EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

CI 45430

(Erythrosine)

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 7th plenary meeting

of 22 June 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

CI 45430 is regulated as a cosmetic colorant in Annex IV of the Cosmetics Directive and permitted together with its lakes and salts. The colorant is currently allowed to be used in all cosmetic products without any restrictions. In September 2006, in order to reduce the intake of iodine from cosmetic products, the Commission proposed to delete CI 45430 from Annex IV if no safety dossier was submitted.

Submission I of the cosmetic colorant CI 45430, with the chemical name Disodium-2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate synonym with Erythrosine sodium, E127; Erythrosine; FD&C Red No. 3 and Food Red 14, was submitted in January 2007 and February 2008.

The colorant CI 45430 is synonym with the hair dye substance CI Acid Red 51 for which no dossier has been submitted and which consequently has been banned (Annex II, n° 1337) for this use according to the hair dye strategy. However, the current dossier applies for a very restricted use in toothpaste products with a maximum concentration of 0.0025% (25 ppm).

2. TERMS OF REFERENCE

1. *Does the SCCS consider CI 45430 safe for consumers when used as a colorant in toothpaste products with a maximum concentration of 0.0025% (25 ppm), taken into account the scientific data provided?*

2. *And/or does the SCCS express any further scientific concern with regard to the use of CI 45430 in toothpaste products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

CI 45430 (INCI name colorant use)
Acid Red 51 (INCI name hair dye use)

3.1.1.2. Chemical names

3',6'-Dihydroxy-2',4',5',7'-tetraiodospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, disodium salt
Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate
Spiro[isobenzofuran-1(3H),[9H]xanthen]-3-one, 3',6'-dihydroxy-2',4',5',7'-tetraiodo-, disodium salt
Tetraiodofluorescein sodium salt

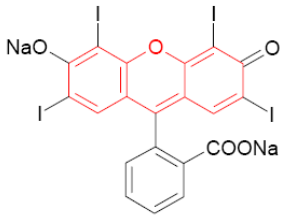
3.1.1.3. Trade names and abbreviations

Sicovit Erythrosine 88 E 127
Erythrosine B
Erythrosine BS
E127
CI 45430
Erythrosine
FD&C Red n° 3
Food Red 14
Japan Red 3
Red n° 3

3.1.1.4. CAS / EC number

CAS: 16423-68-0 (Erythrosine) 1342-25-2 (Acid red 51 /Red No. 3)
EC: 240-474-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{20}H_8I_4O_5$. 2Na

3.1.2. Physical form

Red solid powder, odourless

3.1.3. Molecular weight

Molecular weight: 879.87 g/mol (disodium salt)

3.1.4. Purity, composition and substance codes

≥ 87% (w/w) total colouring matters*

* The colouring matter consists of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate and minor amounts of subsidiary colouring components (less than 4% w/w including fluorescein at levels of less than 20 mg/kg). These minor colouring components are typically by-products of the manufacturing process whereby fluorescein is iodinated in aqueous ethanol.

The rest (≤ 13%) is made up of sodium chloride, sodium sulphate and volatile matter.

3.1.5. Impurities / accompanying contaminants

Table 1 Compositional Specification of Erythrosine

Parameter	**US FDA Specification for FD&C Red No 3**	**EU Specification for E127**	**Erythrosine (supplied by Sensient Colours UK Ltd)**	**Batch AN0756 (Manufactured 08/08/2005)**	**Batch 0709037873 (Manufactured 31/05/2007)**
Identification					
Active colour content	≥ 87%	≥ 87%	≥ 89%	91%	90.8% (89.4%)[2]
Contaminants					
Sodium chloride	≤ 13%	-	≤ 11%	-	12.2%
Sodium sulphate	-	-	-	-	-
Volatile matter	-	-	-	-	-
Arsenic	≤ 3 mg/kg	< 3 mg/kg	≤ 3 mg/kg	< 2 mg/kg	Complies[3]
Lead	≤ 10 mg/kg	< 10 mg/kg	≤ 2 mg/kg	< 3 mg/kg	Complies[3]
Mercury	-	< 1 mg/kg	≤ 1 mg/kg	< 1 mg/kg	< 1 mg/kg
Cadmium	-	< 1 mg/kg	≤ 1 mg/kg	< 1 mg/kg	< 1 mg/kg
Zinc	-	-	-	< 50 mg/kg	-
Heavy Metals total	-	< 40 mg/kg	≤40 mg/kg	< 40 mg/kg	< 40 mg/kg?
Inorganic iodides (calculated as Na_2I)	≤ 0.4 %	< 0.1%	≤ 0.1 %	0 %	0.04 %
Water insoluble matter	≤ 0.2 %	≤ 0.2%	≤ 0.2 %	< 0.2 %	Not found
By-products from the Synthetic Procedure					
Subsidiary colouring matters	-	< 4.0 %[1]	≤ 4.0 %	2.66 %	< 4.0 %
Fluorescein	-	< 20 mg/kg	≤20 mg/kg	< 20 mg/kg	Complies[3]
Unhalogenated intermediates	≤ 0.1 %	-	≤ 0.1 %	-	-
Synthetic intermediates	-	-	≤ 0.4 %	< 0.4 %	Complies[3]
Tri-iodoresorcinol	≤ 0.2 %	< 0.2 %	≤ 0.2 %	Complies[3]	Complies[3]
2-(2′,4′-dihydroxy-3′,5′-diiodobenzoyl) benzoic acid	≤ 0.2 %	< 0.2 %	≤ 0.2 %	Complies[3]	Not found
Monoiodofluoresceins	≤ 1.0 %	-	≤ 1.0 %	-	Not found
Other iodinated fluoresceins	≤ 9.0 %	-	≤ 9.0 %	-	< 1.96 %
Ether extractable matter	-	< 0.2 %	≤ 0.2 %	< 0.2 %	Complies[3]

Parameter	US FDA Specification for FD&C Red No 3	EU Specification for E127	Erythrosine (supplied by Sensient Colours UK Ltd)	Batch AN0756 (Manufactured 08/08/2005)	Batch 0709037873 (Manufactured 31/05/2007)
from solution of pH 7-8					

[1] except fluorescein
[2] gravimetric determination in addition to spectrophotometric assay
[3] compliance with EU Directive 95/45/EC stated by manufacturer

* Subsidiary colouring matters are not reported

3.1.6. Solubility

Solubility in water: approximately 70 g/l at 20°C (DFG, 1991)
Solubility in ethanol: approximately 10 g/l (DFG, 1991)
Solubility in glycerine: approximately 35 g/l (DFG, 1991)

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 4.95 at 25 °C (calculated, Molinspiration, 2007)

3.1.8. Additional physical and chemical specifications

Melting point:	no sharp melting point observed
Boiling point:	/
Flash point:	/
Vapour pressure:	
Density:	0.8 – 1.0 kg/m³ (limit)
Viscosity:	
pKa:	
pH:	7-9 in aqueous solution
Refractive index:	
UV/VIS spectrum (200-800 nm)	λmax 526 nm

3.1.9. Homogeneity and Stability

Based on stability experiments performed at ambient temperature, erythrosine has a shelf-life of 6 years.

The thermal degradation of erythrosine has been monitored over the temperature range 20° C to 350° C (Barbano and Deilavalle, 1984). Free iodide is only released from the colour additive at temperatures above 200° C indicating that the material is stable under the conditions of use.

3.2. Function and uses

CI 45430 (Erythrosine) is a red colour additive that is currently permitted in Europe for use in foods, pharmaceuticals and cosmetics.

CI 45430 (Erythrosine) is listed in Annex IV of the EU Cosmetics Directive 76/768/EEC which permits its use as a colorant in cosmetic products, without a concentration limit for this use. It is, however, not permitted as a hair dye ingredient because a safety dossier supporting its use was not provided under the European Hair Dye strategy[1] and the

[1] http://ec.europa.eu/enterprise/sectors/cosmetics/cosmetic-products/hair-dye-products/safety-strategy/index_en.htm

substance was subsequently banned for this use[2] (Annex II, entry 1337). Erythrosine is historically reported as being used in a range of cosmetic applications including toothpaste, lipstick, eye products, face decorative products, bath & shower products, hair products including sprays & conditioners, shampoos, deodorants, fragrance products, and soap (COLIPA 2006). Following calls for toxicological data on this colour, only the use of this colour in toothpaste products has been actively defended. For this reason this dossier only supports the use of erythrosine in toothpaste products up to a level of 0.0025% and does not relate to its safety in all cosmetic applications.

As a food colorant, erythrosine is added to a range of products to include sweets (Turkish delight), biscuits, glace and tinned cherries and sausages. Following a review of the available scientific data, The European Scientific Committee for Food (SCF) has allocated an acceptable daily intake (ADI) of 0 to 0.1 mg/kg body weight (SCF, 1987). In 1990, the same ADI was assigned by the Joint WHO/FAO Expert Committee for Food Additives (JECFA, 1990), following a re-evaluation of the safety of this colorant. In the EU, Erythrosine is permitted as a colorant in foodstuff[3]. It is limited for use in cocktail cherries and candied cherries up to a maximum level of 200 mg/kg and in Bigarreaux cherries in syrup and cocktails up to 150 mg/kg.

Erythrosine is also approved for use in EU pharmaceutical preparations as a colorant in both solid (capsules/tablets/sugar-coated pills) and liquid (drops/syrups) pharmaceutical preparations. In 1988, The Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) issued a favourable opinion on the use of erythrosine in pharmaceutical products. Erythrosine is typically present at levels of 0.0017 to 0.96 mg/capsule or tablet respectively and at levels of 0.009 to 0.8 mg/mL for oral liquid preparations.

In the US, FD&C Red 3 is permanently listed for use in foods and ingested drugs. In 1990, erythrosine was de-listed by FDA for use in cosmetics and externally applied drugs, following the initial suggested findings that this colorant may induce carcinogenicity in rats. The FDA adopted the Delaney clause which restricts the use of any ingredient, regardless of the amount used, if an ingredient is shown to induce cancer in animals or humans. The FDA did not have an immediate safety concern with this colorant and identified the potential risk based on the data available as about 1 in 100, 000 over a 70 year lifetime. This colorant remains approved for use in the U.S. in foods and ingested drugs.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

The acute toxicity of erythrosine has been determined in a variety of oral studies which had been performed before GLP standards or the relevant OECD test guidelines had been established. Acute dermal or inhalation studies are not available.

3.3.1.1. Acute oral toxicity

Species	Strain	Sex	Details	LD50 [mg/kg]	Guideline GLP	Year/Reference
Mouse	Albino	male	Vehicle: water 10 animals/dose Erythrosine CI 45430 (Coal Tar dye)	1264	Predates OECD; prior to GLP	Roy and Saha, 1981

[2] Commission Directive 2008/88/EC of 23 September 2008 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purpose of adapting Annexes II and III thereto to technical progress. O.J. L 256 24.9.2008 p12

[3] Directive 94/36/EC (European Parliament and the Council of the European Union, 1994)

Species	Strain	Sex	Details	LD50 [mg/kg]	Guideline GLP	Year/Reference
Mouse	Not reported	male	Vehicle: distilled water 5 animals/group Erythrosine BS, ≥ 85% purity	6700	Predates OECD; prior to GLP	Butterworth et al., 1976
Mouse	Not reported	female	Vehicle: distilled water 5 animals/group Erythrosine BS, ≥ 85% purity	6900	Predates OECD; prior to GLP	Butterworth et al., 1976
Mouse	CF1	male	Vehicle: distilled water 10 animal/dose 1000-4913 mg/kg	2558	Predates OECD; prior to GLP	Yankell and Loux, 1977
Rat	CD	female	Vehicle: distilled water 10 animal/dose 2000-4934 mg/kg	2891	Predates OECD; prior to GLP	Yankell and Loux, 1977
Rat	Not reported	male	Vehicle: distilled water 5 animals/group Erythrosine BS, ≥ 85% purity	7400	Predates OECD; prior to GLP	Butterworth et al., 1976
Rat	Not reported	female	Vehicle: distilled water 5 animals/group Erythrosine BS, ≥ 85% purity	6800	Predates OECD; prior to GLP	Butterworth et al., 1976
Rat	Osborne-Mendel	male and female	Vehicle: water 5 animals/sex/group Erythrosine: FD&C Red. No.3, 92-96% purity	1840	Predates OECD; prior to GLP	Hansen et al., 1973a
Rat	Wistar	male	6 animals receiving 2g/kg	> 2000	Predates OECD; prior to GLP	Lu and Lavalle, 1964
Rat	Not specified	Not specified	Vehicle: water Iodine-erythrosine B	> 1000	Predates OECD; prior to GLP	Emerson and Anderson, 1934

3.3.1.2. Acute dermal toxicity

No data available/submitted

3.3.1.3. Acute inhalation toxicity

No data available/submitted

Summary of acute toxicity

As can be derived from the acute oral toxicity studies in rats and mice, the acute oral toxicity of erythrosine is low. After administration of high oral doses of erythrosine, lethargy and depression of motor activity could be observed. No data for the dermal or inhalation routes of exposure are available.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: /
Species/strain: Rabbit, albino

Group size:	4 male animals
Test substance:	Erythrosine CI 45430 (Coal tar dye)
Batch:	not reported
Purity:	not reported
Dose level:	100 mg per square inch area
Controls:	a 1-square inch intact skin area from each animal
Exposure:	48 hours
GLP statement:	no
Date:	1981

100 mg erythrosine was applied to two separate sites of abraded skin (one square inch each) of four male Albino rabbits (age not specified). A further one square inch site served as control. All skin sites were covered by surgical Gauze and rubberised cloth for 24 hrs. After the treatment period, the covers were removed and test material was removed from the skin. Skin reactions were examined at the end of the 24 hr-exposure and 24 hrs thereafter and scored according to the modified method of Draize et al. (1944) as described by Lynch et al. (1978). It was concluded that erythrosine is not a primary skin irritant, because the slight redness observed at the abraded and treated skin sites was not present after 48 hours (Roy and Saha, 1981).

3.3.2.2. Mucous membrane irritation

Eye irritation

Guideline:	/
Species/strain:	Rabbit, strain not specified
Group size:	3 male animals
Test substance:	Erythrosine CI 45430 (Coal tar dye)
Batch:	not reported
Purity:	not reported
Dose level:	100 mg per right eye sac of each animal
Controls:	left eyes of the animals
Exposure:	72 hours
GLP statement:	no
Date:	1981

100 mg erythrosine was instilled into the lower conjunctival sac of the right eye. The left eyes of the animals served as controls. 5 minutes after application, the treated eye was washed with 300 ml distilled water and the cornea, iris and conjunctival mucosa of treated eyes were investigated at 12, 24, 48, and 72 hrs after treatment. At these time points, no adverse ocular reactions were observed (Roy and Saha, 1981).

Guideline:	/
Species/strain:	Rabbit, albino
Group size:	not exactly specified, at least 6
Test substance:	Erythrosine FD&C red No. 3 from Kohnstamm
Batch:	Y 0350 (lake) and X 9973 (colour)
Purity:	not reported
Dose level:	0.2 ml of a 10% aqueous solution twice daily, 5 days per week
Controls:	not stated
Exposure:	4 weeks
GLP statement:	no
Date:	1971

0.2 ml of a 10% aqueous solution of erythrosine was applied repeatedly (twice daily, 5d/week, for 4 weeks) to the conjunctival sac of one eye of a group of at least 6 albino rabbits. One hour after each application the eyes were examined for evidence of staining

and irritation was scored according to Draize. Three days after the last application, two animals of each group (erythrosine colour and erythrosine lake) were killed, upper lids were taken for microscopic examination. Eyeballs and posterior parts were examined grossly for evidence of staining or other abnormalities. Erythrosine colour caused intense colouring of the iris and moderate conjunctival irritation. Staining lasted from 2 to 7 days. Erythrosine lake did not cause severe eye irritation but resulted in spotty staining in some animals and very slight but uniform staining in other animals (Burnett and Opdyke, 1971).

Comment
The protocol selected does not comply with current guidelines and the applied doses (in total 40 mg per day) are quite high. The observation period after the last application was 3 days only. Under these conditions only erythrosine colour exhibited mild irritation. Thus, erythrosine can be regarded as non-irritating to the eye.

Summary of skin and eye irritation

Erythrosine is not irritating to the skin or the eyes.

3.3.3. Skin sensitisation

While no data on sensitisation were contained in the submission dossier, the following studies on skin sensitization could be located from the open literature.

By using a QSAR model (TOPS-Mode) which relates sub-structural molecular descriptors to experimental data derived from the local lymph node assay (LLNA), a weak sensitization potency was predicted for erythrosine (Søsted et al., 2004).
Comment: this study has been undertaken in order to prioritise potentially sensitising substances used as hair dyes for further patch testing and not for hazard identification. As the outcome of the study, a "predicted weak sensitizing potency" is a vague specification. This publication is taken as supporting information but not for a decision of whether or not erythrosine possesses sensitising properties.

Guideline:	predates OECD 406
Species/strain:	Guinea pig, strain not mentioned
Group size:	19 animals
Test substance:	Erythrosine C.I. 45430
Batch:	not given
Purity:	not given
Dose level:	0.1% solution (induction: 10 intradermal injections, 1st challenge after 14 days (intradermally), 2nd challenge after additional 10 days (epidermally, occlusive))
Controls:	sodium chloride as negative, tartrazin as positive control
Exposure:	3 weeks
GLP statement:	predates GLP

Animals received 10 intradermal injections of a 0.1% solution of the colour. The last six injections were given using Freund's adjuvans. After 14 days, a further intradermal injection was given as challenge. A second epidermal, occlusive challenge was given after additional 10 days. After a further intradermal challenge, positive reactions could be observed in 11 of 19 erythrosine-treated animals. After the epidermal challenge, none of the 19 animals exhibited signs of positive reactions. Therefore, a further intradermal challenge was performed later on after which 15 of 19 animals reacted positive. From the results the author concluded, that erythrosine acts via an allergenic mechanism (Maurer, 1979).

Comment

(1) based on the limited reporting and based on the fact, that epidermal challenge did not cause an effect on skin, erythrosine is not regarded as a skin sensitizer.
(2): it was not reported whether dermal application of the dye made detection of eventually occurring skin reactions more difficult.

Guideline:	no corresponding to OECD guideline
Species/strain:	Mouse / Balb/c
Group size:	3 animals
Test substance:	Erythrosine (from Wako Pure Chemical Industries, Ltd. (Osaka, Japan))
Batch:	not given
Purity:	not given
Dose level:	50 µl of a 0.25% solution (Freund's complete adjucans (FCA), test chemical, saline) intradermal, 25 µl of a 0.5% solution topical (vehicle: acetone-olive oil, 4:1, DMSO) on three consecutive days
Controls:	intradermal: vehicle-FCA emulsion; dermal: vehicle alone
Exposure:	9 days (intradermal injection on day 1, five days rest, dermal application daily for 3 consecutive days, day 9: investigation of lymph nodes
GLP statement:	no information

Mice were injected intradermally with the erythrosine-FCA emulsion into two sites of the abdominal skin at both sites of the ventral midline. Five days after injection, test chemical in vehicle was applied to both sides of each ear for three consecutive days. The day after the final dermal application, auricular lymph nodes were excised, pooled, suspended, cultured for 24 hrs and [^{3}H]methylthymidine (^{3}HTdR) incorporation was investigated. Increases in lymph node cell (LNC) number and ^{3}HTdR incorporation relative to controls were determined and expressed as stimulation indices (SIs). Total stimulation indices were obtained by multiplication of SI of lymph node cell proliferation (SI_p) with SI of lymph node cell number (SI_n). A total SI of 3 and above indicates a positive reaction (sensitizer). Results: with a SI_p of 1.15 and a SI_n of 1.35, a total SI of 1.55 was obtained for erythrosine. Thus, erythrosine yielded a negative reaction under the conditions used and was regarded a non-sensitizer (Ikarashi et al., 1996).

Comment
The test used for this study is not a standard test for the determination of skin sensitization. However, by investigating different substances with different sensitizing potential and comparing the results with those from guineas pigs and/or human patch tests, it could be demonstrated, that the methodology (although not validated) properly classified most of the substances investigated. Thus, the results can be used as supporting data within a weight of evidence consideration.

Bär and Griepentrog (1960) have compiled data on the allergenic potential (assessed by guinea pig testing or by the so-called "Läppchentest" in humans). No experimental details are presented. Concerning erythrosine it is reported that guinea pig testing was negative. (Bär and Griepentrog, 1960).

Summary on skin sensitisation
No guideline studies on skin sensitization of erythrosine have been performed. However, there is some information from studies, which have to some extent similarity to guideline studies (Bär and Griepentrog, 1960; Maurer, 1979 and Ikarashi, 1996). From these studies, a low, if any skin sensitisation potential of erythrosine can be deduced. The results can be supported by QSAR considerations (Søsted et al., 2004) and the lack of positive findings in humans after the substance has been used for decades as a food colorant and by the presumably low dermal absorption rate (see section 3.3.4).

3.3.4. Dermal / percutaneous absorption

No studies on dermal absorption of erythrosine have been performed. From physico-chemical data (molecular weight, water solubility and calculated Log Pow value) it can be assumed that dermal absorption is low. These assumptions are confirmed by a dermal carcinogenicity study in ICR mice of both sexes. After repeated (twice weekly for 18 months) dermal application of 0.1 ml of an 1% aqueous solution of erythrosine in distilled water, no indications of systemic bioavailability could be observed (Carson, 1983; Hazleton Laboratories, 1969) (see also section 3.3.7 - carcinogenicity).

Summary on dermal absorption: the dermal absorption of erythrosine can be assumed to be low.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (short-term) oral toxicity

A three-week dietary study has been performed in the rat (Witorsch et al., 1989). However, as this study addressed the effects of erythrosine on the pituitary-thyroid axis (i.e. the study was not intended to be a typical repeat dose study) it is described in section 3.3.12 (Special investigations).

3.3.5.2. Sub-chronic oral / dermal / inhalation toxicity

Repeated dose 56 day oral toxicity study (in mice)

Guideline:	/
Species/strain:	Mouse, CD-1 (Charles River)
Group size:	10 males and 10 females per dose
Test substance:	Erythrosine (FD & C Red No. 3) from H. Kohnstamm & Co Inc
Batch:	AA2459
Purity:	90%
Dose levels:	0, 1.0, 2.0, 3.0 and 5.0% in the diet
Controls:	2 negative control groups (standard laboratory diet)
Exposure:	8 weeks
GLP statement:	predates GLP
Date:	December, 1977

The study was intended as a range finding study for a long-term (carcinogenicity) study. Erythrosine was fed to groups of male and female Charles River CD1 mice. The dietary dose levels of 1.0, 2.0, 3.0 and 5.0% corresponded to 1800, 3700, 6100 and 11100 mg/kg bw/d in females and to 1600, 3600, 5500 and 10000 mg/kg bw/d in males. Individual body weights, food consumption and detailed observations were recorded weekly. Red coloration of the fur and dark red faeces were observed for all treated mice. At the 5.0% dosage level, urine was coloured pink. Necropsy was performed in two animals which died during the study (one male mouse from the control group had a small sized spleen whereas no gross lesions were recorded in one female mouse from the 3% dosing level). Food consumption was comparable among treated and control mice. Male mice at the 2.0%, 3.0% and 5.0% dosage levels showed slightly lower body weight gains compared to controls, whereas female mice exhibited slightly lower body weight gains in all treated groups. As a conclusion, dose levels of 0.3%, 1.0% and 3.0% were recommended for the long-term study. Based on body weight gains, no NOAEL could be derived for female animals. The LOAEL in females was 1.0% erythrosine in the diet (corresponding to 1800 mg/kg bw/d). For male animals, a NOAEL of 2% erythrosine in the diet was derived (corresponding to 3600 mg/kg bw/d) (Jessup et al., 1977).

NOAEL: 3600 mg/kg bw/d in males; LOAEL: 1800 mg/kg bw/d in females based on reduced body weight gain.

