SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Quinoline Yellow (E 104) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Quinoline Yellow (E 104). Quinoline Yellow has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1975, 1978 and 1984, and the EU Scientific Committee for Food (SCF) in 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-10 mg/kg body weight (bw). Studies not evaluated by JECFA and the SCF included a chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats and a study on behaviour in children by McCann et al. from 2007. The latter study concluded that exposure to a mixture of colours including Quinoline Yellow resulted in increased hyperactivity in 8- to 9-years old children. The Panel concurs with the conclusion from a previous EFSA opinion on the McCann et al. study that the findings of the study cannot be used as a basis for altering the ADI. The Panel notes that Quinoline Yellow was negative in in vitro genotoxicity as well as in long term carcinogenicity studies. The Panel concludes that the currently available database on semi-chronic, reproductive, developmental and long-term toxicity of Quinoline Yellow, including a study in rats not apparently taken into consideration by JECFA or the SCF, provides a rationale for re-definition of the ADI. Using the NOAEL of 50 mg/kg bw/day provided by the chronic toxicity and carcinogenicity study with a reproductive toxicity phase carried out in rats and applying an uncertainty factor of 100 to this NOAEL, the Panel establishes an ADI of 0.5 mg/kg bw/day. The Panel notes that at the maximum levels of use of Quinoline Yellow, refined intake estimates are generally well over the ADI of 0.5 mg/kg bw/day.

KEYWORDS

Quinoline Yellow, E 104, D&C Yellow No. 10, Food Colour No. Yellow 13, CAS RN 8004-92-0, 2-(2-quinolyl) indan-1,3-dione-disulphonate, food colouring substance, EINECS number:305-897-5.
SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) has been asked to deliver a scientific opinion re-evaluating the safety of Quinoline Yellow (E 104) when used as a food colouring substance.

Quinoline Yellow (E 104) is a quinophthalone dye allowed to be used as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1975, 1978 and 1984, and the Scientific Committee for Food (SCF) in 1984. Both committees established an ADI of 0-10 mg/kg bw. Quinoline Yellow has also been reviewed by TemaNord in 2002 and evaluated by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) in 2004. The British Industrial Biological Research Association (BIBRA) has issued a report on Quinoline Yellow in 1982 and a Toxicity Profile in 1990. These latter evaluations contain studies not included in the evaluations of the SCF and JECFA, and the review of TemaNord.

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

Toxicokinetic considerations indicate that there is limited absorption of Quinoline Yellow in rats and dogs (an estimated 3-4 % of the administered dose), and that most of an orally administered dose is excreted unchanged via the faeces. Quinoline Yellow is of low acute toxicity. However, the results of short-term, reproductive, developmental and long-term toxicity studies available on Quinoline Yellow indicate that the colour (or its metabolites) is to some extent bioavailable, given that some systemic toxicological findings have been reported in these longer-term studies.

In a long-term study in the mouse, involving in utero exposure, the only significant Quinoline Yellow-related effect was a decrease in white blood cell counts in female mice at the highest dose level of 1500 mg/kg bw/day, a finding which the Panel did not consider as an adverse effect. The Panel considered that the haematological modifications observed in this study were of little biological significance, in the light of the limited effect (22 %), occurring only at the end of the study while no such changes were observed throughout the study (at 3, 6, 9, 12, 18 months) and no change in the ratio of the different cell types was noted.

An oral chronic toxicity and carcinogenicity study in the rat with a reproductive toxicity phase, which was not included in the JECFA evaluations, used dose levels of up to 5 % Quinoline Yellow in the diet. Decreased body weights compared with controls were observed in treated F1 males, but not in females, at dose levels of 2 and 5 % Quinoline Yellow in the diet (reported to be equivalent to 1000 or 2500 mg/kg bw/day). The NOAEL in the adults of the F1 generation was considered by the Panel to be 250 mg/kg bw/day Quinoline Yellow (0.5 % in the diet). The pups of the F0 dams were however reported to display a slightly reduced viability and slightly lower body weight gains during lactation at dose levels of 0.5 % Quinoline Yellow in the diet (reported to be equivalent to 250 mg/kg bw/day). The Panel considered therefore that the NOAEL for the reproductive phase of this study was 50 mg/kg bw/day, based on the reported effects in the F1 pups.

