SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Erythrosine (E 127) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

The Scientific Panel on Food Additives and Nutrient Sources added to Food has re-evaluated the safety of Erythrosine (E 127) when used as a food colouring substance. Erythrosine (E 127) is a xanthene-dye which has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1990 and the EU Scientific Committee for Food (SCF) in 1989. Both committees have established an Acceptable Daily Intake (ADI) of 0-0.1 mg/kg bw/day. Erythrosine is exclusively authorised for use in cocktail and candied cherries, and Bigarreaux cherries (94/36/EC). The Panel considered the weight-of-evidence still showed that the tumorigenic effects of Erythrosine in the thyroid gland of rats are secondary to its effects on thyroid function and not related to any genotoxic activity. Erythrosine-induced rodent thyroid tumours may be considered of limited relevance to humans; an approach which is consistent with previous evaluation of Erythrosine. The Panel considered Erythrosine has a minimal effect in humans at a clinical oral dose of 200 mg daily over 14 days, while a dose of 60 mg daily was without effect (Gardner et al., 1987). The current ADI adopted by the JECFA and the SCF is based on this study. The Panel concurred with their identification of this as the critical study. The 60 mg dose was taken to be the equivalent of 1 mg/kg bw/day. By applying a safety factor of 10 to allow for the small number of subjects used in the study and its relatively short duration, an ADI of 0-0.1 mg/kg bw per day was derived. The Panel concludes that the present database does not provide a basis to revise the ADI of 0.1 mg/kg bw/day. The Panel concluded that at the current levels of use intake estimates for adults on average is 0.0031 mg/kg bw/day and 0.01 mg/kg bw/day at the 95th percentile, and consequently are below the ADI of 0.1

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mg/kg bw/day. The Panel considered there would be no safety concerns at current levels of exposure including other sources of exposure.

KEY WORDS

Erythrosine, E 127, CI Food Red 14, FD&C Red No. 3, CAS Registry Number 16423-68-0, EINECS number 240-474-8, Xanthene, Food colouring substance.

SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Scientific Panel on Food Additives and Nutrient Sources added to Food has been asked to provide a scientific opinion re-evaluating the safety of Erythrosine (E 127) when used as a food colouring substance.

Erythrosine (E 127) is a xanthene-dye which has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1990 and the EU Scientific Committee for Food (SCF) in 1989. Both committees have established an Acceptable Daily Intake (ADI) of 0-0.1 mg/kg bw/day. The ADI was based on a no-effect level for endocrine/hormonal effects of 60 mg/day equivalent to 1 mg/kg bw/day obtained in a 14-day study in human volunteers by Gardner et al. (1987). The applied uncertainty factor was 10. It was also evaluated by the Nordic Council of Ministers.

Erythrosine is exclusively authorised for use in cocktail and candied cherries, and Bigarreaux cherries (94/36/EC).

Specifications have been defined in the EU Commission Directive 2008/128/EC and by JECFA (2006).

The Panel noted that the design of various studies in the previously evaluated dataset would not be in full compliance with current regulatory protocols. However, from the study descriptions available, the Panel considered that these studies were of sufficient quality.

From the studies in rats and humans, the Panel concluded that only a small portion of Erythrosine is absorbed. Tracer studies indicated that no accumulation of Erythrosine, iodine resulting from possible de-iodination of Erythrosine or ring-containing metabolites of Erythrosine accumulate in the thyroid. Erythrosine is excreted almost completely via faeces with unchanged iodine content. In a study in rat and a study in man, increased levels of PBI (in rat and in man) and iodine (in man) were measured in the blood. This might be explained by the circulating Erythrosine in the blood, rather than by an effect of Erythrosine on thyroid hormone levels.

Although some older and more recent in vitro studies showed positive results for the genotoxicity of Erythrosine, there are three negative in vivo genotoxicity studies (mammalian micronucleus, sister chromatid exchange and Comet assay). The weight-of-evidence from the available studies supports the conclusion that Erythrosine is neither genotoxic nor clastogenic in vivo. The SCF, the JECFA and TemaNord evaluation concluded, based on in vivo and in vitro mutagenicity studies available at that time, that Erythrosine did not show any genotoxic activity (SCF, 1989; JECFA, 1986; 1990; TemaNord, 2002). The Panel concurred with these assessments.

When addressing the results of new mutagenicity studies, the available adequate and well reported studies on oral in vivo activity were negative, although clastogenicity has been demonstrated by the *i.p*. route. The Panel considered the weight-of-evidence still showed that the tumorigenic effects of Erythrosine are secondary to its effects on thyroid function and not related to any genotoxic activity.

Although rodents and humans share a common physiology in regard to the thyroid-pituitary feedback system, a number of factors contribute to the greater sensitivity of the rat to long term perturbation of the pituitary thyroid axis which predisposes it to a higher incidence of proliferative lesions in response to chronic TSH stimulation than human thyroid.

Both humans and rodents have nonspecific low affinity protein carriers of thyroid hormone (e.g., albumin). However, in humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin (TBG), which binds T4 (and T3 to a lesser degree); this protein is missing in rodents, birds, amphibians and fish. TBG has 1000 fold greater binding affinity than prealbumin.

Thus species with TBG (humans, primates and dogs) have lower percentages of unbound active T4 (and T3) than species where binding is to albumin and prealbumin (rat, mouse and chicken). As a result, T4 bound to proteins with lower affinity in the rodent is more susceptible to removal from the blood by metabolism and excretion from the body. This difference in T4 half-life results in a 10-fold greater requirement for endogenous T4 in the rat thyroid than in the adult human thyroid. The accelerated production of thyroid hormone in the rat is driven by serum TSH levels that are about 6- to 60-fold higher than in humans. Thus, the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormone. Increases in TSH levels above basal levels in rats could more readily move the gland toward increased growth and potential neoplastic change than in humans.

The male rat is more sensitive to follicular cell hyperplasia and neoplasia as it has higher circulating TSH levels than the females and therefore are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females more frequently develop thyroid cancer.

There are marked species differences in the sensitivity of follicular cell thyroperoxidase enzyme to inhibition. This results in nodule development in species (rat, dog, mouse) sensitive to inhibition but not in species (primate, guinea pig, chicken, human) resistant to inhibition.

Although qualitatively the rat is an indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive, effects on rodents would represent a conservative indicator of potential risk for humans. Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid-pituitary functioning, over a lifetime. The relevance of the experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency, and time) should be considered. In addition, chemically induced effects that are produced by short-term disruption in thyroid-pituitary functioning appear to be reversible when the stimulus is removed.

Since the JECFA and SCF evaluations, no new data are available on chronic toxicity/carcinogenicity. The Panel considered the weight-of-evidence still showed that the tumorigenic effects of Erythrosine are secondary to its effects on thyroid function and not related to any genotoxic activity. Two studies have shown that Erythrosine has an oncogenic effect in the thyroid gland of rats. The weight-of-evidence is that these tumours are elicited by a non-genotoxic mechanism. Erythrosine-induced rodent thyroid tumours may be considered of limited relevance to humans; an approach which is consistent with previous evaluation of Erythrosine. The Panel concurred with this conclusion.

In developmental toxicity studies, Erythrosine does not adversely affect development of young animals at dose levels up to 500 mg/kg bw/day, which is the highest dose level tested for this endpoint. There are no indications, based on the studies evaluated by JECFA and SCF, that Erythrosine can adversely affect male fertility at dose levels up to 2000 mg/kg bw/day, which is the highest dose levels tested (Albridge et al., 1981; Vorhees et al., 1983). Two more recent studies have however indicated that Erythrosine may affect testicular function. The study of Vivekanandhi et al. (2006) indicates that Erythrosine causes decreases in sperm motility at doses of 64 mg/kg bw/day onwards and decreases in sperm counts and increases in sperm abnormalities from 128 mg/kg bw/day onwards. Also the study of Abdel Aziz et al. (1997), evaluated by TemaNord, indicates that testicular function and reproductive performance may be affected by Erythrosine. This is not in line with the results of the other reproductive studies in which Erythrosine did not adversely affect fertility at dose levels up to 2000 mg/kg bw/day. The Panel noted that methodological issues involved in measurement of sperm parameters have been identified (Bell et al., 2010), and that there is no evidence of a functional effect on fertility at much higher dose levels. The Panel also noted that these doses are substantially higher than the NOAEL from the critical study in humans. Therefore the Panel concluded that these studies would not provide a basis for revising the ADI.

However, in a human study (Gardner et al., 1987), increases in serum TSH concentrations, an increased TSH response to TRH, increases in serum PBI and serum iodide concentrations were observed.

The Panel considered that Erythrosine has a minimal effect in humans at a clinical oral dose of 200 mg daily over 14 days, while a dose of 60 mg daily was without effect (Gardner et al., 1987). The current ADI adopted by the JECFA and the SCF is based on this study. The Panel concurred with their identification of this as the critical study. The 60 mg dose was taken to be the equivalent of 1 mg/kg bw/day. By applying a safety factor of 10 to allow for the small number of subjects used in the study and its relatively short duration, an ADI of 0-0.1 mg/kg bw per day was derived.

Altogether, the Panel concluded that the present database on semi-chronic, reproductive, developmental and long-term toxicity, do not provide a reason to revise the ADI of 0-0.1 mg/kg bw/day.

The ADI of 0.1 mg/kg bw/day can for an adult be reached by consumption of 30 g cocktail cherries with the permitted maximum level of 200 mg Erythrosine/kg which is unlikely to occur on a frequent basis. The JECFA, based on evaluation of several national intake estimates, indicated that it is unlikely that long-term intake of Erythrosine will exceed the ADI (JECFA, 2000).

The EU intake monitoring scheme ("SCOOP" report) estimates an adult intake of 0% of the ADI (*i.e.*, negligible) for both children and adults. The JECFA has also assessed national intakes of Erythrosine (JECFA, 2000) where EU estimates were based on model diets constructed for high consumers in the UK. Based on the maximum permitted levels in foodstuffs in the EU and 97.5th percentile food consumption (adjusted for body weight) from dietary records, the estimated intake of high consumers in the UK was 0.01 mg/kg bw/day (representing 13% of the ADI) for adults and 0.05 mg/kg bw/day for children between 1.5 to 4 years (representing 52% of the ADI).

The Panel also noted that the limit test for heavy metals (expressed as lead) 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.

The Panel noted 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, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the framework Directive 89/107/EEC[5] 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[6]of the European Parliament and of the Council of 16 December 2008 on food additives 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.

[5] Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption, OJ L 40, 11.2.1989, p. 27

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

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Erythrosine (E 127) when used as a food colouring substance.

Erythrosine (E 127) is authorised as a food additive in the EU and was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1990 and the EU Scientific Committee for Food (SCF) in 1989.

The Panel on Food Additives and Nutrient Sources added to Food (ANS) 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. The Panel also had access to information submitted to the Scientific Committee on Consumer Safety.

2. Technical data

2.1. Identity of the substance

Erythrosine (E 127) is a xanthene dye with the formula $C_{20}H_6I_4Na_2O_5$ (H_2O). It has a molecular weight of 879.84 g/mol and CAS Registry Number 16423-68-0. Its full chemical name is disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate. Its structural formula is presented in Figure 1:

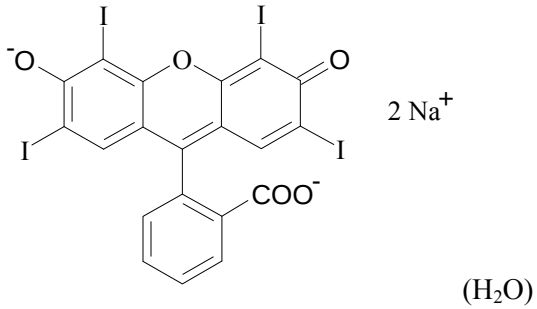

Figure 1: Structural formula of Erythrosine

At least 78 synonyms are in use (ChemIDplus advanced, via internet, 2007). The most commonly used synonyms in published literature are Erythrosine B, CI Food Red 14, FD & C Red No. 3, C.I. 45430, INS No. 127 and Erythrosine sodium (closed form).

Erythrosine is a red odourless powder or granules with a calculated Log P (octanol-water) of 4.95 at 25°C (Molinspiration, 2007) which is soluble in water (≤ 9% w/w) and ethanol.

2.2. Specifications

Specifications have been defined in the Directive 2008/128/EC[7] and by JECFA (JECFA, 2006) (Table 1).

[7] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p.20.

Erythrosine (E 127) consists essentially of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl) benzoate monohydrate and subsidiary colouring matters together with water, sodium chloride and/or sodium sulphate as the principal uncoloured components. Erythrosine is described as the sodium salt. The calcium and the potassium salt are also permitted (2008/128/EC).

The purity is specified as not less than 87% total colouring matters, calculated as the anhydrous sodium salt, including ≤ 4 % subsidiary colouring matters. The remaining 13 % may be accounted for by sodium chloride or sodium sulphate (but this is not mentioned explicitly).