Guideline:	/
Species/strain:	Rat (Carworth Farm E strain SPF rats from Shell Research Ltd.)
Group size:	15 males and 15 females per dose (main study) 10 males and 10 females (supplementary study)
Test substance:	Erythrosine BS (EBS)
Batch:	FD & C Red No. 3, C.I. No. 45430
Purity:	(≥ 85% purity)
Dose levels:	0.25%, 0.5%, 1% and 2% in the diet (main study); 2% in the diet (supplementary study)
Controls:	negative control group (standard laboratory diet) in main and supplementary study
Exposure:	13 weeks (main study); up to 40 weeks (supplementary study)
GLP statement:	predates GLP
Date:	1976

In the main study, erythrosine was fed with diet to groups of male and female rats. Dose levels of 0.25%, 0.5%, 1% and 2% in the diet corresponded to 170, 370, 730 and 1470 mg/kg bw/d in females and 160, 330, 690 and 1750 mg/kg bw/d in males. Body weights and food consumption were recorded weekly. Urinalysis was performed in urine collected during week 13. At autopsy, gross appearance of tissues and weights of selected organs were examined. Further, investigations on haematology (consisting of haemoglobin and methaemoglobin concentrations, packed cell volumes, counts of total erythrocytes, reticulocytes, total and differential leucocytes and erythrocytes containing Heinz bodies) and serum biochemistry were performed. The latter included determination of total serum iodine, total protein-bound iodine, EBS iodine and thyroxine iodine. There were no deaths and the rats appeared to be healthy throughout the study. Weight gain between treated and control animals was comparable. The mean food consumption of animals of both sexes treated with the lowest level of the dye was reduced by 9.5 and 11.5% respectively. The fur and faeces of all treated groups were coloured dose-related. At the two highest dose levels, the urine was coloured red, whereas it was orange at the lower dose levels. There was some distension of the abdomen during most of the study. In males dosed with 0.5% and 2% of the dye in the diet, haemoglobin concentration was reduced. There were no statistically significant differences between treated and control groups in the levels of serum cholesterol, transaminases or urea, in urine parameters and renal function tests. Serum concentrations of total iodine, protein-bound iodine, iodine not bound to protein and EBS iodine exhibited a dose-related increase, whereas the levels of thyroxine iodine were comparable between treated and control groups. Compared to controls, there was a highly significant and dose-related increase in the absolute and relative weights of caecum in treated animals. At the highest dietary levels, there were small increases in the absolute and relative weight of the thyroid glands in both sexes. The weights of the kidneys in females at the 0.25% and 0.5% dose levels (but not at higher dose levels) were lower than those of controls. Pigmentation of the kidney tubules was found in both sexes at the highest dose level and also in males at the 0.5% and 1% doses levels. No histological changes were seen in the thyroid. Based on the renal pigmentation observed at 0.5% erythrosine in the diet, a NOAEL of 0.25% in the diet (corresponding to an intake of 160 mg/kg bw/d in males and 170 mg/kg bw/day in females) was derived. In the supplementary study, haematological investigations were performed, which did not confirm the findings of reduced haemoglobin concentration as seen in the 90d main study (therefore reduced haemoglobin concentration was regarded as a non treatment-related effect) (Butterworth et al., 1976a).

Comment
Increased caecal weights were not considered as being of significance (although this phenomenon had also been observed in another study (Hansen et al., 1973 a)). As explanation, undigested material and changes in bacterial flora have been discussed.

Kidney weight changes in females were not considered as being of significance, because of the unusual small range of kidney weights in the control group.

Guideline:	/
Species/strain:	Pig (Large White strain)
Group size:	3 males and 3 females per dose
Test substance:	Erythrosine BS (EBS)
Batch:	not given
Purity:	85%
Dose levels:	167, 500 and 1500 mg/kg bw/day (the amounts of substance corresponding to each dose level were dissolved in water, added to a part of the daily food ration and given before the main feed)
Controls:	negative control group
Exposure:	14 weeks
GLP statement:	predates GLP
Date:	1976

Erythrosine BS was orally administered to groups of male and female Large White Pigs. Blood for haematological investigations and iodine determinations was taken at weeks 4, 8 and 14. Urine for urinalysis was taken during week 6 and the last week of feeding. At autopsy (week 14), organs were examined for abnormalities, selected organs were weighed and/or preserved in 10% buffered formalin for histopathological examination. All animals appeared healthy throughout the experiment. There were no significant treatment-related effects on body weight except for a slight reduction at the high-dose level. Haematology and urinalyses did not reveal any treatment-related effect. A dose-related increase in thyroid weights was noted, which reached statistical significance in female pigs at 400 and 1500 mg/kg bw/day when compared to controls. No pathological changes in the thyroid were seen, but treated pigs exhibited decreased levels of serum thyroxine when compared with controls. In addition, dose-related increases in the serum levels of protein-bound iodine, unbound iodine and erythrosine-bound iodine were present in all treated groups. Due to increased thyroid weights and decreased serum thyroxine levels at all dosages, a LOAEL of 167 mg/kg bw/day was obtained from this study (Butterworth et al., 1976b).

3.3.5.3. Chronic (> 12 months) toxicity

Dermal

A long-term dermal toxicity study in mice is described in section 3.3.7- Carcinogenicity.

Oral

Guideline:	/
Species/strain:	Rat (Osborne-Mendel)
Group size:	25 males and 25 females per dose
Test substance:	Erythrosine (certified as FD&C Red No. 3)
Batch:	not given
Purity:	95%
Dose levels:	a) 100, 235, 750, 1500 mg/kg bw/twice weekly (dissolved in distilled water) (gavage) b) 0.5%, 1.0%, 2.0% or 4% (with the diet)
Controls:	negative control groups: a) vehicle only, b) standard laboratory diet
Exposure:	a) 85 weeks; b) 86 weeks
GLP statement:	predates GLP
Date:	1973

Erythrosine was administered either for 85 weeks by gavage or for 86 weeks with the diet to Osborne-Mendel rats of both sexes. Gavage levels corresponded to 29, 67, 214, 429 mg/kg bw/day; dietary levels corresponded to approximately 250, 500, 1000 and 2000 mg/kg bw/d. At the end of the treatment periods, the animals were fed control diet until the experiment was terminated at 2 years. Body weight, food intake, deaths and clinical observations were recorded weekly. Prior to treatment and during the study, blood samples were taken for blood count and determination of protein-bound iodine, thyroxine (T4), prothrombin times and erythrosine. At the end of the study (i.e. after 2 years), the major part of the animals was subjected to gross and microscopic examination and a fairly complete series of tissues from some rats in the 4%, 1500 mg/kg and control groups were examined microscopically. At the end of one year, male and female rats given the 4% diet and female rats given the 2% diet showed a statistically significant reduction in body weight (mean weights were: control males: 544 g, males given the 4% diet: 501 g ($p < 0.01$); control females: 351 g, females given the 4% diet: 293 g ($p < 0.001$), females given the 2% diet: 326 g ($p < 0.01$). No consistent differences in red blood cell counts, haematocrit, haemoglobin, reticulocyte counts or other indications of anaemia were observed. Increased values for protein-bound iodine were attributed to circulating erythrosine in the blood serum. In week 16 of the recovery phase, protein-bound iodine levels had returned to control levels in the animals investigated (i.e. the 0.5% and 4% diet groups and the 29 and 429 mg/kg bw gavage groups). Thyroxine-iodine levels were not affected in treated rats. Slight caecal distension occurred in some rats, which were not as pronounced as in another study (Hansen et al., 1973a), maybe due to recovery period after treatment. Diarrhoea was observed in animals fed the 4.0% diet. No gross or microscopic pathology was attributed to administration of erythrosine. No effect on thyroid morphology was observed. The authors conclude that elevated PBI levels were attributed to erythrosine circulating in the blood. Further, a previous study reporting that erythrosine causes anaemia in rats (Bowie et al., 1966) was not confirmed by the results of this study. NOAELs of 1.0% (500 mg/kg bw/d) for females and 2% (1000 mg/kg bw/d) for males were derived for dietary administration based on body weight changes were derived from the dietary part of the study. From the gavage part, the highest dose tested (429 mg/kg bw/d) was taken as NOAEL (Hansen et al., 1973b).

Comment
Although the authors state, that thyroxine-iodine levels were not affected in treated rats, this type of investigation is not described in the experimental and result section of the publication.

Guideline:	/
Species/strain:	Rat (Osborne-Mendel)
Group size:	12 males and 12 females per dose
Test substance:	Erythrosine (certified as FD&C Red No. 3)
Batch:	not given
Purity:	92-96%
Dose levels:	0.5%, 1.0%, 2.0% or 5% (with the diet)
Controls:	standard laboratory diet
Exposure:	2 years
GLP statement:	predates GLP
Date:	1973

Erythrosine was fed to Osborne-Mendel rats for 2 years. Dietary levels corresponded to approximately 250, 500, 1000 and 2000 mg/kg bw/d. Blood was taken for haematology after 3, 6, 14 and 21 months from 3 animals of each group. At autopsy after 2 years, weights of heart, liver, spleen, kidneys and testes from surviving animals were recorded. From 61 animals sectioned microscopically, 21 were investigated in detail and among the remaining 40 animals, investigations were limited to kidney, liver, testes, tumours (if present) and other organs with abnormalities. No effects on mortality, haematology, and

weights of liver, heart, kidney or testes were observed up to the 5.0% dose level. The absolute spleen weights of male animals of the 5% and 2% level were significantly lower than those of controls. Relative spleen weights were significantly reduced in male rats given 0.5, 2 and 5% erythrosine and in females given 5% erythrosine. Significant growth inhibition occurred in both sexes at the 5% level, significantly reduced body weights were also present at week 12 and 27 of this dose level. The only treatment-related gross lesion was caecal distension. Moderate and slight distension was observed at the 5% level and slight distension occurred at the 1.0% and 2.0% levels and no distension was observed at the 0.5% level and in control animals. No treatment-related effects were observed upon histopathological examination. Thus, a LOAEL of 0.5% (corresponding to approximately 250 mg/kg bw/d) erythrosine in the diet was derived for the rat based on reduced relative spleen weight (Hansen et al., 1973a).

Guideline:	/
Species/strain:	Dog (Beagle)
Group size:	3 males and 3 females per dose
Test substance:	Erythrosine (certified as FD&C Red No. 3)
Batch:	not given
Purity:	92-96%
Dose levels:	0.5%, 1.0%, or 2.0% (with the diet)
Controls:	standard laboratory diet (0% erythrosine)
Exposure:	2 years
GLP statement:	predates GLP
Date:	1973

Beagle dogs were fed erythrosine with the diet for 2 years. Dietary levels of 0.5 %, 1.0 % and 2.0 % corresponded to approximately 125, 250 and 500 mg/kg bw/d. Periodic haematological examinations were performed before and during the test period. After 2 years, animals were killed and sections of liver, kidney, heart, lung, gall bladder, spleen, pancreas, adrenal, thyroid, parathyroid, stomach, small and large intestines, brain, rib bone and marrow, testis, prostate, ovary, uterus salivary gland, lymph node and skeletal muscle from the animals receiving the 2 % dose were examined macroscopically, but tissues from other animals were also examined. No effects attributable to the ingestion of erythrosine were observed on body weights, organ weights, or haematology. The following compound-related effects were observed in the animals fed 2% erythrosine in the diet: slight chronic thyroiditis in one male and one female, a slight decrease in the myeloid-erythroid ration in the bone marrow in one male, a slight testicular atrophy in another male and cystic mucoic glands in the gall bladder of a third male. Based on these effects seen in single animals of the 2 % groups (which were regarded as incidental abnormalities by the authors), a NOAEL of 2 % erythrosine in the diet (corresponding to approximately 500 mg/kg bw/d) was derived from this study. (Hansen et al., 1973a).

Comment
Based on the fact that the thyroid is a target tissue of erythrosine toxicity and based on the fact that slight chronic thyroiditis was observed in one male and one female animal of the 2 % dose level (the group size was 3 animals per sex) it is more appropriate to derive a NOAEL of 2 % erythrosine in the diet (corresponding to approximately 250 mg/kg bw/d).

Guideline:	/
Species/strain:	mouse (Mongolian Gerbils)
Group size:	(a) dietary administration: 15-16 animals/sex/dose (b) gavage: 20-26 animals/sex/dose
Test substance:	Erythrosine (certified as FD&C Red No. 3)
Batch:	X3238
Purity:	95 %
Dose levels:	(a) 1.0 %, 2.0 %, or 4.0 % (with the diet)

	(b) 200, 750, or 900 mg/kg bw (twice weekly by gavage)
Controls:	(a) standard laboratory diet (0 % erythrosine; 32 animals per sex)
	(b) vehicle (distilled water) by gavage (33 males; 30 females)
Exposure:	(a) 105 weeks (dietary administration)
	(b) 97 weeks (gavage)
GLP statement:	predates GLP
Date:	1976

Effects of chronic oral administration of erythrosine were investigated in Mongolian gerbils. The substance was either administered orally via diet at dose levels of 0% (32 animals per sex), 1% (15 males, 16 females), 2% (16 males, 15 females) and 4% (15 males, 16 females) for 105 weeks or by two weekly gavage applications (dose volume: 10 ml /kg) of 0 (33 males, 30 females), 200 (20 animals per sex), 750 (22 animals per sex) or 900 (23 males, 26 females) mg/kg bw for a period of 97 weeks. The dietary levels corresponded to approximately 0, 1500, 3000 and 6000 mg/kg bw/d. The gavage levels corresponded to 57, 214 and 257 mg/kg bw/day. Blood was taken before, during the course of the study and at the end of the study. At termination, animals were autopsied, selected organs (heart, liver, spleen, kidneys, testes) were weighed, internal organs of thorax and abdomen and one hind leg were fixed for gross examination and microscopy. Both application regimes did not result in dose-related increases of deaths. Diarrhoea with dye in the faeces was observed in the treated animals. Colouring of the fur could not be detected in the chronic dietary study, possibly because of masking by the natural colour. The mouth area of the animals treated by gavage was coloured pink. Dose-related decreases of body weights were observed in the animals treated via diet. In animals of both sexes receiving the 2 % and 4 % dietary level, relative weights of heart, liver and spleen were statistically significantly decreased. In the animals treated by gavage, body weights and organ weights were not affected. Concerning haematology, slight decreases in haematocrit and haemoglobin values and in leukocyte and reticulocyte counts were observed in some animals and reached statistical significance in some cases. Both modes of administration resulted in a dose-related pigmentation of viscera and fur-covered skin, with dietary administration leading to less coloration. At gross pathology, dose-related changes of the thyroid of animals receiving 1% - 4% dietary application were observed and were characterized by enlargement, increased storage of colloid, follicles associated with foci of microfollicles and focal hyperplasia and intraluminal and interstitial leucocytic infiltration. Although there was slight increase in follicular size in the thyroids of gerbils given 900 and 750 mg/kg bw by gavage, a definite effect comparable to that in the dietary study was not observed. After histopathological examination, no treatment-related effects were observed. Further, erythrosine had no effect on tumourigenesis measured as either incidence of total tumours or of a single tumour type. For dietary administration, a LOAEL of 1 % (corresponding to approximately 500 mg/kg bw/d), for administration via gavage, a NOAEL of 200 mg/kg bw twice weekly (corresponding to 57 mg/kg bw/day) was identified as NOAEL (Collins and Long, 1976).

Comment
The authors of the study state, that they have had limited experience with this animal species (no previous pathological experience, no historical controls for comparison); thus, interpretation of granulomas in the livers of control animals was difficult.

Three further oral chronic toxicity studies (Richter et al., 1982; Brewer et al., 1982 and Brewer et al., 1982) are described in section 3.3.7 - Carcinogenicity

Summary of repeat-dose toxicity studies

A 56 day repeat-dose and subchronic and chronic oral repeat dose studies have been performed in mice, rats, pigs and beagle dogs either by administration via gavage or by administration with the diet. Neither study has been performed in accordance with OECD test guidelines because the studies have been performed before the implementation of the OECD test guideline program. However, the studies were partially performed in compliance

with GLP principles. In general, erythrosine had no pronounced effect of mortality of the animals and animals appeared to be healthy throughout the studies.
In mice and rats, discoloration of fur, mouth area, faeces and – at higher dosage levels- urine has been described. In one rat study, pigmentation of the kidneys was reported (NOAEL: 160 mg/kg bw/d). In mice and rats, erythrosine treatment caused reduction in body weights at the respective upper dose levels tested. The effect was more pronounced after dietary treatment compared to gavage. Statistically significant effects on body weights were not observed in pigs and dogs.
At high doses of erythrosine, increased caecal weights and caecal distension (not accompanied by histological findings) have been described; in addition, diarrhoea was observed in rats and mice at higher erythrosine dose levels. Undigested material and changes in bacterial flora have been discussed as explanation for the caecal effects, which were not considered significant for the toxicological assessment.
Erythrosine-induced organ weight changes were reduced spleen weight in Osborne-Mendel rats (LOAEL: 250 mg/kg bw/d) and dose-related increases in thyroid weights, which were observed in rats (of either sex) and pigs (LOAEL in pigs: 167 mg/kg bw/d). Increased thyroid weight in rats and pigs were not accompanied by histological changes. However, treated pigs exhibited decreased serum thyroxine levels compared to controls (NOAEL: 250 mg/kg bw/d). In Mongolian Gerbils, dose-related changes in gross pathology of the thyroid have been observed after dietary administration of erythrosine. In contrast to other species investigated, in Mongolian Gerbils also statistically significantly decreased relative weights of heart, liver and spleen were reported after dietary administration of erythrosine. Although tissue weights (including thyroid) were not changed in dogs, slight chronic thyroiditis occurred in one male and one female animal at the highest dose tested (2% in the diet).
As far as investigated in the studies, urinalysis and haematology did not reveal significant treatment-related effects in rats, pigs and Beagle dogs. In Mongolian Gerbils, on the other hand, decreases in haematocrit, haemoglobin, leucocyte and reticulocytes were observed in some animals which reached statistical significance in some cases.
As far as investigated, erythrosine caused dose-related increases in serum concentrations of iodine in rats and pigs (which comprised elevations in total serum iodine, iodine bound to protein, iodine not bound to protein and iodine bound to erythrosine). After cessation of erythrosine, levels of protein-bound iodine in the plasma returned to control values (this has only been investigated in rats). Thyroxine-iodine levels were not changed in the rat.
Taken together, most profound and consistently across species observable effects of chronic administration of erythrosine were reduction of body weights (NOAEL: 429 mg/kg bw/d) and (reversibly) elevated levels in serum iodine. Effects on the weights of thyroids were observed for different species. However, only in Mongolian Gerbils, changes thyroid weights were accompanied by histopathological findings (NOAEL: 57 mg/kg bw/d). None of the studies described in this section has been selected for MoS calculation.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Tests for Gene Mutation

In vitro Bacterial Tests

Erythrosine has been tested for mutagenicity in vitro in several GLP-compliant and also in several mutation tests, whose GLP compliance was not reported.

Species/Strain	Guideline/GLP-compliance	Substance, Batch, Purity	Concentrations	Remarks (metabolic activation, positive control)	Findings	Reference
Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100	GLP-compliant, study appears consistent with OECD TG 471	Test compound 21373-9-2 (purity and specifications not reported)	1.0 to 10 000 µg/plate	Aroclor-induced rat liver S9; positive and negative controls included	Negative (with and without metabolic activation)	Jagannath and Myhr, 1984a; FDA Study 66
Salmonella typhimurium strains TA 1535, TA 97, TA 98 and TA 100	GLP-compliance not reported, study appears consistent with OECD TG 471	FD&C Red No.3, lot AC1323 90 % pure	10 to 5000 µg/plate	Rat and hamster liver homogenates; distilled water as negative control	Negative (with and without metabolic activation)	Downie and Matula, 1984, FDA study 78
Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, TA 100	GLP-compliance not reported, study appears consistent with OECD TG 471	FD&C Red No.3, lot and purity not given	1.0 to 10 000 µg/plate	S9 from Aroclor 1254 induced and non-induced adult Sprague-Dawley rats; deionized water as negative control	Some evidence of toxicity was observed at the high dose levels. None of the dose levels increased the number of revertants by a factor of 2 or more.	Lin and Brusick, 1986
Salmonella typhimurium strains TA 92, TA 1535, TA 100, TA 1537, TA 94, TA 98	GLP-compliance not reported, predates OECD Guidelines	Food red 3, lot and purity not given.	Up to 5000 µg/plate	S9 from livers of PCB-pretreated Fischer rats	Negative (with and without metabolic activation)	Ishidate et al., 1984
Salmonella typhimurium strains TA 97a, TA 98, TA 100, TA 102 and TA 104	GLP- and OECD-compliance not reported	Erythrosine BS, lot and purity not given	0 to 2000 µg/plate	Aroclor-induced rat liver S9 or caecal cell-free extracts	Negative; decreased survival was observed in repair-deficient strains (TA 97a, TA 98, TA 100), but not in the repair-proficient strains (TA 102, TA 104)	Lakdawalla and Netrawali, 1988b

Salmonella typhimurium strain TA 1538	GLP compliance not reported, study predates OECD guidelines	Erythrosine BS, lot and purity not given	0.5 and 5.0 mg/ml	rat liver microsomes; negative controls not reported	Negative	Haveland-Smith and Combes, 1980
Salmonella typhimurium TA 1538 and TA 1535	GLP compliance not reported, study predates OECD guidelines	Erythrosine BS, lot and purity not given	0.5 mg/l (fluctuation test with TA 1538) and 1000 µg/plate	microsomes from rats treated with sodium phenobarbitone; no negative controls	Negative in the absence or presence of light	Haveland-Smith et al., 1981
Salmonella typhimurium strains TA 1535, TA 100, TA 1537, TA 1538, TA 98	GLP compliance not reported, study predates OECD guidelines	FD&C Red No.3, lot no. 4561, 93 % purity	50 to 1000 µg/plate	rat liver S9; comparison with historical negative controls and 0.1 or 1.0 mg sodium dithionite	Negative	Brown et al., 1978
Salmonella typhimurium strains TA 1535, TA 100, TA 98, TA 1537	GLP compliance not reported, study predates OECD guidelines	FD&C Red No.3, lot and purity not given	1.0 to 10 000 µg/plate	Aroclor-induced rat liver S9, distilled water as negative control	Negative	Auletta et al., 1977
Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, TA 100	GLP- and OECD compliance not reported	FD&C Red No.3, 90.5 % pure, from ETAD (Ecological and Toxicological association of the Dyestuffs Manufacturing Industry, Basel, Switzerland)	Up to 3333 µg/plate	Aroclor-induced rat and hamster liver S9, methanosulfonate and 3-methycholanthrene as positive controls, solvent controls	Negative	Cameron et al., 1987
Saccharomyces cerevisia strains D5, D7, XV185-14C	GLP-compliance not reported, study appears consistent with OECD TG 481	FD&C Red No.3, lot AC1323 90 % pure	100 to 10 000 µg/ml in distilled water	Rat and hamster liver homogenates; distilled water as negative control	Positive (induction of mitotic gene conversion and reverse mutation)	Downie and Matula, 1984; FDA study 78
Saccharomyces cerevisiae strain D5	GLP-compliance not reported, study predates OECD guidelines, but portions of the test	FD&C Red No.3, lot and purity not given	100 to 10 000 µg/ml	S9 from Aroclor 1254 induced and non-induced adult Sprague-Dawley rats; saline as	Little or no toxicity was observed over the dose range employed	Lin and Brusick, 1986

	appear similar to OECD 481			negative control		
E. coli strains WP2 trp uvrA, WP67 trp uvr polA, WP100 trp uvrA recA	GLP compliance not reported, study predates OECD guidelines	Erythrosine BS, lot and purity not given	0.5, 1.0 and 5.0 mg/ml	Rat liver microsomes, negative controls not reported	Negative	Haveland-Smith and Combes, 1980
Escherichia coli strains WP2 trp, TP2 ttrp uvrA, WP67 trp uvrA polA, TP100 trp uvrA recA, K12 ND lac Z	GLP compliance not reported, study predates OECD guidelines	Erythrosine BS, lot and purity not given	up to 5 mg/ml (microsomal rec and pol assay)	microsomes from rats treated with sodium phenobarbitone; negative controls not reported	Negative	Haveland-Smith et al., 1981
Saccharomyces cerevisiae strain D5	GLP-compliant, study appears consistent with OECD 481	Test material No. 21373-9-2, purity not given	100 to 10 000 µg/ml	Aroclor-induced rat liver S); negative control: distilled water	Negative	Jaganath and Myhr, 1984b (FDA study 67.1)
Saccharomyces cerevisiae strain BZ 34	GLP-compliance not reported, predates OECD Guidelines, study appears consistent with OECD 481	Erythrosine, lot and purity not given	5 mg/ml	No microsomal activation, distilled water as negative control	Negative	Sankaranayanan and Murthy, 1979
Bacillus subtilis strain 168 and strain hcr-9	GLP- and OECD compliance not reported	Eryhrosine BS, lot not given, purified by Sephadex	0 to 1000 µg/ml	Aroclor-induced rat liver S9 or caecal cell-free extracts	Positive in repair-proficient strain 168 in the presence of UV light, negative in repair-deficient hcr-9. Positive effects in 168 decreased in the presence of metabolic activation	Lakdawalla and Netrawali, 1988a

In vitro Mammalian Cell Tests

Test description	Guideline/GLP-	Substance, Batch,	Concentrations	Remarks (metabolic activation, positive	Findings	Reference

	compliance	Purity		control)		
Mammalian cell gene mutation assay (mouse lymphoma, L5178Y cells)	GLP compliance not reported, predates OECD guidelines, but it appeared similar to OECD TG 476	FD&C Red No.3, lot and purity not given	100 to 800 µg/ml without S9; 50 to 400 µg/ml with S9	S9 from Aroclor 1254 induced and non-induced adult Sprague-Dawley rats, deionized water as negative control	Negative	Lin and Brusick, 1986
Mammalian cell gene mutation assay (V79 hamster lung cells)	GLP compliance not reported, predates OECD guidelines, but it appeared similar to OECD TG 476	Erythrosine (Kohnstamm), Lot AC1323, purity > 90 %	100, 200 and 300 µg/ml	primary hepatocytes from male Wistar rats; DMSO as negative control	Negative (non-mutagenic at the HGPRT and Na+, K+, ATPase gene loci)	Rogers et al., 1988
Mammalian cell gene mutation assay (L5178Y TK+/- mouse lymphoma cells)	GLP-compliant, apparently consistent with OECD TG 476	National Cancer Institute test article 43548; purity and further specifications not given	50 to 500 µg/ml	Aroclor-induced rat-liver S9, distilled water as negative control	Positive	Rogers-Back et al., 1985; FDA study 74
Mammalian cell gene mutation assay (L5178Y TK+/- mouse lymphoma cells)	GLP-compliant, apparently consistent with OECD TG 476	National Cancer Institute test article 72391; purity and further specifications not given	60 to 200 µg/ml	Aroclor-induced rat-liver S9, distilled water as negative control	Positive	Kirby et al., 1983
Mammalian cell gene mutation assay (L5178Y TK+/- mouse lymphoma cells)	GLP-compliant, apparently consistent with OECD TG 476	Test material No. 21373-9-2, purity not given	100 to 600 µg/ml (without activation); 35 to 350 µg/ml (with activation	Activating system not specified; distilled water as negative control	Negative	Cifone and Myhr, 1984 (FDA study 67)
Mammalian cell gene mutation assay (L5178Y TK+/- mouse lymphoma cells)	GLP-and OECD compliance not reported	FD&C Red No.3, 90.5 % pure, from ETAD (Ecological and Toxicological association of the Dyestuffs Manufacturing Industry, Basel,	Up to 200 µg/ml	Aroclor-induced rat and hamster liver S9, methanosulfonate and 3-methycholanthrene as positive controls,	Positive at concentrations exerting high toxicity	Cameron et al., 1987