This study, together with several other oral long-term carcinogenicity studies at dose levels up to 2500 mg/kg bw/day in the rat and 7500 mg/kg bw/day in the mouse revealed no evidence of carcinogenicity and a study involving subcutaneous injection of Quinoline Yellow in the rat also provided no evidence of carcinogenic potential.

A lack of genotoxic potential indicated in earlier studies on Quinoline Yellow has been confirmed by more recent guideline compliant studies, comprising a bacterial reverse mutation assay, an in vitro mouse lymphoma L5178Y (TK locus) gene mutation test and an in vivo mouse micronucleus test conducted in NMRI mice. However the Panel noted that the relevance of these recent studies for the
assessment of food grade Quinoline Yellow is unclear as these studies have been carried out with a test substance (Quinoline Yellow) containing a high proportion of the monosulphonate component (85-91%) while the specifications for food-grade Quinoline Yellow indicate that the disulphonate is the main component (>80%) and the monosulphonate only covers 15%. Results obtained by Macioszek and Kononowicz in 2004 indicated however that Quinoline Yellow may have clastogenic and/or aneugenic and DNA-damaging properties, based on positive results obtained in a micronucleus and a Comet assay in vitro. However the Panel noted that several oral long-term carcinogenicity studies with Quinoline Yellow revealed no evidence of carcinogenicity and that the SCF, JECFA, BIBRA, TemaNord and the SCCNFP have also concluded that there is no evidence for carcinogenicity of Quinoline Yellow. The Panel therefore considered that the results of Macioszek and Kononowicz were of uncertain biological significance.

A study by McCann et al. has concluded that exposure to one of two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, in particular Mix B (containing Quinoline Yellow as well as three other colours) was reported to result in increased hyperactivity in 8- to 9-years old, but not in 3-years old children selected from the general population. In 2008, EFSA published an opinion on this McCann et al. study.

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC Panel) concluded that:

- the McCann et al. study provides limited evidence that the two different mixtures of synthetic food colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in children selected from the general population excluding children medicated for Attention-Deficit Hyperactivity Disorder, although the effects were not statistically significant for the two mixtures in both age groups;

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

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

The ANS Panel concurs with these conclusions.

Adverse reactions after oral intake of Quinoline Yellow, mostly taken as part of a mixture of other synthetic colours, have been reported for urticaria and rhinitis. Reports are often characterised by poorly controlled challenge procedures and recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon.

Therefore the Panel concluded that while some sensitivity reactions after Quinoline Yellow intake (urticaria, rhinitis and asthma) have been reported, no conclusion on the induction of sensitivity by Quinoline Yellow could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The Panel concluded that the available database on semi-chronic, reproductive, developmental and long-term toxicity of Quinoline Yellow, including a study apparently not taken into consideration by JECFA or the SCF, provides a basis for re-definition of the ADI. The Panel considered that the long-term chronic toxicity/carcinogenicity study with a reproductive toxicity phase carried out by Biodynamics in rats should be considered as the pivotal study on which to base an ADI. In this study the reported reduced viability and lower body weight gains in pups during lactation at a dose level of Quinoline Yellow of 250 mg/kg bw/day are considered to be indicative of a treatment-related effect, and a NOAEL of 50 mg/kg bw/day is therefore derived from this study. Application of an uncertainty factor of 100 to this NOAEL establishes an ADI of 0.5 mg/kg bw/day.
The dietary exposure to Quinoline Yellow was estimated by the Panel based on the Maximum Permitted Levels (MPLs) of use, by applying the Budget method (Tier 1) with the assumptions described in the report of the Scientific Cooperation (SCOOP) Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day for adults and 13.1 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Quinoline Yellow (Tier 3), as identified by the Panel from the data made available by the FSA, FSFI, AFSSA, UNESDA, CEPS, ELC, and CIAA. For children (1-10 years old), estimates have been calculated for nine European countries (Belgium, France, UK, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Quinoline Yellow exposure estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Quinoline Yellow for European children (aged 1-10 years), ranged from 0.8 to 3.5 mg/kg bw/day and from 1.8 to 9.6 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Quinoline Yellow of 0.9 mg/kg bw/day and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Quinoline Yellow for European children (aged 1-10 years), ranged from 0.45 to 2.0 mg/kg bw/day, and from 1.1 to 4.1 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Quinoline Yellow of 0.5 mg/kg bw/day and of 1.2 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

The Panel concludes that at the maximum levels of use of Quinoline Yellow, refined (Tier 2 and Tier 3) intake estimates are generally well over the ADI of 0.5 mg/kg bw/day established by the Panel.