Table 1: Specifications for Erythrosine according to Commission Directive 2008/128/EC and JECFA (2006).

Purity	Commission Directive 2008/128/EC	JECFA, 2006
Inorganic iodides calculated as sodium iodide	≤ 0.1%	≤ 0.1%
Fluorescein	≤ 20 mg/kg	< 20 mg/kg
Subsidiary colouring matters (except fluorescein)	≤ 4.0%	≤ 4.0%
Water insoluble matter	≤ 0.2%	≤ 0.2%
Ether extractable matter	≤ 0.2%[a]	≤ 0.2%[b]
Arsenic	< 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Zinc	-	≤ 50 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-
Loss on drying at 135° C together with chloride and sulphate calculated as sodium salts	-	≤ 13%
Tri-iodoresorcinol	≤ 0.2%	≤ 0.2%
2-(2,4-dihydroxy-3,5-diodobenzoyl) benzoic acid	≤ 0.2%	≤ 0.2%

[a] from a solution of pH from 7 through 8
[b] from a solution of pH not less than 7

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

The Panel noted that the limit test for heavy metals (expressed as lead) is considered obsolete and is being replaced with limits for individual metals of concern.

According to Directive 2008/128/EC, the above purity criteria for the pure substance also apply to the aluminium lake except that the general method for hydrochloric acid insoluble matter in aluminium lakes is not applicable for this colour and is replaced by a sodium hydroxide insoluble matter method. The aluminium lake should contain no more than 0.5% sodium hydroxide 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 Erythrosine, other than reference to the General Specifications of Aluminium Lakes of Colouring Matters (JECFA, 2006). The Erythrosine used in the production process should comply with the specifications as given above and the aluminium lake should contain no more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% hydrochloride acid-insoluble matter (determined using modified general method for hydrochloric acid insoluble matter applicable for Erythrosine lake), 0.2% ether-extractable matter, 3 mg arsenic/kg and 5 mg lead/kg. Unreacted aluminium chloride may also be present in the final product (not specified).

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

2.3. Manufacturing process

Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride (Mai et al., 2006). Erythrosine may be converted to the corresponding aluminium lake by reacting aluminium oxide with 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 foods

A few methods for the determination of Erythrosine in foods are described in the literature. These methods include High Performance Liquid Chromatography (HPLC) and capillary electrophoresis (Yoshioka and Ichihashi, 2007).

2.5. Stability, reaction and fate in food

Limited information on the reaction and fate of Erythrosine in food is available. 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).

When cherries coloured with Erythrosine are stored in uncoated steel cans, fluorescein is readily formed. The production of fluorescein from Erythrosine occurs in the presence of iron and/or tin and free organic acid as a result of electrochemical reduction in the can (Dickinson and Raven, 1962). This does not occur in coated cans.

2.6. Case of need and proposed uses

Authorised use levels have been defined in the Directive 94/36/EC[8] on colours for use in foodstuffs.

Erythrosine is a synthetic food colouring substance which is permitted in the EU for certain limited uses only. Table 2 summarises the foodstuffs that are permitted to contain Erythrosine up to specified maximum permitted levels (MPLs) set by Directive 94/36/EC.

Table 2: Maximum permitted usage levels of Erythrosine according to European Parliament and Council Directive 94/36/EC

Foodstuffs	Maximum Permitted Level (mg/kg)
Cocktail cherries and candied cherries	200
Bigarreaux cherries in syrup and in cocktail	150

No further data on actual levels of use of Erythrosine are available.

[8] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.09.1994 p. 13.

2.7. Information on existing authorisations and evaluations

Erythrosine has been evaluated several times by JECFA and by the SCF. Both fora have established an Acceptable Daily Intake (ADI) of 0 – 0.1 mg/kg bw/day (JECFA most recently in 1990 and SCF in 1989).

The US Code of Federal Regulation, Title 21, Section 74.303, states that FD & C Red No. 3 (Erythrosine) may be safely used as a colouring in general foods in amounts consistent with Good Manufacturing Practice (GMP) (US Code of Federal Regulation, 2006). In the USA, Erythrosine is permitted for general use, and is commonly used in sweets and foods marketed to children such as candies, popsicles, cake frosting and cake-decorating gels. Concerns regarding the safety of this colour were raised by the United States Food and Drug Administration (US FDA) following the publication of a report indicating that under experimental conditions Erythrosine at high dose levels (4% in the diet) can affect the level of circulating thyroid hormones in rats, thus leading to an increase in the incidence of thyroid tumours. The response of the US FDA in 1990 was withdrawal of permission to use Erythrosine lakes (salts), but not Erythrosine, in all foods, drugs and cosmetics, and to withdraw the use of Erythrosine in cosmetics and externally applied drugs.

In Australia, the Code restricts the use of Erythrosine in foods to preserved cherries known as maraschino cherries, cocktail cherries or glacé cherries. Erythrosine is used to colour these cherries red prior to processing. Food Standards Australia New Zealand (FSANZ) have proposed extending the permitted uses to products such as icing and frostings used in other foods that are more widely consumed (e.g. cakes, biscuits, fancy breads) (FSANZ, 2008).

Erythrosine is used in pharmaceuticals and cosmetics in the EU. The Scientific Committee on Consumer Safety published an opinion on Erythrosine use in toothpaste products in June 2010. GlaxoSmithKline (GSK) considered Erythrosine safe for consumers when used as a colorant in toothpaste products with a maximum concentration of 0.0025% (25 ppm) and estimated exposure from this use to be 0.0002 mg/kg bw/day (GSK, 2008).

2.8. Dietary exposure

Because Erythrosine is only authorised in a few specified foodstuffs, estimating exposure based on the Budget method is inappropriate. It can easily be calculated that the ADI of 0-0.1 mg/kg bw/day can be reached for an adult (assuming 60 kg bw) by consumption of 30 g candied cherries containing the maximum permitted use level of 200 mg Erythrosine/kg.

JECFA evaluated national assessments of intake of Erythrosine from Australia, Brazil, Canada, Japan, New Zealand, the United Kingdom and the United States. The national assessment from the United Kingdom noted that Erythrosine is also widely used in pharmaceutical products in the European Union (> 3000 products in Europe contain Erythrosine according to a report of the European Commission in 1998). Based on the known use levels, the maximum quantity of Erythrosine that could be ingested with a single capsule, sugar-coated pill, or 1 ml of a liquid preparation has been estimated to be 0.013 mg/kg bw/day (assuming 70 kg body weight)(JECFA, 2000). The ADI would be reached only by consuming five to seven pills or capsules or 5-7 ml of a liquid preparation per day, which is considered unlikely on a long-term basis in the UK assessment.

In a more recent evaluation of the North South Ireland Food Consumption Survey (NSIFC) database on a nationally representative sample of adults of the Republic of Ireland and Northern Ireland, aged 18 to 64 years, the intake of those foods containing Erythrosine and the corresponding exposure to Erythrosine from these foods has been calculated. Mean intake of Erythrosine was found to be 0.0031 mg/kg bw/day and 0.01 mg/kg bw/day at the 95th percentile (Gilsenan and Gibney, 2004).

Intake estimates for children are not provided due to a lack of data on cherry consumption in this group.

GSK has estimated that estimated exposure from use of Erythrosine in toothpaste is 0.0002 mg/kg bw/day. There are no usage data for the use of Erythrosine as a colour in pharmaceuticals but the contribution from this source is expected to be low.

The limited authorised uses of Erythrosine preclude Tier 2 or 3 estimates of exposure. The exposure estimates on Erythrosine as indicated above however are in accordance with the Tier 2 approach followed by the Panel and can be considered as valid estimates for Tier 2.

3. Biological and toxicological data

Erythrosine has been evaluated several times by JECFA and the SCF. Erythrosine was also evaluated by TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions and describes the additionally reported new literature data in some more detail.

3.1. Absorption, distribution, metabolism and excretion

JECFA describes several studies on the toxicokinetics of Erythrosine.

Animals

In adult rats, the recovery of Erythrosine (150 mg/rat, dosed by gavage at about 600 to 750 mg/kg bw) in the excreta (faeces and urine) after 5 days was 102%. After intravenous administration of 3 mg Erythrosine/kg bw, an average of 55% of the administered quantity was found in the bile and 1.3% was recovered in the urine in 4 hours. No glucuronic acid conjugation was found (Daniel, 1962).

Erythrosine was found to be metabolically stable in rats; after administration of 500 mg/kg bw Erythrosine, 100% of the amount ingested was excreted with its iodine content intact (Webb et al., 1962).

In rats of both sexes, urinary and faecal excretion of ^{14}C-labelled and ^{125}I-labelled Erythrosine and the distribution of the compound in tissues and body fluids were studied either without pre-treatment or following dosing with unlabelled Erythrosine at dietary levels of 0.5 or 4.0% (equivalent to 250 and 2000 mg/kg bw/day) for seven days. The radioactivity from both radiolabels was excreted predominantly in the faeces, mainly within 48 hours; less than 1% of the dose was excreted in urine. Blood and plasma radioactivity reached maximum levels by one hour, while levels in the liver and kidneys peaked after 4-12 hours. The activity in blood and tissues was very low, suggesting that Erythrosine is not extensively absorbed from the gastrointestinal (GI) tract. Of the tissues examined (liver, kidney, thyroid, brain, and pituitary), the highest levels of radioactivity were found in the liver (maximally 0.145% of the dose of ^{14}C; 0.188% of the dose of ^{125}I). Thyroid residues of ^{14}C were at trace or non-detectable levels, while levels of ^{125}I were detectable but low (maximally approximately 0.01% of the dose), indicating that neither Erythrosine nor its ring-containing metabolites accumulated in the thyroid. The magnitude of the ^{125}I levels in the thyroid was so low that it was not possible to conclude whether the activity resulted from free ^{125}I-iodide in the dose or from ^{125}I-iodide formed by a small degree of metabolic deiodination of Erythrosine. No ^{14}C or ^{125}I was detectable in the brain or pituitary. Small amounts of metabolites, believed to be isomeric diiodo- and triiodofluoresceins, were detected in urine, faeces, plasma, and tissue extracts from the liver and kidney (Obrist et al., 1986).

Protein-bound and total blood iodine levels were elevated in rats given Erythrosine by stomach tubes twice weekly in a chronic study. From experiments with rats and gerbils, JECFA concluded that the elevated protein-bound iodine (PBI) was due to interference by Erythrosine in PBI determinations. In the original publication, it is stated that since the Erythrosine molecule contains a theoretical amount of 56.5% iodine, the elevated PBI values were attributed to presence of Erythrosine in the serum (Bowie et al., 1966; Hansen et al., 1973a), rather than to e.g. elevated levels of T3/T4 (3,5,3'-triiodothyronine/thyroxine (3,5,3',5'-tetraiodothyronine).

In rats, daily high oral doses (> 1 mg) ^{131}I-labelled Erythrosine inhibited uptake of ^{131}I by the thyroid (Marignan et al., 1965).

Erythrosine was orally administered twice weekly to rats in doses of 5, 10, 15 or 50 mg per rat (rats weighing 200-250 g) for six months. Excretion of the dye was mainly in unchanged form in the faeces (Bowie et al., 1966).

Humans

A clinical study evaluated the bioavailability and metabolism of Erythrosine (Ingbar et al., 1983). Five human volunteers (four males and one female, ages 21 to 35 years) received Erythrosine in a milkshake at dose levels of 5, 10, or 25 mg/day in weekly increments for a period of three weeks. The study demonstrated slowly and slightly increasing levels of total serum iodine and PBI associated with the weekly increasing Erythrosine doses. In the other tests for serum T4, T3, Thyroid Stimulating Hormone (TSH) levels, Erythrosine concentration, urinary iodine and Erythrosine excretion, and T3 - resin uptake remained unchanged throughout the three weeks. Increases in serum PBI and total serum iodine during exposure period indicate that a portion of the iodine ingested as Erythrosine appears to be absorbed from the GI tract. The authors considered that the lack of changes in concentration of TSH, T4, and T3 in serum indicated that both the thyroid function and thyroid regulatory mechanisms were unaffected by the ingestion of Erythrosine during a 3-week period at a dose as high as 25 mg/day. Analytical testing of Erythrosine in serum and urine revealed levels less than the limit of detection (0.05 mg/l).

In a further study, three subjects received a single oral dose of 80 mg of ^{131}I- radio-labelled Erythrosine in a milkshake (Ingbar et al., 1984b) Three other subjects consumed a single oral dose of 75 mg of ^{131}I-Erythrosine in a water vehicle. The subject's thyroids were blocked with a saturated solution of potassium iodine to block the uptake of ^{131}I. Stool and urine collections were made, and fasting blood samples were collected. ^{131}I administered as ^{131}I-Erythrosine was eliminated rapidly and nearly completely, whereas whole body ^{131}I content was at levels of 1.0% or less of the administered dose within seven days. No differences were observed between the rates of elimination of Erythrosine administered in the different vehicles and faecal elimination of ^{131}I-Erythrosine was rapid after an initial delay of 24 hours. Faecal recovery of Erythrosine ranged from 80 to 100% with four subjects exhibiting approximately 100% recovery where recoveries of less than 100% were attributed to incomplete collection since radioactivity was not detected in whole body counts, urinary ^{131}I excretion, or serum ^{131}I concentrations. Negligible quantities of ^{131}I appeared in the serum following oral administration but ^{131}I was detected in urine during the first 24 to 48 hours, although these quantities accounted for less than 0.38% of the administered dose in any subject. Serum T3, T4 and TSH concentrations were not affected during the study.