		Switzerland)		solvent controls		
Mammalian cell gene mutation assay Chinese hamster CHL cells)	GLP-compliance not reported, predates OECD Test guidelines, but apparently consistent with OECD TG 473	Food red 3, lot and purity not given.	Up to 0.6 mg/ml	S9 from livers of PCB-pretreated Fischer rats, negative controls not reported	Weakly positive at 48 hrs (according to JECFA, this weakly positive effect might have resulted from osmotic effects)	Ishidate et al., 1984
Micronucleus frequency (V97 hamster lung cells)	GLP-compliance not reported, predates OECD Test guidelines	Erythrosine (Kohnstamm), Lot AC1323, purity > 90 %	50, 100, 200 and 300 µg/ml	primary hepatocytes from male Wistar rats; DMSO as negative control	Increase at the highest dose in the absence of hepatocyte; negative with the addition of hepatocytes	Rogers et al., 1988
Mitotic frequency (V97 hamster lung cells)	GLP-compliance not reported, predates OECD Test guidelines	Erythrosine (Kohnstamm), Lot AC1323, purity > 90 %	50, 100, 200 and 300 µg/ml	primary hepatocytes from male Wistar rats; DMSO as negative control	A dose-related increase in mitotic frequency was observed due to an increase in the number of first mitosis at the expense of later cell divisions.	Rogers et al., 1988
Sister-Chromatid-Exchange (V97 hamster lung cells)	GLP-compliance not reported, predates OECD Test guidelines	Erythrosine (Kohnstamm), Lot AC1323, purity > 90 %	50, 100, 200 and 300 µg/ml	primary hepatocytes from male Wistar rats; DMSO as negative control	Negative	Rogers et al., 1988
Sister-Chromatid-Exchange (Syrian hamster embryo cells)	GLP- and OECD compliance not reported	Erthrosine B from Wako Pure Chemicals, purity not given	0, 11, 33 and 110 µM	No metabolic system, no information on positive or negative controls	Negative	Miyachi et al., 2005
Sister-Chromatid-Exchange (Syrian hamster embryo	GLP- and OECD compliance not reported	Erthrosine B from Wako Pure Chemicals, purity	33 to 330 µM	With and without metabolic activation (post-mitochondrial supernatant from	Negative without metabolic activation, positive at 330 µM in the presence of	Hagiwara et al., 2006

cells)		not given		Sprague-Dawley rats pretreated with Phenobarbital and 5,6-beenzoflavone)	metabolic activation	
DNA repair by HPC/DR (hepatocyte primary culture/DNA repair) in vitro Assay	GLP-compliance not reported, predates OECD Test guidelines	Erythrosine from Allied Chemical Co, purity 93 %	0,001 to 1 mM	Hepatocytes from male Sprague-Dawley rats; 4hr incubation; o-aminoazotoluene as positive control, acid red 14 as negative control	erythrosine did not induce DNA repair in rat hepatocytes up to a concentration of 0.1 mM (a toxic concentration level was achieved at 1 mM	Kornbrust and Barfknecht, 1985
Cell transformation in Fischer rat embryo fibroblasts [4]	GLP-compliance not reported, predates OECD Test guidelines	Food dye red 3 (from FDA), purity 91 %	0.1 to 10 µg/ml	Methylcholanthrene, benzo(a)pyrene and acetone	Negative (erythrosine did not induce cell transformation)	Price et al., 1978

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Test description	Guideline/GLP-compliance	Substance, Batch, Purity	Dosages/ positive and negative controls	Remarks	Findings	Reference
In vivo mouse Micronucleus test (male and female CD-1-mice)	GLP-compliant, predates OECD, but appears to be consistent with OECD TG 474	Test article 21373-9-2; purity and further specifications not provided	24, 80 and 240 mg/kg bw (i.p.); positive controls: triethylenemelamine ; negative controls: deionized water	Harvests were performed 24 and 48 hrs after administration for test compound and 24 hrs after administration for positive and	Negative (no significant increases in bone marrow polychromatic erythrocytes)	Ivett and Myhr, 1984

[4] Although a cell transformation test is not an in vitro genotoxicity test, it is listed here

				negative controls		
Micronucleus test in male B6C3F1 mice (micronuclei in bone marrow polychromatic erythrocytes and in peripheral blood reticulocytes)	GLP-compliance not reported; OECD Guidelines not reported	Erythrosine from BASF, purity approximately 85 %	50, 100 and 200 mg/kg in distilled water for two administrations at intervals of 24 hrs; Mitomycin C as positive control, distilled water as negative control		Negative	Zijno et al., 1994
Micronucleus test in male and female CD-1 mice (micronuclei in bone marrow polychromatic erythrocytes)	GLP-compliance not reported, predates OECD Guidelines	FD&C Red N. 3, FDA grade, (lot and purity not given)	24, 80 and 240 mg/kg bw (i.p.), positive controls: triethylenemelamine ; negative controls: deionized water	Harvests at 24 and 48 hrs after administration	Negative (no significant increases in micronucleus frequencies)	Lin and Brusick, 1986)
DNA repair by HPC/DR (hepatocyte primary culture/DNA repair) invivo/in vitro Assay	GLP-compliance not reported, predates OECD Test guidelines	Erythrosine from Allied Chemical Co, purity 93 %	200 mg /kg (gavage of an aqueous solution)	Hepatocytes from male Sprague-Dawley rats, killed at 2 or 15 hrs after administration of substance	Negative(no induction of DNA repair)	Kornbrust and Barfknecht, 1985
SCE in peripheral blood lymphocytes of male B6C3F1 mice	GLP-compliance not reported; OECD Guidelines not reported	Erythrosine from BASF, purity approximately 85 %	50, 100 and 200 mg/kg in distilled water for two administrations at intervals of 24 hrs; Mitomycin C as positive control, distilled water as negative control		Negative	Zijno et al., 1994
Chromosome aberrations in bone marrow of adult male rats (rattus norvegicus)	GLP-compliance not reported; OECD Guidelines not reported	No information	0, 0.08 and 0.4 g/kg diet /d for 60 d	No positive or control	Positive Mitotic indices statistically significantly increased at the	Mekkawy et al., 2000

					lower dose, statistically significantly decreased at the high dose	
Chromosome aberrations in Swisss-albino mice	GLP-compliance not reported; OECD Guidelines not reported (only abstract available)	No information (only abstract available)	Control, low, intermediate and high dose: intermediate and control reversal; 30d oral administration	Only abstract available, not details	Positive Significant dose-dependent decrease in cell proliferation; chromosomal aberration were observed less significantly	Devi et al., 2004
In vivo Comet Assay	No corresponding OECD Guideline, GLP compliance not reported	Erythrosine (from Tokyo Kasei Kogyo Industry Ltd), purity not given	10, 100 and 2000 mg/kg (gavage)	no positive control; historical data from untreated animals served as negative controls; 3 hrs for all three dose groups; 24 hrs for a further 2000 mg/kg dose group	Positive in the 3 hr experiment: erythrosine induced DNA-damage in the glandular stomach, colon and urinary bladder	Kawaguchi et al., 2001; Sasaki et al., 2002

Summary of Mutagenicity/Genotoxicity

The mutagenicity/genotoxicity of erythrosine has been investigated in a variety of in vitro and in vivo assays. The genotoxicity and mutagenicity of erythrosine based on the respectively available in vitro and in vivo data has been discussed and reviewed by different scientific bodies (e.g. SCF, 1987; JECFA, 1990, TemaNord, 2002). The different evaluation panels consistently concluded, that erythrosine is not genotoxic. Since the publication of these scientific opinions further studies on the endpoint genotoxicity/mutagenicity have become available. Among these studies, there was one positive in vitro test for chromosomal aberrations (Hagiwara al., 2006) and there were three positive in vivo tests (a Comet assay in mice (Kawaguchi et al., 2001; Sasaki et al, 2002) and two studies investing chromosomal aberrations (Mekkawy et al., 2000; Devi et al., 2004))). In the in vitro chromosomal aberration test by Hagiwara et al. (2006), the percentage of cells with polyploidy or endoreplication was only positive in the presence of metabolic activation, whereas the assay was negative (at even higher erythrosine levels) without metabolic activation. In the COMET Assay performed by Kawaguchi and Sasaki, erythrosine-induced DNA-damage in the glandular stomach, colon and urinary bladder was observed 3 hrs after administration of erythrosine (starting at a dose level 100 mg/kg), but not 24 hrs after administration. The authors discuss, that the effects might be due to saturation of metabolic processes. The findings of this study can be overruled by the outcome of several long-term (and carcinogenicity) studies performed with erythrosine: aside from caecal distension (not accompanied by histopathological changes) and diarrhoea observed in some studies and from gross-staining and granular deposits observed in one rat study (Wilhelm and Ivy (1953), see section 3.3.7-carcinogenicity), no adverse effects or histopathological changes could be observed in glandular stomach, colon and bladder. Devi et al. (2004) stated that effects on cell proliferation were more pronounced compared to effects on chromosomal aberration. The study was not in compliance with the respective OECD protocol, test conditions are unusual with respect to animal strain and regimen of substance administration, lacks positive controls and there are further deficiencies in design of the study and presentation of the results. In the study performed by Mekkawy et al. (2000), neither a dose-relationship nor consistency in the results could be observed. Thus, the new studies available do not provide a clear evidence of a genotoxic potential of erythrosine and do not invalidate previous conclusions of different Scientific Panels on the genotoxic potential of erythrosine. Further, long-term (carcinogenicity) studies and mode of action considerations do not support a genotoxic potential of erythrosine. Based on the data available so far it can be concluded that erythrosine is not genotoxic and not mutagenic.

3.3.7. Carcinogenicity

Guideline:	/ (predates OECD Guidelines)
Species/strain:	mice, Charles-River CD-1
Group size:	60 animals/sex/dose
Test substance:	FD & C Red No. 3
Batch:	AA2459
Purity:	90 %
Dose levels:	0.3 %, 1.0 % and 3.0 % in the diet
Controls:	no positive control group; two negative control groups (240 animals in total; 60 animals per sex/group) receiving basal diet (0 % erythrosine)
Exposure:	104 weeks
GLP statement:	conduct was in compliance with the study protocol requirements, International Research and Development Corporation Standard Operating Procedures and the United States Food and Drug Administration Good Laboratory Practice Regulations of June 20, 1979 (Remark: some phases of the study, however, were performed prior to GLP regulations).
Date:	1981

Erythrosine was administered to groups of animals via diet. Animals were randomly assigned to treatment and control groups. Dietary levels of 0, 0.3 %, 1 % and 3.0 % corresponded to average food consumptions of 0, 424, 1474, and 4759 mg/kg bw/d in male animals and 0, 507, 1834 or 5779 mg/kg bw/d in female animals. The mice were observed daily for signs of overt toxicity, moribundity and mortality. Detailed observations were recorded weekly. Individual body weights and food consumption values were recorded weekly during weeks 1 through 14, biweekly during weeks 16 through 26, and once every 4 weeks thereafter. Haematological studies were conducted after 3, 6, 12, 18 and 24 months of the study.
Pink hair coloration was noted for treated mice. The exposed skin areas of the treated mice were noted as light pink, bright pink and pink (0.3, 1.0 and 3.0% dosage levels, respectively). The urines of male mice at the 1.0% dosage level were light orange in colour. The urines of male and female mice at the 3.0% dosage level were orange and light orange, respectively. Faeces of the treated mice were red in colour when sectioned and smeared.
Statistically significant decreases in body weights were noted for mice at the 3.0% dosage level during several weeks of the study when compared to either control group means. Average food consumption values for mice at the 1.0 % and 3.0 % dosage levels were slightly higher when compared to either control group mean. Survival was similar for control and treated mice.
Haematological values were similar for control and treated mice after 3, 6, 12 and 18 months of the study. At 24 months of study, three females at the 1.0 % dosage level and one male at the 3.0% dosage level showed marked increases in total leucocytes and decreases in haemoglobin, haematocrit and erythrocytes. For two of these females at the 1.0 % dosage level, the leucocytes were predominantly lymphoblastic. Statistically significant decreases were seen in lymphocyte values (24 months) for female mice at the 1.0% dosage level. No compound-related changes were noted in macroscopic or microscopic pathologic examinations. There were no significant changes for total tumours, benign tumours or malignant tumours. A NOAEL of 3.0 % erythrosine in the diet (corresponding to 4579 mg/kg bw/d) was derived for male animals and a NOAEL of 1 % in the diet (corresponding to 1834 mg/kg bw/d) was derived for female animals. (Richter et al., 1981 (FDA study E10, reviewed by Borzella and Hallagan, 1987))

Guideline:	no information
Species/strain:	mice, ICR
Group size:	50 animals/sex/dose
Test substance:	erythrosine
Batch:	no information
Purity:	no information
Dose levels:	1.25 % and 2.5 % in the diet
Controls:	45 animals/sex
Exposure:	18 months
GLP statement:	no information
Date:	1984

Two groups of 7-week old mice weighing 27-38 g were fed diets containing erythrosine for 18 months. Dietary dose levels of 1.25 % and 2.5 % erythrosine corresponded to approximately 1875 and 3750 mg erythrosine/kg bw/d. 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 fed the basic diet free of erythrosine for an additional 6 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 leukaemia, 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 utilized (Yoshii & Isaka, 1984 (reported in JECFA, 1986)).

Comment
The original study report was not available for evaluation. Thus, the summary from JECFA, 1986 was used in order to prepare this report.

Two combined long-term/reproduction studies have been performed in rats whose long-term effects are described together (for effects on reproduction and fertility see section 3.3.8.1 – One generation reproduction toxicity).

Guideline:	/
Species/strain:	rat (Charles-River Albino)
Group size:	F0 generation: 60 males and 60 females per dose level F1 generation: 70 males and 70 females per dose level
Test substance:	Erythrosine (from H. Kohnstamm Co., Inc., New York)
Batch:	AA2459
Purity:	90%
Dose levels:	(a) 0.1%, 0.5%, 1% (dietary levels) (b) 4.0 % (dietary level)
Controls:	0 % erythrosine in the diet for (a): two control groups, (b) one control group
Exposure:	F0 animals: during a 64 d premating period, an up to 15 d mating period, and (for female parentals) during lactation and gestation F1 animals: according to protocol: 30 months or until survival decreased to 10 animals of one sex in any group
GLP statement:	GLP-compliant
Date:	1981

Two GLP-compliant combined long-term/reproductive toxicity studies have been performed in the rat. In the first study (a), erythrosine was investigated at dietary levels of 0.1 %, 0.5 % and 1 % (and two control groups receiving basal diet). In the second study (b), erythrosine was investigated at a dietary level of 4.0 % with a concurrent control group receiving basal diet.
Parental Charles River rats (obtained from Charles River Breeding Laboratories, Wilmington, MA) received test diets for a 64-day premating period (administration of the test substance started after a two-week pretest period of weanling rats) and an up to 15d mating period. Female animals received test diets through gestation and lactation. After parturition, the chronic phase of the study started using randomly selected F1 animals receiving the same diets as their parents for 30 months after in utero exposure. The dietary levels of 0.1 %, 0.5 %, 1 % and 4 % corresponded to 49, 251, 507 and 2645 mg/kg bw/d in males and to 61, 307, 642 and 3029 mg/kg bw/d in females. Deaths, morbidity and clinical signs of toxicity were recorded twice per day, individual body weights were determined weekly during the first 14 weeks, twice weekly for further 12 weeks and monthly thereafter. Ophthalmologic investigations were performed at the beginning and after 3, 6, 12, 18 and 24 months of the chronic phase. Ten animals of each sex and group were randomly selected for haematology, clinical chemistry and urinalysis after 3, 6, 12, 18 and 24 months and at termination of the study. Necropsies were conducted on all animals dying spontaneously, killed moribund or on schedule. A complete histopathological evaluation was performed on all rats from the three control groups, the 1 % and the 4 % groups and on ten rats from each sex and group which were selected for an interim kill at 12 months.
Results: in study (a) there were no consistent significant compound-related effects on appearance and behaviour, mortality, food consumption (food consumption was slightly higher in treated males and females), 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. There were no toxicologically significant variations in organ weights. The incidence of non-neoplastic lesions was comparable between treated and control groups. There was a statistically significant increase in the incidence of thyroid follicular cell hyperplasia in male rats given 1 % erythrosine. Further, 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.
In study (b) there were no consistent significant compound-related effects on appearance and 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 to controls (0/69). The incidence of malignant tumours, including thyroid C-cell and follicular carcinomas, was not statistically different between treated and control animals. No compound-related thyroid lesions could be observed in female animals. There were no compound-related lesions in any other tissue of animals of both sexes. Based on the outcome of studies (a) and (b), erythrosine was considered to induce thyroid follicular tumours in male rats (Brewer et al., 1981 (FDA study E9); Brewer et al., 1982 (FDA study E8) (both reviewed in Borzella et al., 1987, JECFA, 1986 and JECFA, 1990)).

Comment
The original FDA studies E8 and E9 (Brewer et al., 1982 and Brewer et al., 1981) have been published later by Borzella et al., 1987 and have been subject to JECFA evaluations from 1986 and 1990). In the JECFA evaluation from 1990 it has been stated, that the microscopic findings in the thyroid and the statistics used have been reviewed (FD&C Red No. 3 Review Panel, 1987; Federal Register, 1990). 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: an increased incidence of combined adenomas and carcinomas was seen in the males fed 4 % erythrosine in the diet (18/68 compared to 2/68 in control males). A statistically significant increase was also found for combined adenomas and carcinomas in male rats fed 0.1, 0.5 or 1 % erythrosine for 122 weeks (3+3/64, 7+1/66, 1+3/57, respectively, compared to 0+1/128 in control male rats). In the female rats a significant increase in tumour yield was only found in the 1 % group (5+1/68 compared to 1+0/138 in controls).
These two studies are the key studies demonstrating that erythrosine acts on the thyroid by causing thyroid follicular cell hyperplasia and thyroid follicular cell tumours (adenomas and/or a combination of adenoma/carcinoma). Based on mechanistic considerations, a threshold mechanism appears to be plausible which justifies the derivation of a NOAEL. Based on the re-evaluation discussed in JECFA (1990) a LOAEL of 49 mg/kg bw/d for can be derived for males (corresponding to 0.1 % erythrosine in the diet) and a NOAEL of 307 mg/kg bw/d can be derived for females (corresponding to 0.5 % erythrosine in the diet).

Guideline:	/
Species/strain:	Rat, Fischer F344
Group size:	50 animals/sex/dose; 30 control animals/sex
Test substance:	FD&C Red No. 3 (Food red no. 3)
Batch:	not given
Purity:	not given
Dose levels:	1.25 and 2.0 % in the diet

Controls:	0% in the diet
Exposure:	18 months
GLP statement:	/
Date:	1984

The study was preceded by a 6-week subchronic toxicity study in order to determine the appropriate dose level for the carcinogenicity part of the study. 2.5 % erythrosine in the diet was considered as the maximum tolerated dose. For the carcinogenicity part of the study, animals were randomly assigned into treatment groups. During the first 20 weeks, substance was administered in pelleted diet and was given in powdered diet thereafter at dose levels of 1.25 and 2.0 %. All rats of the erythrosine-administered groups were sacrificed after 18 months (contrary to planning) because of lung infection of the animals. Control rats were killed after 24 months. No parameters other than histopathology were reported. Histopathology revealed sporadic cases of spontaneous neoplasms (tumours of genital system, endocrine system, hematopoietic system and digestive system) but their frequencies were similar among animals of erythrosine treated groups and comparable to the controls. No pathological changes were observed in the thyroid glands (Fukunishi et al., 1984).

Guideline:	/
Species/strain:	rat, Sprague-Dawley
Group size:	12 female animals in the 6 month period, 20 female animals in the 12 month period
Test substance:	Food Red no.3
Batch:	(maybe given, Japanese could not be decoded)
Purity:	(maybe given, Japanese could not be decoded)
Dose levels:	2 % Food Red no.3 in the diet
Controls:	0 % Food Red no.3 in the diet
Exposure:	6 or 12 months
GLP statement:	/
Date:	1978

Groups of female Sprague-Dawley were exposed to erythrosine in the diet at dose levels of 0 or 2% 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 (Sekigawa et al., 1978).

Older studies

Guideline:	/ (predates OECD Guidelines)
Species/strain:	mice produced by mixed breeding from five different strains
Group size:	a total of 122 male and female animals (erythrosine group), a total of 168 control animals
Test substance:	Erythrosine J.
Batch:	Schultz no. 887; C.I. no. 773
Purity:	bes. rein
Dose levels:	1 mg /animal/day (added to the daily diet as 2 % aqueous solution)
Controls:	negative control: untreated mice; positive control 1: mice receiving ortho-aminoazotoluene positive control 2: mice receiving dimethylaminoazobenzene
Exposure:	500 and 700 days
GLP statement:	predates GLP

Date: final report completed on 4th August 1945

Mice at the age of 50-100 days were fed colour-containing diets. Some of the animals were sacrificed after a treatment period of 500 days, some animals after 700 days. Each animal that died (or was killed) was investigated pathologically and microscopic dissections were made in case of doubt. In animals receiving erythrosine, no treatment-related pathological findings were reported; the incidence of tumours was comparable to that of negative control animals. In animals receiving positive control substances, liver adenomata were observed as well as general toxic reactions (Waterman and Lignac, 1958).

Comment
The study is of limited value, because it was not performed according to OECD or GLP standards, the strains of animals used were not clearly defined and the study was performed under non-adequate testing conditions (it is stated in the publication that "It should be taken into account that the investigations were made in wartime during the occupation and that condition was responsible for many difficulties with housing and food. Many mice died during the experiment from infectious diseases or had to be killed before the end of their span of life.").

Guideline:	/ (predates OECD guidelines)
Species/strain:	rat, Wistar
Group size:	24 male and 20 female parental animals, 24 male and 16 female offspring (F1) animals
Test substance:	Erythrosine J
Batch:	not given
Purity:	not given
Dose levels:	1 % erythrosine J in the diet
Controls:	125 male and 125 female animals in total
Exposure:	24 months
GLP statement:	/ (predates GLP)
Date:	1965

A combined fertility/carcinogenicity study was performed in Wistar rats. Some of the parental animals (exact number not given) were paired after 6 month of treatment. All parental animals received diet containing colour for 24 months (except for females during lactation). After weaning, 24 male and 16 female offspring animals also received colour-containing diets for 24 months. There were no differences in fertility between parental and offspring animals. No changes in food intake, body weight gains, pathological changes or tumour incidence could be observed between treated and control animals. Under the conditions of the study, erythrosine was not carcinogenic to Wistar rats of both sexes (Oettel et al., 1965).

Comment
Unusual feeding for rats (carrots twice a week), it is not stated, which tissues have been examined in detail. Thus, effects in probably non-investigated tissues might not have been detected.

Guideline:	/ (predates OECD guidelines)
Species/strain:	rat, Wistar
Group size:	5 animals/sex
Test substance:	Erythrosine (FD&C red No. 3)
Batch:	not given
Purity:	not given
Dose levels:	4 % erythrosine in the diet
Controls:	50 animals in total (no colorant in the diet)

Exposure: up to 18 months
GLP statement: / (predates GLP)
Date: 1953

Erythrosine was administered via diet until the time of death. Tissues of the alimentary tract were removed and investigated for the presence of gross staining and granular deposits. Tissues (no specific details given) were investigated for other kinds of pathology. Granular deposits in stomach, small intestine and colon were seen. Hepatic cirrhosis was noted in one case of four rats living up to 12 months. Neither tumours nor hepatic cirrhosis could be observed in control animals. Under the conditions of the study, erythrosine was not carcinogenic to Wistar rats of both sexes (Wilhelm and Ivy, 1953).

Comment
The study is of limited value. Only 5 animals per sex were used and the study was not performed according to any standard protocol.

Other routes

Guideline: / (predates OECD guidelines)
Species/strain: Hamster, Lakeview (LVG)
Group size: 13 animals (no information on sex)
Test substance: Food dye red no. 3
Batch: not given
Purity: 91.1 – 91.3 %
Dose levels: 1 mg s.c and/or i.p.
Controls: 24 LVG hamsters
Exposure: single administration with an observation period of 330 days
GLP statement: / (predates GLP)
Date: 1978

Newborn hamsters were injected either subcutaneously and/or intraperitoneally with a total of 1 mg dye (dissolved in saline, autoclaved and diluted with Eagle's basal medium). Animals were examined weekly by palpation. Necropsies were performed at time of intermittent death, tumour occurrence or at termination (330 days). Selected tissues were removed for histophathologic evaluation. Compared to controls, mortality was increased in animals receiving the dye, but not at a statistically significant level. No significant increase in tumours could be observed after administration of the substance. It was concluded from the study, that Food dye red 3 is probably toxic, but not carcinogenic (Price et al., 1978).