The Panel notes that the specifications of Quinoline Yellow need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components. The Panel also considers that further clarification on the proportion of methylated and unmethylated Quinoline Yellow may be required.

The Panel also notes that the specification for lead in Directive 2008/128/EC ($\leq 10$ mg/kg) appears to be high compared to the JECFA specification ($\leq 2$ mg/kg).

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

Abstract .................................................................................................................................................... 1  
Summary .................................................................................................................................................. 2  
Table of contents ...................................................................................................................................... 5  
Background as provided by the European Commission ........................................................................... 6  
Terms of reference as provided by the European Commission ................................................................. 6  
Assessment ............................................................................................................................................... 7  
1. Introduction ....................................................................................................................................... 7  
2. Technical data..................................................................................................................................... 7  
   2.1. Identity of the substance ............................................................................................................. 7  
   2.2. Specifications .......................................................................................................................... 8  
   2.3. Manufacturing process .......................................................................................................... 10  
   2.4. Methods of analysis in foods ................................................................................................ 10  
   2.5. Stability, reaction and fate in food ........................................................................................ 10  
   2.6. Case of need and proposed uses ............................................................................................ 10  
   2.7. Information on existing authorisations and evaluations ....................................................... 11  
   2.8. Dietary exposure ................................................................................................................... 11  
   2.8.1. Actual levels of use of Quinoline Yellow ............................................................................. 11  
   2.8.1.1. Beverages ................................................................................................................. 11  
   2.8.1.2. Foodstuffs ................................................................................................................ 12  
   2.8.2. Exposure assessment ............................................................................................................ 14  
   2.8.2.1. Crude estimates (Budget Method) ........................................................................... 14  
   2.8.2.2. Refined estimates ..................................................................................................... 15  
3. Biological and toxicological data .......................................................................................................... 17  
   3.1. Absorption, distribution, metabolism and excretion .................................................................. 17  
   3.2. Toxicological data..................................................................................................................... 18  
   3.2.1. Acute oral toxicity ................................................................................................................ 18  
   3.2.2. Short-term and subchronic toxicity ..................................................................................... 18  
   3.2.3. Genotoxicity and mutagenicity .......................................................................................... 19  
   3.2.4. Chronic toxicity and carcinogenicity .................................................................................... 20  
   3.2.5. Reproductive and developmental toxicity ............................................................................. 23  
   3.2.6. Allergenicity, hypersensitivity and intolerance .................................................................... 24  
   3.2.7. Other studies ........................................................................................................................ 24  
4. Discussion ........................................................................................................................................ 25  
Conclusions ............................................................................................................................................ 29  
Documentation provided to EFSA ......................................................................................................... 30  
References .............................................................................................................................................. 30  
Annex A ................................................................................................................................................. 37  
Glossary and abbreviations .................................................................................................................... 39
BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

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

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

---

4 OJ L 40, 11.2.1989, p. 27
Re-evaluation of Quinoline Yellow (E 104) as a food additive

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Quinoline Yellow (E 104) when used as a food colouring substance.

Quinoline Yellow (E 104) is a quinophthalone dye allowed to be used as a food additive in the EU and previously evaluated by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1975, 1978, and 1984 (JECFA, 1975a, 1975b, 1984a, 1984b), and the Scientific Committee for Food (SCF) in 1984 (SCF, 1984). Quinoline Yellow has also been evaluated by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP, 2004) and reviewed by TemaNord (TemaNord, 2002). The British Industrial Biological Research Association (BIBRA) has issued a report and a Toxicity Profile on Quinoline Yellow (BIBRA, 1982, 1990). The SCCNFP evaluation and the BIBRA reports contain studies not included in the evaluations of the SCF and JECFA, and the review by TemaNord.