The studies conducted by Ingbar and colleagues (1983, 1984b) provide supporting data that only a very small fraction of the ingested Erythrosine, approximately 1.0%, is absorbed from the GI tract.

The SCF did not specifically address toxicokinetics.

The TemaNord evaluation (2002) referred to the studies described in the JECFA evaluations. No new toxicokinetic data on Erythrosine were published since the TemaNord evaluation (2002).

The JECFA (1986 and 1988) requested additional kinetic studies on Erythrosine relating the amount of absorption to the amount ingested, which would enable a correlation to be established between blood/tissue levels of Erythrosine and effects on thyroid. Although no such kinetic data were provided in the last monograph addenda (JECFA, 1990), JECFA no longer requested these data since an ADI could be derived from the No-Effect-Level for effects on thyroid in humans.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA describes several acute oral toxicity studies which were conducted in a range of animals.

The LD_{50} values derived from the tests were: mouse, 2560-6800 mg/kg bw; rat, 1840-7100 mg/kg bw; and gerbil, 1930 mg/kg bw (Lu and Lavallée, 1964; US FDA, 1969; Hansen et al., 1973b; Butterworth et al., 1976a; Yankell and Loux, 1977).

The SCF opinion refers to several oral toxicity studies, but no details were given. In the TemaNord evaluation (2002), acute toxicity was not addressed.

No new data on acute oral toxicity have been published since the previous evaluations.

3.2.2. Short-term and subchronic toxicity

Several short-term and subchronic studies were evaluated by JECFA.

Rats

Carworth farm E strain Specified Pathogen Free (SPF) rats (15 animals/sex/group) received 0, 0.25, 0.5, 1.0, or 2.0% Erythrosine in the diet (equivalent to 0, 125, 250, 500, or 1000 mg/kg bw/day) for 90 days. There were no effects attributable to treatment on the rate of body weight gain or food intake or on the results of haematological examinations, serum analyses or renal function tests. Thyroid weight relative to body weight was slightly increased in rats receiving 1.0 and 2.0% Erythrosine. Haematological examination, serum analysis, and renal function tests revealed no effects attributable to Erythrosine. A dose-dependent increase in caecal weights was seen, although histology was normal. A dose-related deposition of pigment, identified as protein-bound Erythrosine, was seen in the renal tubules of males; in females, this effect was seen only at the 2% level. In addition, protein-bound Erythrosine increased in a dose-dependent manner, as did both total PBI and non-protein bound iodine. Although relative thyroid weights were slightly increased at the 1.0 and 2.0% levels, thyroid function was not impaired as indicated by normal histopathology, the absence of effects on serum thyroxine levels, and normal rates of oxygen consumption in treated animals. The no toxic effect level was reported to be 0.25% in the diet, equivalent to a dietary intake of 160 to 170 mg/kg/day (Butterworth et al., 1976a). JECFA reference this study to an unpublished BIBRA report submitted to them (JECFA, 1975) which appears to be the basis for this publication.

Sprague-Dawley female rats (12-20 animals per group) were exposed to Erythrosine in the diet at dose levels of 0 or 0.2% (equivalent to 0 or 100 mg/kg bw/day) for either 6 or 12 months. During the last 12 weeks of the experimental period, a slight decrease of body weight gain was observed in rats exposed for 12 months. Other parameters such as food consumption, haematology, clinical chemistry, urinalysis and organ weights were comparable among treated and control rats in both the 6- and 12-month groups. Sporadic pathological changes were observed in treated and control rats and were not considered treatment related (Sekigawa et al., 1978).

Pigs

Twenty-four pigs (large white strain, 3 animals/sex/group weighing approximately 20 kg each) were fed Erythrosine in their diets at dose levels of 0, 167, 500 or 1500 mg/kg bw/day for 14 weeks. No treatment-related effects were reported for clinical observations, body weight, haematology and urine analysis. The treated pigs exhibited decreased levels of serum T4 compared to controls. There were dose-related increases in the serum levels of PBI, non-PBI, and protein-bound Erythrosine in animals of all treated groups. A dose-related increase in thyroid weight was noted, although the differences were statistically significant only in female pigs at the higher-dose levels (500 and 1500 mg/kg

bw/day) when compared with the controls. None of the treated pigs revealed pathological changes of the thyroid (Butterworth et al., 1976b).

The SCF opinion refers to short-term studies in rats and pigs, but no data are provided.

The TemaNord evaluation cites the studies described by the JECFA and the SCF.

No relevant, more recent, additional short-term/sub-chronic studies were identified.

3.2.3. Genotoxicity

Erythrosine was tested for mutagenic activity and showed a very slight but statistically significant mutagenic effect on *Escherichia coli* at a concentration of 5 mg/ml. It was found that the xanthene molecule itself was the causative factor and that the substituent groups only modify the effect (Lück et al., 1963; Lück and Rickerl, 1960).

No mutagenic activity was observed using *E. coli* WP2 UVrA as the indicator organism (Haveland-Smith and Combes, 1980).

A lack of mutagenic activity of Erythrosine in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 was observed when tested in the Ames test at concentrations ranging from 1 to 10,000 µg/plate with or without metabolic activation (Auletta et al., 1977; Bonin and Baker, 1980; Brown et al., 1978) and similar negative results in the Ames test were obtained in several other studies (Tarján and Kurti, 1982; Ishidate et al., 1984; Jaganath and Myth, 1984a; Muzzall and Cook, 1979).

Erythrosine was tested for the induction of point mutations in the *Salmonella typhimurium* plate-incorporating assay using strains TA1535, TA1537, TA1538, TA98 and TA100. No mutagenic effects were observed. In a modified assay using the addition of flavin mononucleotide to the activation mixture, negative results were also obtained (Cameron et al., 1987). Erythrosine was non-mutagenic in the Ames test in strains TA97a, TA98, TA100, TA102, and TA104 up to a concentration of 2 mg/plate, with or without metabolic activation using rat liver S9 or caecal-cell free extracts. The comutagens harman and norharman (+/-S9) did not affect mutagenicity. A dose-dependent suppression in spontaneous reversion frequencies was observed. Toxicity was observed in the repair-deficient strains (TA97a, TA98 and TA100) but not in the repair-proficient strains (TA102 and TA104). According to the authors, Erythrosine was antimutagenic to benzo(a)pyrene and mitomycin C but not to 4-nitroquinoline-N-oxide and methylmethanesulphonate (Lakdawalla and Netrawali, 1988a).

Erythrosine was negative in the host-mediated rec-assay (Kada et al., 1972) and in a mouse host-mediated assay using *Salmonella typhimurium* strains TA98, TA100 and TA1537 (Tarján and Kurti, 1982).

Erythrosine was inactive in DNA-repair, fluctuation and treat-and-plate assays (Haveland-Smith et al., 1981) and in mitotic gene conversion assays using yeast strain B234 (Sankaranarayanan and Murthy, 1979) and strain D5 (Jaganath and Myhr, 1984b; Matula and Downie, 1984). Reported positive results in a mitotic gene conversion assay using yeast strain D7 (Matula and Downie, 1984) and in a reverse mutation assay in yeast XV185-14C have been called into question (Brusick, 1984).

An in vitro chromosome aberration assay in hamster cells indicated a weak effect of Erythrosine (scoring included gaps, which were not specified). JECFA suggested that this may have resulted from osmotic effects of the high concentration (6 mg/ml) of Erythrosine at which the observations were noted, but although Ishidate et al. (1984) investigated the influence of osmolality on induction of chromosomal aberrations, they did not suggest that this played a role for Erythrosine.

In the mouse lymphoma assay using L5178Y $TK^{+/-}$ cells, Erythrosine (Acid Red 51) at concentrations up to 200 µg/ml was reported positive both assay in the presence and absence of metabolic activation

at concentrations exerting high toxicity (Cameron et al., 1987). Erythrosine was found to be non-mutagenic in a mouse lymphoma L5178Y TK+/- forward mutation assay in the presence and absence of metabolic activation (Cifone and Myhr, 1984) and did not induce cell transformation in rat embryo cells in vitro or in vivo (Price et al., 1978).

Erythrosine was reported to increase the yield of sporulation-minus mutants of *Bacillus subtilis* excision repair-proficient strain 168, approximately equal to 400% at a concentration of 1 mg/ml under ambient light conditions. This effect was not seen in the excision repair-deficient strain her-9. The authors considered that these results indicated that there was no involvement of excision repair in the dye-mediated increase in mutants (Lakdawalla and Netrawali, 1988b). The Panel noted that interpretation of the results was complicated by the high toxicity in both strains.

Erythrosine was tested for genotoxicity in V79 Chinese hamster lung cells. Reduced colony size was seen at 200 µg Erythrosine/ml and more than 90% lethality was seen at 400 µg Erythrosine/ml. Erythrosine was non-mutagenic to V79 cells at the Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and Na^+, K^+, ATPase gene loci, and did not increase the frequency of Sister Chromatid Exchanges with or without rat hepatocyte activation. At 300 µg/ml, Erythrosine produced an increase in micronucleus frequency in the absence of hepatocytes. A dose-related increase in the mitotic frequency was observed due to an increase in the number of first mitosis. Thus, increased genotoxicity was observed only at concentrations well within the range of cytoxicity (Rogers et al., 1988).

Erythrosine did not induce DNA-repair in rat hepatocytes in vitro at concentrations up to 1 mM, or in vivo after an oral dose of 200 mg/kg bw (Kornbrust and Barfknecht, 1985).

Erythrosine was inactive in the mouse micronucleus assay (Tarján and Kurti, 1982), following i.p. administration at up to 240 mg/kg bw in male and female CD-1 mice (Ivett and Myhr, 1984; Lin and Brusik, 1986) and in male B6C3F1 mice following two administrations of up to 200 mg/kg bw 24 hours apart (Zijno et al., 1994). Erythrosine did not induce sister chromatid exchanges in peripheral lymphocytes of male B6C3F1 mice and following two administrations of up to 200 mg/kg bw 24 hours apart (Zijno et al., 1994).

The SCF evaluated several studies in 1989 without providing any details. The SCF mentioned that earlier mutagenicity data based mainly on bacterial assays were largely negative, although there was an indication that the compound could produce gene mutation in yeast, albeit only in the dark, and chromosome damage in vitro; negative results however, were obtained in 2/3 in vivo assays for clastogenicity. Also they mentioned that more recently, negative results have been obtained in assays for point mutations in bacteria and gene mutation in cultured mammalian cells (SCF, 1989).

New literature

Groups of four mice were dosed once orally with Erythrosine, and eight organs (glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow) were analysed in the Comet assay 3 hours and 24 hours after exposure. Erythrosine induced dose-related DNA damage in the glandular stomach, colon and urinary bladder after oral administration of 100 mg/kg bw and 2000 mg/kg bw and in the lung at 2000 mg/kg bw in the groups that were sacrificed 3 hours after exposure. In the groups that were sacrificed 24 hours after exposure, no DNA damage was evident (Kawaguchi et al., 2001; Sasaki et al., 2002). The negative result in the bone marrow was consistent with the negative results in bone marrow chromosomal aberration studies.

Erythrosine (330 µM) induced chromosome aberrations in Syrian Hamster Embryo (SHE) cells in the presence, but not in the absence of metabolic activation. The percentage of cells with polyploidy or endoreduplication was enhanced by Erythrosine (330 µM) in the presence of metabolic activation (Hagiwara et al., 2006). However, in another study Erythrosine (up to 110 µM) did not induce chromosome aberrations in SHE cells in the absence of metabolic activation (Miyachi et al., 2005).

Swiss albino mice were exposed to Erythrosine by oral gavage for 30 days (controls 15 mice; control reversals five mice, low dose (62.5 mg/kg bw/day) 10 mice, intermediate dose (125 mg/kg bw/day) 15 mice, intermediate reversals five mice and high dose (250 mg/kg bw/day) 10 mice. A significant dose-dependent decrease in cell proliferation was observed. The percentage of chromosomal aberrations was significantly decreased in the intermediate and the high dose group (Devi et al., 2004). Only an abstract was available providing limited details.

Male rats (20 animals/dose) were exposed to Erythrosine (0, 0.08 or 0.4 g/kg diet) for 30 days and the animals were sacrificed 6 hours after the experimental period. Changes in mutagenic activities, as an index for evaluating of possible toxic effects, were monitored by measuring chromosomal aberrations of rat bone marrow, nucleic acids and total protein concentrations of rat liver and brain in 10 animals/group. The study found that Erythrosine induced chromosomal aberrations. The mitotic index was statistically increased at the low dose but was significantly decreased at the high dose. Biochemical assays revealed that nucleic acids and total protein concentration was significantly increased in brain and liver by both doses (Mekkawy et al., 2000). There was neither a dose-response nor consistency in the findings and therefore no conclusions can be drawn from this study.