Comment
The thyroid did not belong to the tissues selected for histopathological evaluation. The probably occurrence of thyroid lesions or tumours might have been overlooked by palpitation.

Carcinogenicity after dermal application

Guideline: /
Species/strain: Mice / ICR (Swiss-Webster derived)
Group size: 50 males and 50 females per test group
Test substance: FD & C Red No. 3
Batch: X2527
Purity: 91%
Dose levels: 0.1 ml of a 1% aqueous solution applied twice weekly
Controls: negative control: 0.1 ml distilled water applied twice weekly

	positive control: 0.1 ml acetone containing 10 µg 3,4-benzpyrene applied twice weekly
Exposure:	18 months
GLP statement:	predates GLP
Date:	1969

Erythrosine was applied twice weekly to a clipped skin surface area of approximately 6 cm^2 of male and female mice for a period of 18 months. Skin was not occluded. Negative control animals received distilled water only, positive control animals received 3,4-benzpyrene, dissolved in acetone. Body weights were determined at initiation of the study and monthly thereafter. Animals were observed daily for mortality and twice per week for gross toxicity, the survival was determined after 5, 10, 16 and 18 months. At the end of the study, animals were necropsied. Skin and selected grossly abnormal tissue was taken for histopathological examination. Results from the animals treated with 3,4-benzpyrene demonstrated, that the strain of mice used responded to dermal applications of a known carcinogen. The survival rate in the erythrosine-treated animals was comparable to that observed in vehicle controls (525 days on average versus 507 days on average). Gross lesions suggestive of skin neoplasia were not observed and no treatment-related lesions were determined at other sites. An increased incidence of ectoparasitism and an increased incidence of inflammatory skin lesions could be observed. From the results it can be concluded, that erythrosine does not increase the incidence of neoplasia after long-term dermal application on the skin of ICR (Swiss-Webster-derived) white mice (Hazleton laboratories, 1969; Carson, 1984).

Summary of carcinogenicity

Guideline-compliant carcinogenicity studies have not been performed with erythrosine. However, long-term studies are available which enable to draw conclusions on the carcinogenicity of erythrosine. Two long-term studies in ICR mice (Borzella and Hallagan, 1987) and one long-term study using Fischer rats (Fukunishi et al., 1984) were negative. Further studies, which are of limited value (because of limited number of animals used, poor study description or lack of adherence to modern standards) can be used as supporting information (Oettel et al., 1965, Sekigawa et al, 1978, Waterman and Lignac (1958), Wilhelm and Ivy (1953)): in these studies, there were no indications of a carcinogenic potential of erythrosine.
In experiments, where in utero exposure of rats was followed by long-term administration of high dose levels (4% in the diet) of erythrosine, increased thyroid weights were accompanied by histological changes in the thyroid. Thyroid follicular hyperplasia and thyroid follicular tumours (adenomas and a combination of adenomas and carcinomas) could be observed after long-term administration of erythrosine to male rats (and less prominently, thyroid tumours also occurred in female rats). Thus, thyroid tumours and effects on the thyroid were the most prominent adverse treatment-related effects after administration of erythrosine.

After repeated long term dermal application to mice as well as after single i.p. and s.c. applications to hamsters (followed by an observation period of 330 days) erythrosine was not carcinogenic.

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

Two 1-generation studies (in combination with investigation of neurobehavior/psychotoxicity) have been performed in mice. From an interim report of a 3-generation study in rats, only results for the first generation are available. Therefore, this study is described under "1-Generation studies".

Two combined long-term/reproduction studies (FDA E8 and E9, reviewed by Borzella et al. (1987)) have been performed where the impact of erythrosine after administration to parental animals on reproduction and on the F1 offspring has been investigated.

1-Generation study (in mice)

Guideline:	/
Species/strain:	mouse (Crj:CD-1)
Group size:	10 males and 10 females/dose
Test substance:	Erythrosine (from Tokyo Kasei Co Ltd, Tokyo, Japan)
Batch:	GD 51
Purity:	> 85 %
Dose levels:	0.005 %, 0.0015 %, or 0.0045 % (with the diet)
Controls:	standard laboratory diet (0 % erythrosine)
Exposure:	parental animals: during a 4 week premating period, a 5 d mating period, and (for female parentals) during lactation and gestation Offspring (F1): until an age of 9 weeks
GLP statement:	not reported
Date:	2001

In a combined reproductive/neurobehavioral (for the neurobehavioral part of the study see section 3.3.12. – special investigations) study, erythrosine was administered at dietary dose levels of 0, 0.005, 0.0015 and 0.0045 % to groups of male and female mice. The dose levels corresponded to 7.8, 22.35 and 70.43 mg erythrosine /kg bw/d in males (F0 animals during premating) and 9.68, 27.86 and 82.92 mg erythrosine /kg bw/d in females (F0 animals during premating) . The substance was administered during a 4-week premating period (which started at an age of 5 weeks), a 5-day mating period, and during gestation and lactation. Offspring (F1 generation) received diets until an age of 9 weeks was reached. Parental animal were weighed individually on days 0, 2, 7, 14, 21, 28 and 30 during the preconception period. Litter size, litter weight and sex ration of the offspring was measured at birth; offspring was weighed individually on postnatal day (PND) 0, 4, 14 and 21. Offspring was weaned at the age of 4 weeks and one male and one female were randomly selected from each litter to continue treatment until the age of 9 weeks. Animals were weighed individually at an age of 4, 5, 6, 7, 8 and 9 weeks. In parental animals, the average body weights of male and female mice during the premating and mating periods exhibited some differences from control (numeric values were not given), but no significant substance-related changes in body weights were observed during the lactation and gestation periods. In the F1 generation, no significant adverse effects were observed in litter size, litter weight or sex ration at birth. The average body weight of male offspring in the middle dose group during the lactation period and of female offspring of the middle dose group at PND 0, 4, 7 and 21 were statistically significantly increased. However, these increases were not attributed to substance treatment, because offspring of the middle dose group exhibited a higher birth weight. Therefore, erythrosine had no significant adverse effects on reproductive parameters as far as investigated in this study (Tanaka et al., 2001).

For reproductive effects, a NOAEL of 0.0045 % (70.43 mg/kg bw in males; 82.92 mg/kg bw in females at preconception; 261.34 mg/kg bw/d in females during lactation) was derived from this study (highest dose tested).

1-Generation study (in rats)

Guideline:	/
Species/strain:	rat (Sprague-Dawley) (Laboratory Supply Co., Indianapolis, USA)
Group size:	19 – 22 animals/sex/dose
Test substance:	Erythrosine (from H. Kohnstamm & Co, New York, NY, USA)

Batch:	not reported
Purity:	91 %
Dose levels:	0.25 %, 0.5 %, or 1.0 % (with the diet)
Controls:	standard laboratory diet (0 % erythrosine)
Exposure:	parental animals: during a 2 week premating period, a 1-14 d mating period, and (for female parentals) during lactation and gestation Offspring (F1): until an age of 90 - 110 days
GLP statement:	not reported
Date:	1983

The reproductive (and psychotoxic) effects of erythrosine have been investigated in a 1-generation study in rats. Erythrosine was administered at dietary dose levels of 0, 0.25, 0.5 and 1.0 % (corresponding to approximately 250, 500 and 1000 mg erythrosine/kg bw/d) to groups of male and female Sprague-Dawley rats for two weeks prior to breeding, 1-14 days during breeding, then to females during gestation and lactation. The experimental diets were freely available to the offspring throughout postnatal development up to the age of 90-110 days. Body weights were measured weekly except during breeding. On the day following birth, all litters were examined and data collected on litter size, sex distribution, weight, and number of dead or malformed offspring. A variety of behavioural tests (see section 3.3.12. – special investigations) were determined in the offspring. Further, brain weights of day 90 pp were determined in the offspring. The experiment was replicated after 2 years using the same exposure regimen. Erythrosine produced no effects on paternal or offspring weight or food consumption. No significant effects of any of the measures of reproductive performance (proportion of females producing litters to those bred, the number of small litters, gestation length, litter size, sex ratio) were observed. No malformations were seen upon external examination. Preweaning offspring mortality was significantly increased at the 1.0 % and 0.5 % dose levels in the first experiment, but not in the second. However, the authors ascribe these findings to normal variability in background mortality, because preweaning mortality rates were significantly higher in the control group from the second experiment when compared to the first experiment. No adverse effects on offspring growth or adult regional brain weight were observed. Thus, it was concluded that erythrosine did not adversely affect development of rats under the conditions of the experiments (Vorhees et al., 1983).

A NOAEL of 1.0% erythrosine in diet (corresponding to approximately 1000 mg/kg bw/d) fpr reprotoxic effects was derived from this study (highest dose tested).

1-Generation study (rat)

Guideline:	/
Species/strain:	rat (Charles-River Albino)
Group size:	10 males and 20 females per dose level
Test substance:	Erythrosine (FD&C Red No. 3)
Batch:	CCIC-2
Purity:	not given
Dose levels:	1.25, 12.5, 37.5 and 125.0 mg erythrosine/kg bw/d
Controls:	0 mg/kg bw/d erythrosine
Exposure:	oral via diet; parental animals: during a 2 week premating period, an up to 10 d mating period, and (for female parentals) during lactation and gestation; after a 10 d rest a second F1 generation was breeded Offspring (F1b): treated as parental generation for the subsequent generation
GLP statement:	study performed prior to GLP standards
Date:	1972

An interim report up to the F1 generation of a reproductive toxicity study of erythrosine – initially designed as a 3-generation study – is available. Erythrosine was administered at dietary levels of 0 (control), 1.25, 12.5, 37.5 and 125.0 mg erythrosine/kg bw/d (nominal concentration; in the F0 generation, achieved dose levels were 0, 1.3, 13.9, 43.61 and 135 mg/kg bw/d) Charles-River albino rats (10 males and 20 females per dose level) from the beginning of a 14d premating period (which started at an age of 86 days) until animals were sacrificed. After an up to 10 d mating period, gestation and lactation, the offspring of the F0 parental animals (F1a) was weaned and after a 10 d rest F0 animals were mated again in order to produce a second litter (F1b). F1a animals were sacrificed after weaning, 10 male and 20 female animals of the second litter were selected as parental animals for the subsequent generation.
One F0 parental female in the control and one F0 female of the 12.5 mg/kg group died during treatment. Death was attributed to chronic respiratory infection, i.e. was not considered treatment-related. In the F0 parental animals, no abnormal behavioural reactions were noted in the treated animals. Further, no significant differences between treated and control groups were observed with respect to food consumption, body weights and body weight gains. Mating, fecundity, fertility indices and incidence of parturition were comparable between test and control groups. There were no differences between treated and control animals in numbers of pups delivered. Reduced day 4 and day 14 survival indices as well as a reduction of the lactation index were present in the F1b pups of the 12.5 mg/kg group, but not in the F1a pups. There were no significant differences in body weights between test and control pup body weights. Upon examination of each pup at birth and again at weaning, all progeny were judged to be free of gross external abnormalities (Anonymous, 1972c; FDA study F1).

A NOAEL of 125 mg/kg bw/d (highest dose tested) was derived from this study.

Comment
The study represents an interim report of a 3-generation study which describes the results up to two F1 litters (F1a and F1b) obtained from two matings of F0 parental animals. No report or publication describing the results for subsequent generations/ continuation of the study has been located. However, the study might have been addressed in an abstract (Pierce al., 1974) describing the results of multi-generation reproduction studies with certified colours (including Red No 3) in rats. In this abstract it was stated, that data through the F2b generation litter did not give any indication of adverse effects on reproductive performance (see also section 3.3.8.2. two generation reproductive toxicity studies).

Combined long-term/reproduction studies in rats (for tumours and pathology see section 3.3.7 Carcinogenicity)

Guideline:	/
Species/strain:	rat (Charles-River Albino)
Group size:	F0 generation: 60 males and 60 females per dose level
F1 generation: 70 males and 70 females per dose level	
Test substance:	FD&C Red No 3 Certified 773 Erythrosine (from H. Kohnstamm Co., Inc., New York)
Batch:	AA2459
Purity:	90%
Dose levels:	(a) 0.1%, 0.5%, 1% (dietary levels) (b) 4.0 % (dietary level)
Controls:	0 % erythrosine in the diet for (a): two control groups, (b) one control group
Exposure:	parental animals: during a 64 d premating period, an up to 15 d mating period, and (for female parentals) during lactation and gestation
GLP statement:	GLP-compliant

Date: 1981

Two GLP-compliant combined long-term/reproductive toxicity studies have been performed in the rat. In the first study (a), erythrosine was investigated at dietary levels of 0.1 %, 0.5 % and 1 % (and two control groups receiving basal diet). In the second study (b), erythrosine was investigated at a dietary level of 4.0 % with a concurrent control group receiving basal diet. The dietary levels of 0.1 %, 0.5 %, 1 % and 4 % corresponded to 49, 251, 507 and 2645 mg/kg bw/d in males and to 61, 307, 642 and 3029 mg/kg bw/d in females.
Parental Charles River rats (obtained from Charles River Breeding Laboratories, Wilmington, MA) (60 rats/sex/dose) received test diets for a 64-day premating period (administration of the test substance started after a two-week pretest period of weanling rats) and an up to 15d mating period. Female animals received test diets through gestation and lactation. After parturition, the offspring (F1 pups) were counted, sexed and weighed on designated days during lactation. There were no compound-related effects on male and female fertility indices, gestation lengths, pup survival through weaning, number of live and stillborn pups and pup weights at birth. Mean body weights of pups from the 4 % group were slightly (statistically not significant) lower than those of controls on lactation day 21. There were no treatment-related adverse findings in the litters. Thus, for *fertility* (and reproductive toxicity), a NOAEL of 3029 mg/kg bw/d and 2464 mg/kg/d was derived for female and male rats, respectively (highest dose tested). (Brewer et al., 1981 (FDA study E9); Brewer et al., 1982 (FDA study E8); Borzella et al., 1987).

Comment
The NOAELs obtained for the reproduction and fertility part of these two studies are different from the NOAELs for the chronic toxicity/carcinogenicity part of this study.

In an older study performed by Oettel et al. (1965) (described in section 3.3.7 – Carcinogenicity), no effects of erythrosine on fertility could be observed.

3.3.8.2. Two generation reproduction toxicity

25 colours, among them erythrosine, have been investigated after dietary administrations of multiples of the ADI to rats. No doses higher than 1000 mg/kg/day were used. Data through the F2b litter revealed no indications of adverse effects on reproductive performance (Pierce et al., 1974).

Comment:
The study is poorly described and cannot be taken to derive an NOAEL. Probably the follow up of the interim report of FDA study F1 (Anonymous, 1972c), described in section 3.3.8.1.

3.3.8.3. Three generation reproduction toxicity

Guideline:	/
Species/strain:	rat (Sprague-Dawley, COBS Charles-River CD)
Group size:	25 males and 25 females per dose level
Test substance:	Erythrosine (from H. Kohnstamm Co., Inc., New York)
Batch:	not given
Purity:	90 %
Dose levels:	0.25 %, 1.0 %, and 4 % (dietary levels)
Controls:	0 % erythrosine in the diet
Exposure:	parental animals: during a 69 d premating period, an up to 15 d mating period, and (for female parentals) during lactation and gestation of F1a pups. After weaning of the F1a pups, female parentals were rested for a minimum of 13 days and received then the same dosing regimen for

breeding of a second F1 generation (F1b). F1b animals received test diets for a 100 d premating period and were then mated to produce F2a and b litters.
GLP statement: partly GLP-compliant
Date: Publication submitted in 1990

Erythrosine was investigated in a multi-generation study in Sprague-Dawley rats. The substance was administered at dietary levels of 0 (control), 0.25, 1.0 and 4.0 % to groups of 25 animals per sex and dose. Dietary levels corresponded to approximately 149, 613 and 2957 mg erythrosine/kg bw/d for males (average of 3 generations) and 255, 990 and 4760 mg erythrosine/kg bw/d for females (average of 3 generations). At the age of 31 days, parental (F0) animals received test diets for 69 days prior to mating, during the mating period (up to 15 days), gestation and lactation of the F1a pups. F1a pups were examined for external abnormalities and killed at the end of the 21 day lactation period. After weaning of the F1a pups, F0 parental females were rested for a minimum of 13 days and mated again in order to produce a second litter (F1b). 25 rats/sex/group from the F1b litter were randomly selected as the parental animals for the subsequent generation. Remaining F1b pups and parental F0 animals were examined for external abnormalities and killed. F1 parents received their respective diets until an age of approximately 100 days and were then mated twice to produce F2a and F2b litters. F2a pups were examined for external abnormalities and killed at the end of the 21 day lactation period. 25 rats/sex/group from the F2b litter were randomly selected as the parental animals for the subsequent (third) generation. After selection of the third generation parents, gross autopsies were performed on all F1 parents and histopathology was performed on selected tissues from 5 rats/sex/group. The F2 parents remained on the appropriate diet. At approximately 100 days of age, they were mated to produce F3a and F3b litters. F3a pups were examined for external abnormalities and killed at the end of the 21 day lactation period. In F3b animals, gross autopsies were performed on 10 rats/sex/dose and histology was conducted on selected tissues from 5 rats/sex/dose. All remaining F3b animals and F2 parents were killed. Rats were observed daily for gross signs of toxicity and mortality. Individual body weights were recorded weekly. Body weights of pups were determined on PND 4 and 14 (as total litters) and day 21 (individually). Food consumption was recorded. Complete post mortem examinations were conducted on any F0 generation rat that died spontaneously, on all rats from the F1 generation and 10 rats/sex/dose of the F3b pups. Selected organs from 5 rats/sex/dose of the F1 parental animals and F3b pups (including thyroid) were investigated histopathologically.)
In the highest dose group, mean body weights of both sexes were generally decreased when compared with controls at the end of each segment and occasionally, differences reached statistical significance. Group mean body weight gain during gestation among the female F0, F1 and F2 parents was frequently significantly reduced in the 1% and 4% treatment groups. Food consumption of treated parental animals was not statistically different from that of control animals. Group mean body weights for pups from the high-dose groups was consistently decreased when compared with controls at lactation days 0, 4, 14 and 21 and reached statistical significance on day 21. No compound-related effects were noted after histological examinations of selected tissues from rats from the F1 and F3b generations; there were no compound-related effects on thyroid morphology. No effects on organ weights were noted in these rats. There were no compound-related effects on male and female fertility indices, gestation length, number of stillborn pups or pup survival. Based on reductions in body weights, a dietary level of 0.25 % erythrosine (corresponding to approximately 149 mg/kg bw/day in male rats and 255 mg/kg bw/day in female rats) was derived as NOAEL (Borzella and Hallagan, 1990).

Comment
(1) No effects on reproductive performance and fertility were observed up to the highest dose tested (2957 mg/kg bw/d in males: 4760 mg/kg bw/d in females).
(2) Albridge et al (1981) (FDA study 111) describe essentially the same 3-generation reproductive toxicity study in rats. The same NOAEL of 0.25 % erythrosine in the diet was

derived. However, the following differences between the two study descriptions could be identified: FDA study 111 designates the rat strain used as Charles River CD rats. At the highest dose level, there were only 24 females (instead of 25). The Lot Number of FD&C red Nr. 3 used was AA 2459. Date of submission was 11 December, 1981. The test item had been retrieved in 1977. Results had been submitted in 1982.

Further investigations

Effects on spermatogenesis

Guideline:	no corresponding OECD guideline
Species/strain:	male albino mice (not further specified), 70 animals in total
Group size:	(a) For testicular lactic dehydrogenase isozyme (LDH-X) activity, epididymal sperm count and sperm motility: 10 animals/ group (four groups) (b) For sperm head abnormality test procedure: 6 animals/group (5 groups)
Test substance:	Erythrosine (from H. Kohnstamm & Co. Inc.)
Batch:	not provided
Purity:	not provided
Dose levels:	(a) 68 and 136 mg/kg/d (oral gavage) (b) 340, 680 and 1360 mg/kg/d (oral gavage)
Controls:	negative controls for (a) and (b) distilled water Positive control (a): 18 mg/kg/d cyclophosphamide (CP) Positive control (b): 20 mg/kg bw CP (i.p.)
Exposure:	(a): gavage once daily for 21 consecutive days, termination on day 22 (b): gavage once daily for 5 consecutive days, termination on day 35
GLP statement:	not reported
Date:	1997

(a) LDH-X activity, epididymal sperm count and sperm motility:
Groups of 10 mice were orally gavaged with vehicle, CP or erythrosine once daily for 21 days. The day after, animals were killed and testes and caudae epididymis were dissected. LDH-X activities were determined photometrically in supernatants from testes homogenates using DL-alpha-hydroxycaproic acid as substrate. Spermatozoa were obtained from cauda epidydimis. Sperms were counted by using a haemocytometer, sperm motilities and movements were examined microscopically.
(b) Sperm head abnormality test
Animals were treated once daily for 5 consecutive days and were killed 35 days after the last treatment. Both caudae epidydimis were dissected and minced in saline for diffusion of spermatozoa. Saline containing spermatozoa were fixed on glass slides, stained and examined microscopically.

Results
Oral treatment with 68 and 136 mg/kg/d erythrosine significantly decreased LDH-X activity by 28.2 and 31.4 %, respectively (for comparison, CP reduced LDH-X activity by 33.2 %). Sperm counts in erythrosine-treated animals were inhibited by 50.8 % (low dose) and 33.9 % (high dose). The percentage of motile sperms in erythrosine-treated animals showed decreases by 57 % (low dose) and 80.5% (high dose).
Treatment of animals with 680 and 1360 mg/kg/d erythrosine increased the incidence of sperms with abnormal heads to 157.1 % and 164.7 % of the control levels (Abdel Aziz et. al., 1997).

Comment
The results of the study indicate that oral administration of erythrosine might adversely affect sperm parameters in mice. With respect to sperm counts, no dose-response

relationship could be observed. Impaired fertility would be a consequence of adverse effects on sperms. However, in fertility studies on erythrosine (up to 3 generations), no adverse effects of erythrosine on fertility have been observed.

Effects on testicular function

An abstract describing the effects of erythrosine on testicular function is available: *"In an investigation of the potential adverse effects of the food colorant erythrosine on the spermatogenesis process, fifty male Swiss albino mice were orally intubated with varied dosage (0, 64, 128 and 256 mg /kg. bw) for a period of 30 days. The normal average epididymal sperm counts as well as the percentage of motile sperms were significantly (P< 0.001) inhibited with increased dosage. Moreover, erythrosine was shown to disrupt the normal morphology of the sperm head, a dose dependent increase in the percentage of abnormal sperms (microhead, macrohead, deformed head, broken head, coiled sperms and broken tails) were observed in all the treatment groups. These findings indicate that erythrosine in the used doses has a potential toxic effect on spermatogenesis in mice and in turn it may result in testicular dysfunction and reproductive performance."* (Vivekanandhi et al., 2006)

Comment
The full paper was not available.

Effects on estrogenic activity

Guideline:	/
Species/strain:	rat, strain not specified
Group size:	5
Test substance:	erythrosine (FD & C Red 3)
Batch:	not provided
Purity:	not provided
Dose levels:	250 mg/kg bw twice daily (500 mg /kg bw/day)
Controls:	distilled water
Exposure:	3 days, subcutaneous administration
GLP statement:	/
Date:	1958

Erythrosine, dissolved in water was administered twice daily subcutaneously to five immature female rats (age: 20-22 days) for three days. Control rats received water. On the fourth days, rats were killed, uteri were excised and weighed. Other organs (not further specified) were taken and examined for gross abnormalities. There was no statistically significant difference between uterine weight of control and erythrosine treated animals. Thus, under the conditions of this experiment, no estrogenic activity of erythrosine could be observed (Graham and Allmark, 1958).

Summary on reproductive toxicity

No guideline-compliant reproductive toxicity studies on erythrosine are available. However, reproductive toxicity of erythrosine has been investigated using different study designs yielding information on the effects of erythrosine administration on one or further subsequent generations. One-generation data are available from the outcome of the one-generation part of a three-generation study in CD rats, from two combined long-term repeat-dose/reproductive toxicity studies in rats (reproductive and fertility NOAELs were at the respective highest dose levels tested, i.e. 3029 mg/kg bw/d for female rats and 2464 mg/kg/d for female and male rats, respectively) and two combined reproductive/neurobehavioural studies (one performed in mice (yielding a NOAEL at the highest dose tested (0.0045 % in the diet, 70 mg/kg bw/d)), one performed in rats

(yielding a NOAEL at the highest dose level tested (1% in the diet, 1000 mg/kg bw/d))). Further an abstract about a 2-generation study and a full description of a three-generation study in rats are available. NOAEL derived from the three generation study for foetal and postnatal effects were 149 mg/kg bw/day in male rats and 255 mg/kg in female rats. The results from reüroductive toxicity studies demonstrate that erythrosine does not adversely affect fertility, reproduction or post-natal parameters in rats and mice. Additional studies indicated that erythrosine lacks estrogenic activity but that it is capable of adversely affecting testicular function and certain sperm parameters in mice). The impact of erythrosine on sperm parameters might be subject for further clarification. However, the results on sperm parameters and testicular functions do not concur with the outcome of the different studies on fertility, reproduction and postnatal parameters in rats and mice from which it can be deduced that erythrosine does not represent a reproductive toxicant.

