highest analytical value, is lower than or equal to the actual MPL, then the actual MPL is used by default.

b. If analytical and current use level data are available, priority is given to the use level data, even if analytical values are higher; the figure is rounded up to the nearest integer.

c. If no use level data are available because no uses were reported (use level = 0) or industry was not asked, the choice is made between the highest analytical value or the MPL:

   i. If more than 10 analytical data are available, the highest value is used;

   ii. If less than 10 analytical data are available, the MPL is used.

d. If no data were reported by the industry, the MPL is used by default.

e. If the highest use level or the highest analytical data are higher than the proposed adjusted QS values, priority is given to the highest use level/analytical data

3. Tiered approach to intake estimation.

The basic principles of the stepwise approach for estimates of additives' intakes involve, for each successive Tier, further refinement of intakes from the conservative estimates that form the First Tier of screening until more realistic estimates that form the Second and Third Tiers (EC, 2001).

The three screening tiers performed both for children and adult population are:

a. Tier 1: Estimates are based MPLs of use, as specified in the Directive 94/36/EC on food colours and the principles of the Budget method.
b. Tier 2: Estimates are based on MPLs of use, as specified in the Directive 94/36/EC on food colours, adjusted for *quantum satis* usages, and national individual food consumption data.
c. Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.

GLOSSARY/ABBREVIATIONS

ADI	Acceptable Daily Intake
ADHD	Attention-Deficit/Hyperactivity Disorder
ADME	Absorption, Distribution, Metabolism, and Excretion
AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
ANS	Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
CEPS	European Spirits Organisation
CIAA	Confederation of the Food and Drink Industries of the EU
DAD	Diode Array Detection
DBPC	Double-Blind, Placebo-Controlled
DG SANCO	The Directorate General for Health and Consumers
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
ELC	The Federation of European Food Additives, Food Enzymes and Food Culture Industries
EU	European Union
EXPOCHI	Refers to the EFSA Article 36, 2008 call for Proposals Focused on Children and Food Consumption
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GHA	Global Hyperactivity Aggregate
HPLC	High-Performance Liquid Chromatography
JECFA	Joint FAO/ WHO Expert Committee on Food Additives
LOD	Limit of Detection
LOQ	Level of Quantification

MCS	Multiple Chemical Sensitivities
MPL	Maximum Permitted Level
MTDI	Maximum Theoretical Daily Intake
MTT	Methyl thiazolyl diphenyl-tetrazolium bromide (thiazolyl blue tetrazolium bromide)
NOAEL	No Observed Adverse Effect Level
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NRU	Neutral Red uptake
PARNUTS	Foods for Particular Nutritional Purposes
PBL	Peripheral Blood Lymphocytes
PND	Post Natal Day
QS	*Quantum Satis*
RCT	Randomized Control trial
RNA	Ribonucleic Acid
SCE	Sister Chromatid Exchange
SCF	Scientific Committee for Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
TWI	Tolerable Weekly Intake
UDS	Unscheduled DNA Synthesis
UNESDA	Union of European Beverage Associations