Based on the available data the Panel considered that that Erythrosine is not of concern with respect to genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

JECFA evaluated several studies in mice, rats, gerbils and dogs.

Mice

Mice (a total of 122 male and female mice aged 50-100 days, produced by mixed breeding from five different strains) were given a diet containing 1 mg Erythrosine/day for up to 700 days. Tumour incidence was not significantly greater than in the negative controls (Waterman and Lignac, 1958).

Mice (70 animals) were fed Erythrosine at 1 or 2% in the diet (equivalent to 1400 or 2900 mg/kg bw/day). Because of the small number of animals surviving the experiment and the small number of tumours found, no effect on tumour formation could be attributed to the colour (US FDA, 1969).

Charles River CD-1 mice (60 animals/sex/dose) were exposed to Erythrosine in the diet at dose levels of 0, 0.3, 1.0, or 3.0% for 24 months, equivalent to on average 0, 424, 1474, or 4759 mg/kg bw/day for males and 0, 507, 1834, or 5779 mg/kg bw/day for females respectively. With the exception of significantly decreased body weights (throughout the entire study) of males and females at the 3.0% dose level, other investigated parameters (mortality, food intake, haematology, gross pathology and histopathology) were not adversely affected by Erythrosine treatment at any dose level (Richter et al., 1981). These data have now been published in Borzelleca and Hallaghan (1987). There was a significant increase in lymphocytic lymphoma in male mice in the lowest dose group. However, since no dose-effect relationship was observed, this was considered to be of no toxicological significance (Borzelleca and Hallaghan, 1987).

ICR mice (7-week old, 27-38 g, 50 animals/sex/dose) were fed diets containing Erythrosine at dose levels of 1.25 or 2.5% (equivalent to 1786 or 3573 mg/kg bw/day) for 18 months. The mice received Erythrosine in cube diet for the first 20 weeks, and thereafter the Erythrosine was mixed with the basic powder diet. All animals in the experimental groups were then fed the basic diet free of Erythrosine for an additional six months, after which they were sacrificed and autopsied. Mortality was greater among animals exposed to Erythrosine than among the controls (approximately 61% of the animals died in the 2.5% group, 59% in the 1.25% group, and 36% in the control group). Body weight gains were not adversely affected by Erythrosine ingestion. Animals in both experimental groups exhibited a high incidence of lymphocytic leukemia, and sporadic cases of pulmonary adenomas were observed. The frequency of both lesions was in the range spontaneously-occurring in this strain of mice. The

results indicate that Erythrosine was not carcinogenic to ICR mice under the experimental conditions utilised (Yoshii and Isaka, 1984).

Rats

Rats (5/sex) were fed Erythrosine at a level of 4% of the diet (equivalent to 2000 mg/kg bw/day) for up to 18 months. Gross staining was observed in the glandular stomach and small intestine, and granular deposits were observed in the stomach, small intestine, and colon. Hepatic cirrhosis was noted in one out of four rats living up to 12 months. None of the rats fed Erythrosine developed treatment related tumours. The study reports that fifty control animals observed for 20 months or more failed to develop tumours or hepatic cirrhosis (Willheim and Ivy, 1953).

Osborne-Mendel rats (weanling, 12/sex/dose) were fed 0, 0.5, 1.0, 2.0, or 5.0% Erythrosine in their diet (equivalent to 0, 250, 500, 1000 or 2500 mg/kg bw/day) for two years. Growth depression was observed in rats given 5% Erythrosine. Relative spleen weight was depressed in all male test groups and in females at the 5% level. Slight caecal enlargement was noted at 1%, which increased with dose, but the histology of the enlarged ceaca was normal. No other gross or histopathological findings related to colour administration were noted (Hansen et al., 1973b).

Rats (100-days old, 25/sex/dose; control group 50/sex/dose) were fed 0, 0.5, 1.0, 2.0, or 4.0% Erythrosine in their diets (equivalent to 0, 250, 500, 1000, or 2000 mg/kg bw/day) for 86 weeks, and rats (100-days old, 25/sex/dose) were intubated twice a week for 85 weeks with 0, 100, 235, 750, or 1500 mg Erythrosine/kg bw. After these treatments, the animals were then kept on normal diets for the remainder of the two years of the study. Body weight decreases were seen at the 2 and 4% levels. Elevated PBI values, due to interference by Erythrosine with PBI determinations rather than due to thyroid dysfunction, were seen. In the original publication, it is stated that since the Erythrosine molecule contains a theoretical amount of 56.5% iodine, the elevated PBI values were attributable to Erythrosine present in the serum. T4 iodine levels were not affected. There were no other haematological differences and no anaemia was seen. No adverse gross pathology was noted; histopathology examinations did not show any colour-related abnormalities (Hansen et al., 1973a).

Charles River CD weanling rats (70 rats/sex/dose) were fed Erythrosine in the diet at levels of 0.1, 0.5, or 1.0%, corresponding to 49, 251, or 507 mg Erythrosine/kg bw/day for males and 61, 307, or 642 mg Erythrosine/kg bw/day for females for 30 months after *in utero* exposure. Two concurrent control groups (70 animals/sex/group) received no colour in the diet. There were no consistent significant compound-related effects during the *in utero* phase. In the main study, there were no consistent significant compound-related effects on the following: physical observation, behaviour, mortality, food consumption, haematology, clinical chemistry, urinalysis, or ophthalmological findings. Mean body weights of control and treated rats did not differ significantly during the exposure period. The gross pathological changes that were noted could not be attributed to treatment with Erythrosine. The incidence of non-neoplastic lesions was comparable between treated and control groups. There was a statistically significant increase in the incidence of benign thyroid tumours (follicular adenomas): 6/68 in the 1.0% female test group versus 0/140 in the control group. The incidence of malignant tumours in rats of treated groups was comparable with that of the controls (Brewer et al., 1981).

Two groups of Charles River CD weanling rats (70/sex/dose) were given Erythrosine in the diet at dose levels of 0 or 4.0% for a period of approximately 29 months after *in utero* exposure. The average consumption of the Erythrosine was 2465 mg/kg bw/day for males and 3029 mg/kg bw/day for females. There were no consistent significant compound-related effects on the following; physical observations, behaviour, mortality, food consumption, haematology, clinical chemistry, urinalysis, or ophthalmological findings. Mean body weights of treated rats (both sexes) were slightly lower throughout the study than those of the control rats. These differences were statistically significant except at weeks 3-5 and 122 (males) and at weeks 0-4, 6 and 114 (females). The mean absolute and relative thyroid weights of treated males were more than twice those of the controls. Histopathological examination revealed that the incidence of thyroid hyperplasia (follicular and C-cell) was significantly

increased in treated males. There was a statistically significant increase in the incidence of follicular adenoma of the thyroid in treated male rats (16/68) when compared with the controls (0/69). The incidence of malignant tumours, including thyroid C-cell and follicular carcinomas, was comparable among treated and control rats (Brewer et al., 1982).

F344 rats (6-weeks old, pathogen-free, 50 animals/sex/dose) were fed diets containing Erythrosine at levels of 1.25 or 2.5% (equivalent to 625 or 1250 mg/kg bw/day) for 18 months. The control group consisted of 30 animals/sex that received a diet free of Erythrosine. For the first 20 weeks of treatment, Erythrosine was given in pelleted diet and for the remaining treatment period it was given in powder diet. Rats were sacrificed 18 months (control rats 24 months) after the start of the study. No parameters other than histopathology were reported. Histopathological examinations revealed sporadic cases of spontaneous neoplasms (tumours of the genital system, endocrine system, haematopoietic system and digestive system), but their frequencies were similar among animals in the Erythrosine-treated groups and they were comparable to the controls. No pathological changes were observed in the thyroid glands (Fukunishi et al., 1984).

The results of the two long-term feeding studies in rats after *in utero* exposure to Erythrosine (Brewer et al., 1981; Brewer et al., 1982) that were reviewed by JECFA at the thirtieth meeting have now been published (Borzelleca et al., 1987). In the statistical analyses thyroid follicular cell adenomas and carcinomas were treated as separate tumour classes. The authors' conclusion remains that Erythrosine at a level of 4% in the diet for 128 weeks induces an increased incidence in thyroid follicular cell adenomas in male rats (15/69) compared to controls (1/69). The incidence of thyroid follicular cell carcinomas (3/69) was not statistically significantly different from the control value (2/69). In females at the 4% level the incidence of thyroid follicular cell adenomas (5/69) or carcinomas (0/69) were not different than the controls (0/69 and 0/69, respectively). In female rats fed 0.1, 0.5, or 1% Erythrosine in the diet, a numerical increase in adenomas was observed (1/67, 3/68 and 5/69, respectively compared to 1/139 for control females), but the increases were not statistically significant. The incidences of females with carcinomas were 0/67, 0/68 and 1/69 compared to 0/139. In the males, at the 0.1, 0.5 and 1.0% Erythrosine dose levels the incidence of adenomas (0/67, 2/68 and 1/69 compared to 0/139) and carcinomas (3/67, 1/68, and 3/69 compared to 0/139) were not considered significantly different.

The microscopic findings in the thyroids from the above-mentioned studies and the statistics used have been reviewed (FD & C Red No. 3 Review Panel, 1987; Federal Register, 1990). This original study report is not accessible, therefore the figures mentioned below have not been verified (and differ from the figures of Borzelleca et al., 1987 which appears to be the published version of this study). Slight discrepancies in the diagnoses of adenomas/carcinomas were reported. When the combined incidence of adenomas and carcinomas was used in the statistical evaluation, the following results were obtained: as might be expected an increased incidence of combined adenomas and carcinomas was seen in the males fed 4% Erythrosine in the diet (18/68) compared to control males (2/68). A statistically significant increase was also found for combined adenomas and carcinomas in male rats fed 0.1, 0.5 or 1.0% Erythrosine for 122 weeks (3 adenomas and 3 carcinomas in 64 rats, 7 adenomas and 1 carcinoma in 66 rats, 1 adenoma and 3 carcinomas in 57 rats, respectively, compared to 0 adenomas and 1 carcinoma in 128 control male rats). In the female rats, a significant increase in tumour yield was only found in the 1.0% group (5 adenomas and 1 carcinoma in 68 rats compared to 1 adenomas and 0 carcinomas in 138 control female rats). JECFA agreed that it was appropriate to combine thyroid follicular-cell adenomas and carcinomas in the statistical analysis, in view of evidence that adenomas are an earlier stage of carcinomas in the thyroid.

To investigate whether the thyroid tumours found after chronic feeding of Erythrosine to male rats at a dose level of 4.0% in the diet (corresponding to 2000 mg/kg bw/day) as described above, resulted from excess iodine (either as a contaminant of the colour or as iodine metabolised from the colour) or from another non-iodine-related property of Erythrosine, Charles River CD rats (35 animals/sex/dose) were exposed continuously for 27 weeks as follows:

Group 1 - unadulterated diet.

Group 2 - 80 mg of sodium iodide/kg of diet.

Group 3 - purified Erythrosine at 4.0% in the diet.

Group 4 - purified Erythrosine at 4.0% in the diet plus 80 mg of sodium iodide/kg of diet.

Group 5 - purified Erythrosine at 4.0% in the diet plus 160 mg of sodium iodide/kg of diet.

Group 6 - commercial Erythrosine at 4.0% in the diet.

The feeding of commercial Erythrosine at a level of 4% in the diet produced hyperthyroidism. TSH and T4 were elevated, while T3 concentrations were depressed. Changes in clinical chemistry parameters, body weight, and food consumption were also indicative of hyperthyroidism. Additional purification of the commercial preparation of Erythrosine to remove free iodide did not modify these effects. These responses were not found after feeding a diet spiked with sodium iodide only. This study demonstrated that thyroid changes observed in this and former studies are associated with increased TSH concentrations. However, the mechanism for these effects of Erythrosine cannot be determined from the results of this study (Couch et al., 1983).

Rats (20 animals) received weekly subcutaneous (s.c.) injections of 1 ml of a 5% aqueous solution of Erythrosine for 596 days (85 weeks), corresponding to in total 2.65 g Erythrosine/animal. Seven rats survived 300 days or more. No tumours were observed (Umeda, 1956).

Rats (18 animals) received weekly s.c. injections with aqueous solutions of Erythrosine at 12 mg/animal for two years. No tumours, neither at the injection sites, nor in other parts of the body, were observed (Hansen et al., 1973b).

An additional study in rats was evaluated by TemaNord and by the Scientific Committee for Medicinal Products and Medicinal Devices (SCMPMD). Erythrosine used at 4% in the basal diet and administered for 19 weeks significantly promoted the development of thyroid tumors in partially thyroidectomised rats given N-bis (2-hydroxypropyl) nitrosamine, but had no significant effects in non-thyroidectomised rats (Hiasa et al., 1988).