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

2. Technical data

2.1. Identity of the substance

Quinoline Yellow (E 104), CAS Registry Number 8004-92-0, is a quinophthalone dye which is marketed commercially as a mixture of mono-, di- and trisulphonic acid derivatives (see also specifications), with the following structural formula (principal component):

![Figure 1. Structural formula of Quinoline Yellow (principal component)](image)

The disulphonic acid derivative, disodium salt, full chemical name disodium 2-(1,3-dioxoindan-2-yl)quinoline-6,8-disulphonate, has the molecular formula C_{18}H_{9}NO_{8}S_{2}Na_{2} and a molecular weight of 477.38 g/mol. While JECFA has reported that Quinoline Yellow exists in both an unmethylated and methylated form (the methyl group being in the 7-position of the molecule) (JECFA, 1975b, 1984a), in its last revision of the specifications the unmethylated disulphonic acid was considered to be the principal component (JECFA, 2006). The US Environmental Protection Agency (EPA) Substance Registry System describes the molecular formula as unspecified.

At least 28 synonyms are in use (ChemIDplus advanced, via internet, 2006). The Colour Index No. is 47005 and the most commonly used synonyms in published literature are Quinoline Yellow, C.I. Acid
Re-evaluation of Quinoline Yellow (E 104) as a food additive

Yellow 3, Food Colour No. Yellow 13, INS No. 104 and F.D. & C. Yellow No. 10. The synonym given by JECFA is C.I. Food Yellow 13.

Quinoline Yellow is soluble in water, slightly soluble in ethanol and practically insoluble in vegetable oils (Merck Index, 2006).

2.2. Specifications

Specifications have been defined in Commission Directive 2008/128/EC (EC, 2008) and by JECFA (2006) (Table 1). In these specifications, Quinoline Yellow is stated to consist essentially of a mixture of monosulphonates, disulphonates (principal component), and trisulphonates and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Quinoline Yellow is described as the sodium salt, but the calcium and the potassium salts are also permitted (EC, 2008).

In both the JECFA (2006) and the EC (2008) specifications, the purity is given as not less than 70 % total colouring matters, calculated as the sodium salt. The remaining material is described as consisting predominantly of sodium chloride and/or sodium sulphate as the principal uncoloured components although the existing specifications do not include a specific maximum permitted level for these components. Of the not less than 70 % total colouring matters, the following composition is specified:

- not less than 80 % shall be disodium 2-(2-quinolyl)indan-1,3-dione-disulphonate
- not more than 15 % shall be sodium 2-(2-quinolyl)indan-1,3-dione-monosulphonate
- not more than 7 % shall be trisodium 2-(2-quinolyl)indan-1,3-dione-trisulphonate

In addition, the specifications allow for < 4.0 % subsidiary colouring matters, originating from the manufacturing process, < 0.5 % organic compounds other than colouring matters, comprising 2-methylquinolinesulphonic acid, phthalic acid, 2,6-dimethylquinoline and 2,6-dimethylquinolinesulphonic acid, and < 0.01 % of unsulphonated primary aromatic amines (calculated as aniline but not further identified).