3.3.8.2. Teratogenicity

Rat – drinking water

Guideline:	/
Species/strain:	rat (Osborne-Mendel)
Group size:	41 – 42 pregnant animals per dose group
Test substance:	Erythrosine (from H. Kohnstamm Co., Inc., New York)
Batch:	X3238
Purity:	95 %
Dose levels:	0.05 %, 0.1 %, 0.2 % and 4 % in drinking water
Controls:	0 % erythrosine in the drinking water; 46 pregnant animals
Exposure:	during gestation days 0-20
GLP statement:	not reported
Date:	1992

Erythrosine was administered to 12 to 21 week-old pregnant female animals via drinking water containing 0 (control), 0.05, 0.1, 0.2 or 0.4 % erythrosine (corresponding to daily doses of 0, 64, 121, 248 and 472 mg/kg bw/d) on gestation days 0-20 (41 – 46 animals/group).
Animals were observed daily for mortality, moribundy and any deviations from normality. Weights and drinking water consumption were determined daily, feed consumption was determined weekly. On day 20 of gestation, animals were examined for gross abnormalities, killed and Caesarean sections were performed. Uteri were examined for resorption sites, number of corpora lutea and number of implantation sites. Foetuses were examined.
Compared to controls, erythrosine-treated animals consumed less fluid than controls, but these changes were not dose-related and reached statistical significance only occasionally. Four animals of the 0.4 % group rejected their drinking water, leading to decreases in body weights and finally death of three animals (the surviving animals were used to test palatability by replacing test substance with distilled water). It could be demonstrated in the study that rejection of test fluid was due to palatability. Compared to controls, food consumption was statistically significantly increased in the 0.2% group. No significant treatment-related effects on body weight gains were observed. Pregnancy rate was high in all groups. No marked differences in reproductive findings were observed between groups at the time of caesarean sections. Implantation efficiency, foetal viability and foetal development (foetal weight and length or visceral development) were similar in all groups. Specific external variations in live foetuses showed no dose-related effects. Some statistically significant increases in skeletal (sternebral) variations were observed, but were not dose-related and considered to be random. The study demonstrated that erythrosine up to dose levels of 472 mg/kg bw/d was not teratogenic or toxic to rats (Collins et al., 1993a).

A NOAEL of 472 mg/kg bw/d for maternal and foetal effects was derived from this study (highest dose tested).

Rat - gavage

Guideline:	/
Species/strain:	Rat / Osborne Mendel
Group size:	40 – 44 female animals/group
Test substance:	Erythrosine (from H. Kohnstamm Co., Inc., New York)
Batch:	X3238
Purity:	95 %
Dose levels:	15, 30, 100, 200, 400, 800 mg/kg on GD 0-19
Controls:	distilled water (vehicle control)
Exposure:	GD 0-19
GLP statement:	not reported
Date:	1993

Aqueous solutions of erythrosine were administered to 13 – 19 week-old pregnant female animals on GD 0 – 19. Animals were weighed and observed daily, feed consumption was measured weekly. On gestation day 20, animals were examined for gross abnormalities, Caesarean sections were performed and uteri were examined for the presence and position of resorption sites, foetuses, number of corpora lutea and number of implantation sites. Live foetuses were weighed, sexed, examined for external malformations and crown-rump length was determined. Half of the foetuses were examined for skeletal variations and half of the animals were examined for internal visceral variations.
No behavioural changes were observed in the treated animals. Clinical and necropsy findings were unremarkable and not dose-related. Feed consumption was greater in treated animals, but the increases were not related to dosage. Pregnancy rate in all dose groups was at least 85 %. Erythrosine had no effects on implantation efficiency or on the mean numbers of implants per female. In the 400 mg/kg dose group, there was an increased number of early and late deaths per litter which was of borderline significance; there were fewer combined early and late deaths per litter (borderline significance). The mean number of viable male foetuses per litter was higher in the 400 and 800 mg/kg groups (borderline significance).
External foetal development, foetal weight and foetal length from crown to rump were not affected by treatment. No treatment–related effects on skeletal and visceral development were observed. Up to the highest dose level tested (800 mg/kg/d) erythrosine was not foetotoxic and not teratogenic (Collins et al., 1993b).

A NOAEL of 800 mg/kg bw/d for maternal and foetal effects was derived from this study (highest dose tested).

Rat - gavage

Guideline:	/
Species/strain:	Rat / Charles-River Albino
Group size:	21 per dose
Test substance:	FD & C Red No. 3
Batch:	CCIC-2
Purity:	93 %
Dose levels:	Final concentrations of 0.5, 1.67 or 5.0 % (w/v) of the substance in 1.5% aqueous methylcellulose
Controls:	Sodium salicylate as positive control/ aqueous methylcellulose as negative control
Exposure:	GD 6-15
GLP statement:	predates GLP standards
Date:	1972

Pregnant female animals received erythrosine daily from GD 6 - 15 at final concentrations of 0.5, 1.67 or 5.0 % (w/v) of the substance in 1.5 % aqueous methylcellulose (corresponding to 25, 85 and 250 mg/kg/d). Three control groups (comprising 20, 21 and 22 animals) received vehicle only and two positive control groups (21 animal/group) received 200 mg/kg bw / day of sodium salicylate from day 6 of gestation until day 15 of gestation. Mean group body weights were recorded on day 0 (date of insemination), 6 and 15 of gestation and at sacrifice; mortality and observations were recorded daily. On day 20 of gestation, caesarean sections were performed; uteri were investigated for implantation sites, resorption sites, abnormalities and number of viable foetuses. Foetuses were weighed and examined for external, internal and skeletal abnormalities.
Foetuses from the positive control groups exhibited teratogenic findings demonstrating the susceptibility of the animal strain to a teratogenic agent. There were no deaths among the maternal control and erythrosine-treated animals. Erythrosine treatment did not affect the body weight gains of the maternal animals during gestation, adverse reactions were not observed and animals were free of gross uterine abnormalities. Erythrosine treatment did not affect foetal mortality, foetal body weights were within the normal expected ranges for all groups. The number of foetuses with external abnormalities in the treated groups did not differ from that in the control groups. There were no skeletal abnormalities and due to erythrosine treatment, internal examinations revealed no marked differences to controls. From the results of this study, erythrosine was not teratogenic after gavage administration to rats up to the highest dose tested (250 mg/kg bw/day) (Anonymous, 1972 a (FDA study G1)).

A NOAEL of 250 mg/kg bw/d for maternal and foetal effects was derived from this study (highest dose tested).

Rabbit – oral gelatine capsules

Guideline:	/
Species/strain:	Rabbit (New Zealand Albino)
Group size:	17 per group
Test substance:	FD & C Red No. 3
Batch:	CCIC-2
Purity:	92.8 %
Dose levels:	12.5, 40.0, 125.0 mg/kg bw/d on GD 6-18 in gelatine capsules
Controls:	negative control: vehicle; positive control: 37.5 mg/kg thalidomide
Exposure:	GD 6-18
GLP statement:	conducted prior to OECD standards
Date:	1972

Pregnant female animals received gelatine capsules containing 12.5, 40.0 and 125.0 mg/kg bw erythrosine daily from day 6 of gestation until day 18 of gestation. Three control groups (17 animal/group) received empty gelatine capsules and two positive control groups (17 animals/group) received 37.5 mg thalidomide/ kg bw/day from day 6 of gestation until day 18 of gestation. Animals were observed daily for signs of toxicity. On gestation day 29, animals were sacrificed and uteri were examined. The numbers of implantation sites, resorption sites, and live or dead foetuses were determined. Viable foetuses were observed for 24 hours. All foetuses were examined by dissection; skeletal tissue was examined for malformations.
The results obtained with the positive control groups demonstrated the susceptibility of the animals to a teratogenic agent. There were not treatment-related findings on body weights during gestation and no deaths or abnormal behavioural reactions could be observed. The percents of foetal survivals (as indicated by the number of live foetuses per 100 implantations sites) were 92, 80 and 90 for the vehicle control groups and 79, 60, and 56 for the groups treated with 12.5, 40.0 and 125.0 mg/kg bw/day erythrosine, respectively. There were no differences in foetal body weights among control, positive control and

erythrosine-treated groups. No external abnormalities due to treatment with erythrosine were observed in the foetuses. 24-hr survival was not affected by prenatal erythrosine exposure. No gross internal abnormalities and no treatment-relates skeletal malformations were noted 2 after prenatal erythrosine exposure (Anonymous, 1972 b (FDA study G2).

A NOAEL of 125 mg/kg bw/d for maternal and foetal effects was derived from this study (highest dose tested).

Rat and rabbit - gavage

An abstract is available which further demonstrates that erythrosine has no teratogenic potential (Burnett et al., 1974). Erythrosine (not further specified) has been administered by gavage during organogenesis to rats and rabbits (no information on number and strain of animals). There was no evidence of skeletal or soft tissue abnormalities in the foetuses.

Comment
Due to the poor description, the study cannot be taken into account for risk assessment.

Summary of Teratogenicity

The teratogenicity of erythrosine has been investigated in four oral studies (one drinking water study in Osborne-Mendel rats, two oral gavage studies (one in Osborne-Mendel and one in Charles River albino rats) and one study by administration in gelatine capsules in white New Zealand rabbits) up to dose levels of 800 mg/kg bw/d. Up to the highest dose level tested, no evidence of maternal or foetal toxicity following erythrosine exposure could be observed. Thus, it can be concluded that erythrosine is not a teratogen in rats or rabbits.

3.3.9. Toxicokinetics

Guideline:	/
Species/strain:	Rat/Wistar (Shizuoka Agricultural cooperative Association for Laboratory animals)
Group size:	4 animals /group
Test substance:	Erythrosine (from Daiwa Dyestuff MFG Go., Ltd, Saitama)
Batch:	not given
Purity:	approximately 85%
Dose levels:	0.027 % in the diet corresponding to 1600 µg iodine/day
Controls:	positive controls receiving iodine-rich food (iodine levels ranging between 1970 and 2200 µg/day)
Exposure:	1 week of exposure, 4 days of excreta collection
GLP statement:	no
Date:	1987

The bioavailability of iodine from erythrosine in comparison to various iodine-containing foods was investigated in rats and humans (the human part of the study is described in section 3.3.11 – Human data). Spray dried ordinary egg with an iodine content of 1.2 µg/g served as control. Animals received foods and erythrosine with the diet, respectively, for one week. Iodine intake from erythrosine corresponded to approximately 1600 µg iodine/day, iodine intake from iodine-rich food ranged between 1970 and 2200 µg/day. After one week faeces and (in case of erythrosine administration) urine were collected over 4 days. At the end of the experiment, animals were killed after an overnight fasting and faeces, urine, serum, liver and kidneys were analyzed for iodine.
There were no differences in body weight gain, food intake and weights of the liver, kidneys, epididymal adipose tissues and thyroid glands among the different treatment groups. There were no differences in iodine contents of livers and kidneys between control

and erythrosine fed animals. Serum iodine content in erythrosine-fed animals, however, was increased compared to control animals (128 µg/l versus 48 µg/l).
The apparent absorption of iodine was calculated from the respective iodine intake and the amounts of iodine excreted via faeces and urine. Almost all (1560 µg/day) of the iodine administered via erythrosine (1600 µg/day) was excreted via faeces, only 8.4 µg/day were excreted via urine. From iodine-containing foods on the other hand, much lower amounts of iodine were excreted via faeces und urinary iodine excretion was considerably higher. From the results the authors conclude that the bioavailability of iodine from erythrosine is very low (3% in rats) compared to the bioavailability of iodine from iodine-rich foodstuff (Katamine et al., 1987).

Comment
The possibility of biliary excretion has not been addressed in this study. The methodology of iodine determination has not been explicitly described.

Guideline:	/
Species/strain:	Rat (Sprague-Dawley)
Group size:	5 in the experiment with radiolabelled erythrosine
Test substance:	a) Pink cereal containing erythrosine b) Radiolabelled (^{131}I) erythrosine
Batch:	not given
Purity:	not given
Dose levels:	a) diet (cereal) containing 5.2 to 13.9 µg iodine from erythrosine per g cereal b) 0.625 mg erythrosine/rat and 2.125 mg erythrosine/rat
Controls	Yellow cereal with an iodine content between 0.2 and 0.4 µg iodine per g cereal
Exposure:	Dietary studies: 3- 5 weeks; radiolabelled experiments: following acute dosing
GLP statement:	Conducted prior to GLP standards
Date:	1971

In the dietary part of the study, animals received test diets for 5 weeks (2 experiments with a group of male and a group of female rats) or 3 weeks (female rats). At the end of the experiments, Na^{131}I was injected i.p. and the urinary excretion, thyroidal uptake of radioactivity and serum ^{131}I (total, protein-bound and non-protein-bound) were determined for a 24 hr-period. The thyroidal uptake of ^{131}I and protein-bound radioactivity were markedly reduced after intake of erythrosine-containing diets, whereas non-protein-bound radioactivity was elevated. Urinary ^{131}I excretion was higher in erythrosine-fed animals compared to controls statistical significance was only reached in the female animals. The authors state that these results pointed to significant de-iodination of erythrosine.
In the second part of the study, excretion and distribution of ^{131}I from ^{131}I-labelled erythrosine (24.2 µCi/µmol) was investigated in groups of 5 female animals receiving 0.625 or 2.125 mg erythrosine. In the two experiments, 29.8 and 26.6 % of ^{131}I from erythrosine were excreted as iodide via urine within 48 hours. Urinary excretion of radioactivity from erythrosine could not be detected within the first 24 hrs after administration. 56 to 64 % of the administered radioactivity was excreted via faeces within 48 hours. A small fraction (no quantitative figures given) of radioactivity from radiolabelled erythrosine could be detected in the thyroid glands, which apparently did not originate from the intact erythrosine molecule (Vought et al., 1972).

Comment
(1) For some parts of the first study (e.g. determination of protein-bound radioactivity) and for the second part of the study, rather antiquated methods were applied used for determination of radioactivity (e.g. paper chromatography followed by scintillation counting for the second part of the study).

(2) the finding of radiolabel in the thyroid is in contrast to a study from Obrist et al (1986), reported in JECFA (1986).

A further study was not available to the rapporteur. The description of the study is taken from JECFA, 1984

Guideline:	/ (not mentioned)
Species/strain:	rat, (strain not mentioned)
Group size:	not mentioned
Test substance:	^{14}C-labelled erythrosine, ^{125}I-labelled erythrosine and unlabelled erythrosine
Batch:	not given
Purity:	not given
Dose levels:	dose levels of radiolabelled erythrosine: not explicitly stated
Pre-treatment with	a dietary concentration of 0.5 or 4.0 % erythrosine for 7 days; no information on mode of administration and dosage of radiolabelled erythrosine
Controls:	no information
Exposure:	no information
GLP statement:	not mentioned
Date:	1986

The urinary and faecal excretion of ^{14}C-labelled and of ^{125}I-labelled erythrosine were studied in rats of both sexes either without pre-treatment or following dosing with unlabelled erythrosine at dietary levels of 0.5 or 4.0% for 7 days. The distribution of the compound in tissues and body fluids was also studied. 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 1 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 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 ^{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 (described in JECFA, 1986)).

Guideline:	/
Species/strain:	rat, Osborne-Mendel
Group size:	6 male animals/group
Test substance:	FD&C Red No.3
Batch:	not given
Purity:	not given
Dose levels:	0.5 to 500 mg/kg bw in four log-spaced doses (gavage dye metabolism studies); 500 mg/kg bw (dye recovery studies); 3 mg/kg bw i.v. (dye excretion studies)
Controls:	no information
Exposure:	single gavage and i.v. administrations, collection of samples over a period of up to 5 days after administration
GLP statement:	Conducted prior to GLP standards
Date:	1962

Metabolism and excretion of erythrosine was investigated after single oral administration of different amounts of the substance. In dye metabolism studies, urine specimens were collected within a 2-4 hr period after oral (gavage) administration of four different doses of erythrosine; bile specimens were taken 2 hr after administration. In dye excretion studies, bile-duct cannulated rats received 3 mg/kg bw via the femoral vein, urine and bile was collected over a 2 hr period. For dye recovery studies, animals received 500 mg erythrosine/kg bw by gavage and excreta were collected until dye excretion was no longer detectable. Samples were examined by paper chromatography (with and without enzymatic cleavage). Chromatographic analysis of excreta revealed, that the dyes were metabolically stable and that glucuronidation did not take place. The presence of dye in either bile or urine within 2-4 hours after oral administration pointed to systemic absorption (no quantitative data given). After i.v. administration, the major part (average: 55 %; range: 50.4 – 58.0 %) of the administered dose was recovered in bile, whereas on the average, 1.3 % (range: 0.8 – 1.8 %) of the dose could be determined in the urine within 2 hours after administration. and urine within a period of 2 hrs. The dye recovery part of the study revealed, that 101.9 % of the applied dose could be recovered from excreta within 5 days after oral administration (Webb et al., 1962).

Guideline:	/
Species/strain:	male rats and rabbits (no data an strains, only results for rats are presented)
Group size:	1-4 animals/group
Test substance:	FD&C Red No.3
Batch:	not given
Purity:	formulated according to FDA requirements from 1938
Dose levels:	target dose: 0.5 mg/kg bw
Controls:	no information given
Exposure:	single gavage administration, collection of excreta for at least 3 days after administration
GLP statement:	Conducted prior to GLP standards
Date:	1962

Animals received aqueous suspensions of erythrosine by stomach tube. Urine was collected for at least 3 days after dosing and analyzed by spectrofluorometry. After oral administration of 64 – 156 mg erythrosine/kg bw, 55 – 72 % of the administered dose was excreted via faeces, no colour could be identified in the urine by the analytical method applied. A small amount of colour (0.44 to 1.7 %) could be determined in the bile (Daniel, 1962).

Comment
The methodology of bile collection and analysis was not described in the paper.

Summary of toxicokinetics

From animal studies described in this section and from human studies described in section 3.3.11 (Human data) the following conclusions can be drawn with respect to the toxicokinetic behaviour of erythrosine:

No guideline-compliant investigation of erythrosine toxicokinetics has been performed. Data addressing certain aspects of toxicokinetics after oral (single and repeat-dose administration) administration are available from animal studies (different rat strains were used in the different studies) and from studies performed in human volunteers.

Except for one rat study (Vought et al., 1972) animal as well as human studies point to the fact, that oral bioavailability of erythrosine is low. This conclusion is mainly based on results

obtained from determination of urinary and/or faecal excretion of iodine and/or erythrosine after ingestion. While only minor amounts of ingested erythrosine/iodine are excreted via urine, the major part is excreted via faeces, which led to different, albeit mainly coherent findings with regards to oral bioavailability/absorption (Katamine et al., 1987: oral absorption not higher than 1 %, Ingbar, 1984: urinary excretion of erythrosine after intake of radiolabelled substance: 0.38% maximally, radioactivity in faeces: 80 – 103%, maximum body retention detected in a 14d observation period: 1.7 %). The findings of the Ingbar (1983) study indicate that low amounts absorbed after repeated oral administration of erythrosine (based on increased serum iodine levels), are not associated with effects on thyroid parameters. In a more poorly described study (Bernstein et al., 1975) it was concluded that oral bioavailability of erythrosine is not higher than 7.8 %. From one poorly described study it can be assumed, that a small fraction of bioavailable erythrosine (up to 1.7 %) can be excreted via bile. Low bioavailability (about 1%) of erythrosine was also obtained after comprehensive pharmacokinetic evaluation of data from studies yielding AUC (area under the curve) values (Poulsen, 1993).
Investigations in humans demonstrate rapid and quantitative elimination of erythrosine within 14 days, indicating low potential for accumulation. The lack of accumulation is further supported by results from tissue distribution analysis in animal experiments. Erythrosine does not appear to be distributed widely and to a great extent into tissues. Amounts of radioactivity from radiolabelled erythrosine well below 1 % (of the applied radioactivity) could be determined in the liver; even lower amounts (about 0.01 %) were detectable in the thyroid. Apparently, there was no distribution into brain or pituitary. Some studies pointed to the fact, that minor amounts of ingested erythrosine might be metabolized. However, no quantitative figures on the extent of metabolism can be derived, and structural identification of possible metabolites is not possible based on the available studies.
As a worst case assumption, 10 % oral absorption (which might also cover insufficiently investigated biliary excretion) can be assumed, however, the majority of studies points to 1 % oral absorption.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

After sun exposure, a 63 year-old woman developed eczema in uncovered regions of her body which disseminated into erythroderma. After further periods of sun exposures, episodes of erythroderma appeared again, associated with exudative lesions and fever. After a scratch test with food colours (erythrosine was amongst them), erythrosine caused a strongly positive reaction 48 hrs after testing. Further investigation revealed that the patient had received eosin therapy earlier (eosine represents a brominated derivative of fluorescein, i.e. it belongs to the family of fluorescein as erythrosine does). Thus, eosine therapy might be the reason for the skin lesions/reactions after sun exposure. It might be possible that other fluoresceins such as erythrosine might also be capable of eliciting such reactions (Castelain and Piriou, 1978).

Photosensitisation of erythrosine has been investigated in bacterial cultures of streptococcus mutans (S. mutans) grown on a microfilm. The study was aimed at determining the cell-killing potential of erythrosine (in comparison to already well characterised photosensitisers) on in vitro grown biofilms in photodynamic therapy (PDT) (aside from the treatment of localized tumours, PTD is used to selectively kill bacteria and thus, the possibility of using erythrosine in PTD for treatment of oral malignancies (e.g. for the control and treatment of dental plaques) has been addressed). Biofilms were grown in a constant depth film fermenter and erythrosine was localised by confocal laser scanning microscopy after 15 min incubation time. Antibacterial photodynamic activity was investigated in biofilms of different ages (growth times). For comparison between different photosensitizers, 15 min incubations were performed with 22 µM of the respective photosensitiser (erythrosine, methylene blue and photofrin). The bacterial-killing potential was investigated after 15 min irradiation with a 400 W light source in the wavelength ranges of 500-550 nm for erythrosine and 600- 650

nm for methylene blue and photofrin, respectively by determining bacterial survival and comparison with controls (photosensitiser alone, light alone after incubation with medium only, incubation with medium only).

Results
Erythrosine was primarily localized in the biomass of the biofilm. Compared to methylene blue and photofrin, erythrosine is a statistically significantly more potent photosensitiser (Wood et al., 2006).

Comment
Photosensitizing activity of erythrosine towards bacteria has been observed at a wavelength range between 500 and 550 nm, which is different from the UV-A (310 – 400 nm) and UV-B range (290- 310 nm) of wavelengths relevant in human real-life-situation.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

Photomutagenicity

Erythrosine was non-mutagenic in the Ames test in strains TA97a, TA98, T102, and TA 104 when using a concentration of 2 mg erythrosine/plate, with or without metabolic activation by 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 (phototoxicity) was observed in the repair-deficient strains (TA97a, TA98 and TA100) but not in the repair-proficient strains TA102 and TA104 (Lakdawalla and Netrawali, 1988a).

Fluorescein dyes, including erythrosine, were evaluated in DNA-repair, fluctuation, and treat-and plate assays under light and dark conditions. Erythrosine was inactive under all conditions (Haveland-Smith et al., 1981)

Summary of photo-induced toxicity

Erythrosine is considered to be neither phototoxic nor photomutagenic.

3.3.11. Human data

Guideline:	/
Group size:	5 men aged between 21 and 28 years
Test substance:	Erythrosine (from Daiwa Dyestuff MFG Go., Ltd, Saitama)
Batch:	not given
Purity:	approximately 85%
Dose levels:	4900 µg iodine
Controls:	positive controls: groups of 3-5 men (aged 35 – 45 years) receiving iodine-rich food (at iodine levels ranging from 1080 – 3840 µg); negative controls: urine samples from the pre-experiment period
Duration:	single administration; collection of urine for 48 hr
GLP statement:	no
Date:	1987

Urinary iodine excretion after intake of different iodine-rich food items and after erythrosine intake was investigated in male men after informed consent. Before and during the testing period, subjects were asked to abstain from iodine-rich food (except for the test meals). After a 2-day preliminary period, three to five men (age: 35 – 45 years) received a meal containing iodine-rich food yielding intake levels between 1080 and 3840 µg iodine. Five men (age: 21- 28 years) received 10 mg erythrosine resulting in an iodine intake from the colorant of 4900 µg. Pooled urine samples from the collection periods 0-4, 4-12, 12-24, 24-36 and 36-48 hrs were analyzed for iodine contents. In the subjects receiving erythrosine,

plasma levels of iodine were determined 4 hrs after intake. Urinary iodine excretion of the pre-experiment period (i.e. during consumption of low-iodine food items) served as control. Serum iodine concentrations were not increased 4 hrs after intake of erythrosine. After intake of iodine-rich food, the cumulative urinary excretion of iodine over 48 hrs ranged between 10 and 102 % of the iodine intake. After oral intake of erythrosine, on the other hand, only 1 % of the ingested iodine was excreted over a period of 48 hrs. From these results the authors concluded that the bioavailability of iodine from erythrosine is very low (Katamine, 1987).

Comment
(1) It is not stated in the publication whether serum iodine levels were also determined for the pre-experiment period. Thus, it is not clear whether serum concentrations of iodine were not increased compared to controls after erythrosine intake. Urine has not been analyzed for the intact erythrosine molecule.
(2) An ethical approval of the study has not been documented.