Gerbils

Mongolian gerbils (approximately 6-months old, 15-16 animals/sex/group) were fed diets containing Erythrosine at levels of 1.0, 2.0, or 4.0% for 105 weeks. Control groups (31 animals/sex) were fed diets free of Erythrosine. Animals of all treated groups exhibited a statistically significant dose-related decrease in body weight gain when compared with the controls. In general, there were slight, and in some isolated cases significant, depressions of haematocrit and haemoglobin values and leucocyte and reticulocyte counts in animals of treated groups. The relative weights of heart, liver, and spleen were significantly decreased in animals of both sexes at the two high-dose levels. Dose-related changes such as enlargement of follicles and, in some cases focal hyperplasia, were observed in the thyroids of treated animals. Histopathology did not reveal any treatment-related effects (Collins and Long, 1976).

Mongolian gerbils (approximately 6-months old, 20-24 males, unclear if also females are included) received Erythrosine (dissolved in water) by stomach intubation, at dose levels of 200, 750, or 900 mg/kg bw per dose twice weekly for 97 weeks. Controls (32 animals/sex) were intubated with distilled water only. The dosages were administered in a volume of 10 ml/kg bw. No treatment-related adverse effects were observed for investigated parameters such as clinical toxicity, mortality, body weight gain, haematology, organ weights, gross pathology or histopathology (Collins and Long, 1976).

Dogs

Two-year feeding studies were conducted with Beagle dogs (3 animals/sex/dose) at levels of 0, 0.5, 1.0, or 2.0% (equivalent to 0, 250, 500 or 1000 mg/kg bw/day) Erythrosine in the diet. All dogs survived the study. No gross or microscopic pathological changes related to colour administration were observed (Hansen et al., 1973b).

The 1989 SCF opinion does not contain a detailed discussion of chronic toxicity or carcinogenicity but concludes that based on the available mutagenicity data, the oncogenicity observed was due to hormonal effects, and that whilst it was not possible to define a NOAEL from the animal data, a human NOAEL for hormonal changes could be identified from clinical data. The TemaNord evaluation refers to the JECFA evaluation on this subject.

No more recent data on chronic toxicity or carcinogenicity of Erythrosine are available.

3.2.5. Reproductive and developmental toxicity

JECFA evaluated two reproductive and developmental toxicity studies.

Groups of Charles River CD rats (23-25 animals/sex/group) received Erythrosine in the diet at dose levels of 0, 0.25, 1.0, or 4.0% (equivalent to 0, 125, 500, or 2000 mg/kg bw/day) for 3 consecutive generations. Mating was after 69 days of Erythrosine exposure for the F0 parental rats. The study showed that during the gestation period slight to moderate reductions in mean maternal body weight gain were noted in females of all generations at the 1.0 and 4.0% dose levels. Slight to moderate reductions in mean pup body weights were recorded at the 4.0% level on lactation days 0, 4, 14, and 21 in all generations. These reductions were statistically significant only on lactation day 21. There were no consistent compound-related effects on the reproductive performance of males and females and pup survival at any dose level in any generation (Albridge et al., 1981).

The SCF opinion mentioned that multigeneration reproduction and teratology studies were available, but details were not provided.

In the TemaNord evaluation, two additional studied were evaluated.

In rats exposed to Erythrosine at doses of 0, 15, 30, 100, 200, 400, or 800 mg/kg bw/day (by gavage) or 0, 0.05, 0.1, 0.2 or 0.4% in drinking water (equivalent to 0, 64, 121, 248 or 472 mg/kg bw/day) on day 0 – 19 of gestation, Erythrosine was neither fetotoxic nor teratogenic (Collins, 1993). In the SCMPMD evaluation (SCMPMD, 1998) similar doses are reported not to be teratogenic in rabbits (Burnett et al., 1974).

In the SCMPMD evaluation, an additional study in rats is described. Oral administration of Erythrosine (1 mg/kg bw/day) to rat pups during their first month of postnatal life failed to elicit any significant effect on the activity or cognitive performance (Golderning et al., 1981).

Swiss male albino mice (10 mice/group) were given Erythrosine daily by gavage at levels of 64, 128 and 256 mg/kg bw/day for 30 days. The control group was given distilled water orally. With increase in dosage of Erythrosine, a decrease in sperm count was observed from the mid dose onwards. Sperm motility was significantly decreased in all treatment groups. Also, a significant increase in sperm abnormalities was observed for the intermediate and the high dose (Vivekanandhi et al., 2006).

Additionally, studies that evaluated the lifetime toxicity/carcinogenicity potential of Erythrosine in rats also evaluated the reproductive toxicity and post natal parameters of the F0 and F1 generation rats (Brewer et al., 1981 (FDA Study E9); Brewer et al., 1982 (FDA Study E8); reviewed by Borzelleca et al., 1987). These studies included both an *in utero* and an F1 phase. In the *in utero* phase, Erythrosine was administered to F0 rats (60 animals/sex group at 0.0) (2 control groups), 0.1, 0.5, or 1.0% (original study) or 0.0 or 4.0% (high-dose study) for 2 months prior to mating.

No adverse effects related to the administration of Erythrosine were observed on fertility, gestation, parturition, lactation, pup survival through weaning or numbers of live and stillborn pups. The reproductive and fertility NOAEL from these studies is the highest dose tested (4% in the diet), approximately 3029 mg/kg bw/day for females and 2464 mg/kg bw/day for males.

An earlier three-generation study which pre-dated GLP regulations was conducted in albino rats. Erythrosine was administered to groups of rats in the diet at levels of 0, 1.25, 12.5, 37.5 and 125 mg/kg bw/day of Erythrosine (20 females and 10 males/dose) in a three-generation reproduction study. No adverse effects on fertility, litter size, litter viability, or post partum development were observed (Anonymous, 1972c).

Vorhees and colleagues (1983) conducted two experiments to evaluate developmental toxicity and psychotoxicty of Erythrosine in rats. Groups of 18-22 pairs (males and females, weighing 200 to 220 g) of adult Sprague-Dawley rats were fed diets containing Erythrosine at levels of 0, 0.25, 0.5 or 1.0% for 2 weeks before mating and during the mating period. The diets were continued for the females throughout gestation and lactation and were provided continuously to their offspring until they reached 90 to 100 days of age. Two years later, this study was replicated using the same dose groups and number of animals per group. In both experiments, parental animals were evaluated for weight and food consumption and females for reproductive success. The offsprings were assessed for effects on behaviour, weight, food consumption, physical development and brain weight. Erythrosine produced no effects on paternal or offspring weight or food consumption. Pre-weaning offspring mortality was significantly increased at the 0.5% and 1.0% dose levels in the first experiment, but not in the second. Mean litter size was not adversely affected by Erythrosine in both experiments. No dose-dependent effects on behaviour were replicated across the two experiments. Based on these data, the authors concluded that there was no evidence indicating that exposure to Erythrosine *via* dietary exposure at levels as high as 1.0% is psychotoxic to developing rats.

Tanaka (2001), administered Erythrosine in the diet to Crj:CD-1 mice (10 animals/sex/group) at 0 (control), 0.005, 0.015 and 0.045% from five weeks of age in the F0 generation to 9 weeks of age in the F1 generation. Animals were monitored for changes in selected reproductive and neurobehavioural parameters. No adverse effects on litter size, litter weight, or sex ratio at birth were reported. The average body weight of the offspring was significantly increased in the middle-dose group in both sexes during the lactation period, but the author attributed these changes to body size at birth rather than to Erythrosine treatment. No parameters of behavioural development showed any significant adverse effects in either sex in the lactation period. In movement activity of exploratory behaviour, several parameters were significantly changed in the high-dose group (67-261 mg/kg bw/day). These effects were dose-related in adult females in the F0 and F1 generations and in male offspring in the F1 generation. Based on these data, Tanaka concluded that the dose levels of Erythrosine in the study produced few adverse effects in reproductive and neurobehavioural parameters in mice.

Abdel-Aziz et al. (1997) examined the potential adverse effects of Erythrosine on the spermatogenesis process in adult mice as measured by the incidence of sperms with abnormal heads, sperm count and motility, and lactic dehydrogenase isozyme (LDH-Z) activity (a pachytene spermatocyte marker of testicular toxicity). LDH-Z activity was significantly decreased to 71.8% and 68.6% of the control value after oral administration of Erythrosine at doses of 68 and 163 mg/kg bw, respectively. Normal average epididymal sperm count was decreased by about 50 and 33.9% at the low and high-dose levels. Sperm motility was likewise reduced by 57 and 80.5%. Erythrosine, administered daily at doses of 680 and 1360 mg/kg bw (10 and 20% of its LD_{50}) for five days significantly disrupted sperm head morphology. The incidence of sperms with abnormal heads was increased by 57 and 65%, respectively. According to the authors, the induced increase in sperm abnormalities could enhance the spermatogenic dysfunction and germ cell mutagenicity, indicating to authors that at the doses used, Erythrosine has a potential toxic effect on spermatogenesis in mice and in turn, it may affect its testicular function and reproductive performance.

The Panel noted that methodological issues involved in measurement of sperm parameters have been identified (Bell et al., 2010), and that there is no evidence of a functional effect on fertility at much higher dose levels. The Panel also noted that these doses are substantially higher than the NOAEL from the critical study in humans. Therefore the Panel concluded that these studies would not provide a basis for revising the ADI.

In addition, older studies reported in the published literature provide supporting evidence that Erythrosine does not adversely affect reproductive parameters. No effect on fertility was observed when Erythrosine was administered at 1% in the diet of Wistar rats for two years (Oettel et al., 1965) and subcutaneous injections of in aqueous solution (250 mg/kg bw twice daily for three days) to five young rats had no oestrogenic activity (as measured by uterine weight) (Graham and Allmark, 1959). Similarly, as per abstract data reported by Pierce and colleagues (1974), no adverse reproductive effects were seen in a multi-generation study in rats.

As reported by the US FDA, several studies have evaluated the teratogenic potential of Erythrosine in rats following administration in the drinking water as well as following administration *via* oral gavage (Collins et al., 1993a; Collins et al., 1993b). Pregnant Osbourne-Mendel rats were dosed with solutions of 0.05, 0.1, 0.2 or 0.4% (corresponding to daily doses of 64, 121, 248 or 472 mg /kg bw) Erythrosine in drinking water, or doses of 0, 15, 30, 100, 200, 400 or 800 mg Erythrosine/kg bw/day) by gavage on gestation days 0 to 20. Dams were sacrificed on gestation day 20. In the drinking water study (Collins et al., 1993a), Erythrosine-treated animals consumed less fluid than controls, but these changes were not dose-related and only occasionally reached statistically significance on random days. In the 0.2% group, rats consumed significantly more feed than the controls during gestation. Maternal weight gain was not significantly affected in any group, and no dose-related changes were seen in maternal clinical findings, implantations, fetal viability, fetal size (weight and length) or visceral development. No dose-related teratogenesis was seen. Some statistically significant increases in skeletal variations were observed, however these changes were not dose- related and were considered to be random. Based on these data, it was concluded that Erythrosine was neither fetotoxic, teratogenic, nor maternally toxic at the dose levels tested in drinking water and the maternal and fetal NOAEL is considered to be 472 mg/kg bw/day.

In the gavage study (Collins et al., 1993b), feed consumption was significantly increased at the 400 mg/kg bw/day dose level, while increases at the 30 and 800 mg/kg bw/day levels were borderline significance. A significant increase in maternal weight gain was seen in females treated with 30 mg/kg bw/day on days 0 to 7; however, this was considered a random occurrence. No dose-related changes were seen in maternal clinical findings, implantations, fetal viability, or fetal size (weight and length). Likewise, no teratogenic effects were seen, and neither skeletal nor visceral development was affected. Based on these data, Erythrosine was neither fetotoxic, teratogenic, nor maternally toxic at the oral gavage levels tested and the maternal and foetal NOAEL was considered to be 800 mg/kg bw/day.

In a study that pre-dated GLP regulations, pregnant rats (15 to 19 per group) were administered 25, 85, and 250 mg Erythrosine/kg bw/day by gavage on days 6 to 15 of gestation (Anonymous, 1972a). Animals were sacrificed on gestation day 20. Erythrosine had no effects on body weight, general appearance, or mortality of dams as compared to controls. Likewise, there were no Erythrosine related effects on fetal mortality, fetal weight, external or internal foetal development or skeletal development.

Similarly, no adverse effects were seen on body weight gain, mortality of dams, fetal survival, fetal body weights, internal and external foetal development, and skeletal development in rabbits upon sacrifice on gestation day 29 following the administration of 12.5, 40 or 125 mg Erythrosine/kg bw/day from days 6 to 18 of gestation (Anonymous, 1972).

Abstract data in the published literature provide supporting evidence that Erythrosine has no teratogenic potential. As reported by Burnett and colleagues (1974), the teratogenic effects of 25 certified colours, including Erythrosine, were evaluated in rats and rabbits. Results for individual colours were not reported. The colours were administered *via* gavage during organogenesis at doses

based on the highest No-Effect Level in rats and dogs in prior 2-year feeding studies. No skeletal or soft tissue abnormalities were seen with any of the colours.