The Panel noted that if the existing specifications could be extended to include < 30 % sodium chloride and/or sodium sulphate as the principal uncoloured components, 99.5 % of the material would be accounted for. The Panel considered that further clarification on the proportion of methylated and unmethylated Quinoline Yellow would be informative, however it was noted that both JECFA and the SCF considered that data on both derivatives could be used in the toxicological evaluation of either forms of Quinoline Yellow (JECFA, 1984b; SCF, 1983).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colouring matters:</td>
<td>≥ 70 %</td>
<td>≥ 70 %</td>
</tr>
<tr>
<td>Of which:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disodium 2-(2-quinoly)indan-1,3-dione-disulphonate</td>
<td>≥ 80 % ≤ 15 % ≤ 7 %</td>
<td>≥ 80 % ≤ 15 % ≤ 7 %</td>
</tr>
<tr>
<td>sodium 2-(2-quinoly)indan-1,3-dione-monosulphonate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trisodium 2-(2-quinoly)indan-1,3-dione-trisulphonate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water insoluble matter</td>
<td>≤ 0.2 %</td>
<td>≤ 0.2 %</td>
</tr>
<tr>
<td>Subsidiary colouring matters</td>
<td>≤ 4.0 %</td>
<td>≤ 1.0 %</td>
</tr>
<tr>
<td>2-methylquinoline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-methylquinoline-sulphonic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phthalic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-dimethylquinoline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-dimethylquinoline sulphonic acid</td>
<td></td>
<td>≤ 0.5 %</td>
</tr>
<tr>
<td>2-(2-quinolyl)indan-1,3-dione</td>
<td>≤ 4.0 mg/kg</td>
<td>≤ 4.0 mg/kg</td>
</tr>
<tr>
<td>2-[2-(6-methylquinolyl)]-1,3-indandione</td>
<td>≤ 4.0 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Unsulphonated primary aromatic amines</td>
<td>≤ 0.01 % (calculated as aniline)</td>
<td>≤ 0.01 % (calculated as aniline)</td>
</tr>
<tr>
<td>Ether extractable primary aromatic amines</td>
<td>≤ 0.2 % (under neutral conditions)</td>
<td>≤ 0.2 %</td>
</tr>
<tr>
<td>Arsenic</td>
<td>≤ 3 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>≤ 10 mg/kg</td>
<td>≤ 2 mg/kg</td>
</tr>
<tr>
<td>Mercury</td>
<td>≤ 1 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td>≤ 50 mg/kg</td>
</tr>
<tr>
<td>Cadmium</td>
<td>≤ 1 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Heavy metals (as Pb)</td>
<td>≤ 40 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

The Panel noted that the specification for lead in Directive 2008/128/EC (≤ 10 mg/kg) appears to be high compared to the JECFA specification (≤ 2 mg/kg).

The Panel noted that the specifications on the purity of Quinoline Yellow permit concentrations of unidentified unsulphonated aromatic amines such as aniline to be present in concentrations of up to 100 mg/kg Quinoline Yellow. Given the maximal allowed concentration of Quinoline Yellow that can be added to food (500 mg/kg food), the concentration of these unidentified unsulphonated primary aromatic amines in food may reach 50 μg/kg food.

The Panel also noted that the specifications on the purity of Quinoline Yellow allow organic compounds other than colouring matters, including 2-methylquinoline and/or 2,6-dimethylquinoline (sulphonated or unsulphonated) to be present in Quinoline Yellow at a maximum concentration of up to 5000 mg/kg. Given the maximal allowed concentration of Quinoline Yellow that can be added to food (500 mg/kg food) the concentration of these compounds in food may reach 2.5 mg/kg food.

According to EU legislation (EC, 2008), the above purity criteria for the pure substance also apply to the raw material from which the aluminium lake is produced. In addition, the aluminium lake should contain no more than 0.5 % hydrochloric acid (HCl)-insoluble material and no more than 0.2 % ether-extractable material under neutral conditions. Unreacted aluminium oxide may also be present in the final product (quantity not specified). There are no additional specification requirements for the aluminium lake (EC, 2008). JECFA does not give specifications for the aluminium lake of Quinoline Yellow, other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004) which are essentially similar to those in Directive 2008/128/EC (EC, 2008).

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium, for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA,
Re-evaluation of Quinoline Yellow (E 104) as a food additive

2008b), and therefore that specifications for the maximum level of aluminium in the aluminium lake of Quinoline Yellow may be required.

2.3. Manufacturing process

Quinoline Yellow is manufactured by sulphonating 2-(2-quinolyl)indane-1,3-dione or a mixture containing about two-thirds 2-(2-quinolyl)indane-1,3-dione and one third 2-(2-(6-methylquinolyl))indane-1,3-dione (JECFA, 2006). Quinoline Yellow may be converted to the corresponding aluminium lake under aqueous conditions by reacting aluminium oxide with the colouring matter. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with sodium carbonate or sodium bicarbonate or aqueous ammonia. Following lake formation, the product is filtered, washed with water and dried (JECFA, 2004).