Guideline:	/
Species/strain:	5 healthy human volunteers, 4 male, 1 female, aged 21-35 years
Group size:	
Test substance:	Erythrosine
Batch:	not provided
Purity:	not provided
Dose levels:	5 mg erythrosine/day during the second week, 10 mg erythrosine/day during the third week, 25 mg erythrosine/day during the fourth week
Controls:	obtained from the (first) week without erythrosine administration
Exposure:	3 weeks
GLP statement:	/
Date:	1984

Human volunteers were hospitalized for 31 days and received controlled diets (with respect to iodine-, protein-, fat- and carbohydrate intake) including a daily milk shake. After a 3-day equilibration period, the testing period consisting of four weeks of different treatments started. During the first week (week 1) (this period was used in order to obtain control samples) subjects received milk shake without erythrosine. Afterwards, subjects received milk shakes supplemented with 5 (week 2), 10 (week 3) and 25 (week 4) mg erythrosine (corresponding to an additional daily iodine intake of 3, 5 and 15 mg in week 2, 3 and 4, respectively. Urine was collected in 24 hr-intervals for measurement of total iodine. Blood samples were taken daily for serum chemistry and for determination of serum thyroxine (T4), 3,5,3'-triiodothyronine (T3), thyrotropin (TSH), total serum iodine, plasma-bound iodine (PBI) and for determination of resin T3 uptake (as a thyroid function test).
Serum T3 and T4 concentrations were not significantly altered compared to control values by treatment with erythrosine (a small decrease in T3 could be observed after erythrosine treatment). Resin T3 uptake and Serum TSH concentration were not influenced by the administration of erythrosine. In contrast, serum total iodine and plasma-bound iodine both increased progressively until the end of the study (total serum iodine: from 52 to 84 µg/l (mean values); PBI: from 42 to 67 µg/l (mean values). Little if any effect was observed of erythrosine ingestion on urinary iodine excretion. Under the analytical conditions applied, levels of erythrosine in serum and urine were below the minimum detectable level of 0.05 ppm. Clinical chemistry of serum samples revealed decreases in total protein and albumin and a slight increase in serum phosphorus concentrations. From the results it can be deduced that thyroid function in humans is not affected by the dosing regimen applied (Ingbar et al., 1983).

Comment
(1) No quantitative figures can be derived with regard to bioavailability and metabolism of erythrosine (although stool had been collected, analysis of eryhrosine in faeces was not

possible due to technical reasons). However, the slight increase in serum PBI and the negligible change in iodine excretion during erythrosine ingestion indicate that only a small fraction of erythrosine was absorbed and that the absorbed amount was de-iodinated to a limited extent.
(2) an ethical approval of the study has not been documented.

Guideline:	/
Species/strain:	5 human volunteers, one of them being studied twice, four males aged 22-42 years, one female, aged 26 years
Group size:	
Test substance:	Erythrosine
Batch:	not provided
Purity:	not provided
Dose levels:	75 (lemonade) or 80 mg erythrosine (admixture of ^{131}I-labeled erythrosine to unlabelled dye)
Controls:	/
Exposure:	single oral exposure either with a milkshake or with lemonade
GLP statement:	no
Date:	1984

Human volunteers received single oral doses of erythrosine, either in a milkshake or in lemonade after an overnight fast. Before the beginning, written informed consent was obtained. The study had been approved by the institutional Human Studies and Radiation Safety Committees. Erythrosine consisted of an admixture of ^{131}I-labeled erythrosine to non-labelled erythrosine. The uptake of ^{131}I into the thyroid was blocked by administration of a saturated solution of potassium iodide. Immediately after the erythrosine intake, body content of ^{131}I was determined by means of a whole body counter, determinations were repeated daily (except on weekends) throughout the duration of the study (21 d). Complete stool and 24 hr urine collections were taken. Fasting blood samples were obtained every morning for measurement of serum thyroxine (T4), 3,5,3'-triiodothyronine (T3) and thyrotropin (TSH).
Whole body counts demonstrated that for both types of administration (lemonade and milk-shake) radioactivity was eliminated rapidly and almost completely within 14 days. The maximum body retention determined within the observation period was 1.7% of the administered radioactivity. Concurrent with the rapid decline of radioactivity in whole bodies, faecal excretion of ^{131}I could be observed. After an initial delay of about 24 hr, between 80 and 103 % of the applied radioactivity could be recovered from faeces which was in line with extremely small (if any) amounts of radioactivity determined in the urines or in plasma samples. The cumulative urinary excretion of ^{131}I did not exceed 0.38 % of the administered radioactivity within 48 hr after administration and was below detectable (or background) levels thereafter. According to the authors (remark: only graphs (diagrams) without giving numbers are presented) Serum T4 and T3 concentrations were not significantly altered compared to the values obtained before administration of erythrosine. From the results of this study it can be concluded that under the conditions of this experiment (i.e. blocking of thyroid iodine uptake), systemic bioavailability of a single oral administration of erythrosine is extremely low and that almost complete excretion occurs via faeces. As the results are based on determination of radioactivity, no final conclusions on metabolism (e.g. cleavage of iodine from the intact erythrosine molecule) of erythrosine can be drawn from this study. Further, it can be assumed, that thyroid function is not affected under the conditions of this experiment (i.e. within an observation period of short duration) (Ingbar et al., 1984b).

Guideline:	/
Species/strain:	male healthy human volunteers, aged between 22 and 38 years
Group size:	10 per dose

Test substance:	Erythrosine (from Certified Colour Manufacturers Association)
Batch:	Lot AC 7172
Purity:	not given
Dose levels:	20, 60 and 200 mg/day
Controls:	pre-exposure values served as control values
Exposure:	14 days
GLP statement:	/
Date:	1987

Volunteers received single daily oral doses of erythrosine in gelatine capsules for 14 days. Before the first administration, 24 hr urine samples had been collected (this day 1 urine sample was taken for urinalysis in order to obtain "baseline values"). Further urine samples, collected over 24 hr periods were used for urinalysis on day 8 and 15. On day 1, 8 and 15 serum was taken for determination of T4, T3, reverse T3 (rT3), T3 charcoal uptake (as a thyroid function test), TSH, protein-bound iodide, total iodide, complete blood cell counts and blood chemistry (called SMA-20). Serum samples obtained on day 1 were taken in order to derive "baseline values". After blood collection on day 1, a thyrotrophin releasing hormone (TRH) test was performed.
TRH testing can demonstrate early, minimal disruption of the pituitary-thyroid axis before changes in basal serum TSH and thyroid hormone levels are evident.
For the TRH test, individuals received a single bolus dose 500 µg TRH and samples for determination of thyroid stimulating hormone (TSH) were taken at 15 min intervals for 1 hr. The TRH test was repeated on day 15. All results from complete blood count, blood chemistry and urinalysis obtained at day 1, 8 and 15 were considered normal.
There were no significant changes in serum T4, T3, rT3, and T3 charcoal uptake values at any dose. In men receiving 200 mg erythrosine/day, the mean basal serum TSH concentration increased significantly from 1.7 ± 0.1 on day 1 to 2.2 ± 0.1 µU/ml on Day 15 ($p < 0.05$) and the mean peak TSH increment after TRH increased from 6.3 ± 0.5 to 10.5 ± 1.0 µU/ml ($p < 0.05$). There were no significant changes in basal or peak TSH responses in the men receiving 20 or 60 mg erythrosine/day. Significant dose-related increases in serum total iodide and PBI concentrations occurred during all three doses, and significant dose-related increases in urinary iodide excretion occurred during the 60 and 200 mg/day erythrosine doses. The authors concluded, that the increase in TSH secretion induced by erythrosine was related to the antithyroid effect of increased serum iodide concentrations, rather than a direct effect of erythrosine on thyroid hormone secretion or peripheral metabolism.
Certain aspects of this study, including statistical analysis have been questioned, the lack of a control group has been criticised. From a recalculation addressing two endpoints (basal TSH concentration and maximum TSH increment after TRH provocation), Crump and Farrar (1987) suggested that there is no statistical evidence for effects on basal TSH by erythrosine at dose levels between 60 and 200 mg/day. For maximum TSH increment after TRH stimulation, there is some evidence of an effect at the highest dose (a slight but significant increase in the top dose group). Thus, a NOAEL of 60 mg/day was derived (for a 60 kg person this would be 1.0 mg/kg bw/day) (Gardner et al., 1987; Crump and Farrar, 1987).

Comment
(1) Crump and Farrar (1987) calculated initial differences between treatment groups; further analysis suggests that subjects have probably not been allocated randomly to treatment groups (i.e. treatment groups differ with respect to certain variables).
(2) Gardner et al. suggest that the increase of TSH secretion is rather due to increased levels of serum iodide than to the erythrosine molecule itself. However, in view of the results from toxicokinetic studies it seems to be unlikely that increased serum iodide is a consequence of erythrosine metabolism. Thus, it remains to be clarified whether and by which mechanism erythrosine might cause elevated serum iodide levels. A possible explanation has been given be Minegishi et al. (1986), see section 3.3.12- Special investigations.

(3) The study had been approved by the Virginia Commonwealth University Committee on the conduct of human Research.
(4) For better interpretation of the results, JECFA (1986) reports on a further study (Paul et al. (1987) which had been undertaken in order 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 daily for 14 days; the doses were selected to correspond to the amounts of iodine that might be bioavailable from the doses of erythrosine used in the study by Gardner et al., 1987. Following administration of 1500 µg/day there were small but significant decreases in serum T4 and serum T3 concentration, a small compensatory increase 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." From this, JECFA (1988) commented: "Additional human studies confirmed that erythrosine is poorly absorbed. The data did not indicate the mechanism by which erythrosine exerted its effect on the thyroid. However, it appeared that inorganic iodine per se was not the causative agent."

Guideline:	/
Species/strain:	6 euthyroid human volunteers, aged 24 – 68 years
Group size:	6
Test substance:	erythrosine
Batch:	not provided
Purity:	not provided
Dose levels:	erythrosine corresponding to 1.67 mg iodine/day
Controls:	values obtained prior to treatment served as positive controls
Exposure:	10 days
GLP statement:	/
Date:	1975

Human volunteers received a diet of fixed composition. After 5 days on the diet, they ingested 1.67 mg (1.91 µmol) erythrosine per day (divided into four equal portions) for 10 days. For the whole study duration, 24-hr urines were consecutively collected for determination of iodine and creatinine. Before and after the end of the erythrosine ingestion period, PBI, T4 and total iodine in serum were measured and plasma inorganic iodine was calculated before and after 8 days of erythrosine ingestion. Thyroidal 24-hr radioiodine uptakes (^{131}I) were measured before and after 6 days of erythrosine ingestion. No statistically significant increase in plasma inorganic iodine and total iodine in serum could be observed after erythrosine intake when compared to values obtained before erythrosine intake (comment: which is in contrast to previous findings with longer and/or higher dose applications of erythrosine). 24 hr uptakes of ^{131}I, PBI and T4 levels remained unchanged. Based on clinical aspects of thyroidal iodine metabolism the authors concluded that the oral bioavailability of erythrosine was not higher than 7.8 % (Bernstein et al., 1975).

Comment
The study was not approved by an ethical committee.

Guideline:	/
Species/strain:	5 euthyroid human volunteers (male), no information on age
Group size:	3 men (group 1) and 2 men (group 2)
Test substance:	#2 pink gelatine capsules containing erythrosine (amount not specified)
Batch:	not provided
Purity:	not provided
Dose levels:	not provided
Controls:	values obtained prior to treatment served as positive controls
Exposure:	group 1: 6 capsules/d for 6 weeks; group 2: 12 capsules/d for 6 weeks
GLP statement:	/

Date: 1970

Occasioned by a case report where a patient receiving medication with erythrosine-containing capsules, the effect of erythrosine-containing capsules on radioactive iodine (RAI) uptake, protein-bound iodine (PBI) levels and T3 uptakes has been investigated in euthyroid men. Tests were performed before treatment, after a 6-week intake period and 6 weeks after the treatment had been finished. There was no difference in PBI values between subjects taking 6 and 12 capsules. There was a statistically significant increase of PBI levels after 6 weeks of treatment, but values did not exceed the normal range. There was no significant difference between the RAI uptakes at the three time points of investigation; however a tendency to lower levels at 6 weeks was noted. There was no significant difference in T3 uptakes between the three time points, although there was a general trend towards higher T3 uptake during the course of the study (Haas, 1970).

Comment
(1) The study is of limited value for assessment, because erythrosine intakes are not documented and because irregularities in capsule intakes by the volunteers occurred.
(2) Approval by an ethical committee has not been documented

A further study investigated the effects of erythrosine-containing Dopa and lithium capsules on PBI and thyroxine levels in humans. Serum concentrations of thyroxine and PBI were determined in five patients who had received Dopa (8-12 capsules daily for 2-6 months) and in five patients receiving lithium carbonate therapy (4-6 capsules daily for 2-4 months). Iodine content of the capsules was 28 µg and 38-45 µg for 250 and 500 mg Dopa, respectively, and between 65 and 78 µg for 300 mg lithium carbonate. Serum PBI concentrations in patients receiving capsules with Dopa were elevated while values for patients treated with lithium carbonate were normal. Thyroxine concentrations were normal for both groups, however a significant increase in PBI-thyroxine difference was observed in patients treated with lithium carbonate (Bora, 1969).

Comment
(1) due to vague exposure description, erythrosine intake cannot be quantified. Thus, the study is of limited value for toxicological assessment.
(2) approval by an ethical committee has not been documented.

Summary of Human data

Human studies on erythrosine have been performed in order to address the aspects 'toxicokinetics' and 'thyroid function'. Human studies have been performed either as single dose experiments or as experiments using repeated administration schemes (with durations up to 3 weeks). With respect to toxicokinetics, the studies pointed to a low bioavailability and rapid elimination of erythrosine: after single exposure to ^{131}I-labelled erythrosine, radioactivity from was completely eliminated within 14 days, the major part of radioactivity could be recovered from faeces. In studies addressing thyroid function, the influence of erythrosine on serum T3, T4, TSH or thyroid function (e.g. resin T3 uptake test) as well as iodine in serum (e.g. total iodine, PBI) and/or clinical chemistry have been investigated.
Dose-related increases in serum total iodine and PBI were observed in two of three studies, where this association has been investigated. As far as clinical chemistry was investigated, decreases in total protein and albumin and a slight increase in serum phosphorus concentrations were observed. There were no statistically significant changes in T3, T4 or T3 uptake levels under the conditions applied for the human studies. However, for TSH response after TRH stimulation, there was a slight but statistically significant increase in men receiving 200 mg erythrosine/day for 14 days. Based on these findings, a NOAEL of 60 mg/kg bw/d (corresponding to 1 mg/d) was derived for humans.

3.3.12. Special investigations

3.3.12.1 Special investigations addressing thyroid function

Guideline:	/
Species/strain:	Rat, Sprague-Dawley BR (Harlan)
Group size:	initially 13 animals/group
Test substance:	FD&C Red 3
Batch:	/
Purity:	not given
Dose level:	0.5, 1.0 and 4.0 % (diet)
Controls:	(a) 0 % erythrosine in diet (b) 100 mg/kg bw/d NaI as positive control (c) 1000 mg/kg bw/d fluorescein
Exposure:	3 weeks
GLP statement:	no
Date:	November, 1984

Groups of animals (initially 13 animals per group) were fed diets containing, 0.5%, 1 % and 4 % erythrosine for three weeks (corresponding to approximately 300, 600 and 2400 mg/kg bw/d). Additional groups received NaI (100 mg/kg/day) or fluorescein (1000 mg/kg/day). Control animals were fed normal diet. After the feeding period, effects on the pituitary-thyroid axis were investigated by an in vivo TRH (thyrotrophin releasing hormone) provocative test, in which a bolus level of TRH at a dosage of 1 mg/kg bw was injected into the vena cava of Nembutal-anesthetized animals. Blood samples were taken before and 5 and 10 min after TRH injection and TSH, serum T3, serum T4 and T3 resin uptake (as a thyroid function test) were determined in plasma samples. A dose-dependent increase in serum T4 levels was seen in the erythrosine treated animals, statistically significant at the 1 % and 4 % erythrosine levels. Free T4 indices (but not free T3 indices) were significantly increased at the 1 % and 4 % erythrosine levels. Further, at the highest dose of erythrosine, T3 levels were significantly increased, whereas T3 resin uptakes were significantly decreased. An exaggerated TSH response to TRH was seen in rats fed 4 % erythrosine in the diet, because serum TSH levels measured 10 min after TRH injection were 80 % greater than those of control rats. The exaggerated TSH response indicated less thyroid hormone inhibition of the pituitary. The exaggerated TSH response after injection of a bolus of TRH is consistent with a break in the normal thyroid-pituitary feedback relationship, secondary to deiodination of the T4 molecule at a peripheral site and/or the pituitary gland. The authors discuss, that disruption of the thyroid regulatory negative feedback mechanism does not arise from a conventional antithyroid mechanism.
Comparable effects were not observed following ingestion of fluorescein which indicated that effects on the pituitary-thyroid axis were mediated by erythrosine (or an iodinated metabolite). Further iodide administration did not enhance the TSH response to TRH (although T4 und T3 levels were elevated). Therefore the authors suggest that the observed effects of erythrosine on TSH release were due to the intact erythrosine molecule or an iodinated metabolite thereof, but not to the fluorescein body or iodide. As significant elevations in serum T4 were observed at 1 % erythrosine in the diet, a dietary level of 0.5 % was derived as the NOAEL (corresponding to 300 mg/kg bw/d) (Witorsch et al., 1984; Jennings et al., 1990).

Comment
The significant increase in T3 levels is in contrast to other studies (e.g. Ingbar et al., 1984a), however, the authors (Witorsch et al., 1984) give some explanations for this findings (probably high baseline levels of T3 (baseline levels of T3 have not been determined) , conditions of the experiment (transport of rats during the feeding schedule)).

Guideline: /

Species/strain:	Rat, Sprague-Dawley
Group size:	35 animals/sex/group (plus 10 males and 10 females for health status evaluation)
Test substance 1:	FD&C Red No. 3
Test substance 2:	Sodium Iodide Dihydrate (abbreviated as NaI)
Batch/Purities of test substance 1:	
	(a) Purified FD&C Red No. 3, (used for groups 7-12): Lot 9991 (89.84 % purity), lot 9997 (90.6 % purity) and lot AC5835 (purity 97.7%)*)
	(b) Commercial FD&C Red No. 3, (used for groups 13 and 14): Lot AA2459 (91.1 % purity), lot AC4862 (93.0 % purity)*)
Batch/purity of test substance 2:	
	Lot JJ5215BJ (> 99% purity) (used for groups 5-12)
Dose levels:	group 5 and 6: 80 µg of NaI (sodium iodide)/g of diet group 7 and 8: purified erythrosine at 4.0% in the diet group 9 and 10: purified erythrosine at 4.0% in diet plus 60 µg NaI/g diet group 11 and 12: purified erythrosine at 4.0% in diet plus 140 µg NaI/g diet group 13 and 14: commercial erythrosine at 4.0% in the diet
Controls:	1. ethanol (vehicle) control (20 animals/sex/group) (group 1 and 2) 2. basal diet without test substance (20 animals/sex/group) (group 3 and 4)
Exposure:	27 weeks
GLP statement:	in compliance
Date:	1983

*) different batches were used during the different stages of the studies, batches were controlled for iodide content

A study was undertaken to investigate whether the thyroid tumours found after in utero exposure followed by chronic feeding of commercial FD&C Red No. 3 to male rats at a dose level of 4.0% in the diet resulted from excess iodine (either as a contaminant of the colour or as iodine metabolized from the colour) or from another non-iodine-related property of erythrosine. The study was composed of different dose groups, receiving either NaI (in amounts present in commercial FD&C Red No. 3 in order to see whether NaI impurities would be the reason for the observed thyroid effects), purified colour (low NaI content: a milder effect in this group would suggest that the changes were due to contaminants whose content can be lowered by purification), purified colour plus two different levels of NaI (in order to see whether the effects after administration of commercial FD&C Red No. 3 were due to it's NaI content) and commercial FD&C Red No. 3 (to see whether the previously observed thyroid effects were due to removable non-iodide contaminants). Control groups receiving ethanol were included, because ethanol was used to obtain a uniform incorporation of NaI into the diet.
Animals were randomly assigned into treatment groups. During the study, clinical signs and mortalities were checked daily. Body weights were determined at the beginning and the end of the study and weekly throughout the study. Food intake was determined weekly. At study weeks 4, 7, 10, 14, 18, 22 and 27 blood was taken from 5 control and 10 treated animals for determination of T4, TSH, T3 uptake, clinical chemistry, PBI and plasma iodide (PI). All incidentally dying and all sacrificed animals were necropsied. External and internal macroscopic examinations were performed. Weights of tissues from all treatment and control groups were determined, thyroids were evaluated histologically.
Results: clinical observations revealed no treatment-related effects. Food consumption increased in animals receiving the colour. The group mean body weights for animals which were solely treated with NaI were equivalent to those of controls. Animals receiving colour had consistently lower body weights compared to controls. Gross pathological examinations revealed no test-material related effects. There were significant increases in thyroid weights

and thyroid/body weight ratios in NaI as well as colour-treated animals. No histopathological changes in thyroids and no thyroid tumours could be observed. There were no consistent differences between control and NaI only groups with respect to differences in thyroid parameters, clinical chemistry or gross pathology. Both PBI and total iodide were significantly higher compared to controls at all times in all groups receiving colour. Total iodine was also significantly higher in animals receiving colour. In the NaI only groups, PBI increased gradually from week 14 until the end of the study. Total iodine levels were significantly greater than in controls in both sexes of NaI treated animals. Triglyceride levels and free fatty acid levels were increased in groups fed colour, no consistent changes in cholesterol levels could be observed.
Thyroxin (T4) levels in all groups receiving colour were significantly elevated above controls (occasionally elevated levels were observed in NaI-only groups). The degree of elevation observed in the colour-fed animals would be expected to cause a mild state of hyperthyroidism.
T3 (3,5,3'-triiodothyronine) uptake levels were depressed in all groups receiving colour beginning at week 10. Some evidence of repression was observed in females receiving NaI.
TSH levels were generally elevated in all groups fed colour.

Interpretation
The results demonstrate that changes in thyroid parameters (and tumours) observed after long-term administration of erythrosine to rats cannot solely be explained by changes in thyroid parameters due to NaI contamination of the colour. Purified colour and commercial colour caused the same patterns of changes in thyroid parameters and clinical chemistry. Thus, components removed by purification of the colour (including 60 ppm NaI) do not explain the observed changes. Addition of NaI to the purified colour (60 – 140 ppm) did not affect the results. Thus, if the changes observed were caused by free iodide and not by the colour per se, then an amount of liberated iodine (metabolically or by other cleavage pathways) from the erythrosine molecule would be required that supplementation by 60 or 140 ppm NaI would not influence the results. Thyroxin (T4) levels in all groups receiving colour were significantly elevated above controls (occasionally elevated levels were observed in NaI-only groups). The degree of elevation observed in the colour-fed animals would be expected to cause a mild state of hyperthyroidism. The observed T3 reductions are not impressive in terms of changes reported in the literature. However, they are consistent with elevated levels of T4. The observed elevation in TSH levels might be a contributing factor to explain thyroid hyperplasia and thyroid tumours as observed after long-term feeding of an erythrosine diet containing 4 % of the colorant.
Increased food consumption and decreased body weights are consistent with a higher metabolic rate induced by elevated T4 levels. No final conclusions on the mode of action of erythrosine and the toxicologically relevant species causing the observed effects on thyroid parameters and/or thyroid can be drawn from these investigations. However, it can clearly be stated that effects are due to colour intake (and not iodine-containing impurities). There is evidence, that oral administration of erythrosine, irrespective of its purity, can lead to hyperthyroidism, which is supported by elevated serum concentrations of T4 and TSH, the albeit non-pronounced depression of T3, combined with lowered body weights, increased food consumption, increased thyroid weights and change in certain clinical-chemical parameters. These observation support the hypothesis, that long-term dietary administration of erythrosine to rats produces a situation of hyperstimulation of the thyroid which could ultimately end-up in thyroid tumours (Couch et al., 1983).

Comment
From JECFA (1986), it can be seen that thyroids from the Couch et al. (1983) study were subjected to ultrastructuiral examination by electron microscopy (Capen CC, sine data a; the document is not available to the rapporteur): *"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."

Guideline:	/
Species/strain:	Rat / Sprague-Dawley
Group size:	15 male rats / group, further division into groups of 5 animals after 6 months
Test substance:	FD&D Red No. 3
Batch:	not reported
Purity:	not reported
Dose levels:	0.25 %, 0.5 %, 1.0 %, 2 % and 4 % in the diet
Controls:	animals receiving 0% FD&C red No. 3 in the diet
Exposure:	7 months
GLP statement:	not given
Date:	1984

Groups of rats received erythrosine at dietary dose levels of 0.25%, 0.5%, 1.0 %, 2 % and 4 % for six months. The dose levels correspond to approximately 125, 250, 500, 1000 and 2000 mg/kg bw/d. Control rats received diet without erythrosine. After 6 months, groups were further subdivided into 3 groups of 5 rats, respectively. During the subsequent month, one subgroup received 15 µg T3/mg bw/day subcutaneously, one subgroup received the vehicle saline subcutaneously and the third group received no subcutaneous injection. Measurements of daily faecal and urinary iodine excretion were performed during the 7th month of the study in four rats of the control, 0.5 % erythrosine and 4 % erythrosine group. Blood samples for determination of TSH, T3, T4 and rT3 were obtained prior to initiation of the study and monthly thereafter until termination of the study. At termination of the study, animals were sacrificed. Thyroids were removed for ultrastructural (light and electron microscopic) investigations, livers and pituitary glands were removed for investigation of in vitro T4 metabolism. Radioimmunoassays were applied for determination of TSH, T4, T3 and rT3, in vitro T4-metabolism was carried out in tissue from the animals receiving 0.0%, 0.5% and 4% of the substance. Tissue homogenates were incubated with ^{125}I-T4 and supernatants from the precipitated reaction mixtures were analyzed for ^{125}I-labelled contents by paper chromatography.