3.2.6. Sensitivity

None of the previous evaluations discuss intolerance or allergenicity, nor were any studies identified which had been published since the TemaNord evaluation.

JECFA refers to one study with guinea pigs in which Erythrosine had no sensitisation activity. However, it is unclear which type of study was performed.

The SCMPMD refers to one study in which it was shown that Erythrosine can increase the release of serotonin by leukaemic basophils in vitro, which means it might increase the intensity of immediate-type allergic reactions (Tanaka et al., 1995). The SCMPMD indicates that this finding is quite insignificant.

A case study was reported, in which a patient suffered from hypersensitivity to denture materials. This could be due to the use of Erythrosine in these materials, but is not completely clear from this study (Barclay et al., 1999).

Erythrosine was able to provoke an experimental iodine allergy in guinea pigs. The authors refer to several studies in which Erythrosine has been reported to be able to provoke hypersensitivity reactions (Sugihara et al., 2004).

TemaNord mentions that Erythrosine has been reported to induce hyperactivity in children, but that this has not been sufficiently documented. Furthermore, they indicate that in vitro studies have shown that high concentrations of Erythrosine can inhibit brain tissue ATPases and active re-uptake of neurotransmitters (Mailman and Lewis, 1981; Mailman et al., 1980). This has been postulated to be the underlying mechanism for hyperactivity. However, Erythrosine has not been documented to penetrate the blood brain barrier to give rise to significant brain concentrations, therefore TemaNord concluded that, "*also taking into consideration the very low level of bioavailability, this effect on behaviour seems to be only of academic interest*".

Four groups of young male adult Charles Foster rats were subdivided into five subgroups (a total of 20 subgroups) which orally received 0, 1, 10, 100 and 200 mg/kg bw Erythrosine in 0.5 ml distilled water. Group 1 rats (8-12 rats per subgroup) were tested for motor activity at various times between 0-9 hours post-dose. Group 2 rats (4-6 rats per subgroup per time) were used for measurement of neurobiochemical parameters at 2 and 7 hours post-dose. Group 3 rats (12 rats per subgroup) were used for measurement of pargyline- induced serotonin (5HT) accumulation and 5-Hydroxyindoleacetic acid (5-HIAA) declination rates. Group 4 rats (8-12 rats per subgroup) were treated with specific Monoamine oxidase inhibitors (MAOIs) (clorgyline and deprenyl, 5 mg/kg bw *i.p.*) 10 minutes after Erythrosine dosing and were tested for motor activity every 30 minutes between 0-9 hours post-dose. Motor activity was assessed by the number of rearing events in a five minute observation period.

There was no effect on motor activity after 1 mg/kg bw Erythrosine, but there was a dose-related decrease in motor activity at the other doses, with a maximum decrease at 2 hours post dose. This decrease was no longer significantly different at 3-4 hours post dose in the 10 mg/kg bw group and at 7 hours post dose in all dose groups. These effects were abolished by administration of MAOIs. There was a decrease in levels of 5HT in medullary pons, hypothalamus and hippocampus at 2 hours but not at 7 hours, at those doses where effects were observed in motor activity (Dalal and Poddar, 2009). The effects seen on behaviour and neurobiochemical parameters at higher doses were transient and returned to normal levels within 7 hours post dose. Therefore, this study does not affect the current assessment, nor does it indicate a need to revise the ADI.

3.2.7. Special studies on thyroid function and morphology

Since the oncogenic effect of Erythrosine can be attributed to its hormonal effects, several studies in rats and humans were evaluated by the JECFA.

Rats

Thyroid glands from the Primate Research Institute 27-week toxicity study in rats (see Couch et al., 1983, section 3.2.4.) were subjected to ultra-structural examination by electron microscopy. Rats fed Erythrosine were reported as displaying hypertrophy of follicular cells with increased development of synthetic and secretory organelles (rough endoplasmic reticulum, Golgi apparatuses, and long microvilli). These changes were interpreted as representing mild to moderate stimulation of follicular cells consistent with elevated serum T4 levels. Lysosomal bodies in rats receiving Erythrosine were described as being larger, more irregular, and electron dense than controls, and appeared to be closely associated or fused with the limiting membrane of colloid droplets, a process involved in secretion of thyroid hormones. The degree of thyroid stimulation and the accumulation of colloid droplets and lysosomes in follicular cells were stated to be greater in male than in female rats fed commercial Erythrosine. Ultrastructural indications of long-term thyroid stimulation appeared greater in rats fed commercial Erythrosine than in rats fed purified colour with supplemental iodide (Capen, *sine data* a, as cited by JECFA, 1986).

Thyroids from rats fed 0, 0.25, 0.5, or 4.0% Erythrosine in the diet (equivalent to 0, 125, 250 or 2000 mg/kg bw/day) in a 7-month study of thyroid function (Ingbar et al., 1984a) were subjected to examination by electron microscopy. Thyroid follicular cells from rats fed Erythrosine were reported as displaying ultra-structural features of a dose-dependent stimulation of synthetic and secretory activity, most marked in rats fed 4% Erythrosine and indicated by hypertrophy of follicular cells with increased development of secretory organelles. These features were considered to be generally consistent with a response to long-standing TSH stimulation. The ultrastructural changes were reversible by administration of T3 during the last month. A dose-dependent accumulation of numerous lysosome-like bodies observed in follicular cells of treated rats were considered not to be an expected response to TSH stimulation alone. Similar, but less marked changes, in follicular cell stimulation and accumulation of lysosome-like bodies were stated to be present in rats fed Erythrosine at dietary levels of 0.25 and 0.5% (Capen, *sine data* b, as cited by JECFA, 1986).

Three groups of 160 male Sprague-Dawley rats were administered Erythrosine at dose levels of 0.0, 0.25 or 4.0% in the diet (equivalent to 0.0, 150 or 2500 mg/kg bw/day) for 60 days. Physical observations and body weight and food consumption measurements were performed on all animals pre-test and at weekly intervals during the treatment period. Necropsy was performed with up to 20 animals per test group at days 0, 3, 7, 10, 14, 21, 30 and 60. Serum was prepared from blood samples taken from the abdominal aorta at each sacrifice interval and analysed by radioimmunoassay for TSH, T4, T3 and reverse T3 (rT3)[9]. Thyroid and pituitary glands were weighed at each interval and organ/body weight ratios were calculated. Gross post-mortem examinations were conducted on the thyroid and pituitary glands only. Three rats receiving 4.0% Erythrosine in the diet died spontaneously during the second week of the study. The animals receiving 4% Erythrosine in the diet lost weight during the first week of the study and the mean body weights were significantly lower than control values throughout the study (13% at week one and 17% at week 8). Food consumption of the animals receiving 4.0% Erythrosine in the diet was significantly lower than the control value at week one, but after week two it was comparable. This probably reflected a palatability issue during the first two weeks. The absolute pituitary weights of males receiving 4% Erythrosine were statistically significantly lower than control values at days 7, 10, 14, 21 and 60. The differences were considered to reflect the body weight differences between the high-dose animals and the controls. The absolute thyroid/parathyroid weights of the rats at the 4% level were generally lower than the control values, but the differences were slight and may be due to the body weight differences between these groups.

[9] reverse triiodothyronine; 3,3',5'-triiodothyronine.

The relative weights of these organs were significantly greater at day 21; otherwise relative weights were only slightly greater and not significant. Thyroid/parathyroid absolute and relative weights of the rats fed 0.25% Erythrosine were significantly lower at day 60, otherwise they were comparable to controls. Gross post-mortem examinations of thyroid and pituitaries did not show treatment-related changes (Kelly and Daly, 1988).

The analysis of serum hormone levels in the rats of the study described above revealed the following: there was a change (slight increase) in serum TSH levels in the control rats during the 60-day experimental period. The baseline (day 0) TSH level was significantly lower than the levels on days 21, 30, and 60. In the 0.25% group, serum TSH concentrations were significantly increased over baseline (day 0) at days 14, 21, 30 and 60. When compared to the TSH levels in control animals, a significant increase was observed at days 21, 30 and 60 in the 4.0% group. In the 4.0% group the TSH levels were significantly increased over the baseline (day 0) level and the corresponding control levels at all time points. When compared to the 0.25% group the serum TSH levels in the high dose group were significantly greater at days 3, 7, 10, and 14. Serum T4 concentrations were increased over baseline and control values at days 10 and 14 in the 0.25% group, while in the 4.0% group the serum T4 concentrations were increased at all time points. Furthermore, the high dose animals had significantly greater T4 concentrations than the low dose animals at days 7, 10, 21, 30 and 60. Serum T3 concentrations in the low dose rats were comparable to the control values except for a decrease at day 30. In the high dose rats serum, T3 concentrations were significantly lower than baseline (day 0) and control values at all time points. In addition, serum T3 concentrations were decreased compared to those of the low dose animals on days 3, 10, 14, 21, 30, and 60. Serum rT3 concentrations were increased above baseline (day 0) in the low dose group at days 7, 10, 14, 21, 30 and 60; and increased above control values at days 10, 14 and 21. A marked increase in serum rT3 over controls and low dose animals was seen in the high dose group at all time points. The results indicate that the ingestion of a dietary concentration of 4% Erythrosine induces a rapid and sustained increase in serum TSH, T4, and rT3 and a comparable decrease in serum T3 concentrations, and that these changes are also induced, but are less pronounced, after administration at dose levels of 0.25% Erythrosine in the diet. These findings are consistent with an inhibition by Erythrosine of the deiodination in the 5'-position of T4 and rT3, resulting in a decreased production of T3 from T4 and a decreased deiodination of rT3, respectively (Braverman and DeVito, 1988). The Panel considers that the changes observed in some hormone levels at some time points in the 0.25% group would be consistent with the changes at the higher dose but are unlikely to result in functional disturbances and should be regarded as a NOAEL.

Three groups of 80 male Sprague-Dawley rats were administered Erythrosine at dose levels of 0.0, 0.03, 0.06 and 4.0% in the diet for a maximum of 60 days (corresponding to 0.0, 17.5, 35.8, and 2671.7 mg/kg bw/day, respectively). Control animals (100 males) received standard laboratory diets. Physical observations, body weight and food consumption measurements were performed on all animals pre-test and at weekly intervals during the study period. For the determination of baseline data, 20 control animals were bled for radioimmunoassays of TSH, T4, T3, and rT3 and sacrificed on test day 0, prior to the initiation of dosing. Additional necropsy intervals were staggered so that on days 7, 21, 30 and 60, an additional 20 animals per group at each interval were bled for radioimmunoassay samples. Brain, pituitary and thyroid were weighed and organ to body and organ to brain weight ratios were calculated for all animals. Gross post mortem examinations were performed on the thyroids, pituitary and brains of all animals. In the animals receiving 4% Erythrosine in the diet, a substantial loss of body weight and decreased food consumption during week one of the study, probably due to poor palatability of the diet, resulted in statistically significantly lower body weights of the animals throughout the study period. The absolute and relative thyroid: parathyroid weights of the animals receiving 4.0% Erythrosine were increased at days 21 and 30, and at day 60 (relative organ to body weight ratio). The absolute and relative (organ to brain weight ratio) pituitary weights of animals at the 4.0% level were lower than control values at day seven. In the 0.03% Erythrosine group, absolute and relative thyroid to parathyroid weights were greater than corresponding control values at days 21 and 30, but comparable to control values at days 7 and 60. Thus, no consistent and dose-related changes in organ weight, absolute or relative, were found at the lower doses. Gross post-

mortem examination of the thyroid, pituitaries and brain did not reveal any treatment related effects (Kelly and Daly, 1989).

The analysis of serum hormone levels in the rats of the study above revealed the following: in the 0.03% and 0.06% groups there were no significant changes in serum TSH, T4, T3, and rT3 concentrations during the 60-day treatment period. In the 4.0% group, TSH concentrations were significantly greater than the corresponding control values at days 21, 30, and 60. A 41% increase after seven days was not statistically significant compared to the control value. Serum TSH concentrations in the 4% group were significantly greater than those of the 0.03% group at days 21, 30, and 60, and the 0.06% group at day 30. In the 4.0% group, serum T4 concentrations were slightly elevated above controls during the treatment period. However, the increase was only statistically significant on day 30. In the high dose animals, serum T3 concentrations were significantly lower than controls at all time points. Serum rT3 concentrations were markedly increased in the high dose animals compared to controls or animals fed 0.03% and 0.06% Erythrosine at all time points (Braverman and DeVito, 1989).