2.4. Methods of analysis in foods

Several methods for the determination of Quinoline Yellow in foods are described in the literature, of which variations of High Pressure Liquid Chromatography (HPLC) appear to be most generally employed.

2.5. Stability, reaction and fate in food

In general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) may affect the colour (Scotter and Castle, 2004).

2.6. Case of need and proposed uses

Maximum permitted use levels have been defined in the EU legislation by Directive 94/36/EC on food colours (EC, 1994). Currently, Quinoline Yellow is authorised for use in the EU with a maximal allowed use level of 50 to 500 mg/kg food for various foodstuffs. Quinoline Yellow is also allowed in beverages at levels up to 200 mg/L. Table 2 summarises those beverages and foodstuffs that are permitted to contain Quinoline Yellow up to specified Maximum Permitted Levels (MPLs) set by EC legislation (EC, 1994).


<table>
<thead>
<tr>
<th>Beverages</th>
<th>Maximum permitted level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic flavoured drinks</td>
<td></td>
</tr>
<tr>
<td>Americano</td>
<td></td>
</tr>
<tr>
<td>Bitter soda, Bitter vino</td>
<td></td>
</tr>
<tr>
<td>Liquid food supplements/dietary integrators</td>
<td></td>
</tr>
<tr>
<td>Spirituous beverages</td>
<td></td>
</tr>
<tr>
<td>Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails</td>
<td></td>
</tr>
<tr>
<td>Fruit wines, cider and perry</td>
<td></td>
</tr>
</tbody>
</table>
### Information on existing authorisations and evaluations

Quinoline Yellow has been evaluated previously by JECFA (1975a; 1975b, 1978; 1984a, 1984b) and the SCF (1984). Both committees established an ADI of 0-10 mg/kg bw.

Quinoline Yellow has also been evaluated by the SCCNFP (2004), and BIBRA has issued a report and a Toxicity Profile on Quinoline Yellow (BIBRA, 1982, 1990). Quinoline Yellow has not been authorised by the US Food and Drug Administration (FDA) as a colour additive and hence is not permitted in food in the USA.

### Dietary exposure

#### Actual levels of use of Quinoline Yellow

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

#### Beverages

For non-alcoholic flavoured drinks, a survey conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggested that the highest current use level of Quinoline Yellow in beverages was 80 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009 gives a range