Results
Although mean serum TSH values were higher in the 4% erythrosine group than in the control or 0.5% groups over the first 6 months of the study, the differences were not statistically-significant due to pronounced interanimal variation at all time points. 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 base-line and control values in animals receiving 4% erythrosine during the 6 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 in all animals that had received injections of T3 during the seventh month of the study were too low to be measured. Serum T3 concentrations in all 3 subgroups 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 since significant depressions relative to controls were seen only at the 1- and 2-month time periods. In animals receiving 4% erythrosine, serum rT3 concentrations increased approximately 7-fold and remained elevated throughout the 6 months of the study. In both control and 0.5% groups, serum rT3 concentrations did not

differ significantly from base-line values or from each other. Serum rT3 was undetectable in animals receiving injections of T3 during the final month of the study. Daily urinary iodine excretion averaged 3.5, 60.8, and 456.5 µg in the control, 0.5%, and 4.0% groups, respectively. Corresponding values for faecal iodide content were 28.2, 31.8, and 175.8 µg/sample.
In liver homogenates of rats treated with erythrosine, decreased ^{125}I-T4 degradation and ^{125}I-T3 generation could be observed. In the pituitary, ^{125}I-T4 degradation and ^{125}I-T3 generation appeared to be higher in the two higher dose erythrosine groups than in controls, though no dose-related statistically significant differences were observed.
The authors concluded, that these findings were consistent with an inhibition of type I 5'-monodeiodination of T4 to T3, followed by an activation of TSH secretory mechanisms in the pituitary. Investigation of thyroids by electron microscopy demonstrated that thyroid follicular cells in rats administered erythrosine had ultrastructural evidence of a dose-dependent stimulation of synthetic and secretory activity. This was most evident in rats fed 4%, and was indicated by hypertrophy of follicular cells with increased development of secretory organelles in the cytoplasm. Many of the ultrastructural changes were consistent with a response to long-standing pituitary TSH stimulation. Less marked changes in follicular cell stimulation and accumulation of lysosome-likebodies were present in rats at dietary levels of 0.25 and 0.5 % erythrosine. Ultrastructural evidence of thyroid follicular cell stimulation was by erythrosine was reversible by administration of exogenous T3 during the last experimental month (Ingbar et al., 1984a).

Comment
The description of electron microscopy was described in an Appendix attached to the Ingbar, 1984a study. In JECFA, 1986 this is cited as Capen, C.C. (sine data b). Untitled report to the Certified Color Manufacturers Association, Inc., Washington DC, USA.

From JECFA (1986) the information is available, that identical studies (Ingbar et al., 1984a) have been performed using female rats (the study report is not available to the rapporteur):
"In identical studies on female rats to those outlined above, similar results were obtained in that erythrosine at dietary concentrations of 4 % caused an increase in serum T4, rT3, and TSH concentrations, a decrease in hepatic deiodination of T4 to T3, and an increase in deiodination 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)."

Guideline:	/
Species/strain:	rat (Sprague-Dawley)
Group size:	80 animals per dose group
Test substance:	FD&C red No. 3
Batch:	not reported
Purity:	not reported
Dose levels:	0.03 %, 0.06 % and 4.0 % in the diet
Controls:	20 animals sacrificed on day 0 of the study and an additional group of animals receiving 0 % FD&C red No. 3 (erythrosine) in the diet
Exposure:	60 days
GLP statement:	not given
Date:	1989

Male rats were divided into four groups and received 0, 0.03, 0.06 and 4.0 % of the substance with the diet (corresponding to 0.0, 17.5, 35.8 and 2671.7 mg/kg bw/day). 20 additional untreated rats were sacrificed on day 0 of the study in order to obtain baseline values. At days 7, 21, 30 and 60, twenty animals from each group were injected with ketamine and sacrificed. Serum was taken for determination of TSH. In the 0.03 and 0.06

% groups, no significant changes in TSH levels were observed compared to controls. In the 4 % group, increases in serum TSH concentrations were present, reaching statistical significance (compared to controls) on day 21, 30 and 60. In the 4.0% group, serum T4 concentrations were slightly elevated above controls during the treatment period (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 The authors conclude that the results are consistent with the inhibition of the peripheral outer ring (5') de-iodination of T4 and rT3 by erythrosine. The rise in serum TSH is believed to be a compensatory response to the decrease in serum concentration of metabolically active T3. For effects on serum TSH in rats, a NOAEL of 0.06 % in the diet (corresponding to 35.8. mg/kg bw/day) was derived from this study (Braverman and DeVito, 1989).

Comment
(1) From JECFA (1990) the information is available, that further investigations on the animals have been performed (Kelly and Daly, 1989; the original study is not available to the rapporteur).
Physical observations, body weight and food consumption measurements were performed on all animals before the test and at weekly intervals during the study period. From 20 animals bled for radioimmunoassays on days 7, 21, 30 and 60, brain, pituitary and thyroid were taken, 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 to 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.
(2) From JECFA (1990) the information is available, that the studies (Braverman and de Vito, 1989 and Kelly and Daly (1989)) were preceded by a study of similar design performed by the same authors ((Braverman and de Vito (1988) and Kelly and Daly, (1988); the original studies are not available to the rapporteur): "*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). 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. 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).”

Guideline:	/
Species/strain:	Rat, Sprague-Dawley
Group size:	10 – 15 animals/dose
Test substance:	Erythrosine-A (from San-ei Chemical Industries Co., Ltd., Osaka Japan) Erythrosine-B (from Benifjui Chemical Industries Co., Ltd, Tokyo Japan)
Batch:	not given
Purity:	Erythrosine-A (ER-A): 98.4 % Erythrosine-B (ER-B): 98.1 %
Dose levels:	0.25, 1.0 and 4.0 % of either ER-A or ER-B in the diet
Controls:	0 % in the diet
Exposure:	2 weeks
GLP statement:	/
Date:	1986

Animals received either ER-A or ER-B via diet. The two substances differed with respect to particle size: ER-A represents a fine crystalline powder with particle size ranging between 5-40 µm, whereas ER-B represents a crystalline powder with a particle size ranging between 50 and 150 µm. After the two week feeding period, animals were killed, bled and serum was separated for determination of T3, T4, and TSH. Thyroids were removed and mitochondrial-microsomal fractions were prepared in order to determine thyroid peroxidise (TPO) activity.

Results: animal behaviour and general appearance were normal for all rats throughout the study. Body weight gains of rats fed ER at the 4 % level were significantly less than those of the controls. Food consumption was lower in animals receiving the 4 % ER diets. The level of T4 in serum was increased in the 0.25, 1.0 and 4 % ER-A-treated animals (statistically significant at the 4 % dose) and in the 1 % group of the ER-B treated animals. The serum levels of T3 in the ER-treated rats were decreased over a range of doses and were statistically significantly less than those of controls in the 1 % and 4 % groups of the ER-A treated animals and in the 4 % group of the ER-B treated animals. Serum TSH-levels did not show statistically significant differences between groups, because of large deviations. TPO activities of the treated animals were reduced over a range of doses and were statistically significantly less compared to controls for the 1 % and 4 % ER-A groups and in the 1 % ER-B group. Acetone-ether extracts of the sera were analyzed by thin-layer chromatography (TLC). Bands for erythrosine and triiodofluorescein were identified, indicating that iodine is cleaved from the erythrosine molecule (no quantitative figure given). In the sera obtained after 2 weeks treatment of the 4 % animals, the ratios of erythrosine to triiodofluorescein were 1.4 and 2.5 in ER-A and ER-B treated animals, respectively. From the results it was deduced, that particle size has an influence on the biological effects of ER (the smaller the particle size, the easier the release of the dye, the easier its bioavailability). The effects of ER on TPO activities might be an explanation for the changes of thyroid parameters after ER administration: TPO contributes to the organification of iodide and to the condensation of iodothyrosines to T3 and/ or T4 (Minigeshi et al., 1986).

Guideline:	/
Species/strain:	Rat/Wistar
Group size:	12 – 20 male animals
Test substance:	Red 3 (Sanei Chemical Ind., Osaka)
Batch:	not given
Purity:	not given
Dose levels:	4 % in the diet
Controls:	different control groups as described below
Exposure:	19 weeks
GLP statement:	not reported
Date:	1988

The study was undertaken in order to investigate the effects of erythrosine and thyroidectomy on the development of thyroid tumours. At the beginning of the experiment N-bis(2-hydroxypropyl)nitrosamine (DHPN) was injected i.p. at a dose of 2.8 mg/kg bw, control animals received an injection of 1 ml saline/ kg body weight. After 1 week when animal received basal diets, animals were further subdivided into groups of animals, whereby three groups received 4 % Red 3 via diet. After 4 weeks, the left thyroid lobe was resected in two groups of animals. For the investigation of thyroid effects of Red 3, the following groups were assigned:

a) DHPN, thyroidectomy, Red 3 (12 animals)
b) DHPN, no thyroidectomy, Red 3 (19 animals)
c) DHPN, thyroidectomy, no dye (11 animals)
d) DHPN, no thyroidectomy, no dye (20 animals)
e) No DHPN, no thyroidectomy, Red 3 (19 animals)
f) No DHPN, no thyroidectomy, no dye (20 animals)

At termination of the experiment after 20 weeks (19 weeks of colour feeding), animals were bled and killed. Liver, lungs, kidneys and thyroid glands were conserved, but only thyroid glands were investigated histologically. Serum T4, T3 and TSH was determined in five animals from each group.

Results: Final mean body weights were lower in Red 3 treated rats compared to untreated controls (statistically significant in group (a) only). Red 3-treated rats had higher thyroid weights (statistically significant in group (e) compared to group (f) and in group (a) compared to group (c). Mean relative liver weights were significantly lower in group (a) compared to group (c), but not in group (b) compared to group (d). The incidences of thyroid follicular adenomas were higher in animals of groups (a) and (b) compared to group (d) (statistically significant in group (a). Serum TSH levels were statistically significantly elevated in groups (a) and (e) but not in group (b). Serum T4 levels were statistically significantly elevated in group (b) compared to group (d) and in group (e) compared to group (f). Group (a) was significantly different from group (c). Serum T3 levels were lower in group (a) compared to group (c) and ssignificantly higher in group (e) compared to group (f). The investigations demonstrate, that Red 3 promotes the development of thyroid tumors in partially thyroidectomised male Wistar rats treated with DHPN, but not in non-thyroidectomized rats (Hiasa et al., 1988).

The *in vitro* effect of erythrosine on [^{125}I]T4 and [^{125}I]T3 was investigated in liver homogenates from male Sprague-Dawley rats pretreated with erythrosine (rats received erythrosine at dose levels of 2.5 (for 7 days), 10 (for 2 days), 50 (for 2 days) and 250 (for 27 days) mg/kg bw/d, i.p.). For comparison, the influence of fluorescein (the non-iodinated analogue of erythrosine) was also investigated at 250 mg/kg bw/d for 7 days. Incubation products were analyzed by paper chromatography.
Results: erythrosine produced a dose-dependent inhibition of [^{125}I]T4-deiodination, paralleled by an inhibition of [^{125}I]T3 formation. No effect on serum T3 production was observed after i.p. administration of 2.5 mg/kg bw/d for 7 days. At higher doses of erythrosine, reduction T4-deiodination exceeded the reduction of T3 formation pointing to the fact, that pathways of T4 metabolism not leading to T3 formation were also inhibited (e.g. formation of rT3). As fluorescein did not induce comparable effects it was deduced, that iodine present in the erythrosine is a prerequisite for the observed effects. The data demonstrate that thyroid-related effects of erythrosine might be explained by the inhibition of the conversion of T4 to T3 (which consequently leads to reduced serum T3 concentration) resulting in an increased stimulation of the thyroid by TSH (Ruiz and Ingbar, 1982).

A study was undertaken in order to determine, whether erythrosine due to its high content in iodine (60.7%) could modify the metabolism of the thyroid. The authors wanted to assess the hypothesis that erythrosine after oral administration could liberate iodine metabolically.
Results: After repeated exposure (4 to 17 days) of high doses of erythrosine (80 to 400 mg/day) a significant reduction in radioiodine fixation was observed (i.e. the uptake by the thyroid of ^{131}I given orally to rats was inhibited in erythrosine treated animals). With lower doses (3 to 5 mg/d) during 1 month, this reduction was less significant (a slight hypofixation may be observed in young animals). 1 to 2 mg erythrosine is considered by the authors as the detection limit of the test. The observed modification of thyroid metabolism may be due to free iodine as an impurity in the dye or due to a release of iodine after metabolism of erythrosine. After high purification of erythrosine, high doses were again administered to animals. The hypofixation of radiolabelled iodine still exists but in a lower proportion compared with the non purified dye. When administered at low doses, no difference between purified and non purified dye has been observed, probably because of the detection limit of the test. In conclusion, it seems that non sufficiently purified erythrosine may induce modification in thyroid metabolism. After purification, this perturbation may be reduced. Modification of thyroid metabolism still exist at low doses (but > 5mg/kg which was the limit of the test) after long term exposure (Marignan, 1965).

Comment
(1) The results of Marignan (1965) are in contrast to the investigation by Couch et al. (1983) in that slight differences are observed between highly purified and non-purified erythrosine

(2) no information on the strain of animals can be given, because the respective page was missing in the publication submitted.

3.3.12.2 Special investigation addressing intolerance and allergenicity

Poulsen (1980) states that erythrosine when used as a food additive (i.e. after oral intake) has been reported to cause intolerance, which is not based on an immunological reaction involving antigens and antibodies. Also Tema Nord (2002) recognizes that hypersensitivity reactions have been reported, but that no further details are available.

A case study was reported, in which a patient suffered from hypersensitivity to denture materials. It is not completely clear from the study, whether this was definitively due to the erythrosine content of the material (Barclay et al., 1999).

A more recent study describes that erythrosine is able to provoke an experimental iodine allergy in guinea pigs (Sugihara et al., 2004).

SCMPND (1987) considers a study as insignificant (Tanaka et al., 2005, the study is not available to the rapporteur) where it is described 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.

SCMPND (1987) also summarizes adverse reactions in humans when erythrosine is used as a colorant in medicinal products: *"Safety problems have never been encountered with E127 erythrosin. In those cases in which adverse reactions to the product have been reported, including allergic reactions to products containing erythrosine (E 127), it has not been possible to determine whether the reaction was provoked by the colouring agent or the active ingredient of the product (EMEA report, 1998). Erythrosin is considered to be an uncommon cause of clinically severe bronchocontriction in perennial asthmatics (Weber et al., 1979)."*

3.3.12.3 Special investigations addressing behavioural/neurotoxic aspects

Behavioural neurotoxicity in mice

Guideline:	/
Species/strain:	mouse (Crj:CD-1)
Group size:	10 males and 10 females/dose
Test substance:	Erythrosine (from Tokyo Kasei Co Ltd, Tokyo, Japan)
Batch:	GD 51
Purity:	> 85 %
Dose levels:	0.005 %, 0.0015 %, or 0.0045 % (with the diet)
Controls:	standard laboratory diet (0 % erythrosine)
Exposure:	parental animals: during a 4 week premating period, a 5 d mating period, and (for female parentals) during lactation and gestation Offspring (F1): until an age of 9 weeks
GLP statement:	not reported
Date:	2001

In a combined reproductive/neurobehavioral (for the reproductive part of the study see section 3.3.8 – reproductive toxicity) study, erythrosine was administered at dietary dose levels of 0, 0.005, 0.0015 and 0.0045 % to groups of male and female mice. The dose levels corresponded to 7.8, 22.35 and 70.43 mg erythrosine /kg bw/d in males (F0 animals during premating) and 9.68, 27.86 and 82.92 mg erythrosine /kg bw/d in females (F0 animals during premating).
The substance was administered during a 4-week premating period (which started at an age of 5 weeks), a 5-day mating period, and during gestation and lactation of the offspring (F1

generation) until the offspring reached an age of 9 weeks. The following functional and behavioural developmental parameters were measured for all individual offspring animals: surface righting (on PND 4 and 7), negative geotaxis (on PND 4 and 7), cliff avoidance (on PND 7), swimming behaviour (on PND 4 and 14) and olfactory orientation (on PND 14). The exploratory behaviour of 8 week-old parental animals and 3- and 8-week old F1 offspring mice was investigated in an animal movement analyzing system.
In parental animals, the number of turning females was significantly and in a dose-related manner increased in the high dose group of the exploratory behaviour experiment. Other parameters investigated in this part of the study (number of movements, movement time, number of horizontal activities, total distance, number of vertical activities, vertical time, average distance, average speed and number of defecations) were not affected.
In the offspring, no adverse effects on functional neurobehavioural parameters were observed. Concerning the exploratory behaviour, several parameters were changed in animal receiving the highest dose of erythrosine (significantly reduced horizontal activities and significantly increased total distance of males at week 3; increases of number of movements, movement time (statistically significant), total distance (statistically significant), average distance and average speed (statistically significant) of females at week 8). For some of the parameters (e.g. average distance in males at week 3; number of movements and average distance of females at week 8) dose-relationships could be observed. At a dose level of 0.0045 % erythrosine in the diet, certain neurobehavioural parameters were statistically significantly different from control values. Thus, effects on neurobehavioral parameters, an NOAEL of 0.0015 % erythrosine in the diet (corresponding to approximately 22.35 mg/kg bw/d for males and to 27.86 mg/kg bw/d for females (78.23 mg/kg bw/d during lactation)) were derived from this study (Tanaka, 2001).

Behavioural toxicity / developmental neurotoxicity in rats

Guideline:	/
Species/strain:	rat (Sprague-Dawley) (Laboratory Supply Co., Indianapolis, USA)
Group size:	19 – 22 animals/sex/dose
Test substance:	Erythrosine (from H. Kohnstamm & Co, New York, NY, USA)
Batch:	not reported
Purity:	91 %
Dose levels:	0.25 %, 0.5 %, or 1.0 % (with the diet)
Controls:	standard laboratory diet (0 % erythrosine)
Exposure:	parental animals: during a 2 week premating period, a 1-14 d mating period, and (for female parentals) during lactation and gestation Offspring (F1): until an age of 90 - 110 days
GLP statement:	not reported
Date:	1983

The behavioural/psychotoxic effects of erythrosine have been investigated in a 1-generation study in rats, which did not comply with an existing OECD Test guideline. Erythrosine was administered at dietary dose levels of 0, 0.25, 0.5 and 1.0 % (corresponding to approximately 250, 500 and 1000 mg erythrosine/kg bw/d) to groups of male and female Sprague-Dawley rats for two weeks prior to breeding, 1-14 days during breeding, then to females during gestation and lactation. The experimental diets were freely available throughout postnatal development to the offspring up to the age of 90-110 days of the offspring. Offspring injected daily with 50 mg/kg hydroxyurea on PND 2-10 served as positive control. Behavioural landmarks were evaluated according to the Cincinnati psychoteratogenicity test system for rats. The experiment was replicated after 2 years using the same exposure regimen, but without positive control in the second experiment and using different versions of the Cincinnati psychoteratogenicity test system in the two experiments. No dose-dependent effects on behaviour were replicated across the two experiments. Therefore it was concluded that exposure to erythrosine via dietary exposure at levels as high as 1 % do not cause psychotoxic effects in developing rats (Vorhees et al., 1983).

An abstract is available where dose-related decreases in motor activity and brain regional serotonergic activity has been observed in rats after single oral administration of up to 200 mg/kg erythrosine: *"The present study provides evidence that a single higher dosage (10, 100 or 200 mg/kg, p.o.) of erythrosine administration to young adult male rats reduced motor activity (MA) maximally at 2 h and brain regional (medulla-pons, hippocampus and hypothalamus) serotonergic activity (measuring steady-state levels of 5-HT and 5-HIAA, pargyline-induced 5-HT accumulation and 5-HIAA declination rate and 5-HT receptor binding) under similar experimental condition. The degree of erythrosine-induced inhibition of both MA and brain regional serotonergic activity was dosage dependent. Lower dosage (1 mg/kg, p.o.) did not affect either of the above. Erythrosine (100 or 200 mg/kg, p.o.)-induced MA suppression was also observed in the presence of specific MAO-A inhibitor, clorgyline (5 mg/kg, i.p.) or MAO-B inhibitor, deprenyl (5 mg/kg, i.p.); but their co-application (5 mg/kg, i.p., each) effectively prevented the erythrosine-induced motor suppression. Altogether these results suggest that a single higher dosage of erythrosine (10–200 mg/kg, p.o.) may reduce MA by reducing serotonergic activity with modulation of central dopaminergic activity depending on the brain regions."* (Dalal and Podder, 2009).

Comment:
The full paper was not available for evaluation.

Tema Nord (2002) notes that *"Erythrosine has been reported to induce hyperactivity in children, but this has not been sufficiently documented. In vitro studies have shown that high concentrations of erythrosine can inhibit brain tissue ATPases and active reuptake of neurotransmitters. This has been postulated to be the underlying mechanisms for hyperactivity. However, erythrosine has not been documented to penetrate the blood brain barrier to give rise to significant brain concentrations. So also taking into consideration the very low level of exposure this effect on behaviour seems to be of only academic interest."*

Summary of special investigations addressing thyroid function

Studies addressing thyroid function and biochemical aspects have not been performed according to OECD guidelines. However, GLP-compliance was reported for some of the studies.
These studies have mainly been undertaken (a) in order to get more insight into the biochemical pathways leading to the observed effects of erythrosine on the thyroid and (b) in order to determine the relevant toxicological agent. The latter aspect (b) has been addressed by investigating e.g. fluorescein or NaI or erythrosine of different grades of purities on postulated markers for erythrosine-mediated effects (e.g. thyroid hormones, TSH, thyroid function assays, iodine uptake of the thyroid). From the results it can be deduced, that erythrosine-mediated effects on the thyroid are not likely to be produced by the fluorescein body, by impurities present in the batches used or by iodide (which might be present either from impurities or from a presumed cleavage from the erythrosine molecule). Rather, the results observed point to the fact that erythrosine mediated effects on the thyroid are due to either the intact erythrosine molecule (or an iodinated metabolite thereof).
In order to address mechanistic aspects of erythrosine-induced effects on the thyroid, mainly in vivo animal studies with dietary administration of erythrosine for different durations have been performed. In vivo studies performed in Sprague Dawley rats administering dose levels up to 4 % erythrosine in the diet for either 3 weeks (Witorsch, 1989), 60 days (Bravermann and de Vito, 1988; 1989; Kelly and Daly, 1988; 1989), 27 weeks (Couch et al., 1983), 7 months (Ingbar et al, 1984a; Ingbar et al., 1985) almost consistently yielded the following results: oral administration caused a dose-dependent increase in serum T4 levels and a dose-dependent increase in serum TSH levels. Apart from one study where increased T3 levels have been determined (which might be an artefact) (Witorsch et al., 1989), serum T3 reduction was observed, however less pronounced when

compared to the increase in serum T4 levels. As far as investigated, also serum rT3 levels showed a dose-dependent increase. As far as investigated, also time-dependencies were observed. One study (Ingbar et al., 1984) demonstrated that effects on thyroid hormones were reversible after external administration of T3. Witorsch et al. (1989) in addition investigated the effect of erythrosine on the TSH response to TRH. Exaggerated TSH responses induced by erythrosine point to a less thyroid inhibition of the pituitary. In some of the studies, thyroids were analysed by electron microscopy. In these studies, dose-dependent hypertrophy of follicular cells and increased development of secretory and synthetic organelles were observed.
In in vitro assays (Ingbar et al., 1984; Ruiz and Ingbar, 1982), erythrosine produced a dose-dependent inhibition of T4-deiodination, paralled by an inhibition of T3 formation in rat liver homogenates. A further study demonstrated that erythrosine dose-dependently inhibited TPO.
The results from these mechanistic studies are in line with the following proposed mode of action (MoA) of erythrosine: erythrosine impairs deiodination of T4 to T3. The resulting decrease in T3 stimulates the release of thyrotropin-releasing hormone from the hypothalamus, followed by release of thyrotropin from the pituitary. Sustained increases in the levels of thyrotropin induce hyperstimulation of the thyroid and as a consequence, morphological changes followed by tumorigenic effects (if treatment dose and duration are high) may occur. These results support a thresholded mode of action.

Allergenicity

Reports are available where it is stated that erythrosine is able to cause intolerance, hypersensitivity or allergy after oral intake. No detailed descriptions are given. From one case report available, it cannot be concluded definitely that erythrosine was the causative agent for hypersensitivity. In guinea pigs, erythrosine has been shown to provoke an experimental iodine allergy. However, when erythrosine was used as a drug colorant in general, no safety problems occurred. In cases, where adverse reactions or allergenicity has been reported from a drug, it was not possible to discriminate, whether the effect was due to erythrosine or the (active) ingredient of the drug. Human experience may superimpose the findings in guinea pigs. Allergenicity is not considered as an endpoint of relevance for erythrosine.