Rats were given *i.p.* daily doses of 2.5 - 250 mg Erythrosine/kg bw in vivo prior to preparation of liver homogenates to investigate the effects of Erythrosine on the metabolism of ^{125}I-labelled thyroxine (T4) and triiodothyronine (T3). Erythrosine caused a dose-dependent inhibition of the deiodination of T4 and of the generation of T3. At dose levels of > 10 mg/kg bw/day Erythrosine, the proportionate reduction in the deiodination of ^{125}I-T4 exceeded the reduction in the generation of ^{125}I-T3, indicating that pathways of T4 metabolism other than those leading to T3 formation were also inhibited. The authors concluded that Erythrosine may inhibit the 5-monodeiodination of T4 to reverse T3 (rT3) since Erythrosine also inhibited the 5-monodeiodination of T3 to 3,3'-diiodothyronine. Fluorescein did not exhibit similar effects. It was considered unlikely that Erythrosine would produce similar effects in man in the doses normally ingested. This conclusion was supported by studies in human volunteers given oral doses of Erythrosine of up to 25 mg daily for seven days where no changes in serum T4, T3, or rT3 concentrations were detected (Ruiz and Ingbar, 1982; Garber et al., 1981).

Male Sprague-Dawley rats (15 animals/dose) received 0, 0.25, 0.50, 1.0, 2.0, or 4.0% Erythrosine in the diet (equivalent to 0, 125, 250, 500, 1000, or 2000 mg/kg bw/day) for a period of seven months. After six months each group was divided into three sub-groups of five rats; during the next month one sub-group received 15 µg T3/kg bw/day by s.c. injection, a second sub-group received the saline vehicle, and the third sub-group continued with no injections. Blood samples (orbital puncture) were obtained just before the start of the study and at monthly intervals thereafter, and serum thyroid hormone levels were determined. At termination, pituitary glands and liver segments were excised and in vitro studies of T4 metabolism were performed on these tissues.

Serum TSH levels were variable and, although mean serum TSH values were higher in the 4% Erythrosine group than in the control or 0.5% groups over the first six months of the study, the differences were not statistically significant. Serum TSH concentrations in sub-groups which received injections of T3 during the final month were below detection limits (15 µU/ml). Serum T4 concentrations were elevated relative to baseline and control values in animals receiving 4% Erythrosine during the six months of the study, whereas the values for the control and 0.5% Erythrosine groups did not differ significantly from baseline values or from each other. Serum T4 concentrations were immeasurably low in all animals that had received injections of T3 during the seventh month of the study. Serum T3 concentrations in all three groups decreased significantly with time; additionally, 4% Erythrosine in the diet decreased the T3 concentration and the values were significantly lower than controls at 1, 2, 4, and 5 months. The effects of 0.5% Erythrosine on T3 levels were unclear, significant depressions relative to controls only being observed at the 1- and 2-month time periods and not subsequently. In animals receiving 4% Erythrosine, serum rT3 concentrations increased approximately 7-fold and remained elevated throughout the six months of the study. In both control and 0.5% groups, serum rT3 concentrations did not differ significantly from baseline values or from each other. Serum rT3 was undetectable in animals receiving injections of T3 during the final month of the study.

In vitro metabolism of ^{125}I-labelled T4 was greatly altered in liver homogenates from rats fed 4.0% Erythrosine in the diet, with degradation of T4 decreasing to approximately 40% of values in homogenates of control livers. There was an associated decrease of about 75% in the generation of ^{125}I-iodide and an approximately 80% decrease in the generation of ^{125}I-T3 from ^{125}I-T4. Percentage degradation of T4 and generation of iodide and T3 in liver homogenates from rats fed 0.5% Erythrosine were similar to controls. In vitro metabolism of T4 was studied in pituitary glands from the control, 1, 2, and 4% dose groups. Overall, ^{125}I-T4 degradation and generation of ^{125}I-iodide appeared higher in the two higher Erythrosine dose groups than in controls, but none of the differences were statistically significant. The results were interpreted as indicating that the primary effect of high doses of Erythrosine on thyroid hormones is inhibition of type I 5'-monodeiodination of T4 to T3. As a consequence, TSH secretory mechanisms were activated in the pituitary. The increases in serum rT3 levels were considered to arise from both increased availability of the T4 precursor and inhibition of metabolism of rT3 by 5'-monodeiodination (Ingbar et al., 1984a).

In identical studies on female rats to those outlined above, similar results were obtained in that Erythrosine at dietary concentrations of 4% (equivalent to 2000 mg/kg bw/day) caused an increase in serum T4, rT3, and TSH concentrations, a decrease in hepatic deiodination of T4 to T3, and an increase in deiodination of T4 to T3 in the pituitary. Hepatic generation of T3 from T4 was also diminished following dietary administration of 0.5% Erythrosine, but to a lesser degree and no changes in serum thyroid-related hormones could be detected. No alteration in the metabolism of T4 was observed in liver homogenates from rats receiving 0.25% dietary Erythrosine (Ingbar, 1985).

In both the above studies, ultrastructural examination of thyroids of rats receiving 4% Erythrosine in the diet (equivalent to 2000 mg/kg bw/day) for six months revealed enhanced synthetic and secretory activity consistent with prolonged hyperstimulation by TSH. Less marked changes were seen at the 0.5% dose level (equivalent to 250 mg/kg bw/day) and the changes generally were less in females than males similarly treated.

Sprague-Dawley rats (13/dose) were fed 0, 0.5, 1.0, or 4.0% Erythrosine in the diet (equivalent to 0, 125, 250, 500 or 2000 mg/kg bw/day), 100 mg sodium iodide/kg bw/day, or 1000 mg fluorescein/kg bw/day for three weeks. The rats were then subjected to an in vivo TSH-releasing hormone (TRH) provocative test (100 ng/100 g bw). Of all the treatments, only 4% dietary Erythrosine produced an exaggerated response to TRH; ten minutes after TRH injection, the serum TSH levels were 80% greater than controls ($p < 0.01$). Erythrosine also produced a dose-dependent increase in total serum T4 levels, statistically significant ($p < 0.01$) at the 1% and 4% dose levels and, at the 4% level. Contrary to other studies, there was also a significant increase in serum T3 levels and a significant decrease in T3 resin uptakes. The free T4 indices were significantly elevated after treatment with 1% or 4% dietary Erythrosine, but the free T3 indices were not. The data were taken to indicate that feeding with 4% Erythrosine disrupts the normal negative feedback regulatory mechanism of the pituitary- thyroid axis and the TRH hyper-responsiveness in the presence of elevated serum T4 and T3 levels suggested that the defect did not arise from a conventional antithyroid mechanism. The data also suggested that the effect of Erythrosine on TSH release was due to intact dye or an iodinated metabolite rather than to the fluorescein nucleus (Witorsch et al., 1984).

Humans

Five human volunteers (four males and one female, aged 21-35 years) received Erythrosine in the diet at dose levels of 5, 10, or 25 mg/day in weekly increments for a period of three weeks.

Total serum iodine and PBI increased slowly and slightly in association with the weekly increasing Erythrosine doses. Other tests for serum T4, T3, TSH, Erythrosine, urinary iodine, Erythrosine excretion, and T3-resin uptake remained unchanged throughout the three weeks. Increases in serum PBI and total serum iodine during the exposure period indicate that a portion of the iodine ingested as Erythrosine appears to be absorbed from the gastrointestinal tract. No changes in concentration of TSH, T4, and T3 in serum indicate that both the thyroid function and thyroregulatory mechanisms

were unaffected by the ingestion of Erythrosine during a three-week period at dose levels of 5, 10 and 25 mg/day in weekly increments (Ingbar et al., 1983).

Human volunteers were given single doses of 75-80 mg Erythrosine labelled with ^{131}I in a milk-shake or lemonade; the subjects received a daily dose of five drops of saturated potassium iodide solution to block thyroid uptake of ^{131}I. Daily blood samples were examined for total serum ^{131}I, and for T4 and T3[10]. Negligible quantities of ^{131}I appeared in the serum, never exceeding 0.013% of the dose/l serum, and in none of the studies were the serum T4 and T3 concentrations significantly altered following ingestion of Erythrosine. These studies indicate that at the single oral dose levels used, Erythrosine did not affect thyroid hormone levels (Ingbar et al., 1984b).

No biologically significant increases in plasma inorganic iodine or in urinary iodine were found in six patients (aged 25-68 years, sex not reported) after oral exposure to 1.9 µmole (1.68 mg/day) of Erythrosine for 10 days. In other assays of thyroid function, thyroid radioiodine uptake, levels of thyroxine, and PBI in plasma remained unchanged. No further details were provided (Bernstein et al., 1975).

In a pivotal clinical study, Gardner et al. (1987) examined the effects of Erythrosine on the thyroid function in 30 healthy male subjects. Ten men per group consumed capsules containing 20, 60, or 200 mg/day of Erythrosine for 14 days. Serum T4, T3, reverse T3, T3-charcoal uptake, TSH, PBI, total iodine, and total urinary iodine excretion were measured on days 1, 8, and 15.

Thyrotropin-releasing hormone (TRH) was measured on days 1 and 15. No significant changes in serum T3, T4, rT3 and T3 uptake were seen in any group. Mean basal serum TSH concentration increased from 1.7 on day 1 to 2.2 µU/ml on day 15 ($p < 0.05$) and the mean peak TSH increment after TRH increased from 6.3 to 10.5 µU/ml ($p < 0.05$) in the high-dose group. There were no significant changes in basal or peak TSH responses at the two lower dose levels. Significant dose-related increases in serum total iodide and PBI concentrations occurred in all three groups and significant dose-related increases in urinary iodide excretion occurred in the 60 and 200 mg/day dose groups. The dose-dependent increases in serum and urinary iodide levels indicated that some of the iodine in the Erythrosine molecule is bioavailable. The daily iodide load estimated from the mean 24-hour urinary excretion was approximately 0.5% by weight of the daily dose of Erythrosine. Given the presence of 0.25% sodium iodide in the Erythrosine, the authors attributed about half of this iodide load (e.g. 1000 µg/day at the 200 mg/day dose level) to Erythrosine deiodination. The authors suggested that these data indicated that the increase in TSH secretion was related to the effect of increased serum iodide rather than a direct effect of Erythrosine on thyroid hormone secretion or peripheral metabolism. The authors state that the NOAEL in this clinical study for effects on basal and TRH-stimulated TSH secretion is considered 200 mg Erythrosine per day (Gardner et al., 1987). The Panel considered Erythrosine has a minimal effect in humans at a clinical oral dose of 200 mg daily over 14 days, while a dose of 60 mg daily was without effect.

The Panel noted that some of the kinetic data indicate that Erythrosine is excreted with its iodine intact. It may also be possible that the increase in iodide levels resulted from iodide released from thyroid hormones instead of from Erythrosine, or that Erythrosine in the serum interfered with iodide determinations.

Certain aspects of this study by Gardner and colleagues have been questioned, including the statistical analysis, failure to correct for significant differences between groups in basal and maximal TRH-stimulated and TSH concentrations on day 1, and lack of a control group (Farrar and Crump, 1989). With respect to basal TSH, it was suggested that there was no statistical evidence for variation due to treatment over the dose range studied when appropriate statistical methods were used to control for apparent initial differences among treatment groups. The questions raised and statistical re-evaluation

conducted by Crump and Farrar (1987) suggest that there is no effect of treatment on basal TSH concentration or maximum TSH increment after TRH provocation at doses lower than 200 mg/day.

The Panel considered Erythrosine has a minimal effect in humans at a clinical oral dose of 200 mg daily over 14 days, while a dose of 60 mg daily was without effect. The Panel agreed with the analysis of Crump and Farrar and considered the NOAEL to be 60 mg/day.

Paul et al. (1988) performed a study similar to Gardner et al. (1987) that was designed to determine whether relatively small supplementary amounts of iodine in the diet would affect thyroid function. Normal, euthyroid human subjects received 250, 500, or 1500 µg iodine (I_2) daily for 14 days; the doses were selected to correspond to the amounts of iodide that might be bioavailable from the doses of Erythrosine used in the study by Gardner et al. (1987). Following administration of 1500 µg Erythrosine/day, there were small but significant decreases in serum T4 and T3 concentrations, and small compensatory increases in serum TSH concentrations, and in the TSH response to TRH. However, all values remained within the normal range. In contrast, no changes occurred following daily administration of 250 or 500 µg I_2 (Paul et al., 1988).

The SCF opinion mentions that in vitro studies have shown that Erythrosine inhibits the conversion of T4 to T3 in the liver. The SCF opinion indicates that these studies suggest a mechanism of action for the effects of Erythrosine in producing thyroid hyperplasia. Inhibition of conversion of T4 to T3 will produce reduced tissue and plasma levels of T3, which will in turn reduce the inhibitory effect of T3 on secretion of TSH. The resultant increased secretion of TSH will stimulate the thyroid, leading to hypertrophy, adenoma formation and possibly malignant changes (SCF, 1989). In addition, the SCF mentions in its opinion (1983) that some information on metabolism including the contribution to human iodine intake from the ingested colour is available. However, no further information on these studies is provided.