### Table: Maximum permitted level of use of Quinoline Yellow (E 104) (mg/kg)

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Maximum permitted level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete formulae for weight control intended to replace total daily food intake or an individual meal</td>
<td>50</td>
</tr>
<tr>
<td>Complete formulae and nutritional supplements for use under medical supervision</td>
<td>50</td>
</tr>
<tr>
<td>Soups</td>
<td>50</td>
</tr>
<tr>
<td>Flavoured processed cheese</td>
<td>100</td>
</tr>
<tr>
<td>Fish paste and crustaceans paste</td>
<td>100</td>
</tr>
<tr>
<td>Smoked fish</td>
<td>100</td>
</tr>
<tr>
<td>Savoury snack products and savoury coated nuts</td>
<td>100</td>
</tr>
<tr>
<td>Meat and fish analogues based on vegetable proteins</td>
<td>100</td>
</tr>
<tr>
<td>Jam, jellies and marmalades and other similar fruit preparations</td>
<td>100</td>
</tr>
<tr>
<td>Including low calorie products</td>
<td>100</td>
</tr>
<tr>
<td>Edible ices</td>
<td>150</td>
</tr>
<tr>
<td>Desserts including flavoured milk products</td>
<td>150</td>
</tr>
<tr>
<td>Fine bakery wares</td>
<td>200</td>
</tr>
<tr>
<td>Candied fruit and vegetables, Mostarda di frutta</td>
<td>200</td>
</tr>
<tr>
<td>Preserves of red fruits</td>
<td>200</td>
</tr>
<tr>
<td>Extruded or expanded savoury snack products</td>
<td>200</td>
</tr>
<tr>
<td>Pre-cooked crustaceans</td>
<td>250</td>
</tr>
<tr>
<td>Confectionery</td>
<td>300</td>
</tr>
<tr>
<td>Mustard</td>
<td>300</td>
</tr>
<tr>
<td>Fish roe</td>
<td>300</td>
</tr>
<tr>
<td>Solid food supplements/dietary integrators</td>
<td>300</td>
</tr>
<tr>
<td>Decorations and coatings</td>
<td>500</td>
</tr>
<tr>
<td>Sauces, seasonings, pickles, relishes, chutney and piccalilli</td>
<td>500</td>
</tr>
<tr>
<td>Salmon substitutes</td>
<td>500</td>
</tr>
<tr>
<td>Surimi</td>
<td>500</td>
</tr>
<tr>
<td>Edible cheese rind and edible casings</td>
<td>Quantum satis</td>
</tr>
</tbody>
</table>
of use level from 1 to 60 mg/L (UNESDA, 2009). The UK Food Standards Agency (FSA) conducted an ad hoc survey in which artificial colours were analytically determined in 201 ready-to-drink retail soft drinks selected for being distinctly coloured (FSA, 2003). Quinoline Yellow was found to be present at a level higher than 0.1 mg/L (Limit Of Detection-LOD) in 80 products, with levels varying from < 0.5 to 92 mg/L. In another survey, conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Quinoline Yellow was found to be present at a level higher than 1 mg/L (Limit Of Quantification-LOQ) in ten out of 54 soft drinks; the concentration in these products ranged from 1 to 97 mg/L (FSAI, 2009). The Confederation of the Food and Drink Industries of the EU (CIAA) also reported other current use levels of Quinoline Yellow in soft drinks ranging from 1 to 60 mg/L (CIAA, 2009). The Federation of European Food Additives, Food Enzymes and Food Cultures Industries (ELC) has provided from its UK Member Association, Food Additives and Ingredients Association (FAIA), further data which gives a typical maximum use level for Quinoline Yellow of 13.8 mg/L (ELC, 2009).

For spirituous beverages, including products with less than 15% alcohol, the survey conducted by the FSAI (2009) gave a range of Quinoline Yellow concentrations from 1 to 40 mg/L from the analyses of 14 retail samples. The European Spirits Organisation reported a range of use levels of Quinoline Yellow from 7 to 178 mg/L (CEPS, 2009).

For fruit wines (still or sparkling), cider and perry, the CIAA reported no uses at the date of the survey.

2.8.1.2. Foodstuffs

For confectionery products, the Panel was also provided with data from an ad hoc survey conducted by the FSA, in which artificial colours were analytically determined in 195 retail samples of packaged sweets, selected for being distinctly coloured (FSA, 2002). Quinoline Yellow was found to be present in a total of 116 products with levels varying from 0.7 to 200 mg/kg (LOD <0.5 mg/kg). According to the FSAI data, Quinoline Yellow was found in 85 out of 183 samples, with levels ranging from 2 to 130.1 mg/kg (LOQ of 2 – 5 mg/kg, depending on laboratory) (FSAI, 2009). Data provided by French industries on Quinoline Yellow in sweets showed use levels varying from 14 to 250 mg/kg (unpublished data provided by the French Food Safety Agency (AFSSA)). In addition, the CIAA reported current use levels not higher than 64 mg/kg (CIAA, 2009). Data provided by the ELC (2009), from its UK Member Association (FAIA), give a range of typical low and maximum use levels of Quinoline Yellow from 6 to 100 mg/kg (ELC, 2009).

For candied fruit, vegetables, mostarda di frutta, and preserved red fruits a maximum use level of Quinoline Yellow of 150 mg/kg has also been reported by the CIAA (2009).

For preserved red fruits, the FSAI survey (2009) gave a range of analytical values of Quinoline Yellow from <2 to <5 mg/kg for 10 retail samples.

For decorations and coatings, the CIAA (2009) reported a range of low and maximum use levels of Quinoline Yellow from 150 to 500 mg/kg.

For fine bakery wares, the CIAA (2009) reported a range of low and maximum use levels of Quinoline Yellow from 88 to 146 mg/kg.