Neurobehaviour/hyperactivity

Erythrosine is a colorant and recently some colorants have been discussed as being associated with hyperactivity in children (e.g. McCann et al., 2007). Without giving details or reference to specific investigations, Tema Nord (2002) stated that "Erythrosine has been reported to induce hyperactivity in children, but this has not been sufficiently demonstrated". Two studies investigating neurobehavioral effects (one in rats and one in mice) have been located. Whereas no dose-related behavioural effects were observed in rats up to the highest dose level tested (1 % in the diet), a dietary exposure level of 0.0045 % caused effects in certain neurobehavioural parameters in mice which were statistically significantly different from control. A further study (only abstract available) reports a **decreased** motor activity and serotonergic activity of erythrosine starting at single oral dose levels of 10 mg/kg.
As the effect of interest is **hyperactivity**, the study by Tanaka (2001) (a study with repeat-dose administration) reporting on adverse effects on neurobehavioural parameters (NOAEL 0.0015 % in the diet (corresponding to 22.35 mg/kg bw/d for males and to 27.86 mg/kg bw/d for females) was also considered in MoS discussion (in addition to effects on the thyroid).

3.3.13. Safety evaluation (including calculation of the MoS)

Calculation of margin of safety is based on oral absorption, because this opinion evaluates the safety of erythrosine in dental care products. Thus, absorption through skin is not addressed here.

Three studies have been used for calculation of the MoS. Erythrosine exerts the critical adverse effects (increased incidence of thyroid follicular cell hyperplasia, adenomas and carcinomas in rats receiving – after in utero exposure - 4 % erythrosine in the diet) secondary to effects on thyroid and thyroid function. For effects on the thyroid two studies investigating thyroid parameters have been chosen as basis for MoS calculation (one human study and one animal study). In order to avoid species extrapolation, a human study investigating thyroid parameters after repeat dose exposure has been used as one possibility (Gardner et al., 1987). In this study, a NOAEL of 1 mg erythrosine/kg bw/d has been derived.
As thyroid parameters have thoroughly been investigated in a mechanistic animal (rat) study (Braverman and de Vito, 1989), this study has also been used in parallel for MoS calculation. In this study, a NOAEL of 35.8 mg/kg bw/d has been derived.
Apart from the critical effect on thyroids, a further aspect has been regarded in MoS calculation. As erythrosine is a colorant and as recently some colorants have been discussed as being associated with hyperactivity in children (e.g. McCann et al., 2007), a third possibility of MoS calculation was considered in order to address probable neurobehavioral effects. A neurobehavioral study performed in mice (Tanaka et al., 2001) leading to a NOAEL of 22.35 mg/kg bw/d was taken for MoS calculation.

CALCULATION OF THE MARGIN OF SAFETY

Erythrosine (CI 45430)

Daily exposure from toothpaste[5] mg/d)	**=**	**0.48 g/d (480**
Daily intake erythrosine from toothpaste (0.0025 % erythrosine)	**=**	**0.012 mg**
Typical body weight of human	**=**	**60 kg**
SED (Daily erythrosine intake / body weight) bw/d	**=**	**0.0002 mg/kg**

1. MOS from a human study (Gardener et al., 1987)

No observed adverse effect level (NOAEL) (14 day oral study in humans (Gardner et al., 1987))		**=**	**1 mg/kg/d**
Margin of Safety	**NOAEL / SED**	**=**	**5000**

2. MOS from a mechanistic animal study (Bravermann and de Vito, 1989)

No observed adverse effect level (NOAEL)	**=**	**35.8 mg/kg/d**

[5] **Notes of Guidance, 7th revision, table 2 page 88 (Calculation of the daily exposure to cosmetics using Colipa data [SCCNFP/0321/02]**

Margin of Safety	**NOAEL / SED**	**=**	**175000**

3. MOS for behavioural effects (i.e. non-thyroid effects)

NOAEL behavioural effects: 22.35 mg/kg bw/d (Tanaka et al., 2001)

Margin of Safety	**NOAEL / SED**	**=**	**111750**

Conclusions and uncertainty consideration on MoS calculation:

With respect to the first possibility (MoS based on a human study (Gardner et al., 1987) a criticism of the critical study might be the short duration of the study (14 day study) and the small number of subjects. If accounting for these two aspects of uncertainties by e.g. applying two safety factors of 10 for each of these two aspects of uncertainty, a MoS of 50 would be obtained, which can be regarded as being protective, because species differences do not have to be taken into account for this study (i.e. a MOS of 10 regarding interindividual differences would be sufficient).

The NOAEL of the animal study reported by Braverman and de Vito (1989) was based on effects of erythrosine on serum TSH levels in rats. In this case, aspects of species extrapolation (from rats to humans) have to be considered. It has been broadly discussed (e.g. SCCNFP/0826/04; Choksi et al., 2003) that differences between species exist with respect to thyroid hormones, their metabolism and their response to stimuli. It has been stated that thyroid hormone homeostasis in humans (when compared with rats) might be less susceptible to chemical stimuli. However it cannot be stated a priori whether the rat or the human would be more sensible with respect to the adverse effects of erythrosine: as some authors (Witorsch et al. 1989) claim, that erythrosine might not operate as a "classical" antithyroid agent and as TPO is also affected by erythrosine, further aspects might have to be taken into account when considering species differences. Thus, species differences and interindividual differences have to be covered by the MoS of 100. The MoS of 175000 obtained from the Bravermann and de Vito (1989) study is considerably higher than 100.

A non-thyroid endpoint has also been used for MoS calculation. As it has been claimed by some authors, that certain food colorants might be associated with hyperactivity in children, also the neurobehavioral aspect was addressed in MoS calculation. A mouse study yielding a NOAEL of 22.35 mg/kg bw/d based on some adverse neurobehavioral effects with respect to exploratory behaviour was used for MoS calculation, which yielded a value of 111750. The MoS of 111750 obtained from the Tanaka (2001) study is considerably higher than 100.

Thus, based on three different approaches, high MoS values are obtained for erythrosine.

3.3.14. Discussion

Physico-chemical data
Erythrosine is a red colorant used in foods, pharmaceuticals and cosmetics. At ambient temperatures, it consists of an odourless solid powder with no sharp melting point (due to contaminants and by-products) which is stable for several years. Free iodide is only released at temperatures above 200°C. Erythrosine is soluble in water (approximately 70 g/l at 20°C) and ethanol (approximately 10 g/l) and an aqueous solution yields a pH in the range from 7-9.

Acute toxicity

No data on acute dermal or inhalation toxicity are available. A considerable number of acute oral toxicity studies performed in mice and rats revealed that the acute oral toxicity of erythrosine is low. After administration of high oral doses of erythrosine, lethargy and depression of motor activity could be observed.

Skin / eye irritation
Erythrosine is not irritating to skin or eyes.

Skin sensitization
No guideline studies on skin sensitization of erythrosine have been performed. However, there is some information from studies, which to some extent have similarity to guideline studies. From these studies, a low, if any skin sensitisation potential of erythrosine can be deduced. The results can be supported by QSAR considerations and the lack of positive findings in humans after the substance has been used for decades as a food colorant and by the presumably low dermal absorption rate.

Dermal/percutaneous absorption
No studies on dermal absorption of erythrosine have been performed. From physico-chemical data, from the results of a repeat-dose dermal study and based on the low oral bioavailability it can be deduced, that dermal absorption of erythrosine is low.

Repeated dose toxicity
A 56 day repeat-dose and subchronic and chronic oral repeat dose studies have been performed in mice, rats, pigs and beagle dogs either by administration via gavage or by administration with the diet. In general, erythrosine had no pronounced effect of mortality of the animals and animals appeared to be healthy throughout the studies.
In mice and rats, discoloration of fur, mouth area, faeces and – at higher dosage levels- urine has been described. In one rat study, pigmentation of the kidneys was reported (NOAEL: 160 mg/kg bw/d). In mice and rats, erythrosine treatment caused reduction in body weights at the respective upper dose levels tested. The effect was more pronounced after dietary treatment compared to gavage. Statistically significant effects on body weights were not observed in pigs and dogs.
At high doses of erythrosine, increased caecal weights and caecal distension (not accompanied by histological findings) have been described; in addition, diarrhoea was observed in rats and mice at higher erythrosine dose levels. Undigested material and changes in bacterial flora have been discussed as explanation for the caecal effects, which were not considered significant for the toxicological assessment.
Erythrosine-induced organ weight changes were reduced spleen weight in Osborne-Mendel rats (LOAEL: 250 mg/kg bw/d) and dose-related increases in thyroid weights, which were observed in rats (of either sex) and pigs (LOAEL in pigs: 167 mg/kg bw/d). Increased thyroid weight in rats and pigs were not accompanied by histological changes. However, treated pigs exhibited decreased serum thyroxine levels compared to controls (NOAEL: 250 mg/kg bw/d). In Mongolian Gerbils, dose-related changes in gross pathology of the thyroid have been observed after dietary administration of erythrosine. In contrast to other species investigated, in Mongolian Gerbils also statistically significantly decreased relative weights of heart, liver and spleen were reported after dietary administration of erythrosine. Although tissue weights (including thyroid) were not changed in dogs, slight chronic thyroiditis occurred in one male and one female animal at the highest dose tested (2% in the diet).
As far as investigated in the studies, urinalysis and haematology did not reveal significant treatment-related effects in rats, pigs and Beagle dogs. In Mongolian Gerbils, on the other hand, decreases in haematocrit, haemoglobin, leucocyte and reticulocytes were observed in some animals which reached statistical significance in some cases.

As far as investigated, erythrosine caused dose-related increases in serum concentrations of iodine in rats and pigs (which comprised elevations in total serum iodine, iodine bound to protein, iodine not bound to protein and iodine bound to erythrosine). After cessation of erythrosine, levels of protein-bound iodine in the plasma returned to control values (this has only been investigated in rats). Thyroxine-iodine levels were not changed in the rat.
Taken together, most profound and consistently across species observable effects of chronic administration of erythrosine were reduction of body weights (NOAEL: 429 mg/kg bw/d) and (reversibly) elevated levels in serum iodine. Effects on the weights of thyroids were observed for different species. However, only in Mongolian Gerbils, changes thyroid weights were accompanied by histopathological findings (NOAEL: 57 mg/kg bw/d). None of the studies described in this section has been selected for MoS calculation.

Mutagenicity/Genotoxicity
The mutagenicity/genotoxicity of erythrosine has been investigated in a variety of in vitro and in vivo assays. The genotoxicity and mutagenicity of erythrosine based on the respectively available in vitro and in vivo data has been discussed and reviewed by different scientific bodies (e.g. SCF, 1987; JECFA, 1990, TemaNord, 2002). The different evaluation panels consistently concluded, that erythrosine is not genotoxic. Since the publication of these scientific opinions further studies on the endpoint genotoxicity/mutagenicity have become available. Among these studies, there was one positive in vitro test for chromosomal aberrations (Hagiwara al., 2006) and there were three positive in vivo tests (a Comet assay in mice (Kawaguchi et al., 2001; Sasaki et al, 2002) and two studies investing chromosomal aberrations (Mekkawy et al., 2000; Devi et al., 2004))). In the in vitro chromosomal aberration test by Hagiwara et al. (2006), the percentage of cells with polyploidy or endoreplication was only positive in the presence of metabolic activation, whereas the assay was negative (at even higher erythrosine levels) without metabolic activation. In the COMET Assay performed by Kawaguchi and Sasaki, erythrosine-induced DNA-damage in the glandular stomach, colon and urinary bladder was observed 3 hrs after administration of erythrosine (starting at a dose level 100 mg/kg), but not 24 hrs after administration. The authors discuss, that the effects might be due to saturation of metabolic processes. The findings of this study can be overruled by the outcome of several long-term (and carcinogenicity) studies performed with erythrosine: aside from caecal distension (not accompanied by histopathological changes) and diarrhoea observed in some studies and from gross-staining and granular deposits observed in one rat study (Wilhelm and Ivy (1953), see section 3.3.7-carcinogenicity), no adverse effects or histopathological changes could be observed in glandular stomach, colon and bladder. Devi et al. (2004) stated that effects on cell proliferation were more pronounced compared to effects on chromosomal aberration. The study was not in compliance with the respective OECD protocol, test conditions are unusual with respect to animal strain and regimen of substance administration, lacks positive controls and there are further deficiencies in design of the study and presentation of the results. In the study performed by Mekkawy et al. (2000), neither a dose-relationship nor consistency in the results could be observed. Thus, the new studies available do not provide a clear evidence of a genotoxic potential of erythrosine and do not invalidate previous conclusions of different Scientific Panels on the genotoxic potential of erythrosine. Further, long-term (carcinogenicity) studies and mode of action considerations do not support a genotoxic potential of erythrosine. Based on the data available so far it can be concluded that erythrosine is not genotoxic and not mutagenic.

Carcinogenicity
No guideline-compliant carcinogenicity studies have been performed with erythrosine. However, several long-term studies are available which make it possible to draw conclusions on the carcinogenicity of erythrosine. Two long-term studies in ICR mice and one long-term study using Fischer rats were negative. Further studies, which are of limited value (because of limited number of animals used, poor study description or lack of adherence to modern standards) can be used as supporting information. In these studies, there were no

indications of a carcinogenic potential of erythrosine. In experiments, where in utero exposure of rats was followed by long-term administration of high dose levels (4% in the diet) of erythrosine, increased thyroid weights were accompanied by histological changes in the thyroid. Thyroid follicular hyperplasia and thyroid follicular tumours (adenomas and a combination of adenomas and carcinomas) could be observed after long-term administration of erythrosine to male rats (and less prominently, thyroid tumours also occurred in female rats). Thus, thyroid tumours and effects on the thyroid were the most prominent adverse treatment-related effects after administration of erythrosine. After repeated long term dermal application to mice as well as after single i.p. and s.c. applications to hamsters (followed by an observation period of 330 days) erythrosine was not carcinogenic.

Reproductive toxicity

The reproductive toxicity of erythrosine has been investigated using different study designs yielding information on the effects of erythrosine administration on one or further subsequent generations. One-generation data are available from the outcome of the one-generation part of a three-generation study in CD rats, from two combined long-term repeat-dose/reproductive toxicity studies in rats (reproductive and fertility NOAELs were at the respective highest dose levels tested, i.e. 3029 mg/kg bw/d for female rats and 2464 mg/kg/d for female and male rats, respectively) and two combined reproductive/neurobehavioural studies (one performed in mice (yielding a NOAEL at the highest dose tested (0.0045 % in the diet, 70 mg/kg bw/d)), one performed in rats (yielding a NOAEL at the highest dose level tested (1% in the diet, 1000 mg/kg bw/d))). Further an abstract about a 2-generation study and a full description of a three-generation study in rats are available. NOAEL derived from the three generation study for foetal and postnatal effects were 149 mg/kg bw/day in male rats and 255 mg/kg in female rats. The results from reproductive toxicity studies demonstrate that erythrosine does not adversely affect fertility, reproduction or post-natal parameters in rats and mice. Additional studies indicated that erythrosine lacks estrogenic activity but that it is capable of adversely affecting testicular function and certain sperm parameters in mice). The impact of erythrosine on sperm parameters might be subject for further clarification. However, the results on sperm parameters and testicular functions do not concur with the outcome of the different studies on fertility, reproduction and postnatal parameters in rats and mice from which it can be deduced that erythrosine does not represent a reproductive toxicant.

The teratogenicity of erythrosine has been investigated in four oral studies (one drinking water study in Osborne-Mendel rats, two oral gavage studies (one in Osborne-Mendel and one in Charles River albino rats) and one study by administration in gelatine capsules in white New Zealand rabbits) up to dose levels of 800 mg/kg bw/d. Up to the highest dose level tested, no evidence of maternal or foetal toxicity following erythrosine exposure could be observed. Thus, it can be concluded that erythrosine is not a teratogen in rats or rabbits.

Toxicokinetics

No guideline-compliant investigation of erythrosine toxicokinetics has been performed. Data addressing certain aspects of toxicokinetics after oral (single and repeat-dose administration) administration are available from animal studies (different rat strains were used in the different studies) and from studies performed in human volunteers.

Animal as well as human studies point to the fact, that oral bioavailability of erythrosine is low (maximally 10%). While only minor amounts of ingested erythrosine/iodine are excreted via urine, the major part is excreted via faeces. From one poorly described study it can be assumed, that a small fraction of bioavailable erythrosine (up to 1.7 %) can be excreted via bile. Low bioavailability (about 1%) of erythrosine was also obtained after comprehensive pharmacokinetic evaluation of data from studies yielding AUC (area under the curve) values.

Investigations in humans demonstrate rapid and quantitative elimination of erythrosine within 14 days, indicating low potential for accumulation. The lack of accumulation is further supported by results from tissue distribution analysis in animal experiments. Erythrosine does not appear to be distributed widely and to a great extent into tissues. Amounts of radioactivity from radiolabelled erythrosine well below 1 % (of the applied radioactivity) could be determined in the liver; even lower amounts (about 0.01 %) were detectable in the thyroid. Apparently, there was no distribution into brain or pituitary. Some studies pointed to the fact, that minor amounts of ingested erythrosine might be metabolized. However, no quantitative figures on the extent of metabolism can be derived, and structural identification of possible metabolites is not possible based on the available studies.

Phototoxicity
Erythrosine is considered to be neither phototoxic nor photomutagenic.

Human data
Human studies on erythrosine have been performed in order to address the aspects 'toxicokinetics' and 'thyroid function'. Human studies have been performed either as single dose experiments or as experiments using repeated administration schemes (with durations up to 3 weeks). With respect to toxicokinetics, the studies pointed to a low bioavailability and rapid elimination of erythrosine: after single exposure to ^{131}I-labelled erythrosine, radioactivity from was completely eliminated within 14 days, the major part of radioactivity could be recovered from faeces. In studies addressing thyroid function, the influence of erythrosine on serum T3, T4, TSH or thyroid function (e.g. resin T3 uptake test) as well as iodine in serum (e.g. total iodine, PBI) and/or clinical chemistry have been investigated.
Dose-related increases in serum total iodine and PBI were observed in two of three studies, where this association has been investigated. As far as clinical chemistry was investigated, decreases in total protein and albumin and a slight increase in serum phosphorus concentrations were observed. There were no statistically significant changes in T3, T4 or T3 uptake levels under the conditions applied for the human studies. However for TSH response after TRH stimulation, there was a slight but statistically significant increase in men receiving 200 mg erythrosine/day for 14 days. Based on these findings, a NOAEL of 60 mg/kg bw/d (corresponding to 1 mg/d) was derived for humans.

Special investigations on allergenicity and neurobehaviour

Allergenicity
Reports are available where it is stated that erythrosine is able to cause intolerance, hypersensitivity or allergy after oral intake. No detailed descriptions are given. From one case report available, it cannot be concluded definitely that erythrosine was the causative agent for hypersensitivity. In guinea pigs, erythrosine has been shown to provoke an experimental iodine allergy. However, when erythrosine was used as a drug colorant in general, no safety problems occurred. In cases, where adverse reactions or allergenicity has been reported from a drug, it was not possible to discriminate, whether the effect was due to erythrosine or the (active) ingredient of the drug. Human experience may superimpose the findings in guinea pigs. Allergenicity is not considered as an endpoint of relevance for erythrosine.

Neurobehaviour
Erythrosine is a colorant and recently some colorants have been discussed as being associated with hyperactivity in children (e.g. McCann et al., 2007). Without giving details or reference to specific investigations, Tema Nord (2002) stated that "Erythrosine has been reported to induce hyperactivity in children, but this has not been sufficiently demonstrated". Two studies investigating neurobehavioral effects (one in rats and one in

mice) have been located. Whereas no dose-related behavioural effects were observed in rats up to the highest dose level tested (1 % in the diet), a dietary exposure level of 0.0045 % caused effects in certain neurobehavioural parameters in mice which were statistically significantly different from control. A further study (only abstract available) reports a **decreased** motor activity and serotonergic activity of erythrosine starting at single oral dose levels of 10 mg/kg.
As the effect of interest is **hyperactivity**, the study by Tanaka (2001) (a study with repeat-dose administration) reporting on adverse effects on neurobehavioural parameters (NOAEL 0.0015 % in the diet (corresponding to 22.35 mg/kg bw/d for males and to 27.86 mg/kg bw/d for females) was also considered in MoS discussion (in addition to effects on the thyroid).

Special investigations on thyroid function

After in utero exposure followed by long-term (30 months) exposure to high levels in the diet (4%), erythrosine increased the incidence of thyroid follicular cell hyperplasia, adenomas and some carcinomas in male rats. Mechanistic studies indicated, that this was due to an inhibition of the peripheral conversion of T4 to T3, finally resulting in a long-term increased stimulation of the thyroid by TSH. Chronic TSH stimulation leads to follicular cell hypertrophy in the thyroid.

Lower body weights and higher food intake has been observed in most animal studies performed with erythrosine when dose levels were high (e.g. 4%in the diet). This is in line with changes in metabolic activities due to changes in thyroid parameters/thyroidal pathways.

Changes in thyroid parameters (e.g.T3-, T4-, or TSH levels) were reversible at low doses. At higher doses, thyroid weights increased: Only at even higher doses (exposure durations) increased thyroid weights were accompanied by histological changes in the thyroid. Thus, it can be concluded that stimulation of the thyroid occurs after a certain level of blockage of T4-T3 conversion has been achieved.

Based on these steps and the fact, that a genotoxic potential of erythrosine is unlikely an indirect (secondary) pathway is the most plausible explanation for the thyroid tumours observed in rats. Therefore, the derivation of NOAELs for thyroid effects is justified.

As iodine plays an important role in biochemical pathways associated with thyroid hormones, regulation of thyroidal feedback mechanisms and the thyroid in general, investigations have been performed in order to determine whether iodine-containing impurities, the intact erythrosine molecule itself or iodide liberated from the erythrosine molecule represent the causative agent for thyroid effects observed after administration of erythrosine. It could be demonstrated, that erythrosine effects on the thyroid were not due to iodine containing impurities. Elevated levels of circulating (plasma) iodide have been observed after erythrosine treatment. Due to the high metabolic stability of the erythrosine molecule it appears unlikely that elevated iodide levels are due to cleavage of iodide from the erythrosine molecule. Rather, elevated iodide levels might be due to erythrosine-induced inhibition of thyroid peroxidase (TPO). TPO contributes to the organification of iodide and to the condensation of iodothyrosines to T3 and/or T4.

Further studies have been undertaken in order to determine the effects of erythrosine on humans. With respect to toxicokinetics it could be demonstrated that as in animals, systemic bioavailability of erythrosine is low and that elimination of erythrosine is rapid.
In men receiving 20, 60 and 200 mg erythrosine/day for 14 day there were no significant changes in serum T4, T3, and rT3 values at any dose. Further, it was considered that there were no statistically significant variations of basal TSH over the dose-range studied. There were no significant changes in basal or peak TSH responses in the men receiving 20 or 60

mg erythrosine/day. However, the maximum TSH increment after TRH provocation showed a slight but statistically significant increase at 200 mg/d. Significant dose-related increases in serum total iodide and PBI concentrations occurred during all three doses, and significant dose-related increases in urinary iodide excretion occurred at the 60 and 200 mg/day erythrosine doses. From investigations performed in animals and humans, no firm quantitative conclusions can be drawn with respect to species differences of erythrosine-induced effects on thyroid and/or thyroid hormones. However, based on the observed increase in TSH response after TRH provocation after erythrosine administration to men, qualitatively comparable mechanisms of actions might be operating in experimental animals and humans. It has been broadly discussed (e.g. SCCNFP/0826/04; Choksi et al., 2003) that differences between species exist with respect to thyroid hormones, their metabolism and their response to stimuli. It has been stated that thyroid hormone homeostasis in humans (when compared with rats) might be less susceptible to chemical stimuli. However it cannot be stated a priori whether the rat or the human would be more sensible with respect to the adverse effects of erythrosine: as some authors (Witorsch et al. 1989) claim, that erythrosine might not operate as a "classical" antithyroid agent and as TPO is also affected by erythrosine, further aspects might have to be taken into account when considering species differences. Further, no firm conclusions can be drawn with respect to human variability.

4. CONCLUSION

Based on the data taken into account, the SCCS considers CI 45430 safe for consumers when used as a colorant in toothpaste products with a maximum concentration of 0.0025 % (25 ppm).

Concerning intake from cosmetics (use in toothpastes only), erythrosine has a very high MoS and intake represents only a small fraction of the ADI of 0.1 mg/kg/day. However, aggregate exposure to erythrosine is possible due to other uses (e.g. food, medical products). Exposure from sources different from toothpaste has not been addressed in this opinion.

5. MINORITY OPINION

Not applicable

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7. ABBREVIATIONS

PBI	Plasma-bound iodine
GD	Gestation Day
MoA	Mode of action
MoS	Margin of Safety
PP	Post Partum
PND	Postnatal Day
T3	3,5,3'-triiodothyronine
T4	Thyroxine (3,5,3',5'-tetraiodothyronine)
rT3	reverse T3 (3,3',5'-triiodothyronine)
TSH	Thyroid Stimulation Hormone
TRH	Thyrotrophin Releasing Hormone
TPO	Thyroxin Peroxidase