Iodine bioavailability following Erythrosine administration

Katamine et al. (1987) examined the bioavailability of iodine in humans after ingestion of various iodine-rich foods and food colours. In one group, 3-5 men aged 35-45 years received iodine-rich foods which provided ingested iodine levels that ranged from 1080-3840 µg. A second group of five males aged 21-28 years received 10 mg Erythrosine which provided an ingested iodine level of 4900 µg. In the subjects that received Erythrosine, the serum iodine levels determined 4 hours following Erythrosine intake were not increased and the amount of iodine urinary excretion was very low during 48 hours after ingestion (approximately 1% of the dose administered). In contrast, the subjects which received the iodine-rich foods had urinary excretion levels that ranged from 10% to 102% greater. These data demonstrate that the bioavailability of iodine from iodine-rich foods is much greater than the limited bioavailability of iodine from ingestion of 10 mg Erythrosine.

Six healthy volunteers were given diets containing 1.9 µmol (1.680 µg/day of Erythrosine (7.72 µmol (980 µg) of iodine/day)) for 10 days (Bernstein et al., 1975). No biologically significant increases in plasma inorganic iodine or in urinary iodine excretion were found. In other assays of thyroid function, thyroid radioiodine uptake, levels of thyroxine and PBI in plasma remained unchanged. Based on clinical aspects of thyroidal iodine metabolism, the authors concluded that a maximum of 7.8% of the iodine content of the ingested Erythrosine was bioavailable.

Following a case report of increased protein bound iodide levels and depressed radioactive iodine (RAI) in a patient who received medication in Erythrosine-containing capsules that had approximately 190 µg of iodine, Haas (1970) evaluated ingestion of gelatine capsules containing Erythrosine in five euthyroid subjects. Three subjects ingested six capsules while two ingested an average of 12 capsules. RAI uptake test was performed 24 hours after ingestion of 10 µc of ^{131}I. PBI levels and T3 uptakes were also measured. There was no difference in PBI values from subjects taking 6 or 12 tablets. There was a statistically significant increase in PBI levels after six weeks of treatment, although values remained in the normal range. Six weeks after treatment was discontinued, levels fell significantly but

again remained within the normal range. Although RAI uptakes tended to be lower after six weeks of treatment, this difference was not significant. There was no significant difference in T3 values between the baseline, 6-, and 3-month evaluations, although there was a general trend toward higher T3 uptake during the course of the study (Haas, 1970).

Bora (1969) also reported effects on PBI levels in patients ingesting DOPA capsules containing Erythrosine. The 250 and 500 mg DOPA capsules contained 28 µg iodine/capsule and 38-45 µg iodine/capsule, respectively. The iodine content of the pure DOPA was negligible. Serum PBI concentrations in patients receiving capsules with DOPA were elevated, while values for those treated with capsules containing lithium carbonate were normal. Thyroxine concentrations were normal for both groups. However, a significant increase in the PBI: thyroxine ratio was observed in three of five patients treated with lithium carbonate.

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.

Erythrosine (E 127) is a xanthene-dye authorised as a food additive in the EU (E127) and previously evaluated by the (JECFA most recently in 1990 and the SCF in 1989. Both committees have established an ADI of 0-0.1 mg/kg bw/day. The JECFA and SCF ADI was based on a NOAEL for endocrine/hormonal effects of 60 mg/day equivalent to 1 mg/kg bw/day obtained in a 14-day study in human volunteers (Gardner et al., 1987). The applied uncertainty factor was 10.

Specifications have been defined in the EU Commission Directive 2008/128/EC and by JECFA (2006).

The Panel noted that the design of various studies in the previously evaluated dataset would not be in full compliance with current regulatory protocols. However, from the study descriptions available, the Panel considered that these studies were of sufficient quality. .

From the studies in rats and humans, the Panel concluded that only a small portion of Erythrosine is absorbed. Tracer studies indicated that no accumulation of Erythrosine, iodine resulting from possible de-iodination of Erythrosine or ring-containing metabolites of Erythrosine accumulate in the thyroid. Erythrosine is excreted almost completely via faeces with unchanged iodine content. In a study in rat and a study in man, increased levels of PBI (in rat and in man) and iodine (in man) were measured in the blood. This might be explained by the circulating Erythrosine in the blood, rather than by an effect of Erythrosine on thyroid hormone levels.

Although some older and more recent in vitro studies showed positive results for the genotoxicity of Erythrosine, there are three negative in vivo genotoxicity studies (mammalian micronucleus, sister chromatid exchange and Comet assay). The weight-of-evidence from the available studies supports the conclusion that Erythrosine is neither genotoxic nor clastogenic in vivo. The SCF, the JECFA and TemaNord evaluation concluded, based on in vivo and in vitro mutagenicity studies available at that time, that Erythrosine did not show any genotoxic activity (SCF, 1989; JECFA, 1986; 1990; TemaNord, 2002). The Panel concurred with these assessments.

When addressing the results of new mutagenicity studies, the available adequate and well reported studies on oral in vivo activity were negative, although clastogenicity has been demonstrated by the *i.p*. route. The Panel considered the weight-of-evidence still showed that the tumorigenic effects of Erythrosine are secondary to its effects on thyroid function and not related to any genotoxic activity.

Although rodents and humans share a common physiology in regard to the thyroid-pituitary feedback system, a number of factors contribute to the greater sensitivity of the rat to long term perturbation of

the pituitary thyroid axis which predisposes it to a higher incidence of proliferative lesions in response to chronic TSH stimulation than human thyroid.

Both humans and rodents have nonspecific low affinity protein carriers of thyroid hormone (e.g., albumin). However, in humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin (TBG), which binds T4 (and T3 to a lesser degree); this protein is missing in rodents, birds, amphibians and fish. TBG has 1000 fold greater binding affinity than prealbumin. Thus species with TBG (humans, primates and dogs) have lower percentages of unbound active T4 (and T3) than species where binding is to albumin and prealbumin (rat, mouse and chicken). As a result, T4 bound to proteins with lower affinity in the rodent is more susceptible to removal from the blood by metabolism and excretion from the body. This difference in T4 half-life results in a 10-fold greater requirement for endogenous T4 in the rat thyroid than in the adult human thyroid. The accelerated production of thyroid hormone in the rat is driven by serum TSH levels that are about 6- to 60-fold higher than in humans. Thus, the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormone. Increases in TSH levels above basal levels in rats could more readily move the gland toward increased growth and potential neoplastic change than in humans.

The male rat is more sensitive to follicular cell hyperplasia and neoplasia as it has higher circulating TSH levels than the females and therefore are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females more frequently develop thyroid cancer.

There are marked species differences in the sensitivity of follicular cell thyroperoxidase enzyme to inhibition. This results in nodule development in species (rat, dog, mouse) sensitive to inhibition but not in species (primate, guinea pig, chicken, human) resistant to inhibition.

Although qualitatively the rat is an indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive, effects on rodents would represent a conservative indicator of potential risk for humans. Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid-pituitary functioning, over a lifetime. The relevance of the experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency, and time) should be considered. In addition, chemically induced effects that are produced by short-term disruption in thyroid-pituitary functioning appear to be reversible when the stimulus is removed.

Since the JECFA and SCF evaluations, no new data are available on chronic toxicity/carcinogenicity. The Panel considered the weight-of-evidence still showed that the tumorigenic effects of Erythrosine are secondary to its effects on thyroid function and not related to any genotoxic activity. Two studies have shown that Erythrosine has an oncogenic effect in the thyroid gland of rats. The weight-of-evidence is that these tumours are elicited by a non-genotoxic mechanism. Erythrosine-induced rodent thyroid tumours may be considered of limited relevance to humans; an approach which is consistent with previous evaluation of Erythrosine. The Panel concurred with this conclusion.

In developmental toxicity studies, Erythrosine does not adversely affect development of young animals at dose levels up to 500 mg/kg bw/day, which is the highest dose level tested for this endpoint. There are no indications, based on the studies evaluated by JECFA and SCF, that Erythrosine can adversely affect male fertility at dose levels up to 2000 mg/kg bw/day, which is the highest dose levels tested (Albridge et al., 1981; Vorhees et al. 1983). Two more recent studies have however indicated that Erythrosine may affect testicular function. The study of Vivekanandhi et al. (2006) indicates that Erythrosine causes decreases in sperm motility at doses of 64 mg/kg bw/day onwards and decreases in sperm counts and increases in sperm abnormalities from 128 mg/kg bw/day onwards. Also the study of Abdel Aziz et al. (1997), evaluated by TemaNord, indicates that testicular function and reproductive performance may be affected by Erythrosine. This is not in line with the results of the other

reproductive studies in which Erythrosine did not adversely affect fertility at dose levels up to 2000 mg/kg bw/day. The Panel noted that methodological issues involved in measurement of sperm parameters have been identified (Bell et al., 2010), and that there is no evidence of a functional effect on fertility at much higher dose levels. The Panel also noted that these doses are substantially higher than the NOAEL from the critical study in humans. Therefore the Panel concludes that these studies would not provide a basis for revising the ADI.

However, in a human study (Gardner et al., 1987), increases in serum TSH concentrations, an increased TSH response to TRH, increases in serum PBI and serum iodide concentrations were observed.

The Panel considered that Erythrosine has a minimal effect in humans at a clinical oral dose of 200 mg daily over 14 days, while a dose of 60 mg daily was without effect (Gardner et al., 1987). The current ADI adopted by the JECFA and the SCF is based on this study. The Panel concurred with their identification of this as the critical study. The 60 mg dose was taken to be the equivalent of 1 mg/kg bw/day. By applying a safety factor of 10 to allow for the small number of subjects used in the study and its relatively short duration, an ADI of 0-0.1 mg/kg bw per day was derived.

Altogether, the Panel concluded that the present database on semi-chronic, reproductive, developmental and long-term toxicity, do not provide a reason to revise the ADI of 0-0.1 mg/kg bw/day.

The ADI of 0.1 mg/kg bw/day can for an adult be reached by consumption of 30 g cocktail cherries with the permitted maximum level of 200 mg Erythrosine/kg which is unlikely to occur on a frequent basis. The JECFA, based on evaluation of several national intake estimates, indicated that it is unlikely that long-term intake of Erythrosine will exceed the ADI (JECFA, 2000).

The EU intake monitoring scheme ("SCOOP" report) estimates an adult intake of 0% of the ADI (*i.e.*, negligible) for both children and adults. The JECFA has also assessed national intakes of Erythrosine (JECFA, 2000) where EU estimates were based on model diets constructed for high consumers in the UK. Based on the maximum permitted levels in foodstuffs in the EU and 97.5th percentile food consumption (adjusted for body weight) from dietary records, the estimated intake of high consumers in the UK was 0.01 mg/kg bw/day (representing 13% of the ADI) for adults and 0.05 mg/kg bw/day for children between 1.5 to 4 years (representing 52% of the ADI).

The Panel also noted that the limit test for heavy metals (expressed as lead) 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.

The Panel noted 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

Erythrosine (E 127) is a xanthene-dye authorised to be used as a food additive in the EU and previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees have established an ADI of 0-0.1 mg/kg bw/day.

The Panel concluded that the present database does not provide a basis to revise the ADI of 0.1 mg/kg bw/day.

The Panel concluded that at the current levels of use intake estimates for adults on average is 0.0031 mg/kg bw/day and 0.01 mg/kg bw/day at the 95th percentile, and consequently are below the ADI of 0.1 mg/kg bw/day. The Panel considered there would be no safety concerns at current levels of exposure including other sources of exposure (e.g. toothpaste, pharmaceuticals).

The Panel also noted that the limit test for heavy metals (expressed as lead) is considered obsolete and is being replaced with limits for individual metals of concern.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium and that therefore specifications for the maximum level of aluminium in the lakes are required.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

2. GSK (GlaxoSmithKline Consumer Healthcare), 2008. Submission to the Scientific Committee on consumer products, Erythrosine (CI45430).

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GLOSSARY/ABBREVIATIONS

Aluminium lake	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.
ADI	Acceptable Daily Intake
AFC	EFSA Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food
ANS	Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
GMP	Good Manufacturing Practice
GSK	GlaxoSmithKline
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSANZ	Food Standards Australia New Zealand
GI	Gastrointestina
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
5-HIAA	5-Hydroxyindoleacetic acid
HPLC	High Performance Liquid Chromatography
JECFA	WHO/FAO Joint Expert Committee on Food Additives
LDH-Z	Lactic Dehydrogenase Isozyme
MAOI	Monoamine oxidase inhibitors
MLP	Maximum Permitted Level
NOAEL	No Observed Adverse Effect Level
NSIFC	North South Ireland Food Consumption Survey
PBI	Protein-bound iodine
SCF	Scientific Committee for Food
SCMPMD	Scientific Committee for Medicinal Products and Medicinal Devices
T3	3,5,3'-Triiodothyronine
T4	Thyroxine (3,5,3',5'-tetraiodothyronine)
rT3	reverse T3 (3,3',5'-triiodothyronine)
SHE	Syrian Hamster Embryo
TBG	Thyroxine-binding globulin

TRH	Thyrotropin-releasing hormone
TSH	Thyroid Stimulating Hormone.
TWI	Tolerable Weekly Intake
US FDA	US Food and Drug Administration