For edible ices, the FSAI survey (2009) gave analytical values of Quinoline Yellow ranging from 1 to 6.5 mg/kg for 30 retail samples.

For flavoured processed cheese, edible cheese rind and edible casing, the CIAA reported a typical maximum value for Quinoline Yellow of 0.004 mg/kg.
For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from <1 to <20 mg/kg for 30 retail samples and the CIAA (2009) reported a range of low and maximum use levels of Quinoline Yellow from 2 to 80 mg/kg.

For sauces, seasonings, pickles, relishes, chutney and mustard, the FSAI survey (2009) gave a range of analytical values from 2 to 47 mg/kg for five retail samples; the CIAA (2009) reported a range of low and maximum use levels of Quinoline Yellow from 20 to 80 mg/kg.

For extruded or expanded savoury snack products and savoury snacks products and savoury coated nuts, the CIAA (2009) reported a range of low and maximum use levels of Quinoline Yellow from 0 to 10 mg/kg.

For cocktail cherries and candy cherries, the FSAI (2009) survey gave a range of use levels of Quinoline Yellow from 18 to 34 mg/kg.

For jams, jellies and marmalades, the CIAA (2009) reported maximum use levels of Quinoline Yellow ranging from 12 to 100 mg/kg; the FSAI survey gave a range of analytical values from <2 to 26 mg/kg for five retail samples.

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

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

<table>
<thead>
<tr>
<th>Beverages</th>
<th>Maximum reported use level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic flavoured drinks</td>
<td></td>
</tr>
<tr>
<td>Americano</td>
<td></td>
</tr>
<tr>
<td>Bitter soda, Bitter vino</td>
<td></td>
</tr>
<tr>
<td>Liquid food supplements/dietary integrators</td>
<td></td>
</tr>
<tr>
<td>Spirituous beverages</td>
<td></td>
</tr>
<tr>
<td>Aromatized wines, aromatized wine-based drinks</td>
<td></td>
</tr>
<tr>
<td>Aromatized wine-based drinks and aromatized</td>
<td></td>
</tr>
<tr>
<td>wine-product cocktails</td>
<td></td>
</tr>
<tr>
<td>Fruit wines, cider and perry</td>
<td></td>
</tr>
<tr>
<td>Spirituous beverages</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Maximum reported use level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavoured processed cheese</td>
<td></td>
</tr>
<tr>
<td>Edible cheese casings*</td>
<td>0.004</td>
</tr>
<tr>
<td>Edible ices</td>
<td></td>
</tr>
<tr>
<td>Extruded or expanded savoury snack products</td>
<td></td>
</tr>
<tr>
<td>Savoury snack products and savoury coated nuts</td>
<td></td>
</tr>
<tr>
<td>Complete formulae for weight control intended</td>
<td></td>
</tr>
<tr>
<td>to replace total daily food intake or an</td>
<td></td>
</tr>
<tr>
<td>individual meal</td>
<td></td>
</tr>
<tr>
<td>Complete formulae and nutritional supplements</td>
<td></td>
</tr>
<tr>
<td>for use under medical supervision</td>
<td></td>
</tr>
<tr>
<td>Soups</td>
<td></td>
</tr>
<tr>
<td>Desserts including flavoured milk products</td>
<td></td>
</tr>
<tr>
<td>Sauces, seasonings, pickles, relishes</td>
<td></td>
</tr>
<tr>
<td>Chutney and piccalilli</td>
<td></td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The table above lists the maximum reported use levels of Quinoline Yellow in beverages and foodstuffs used for the refined exposure assessment.
2.8.2. Exposure assessment

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

2.8.2.1. Crude estimates (Budget Method)

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

In the case of Quinoline Yellow, the maximum permitted use level considered for beverages was 200 mg/L. The maximum permitted level considered for solid foods was 500 mg/kg (Table 2).

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In fact, even though Quinoline Yellow may be used in a variety of solid foods that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed solid foods with the same colour added. In the case of beverages, uses are reported for a limited number of beverages; however, some of these may constitute a significant proportion of liquid intake (i.e. non-alcoholic flavoured drinks) with consumer loyalty to a single brand (and therefore to a specific colour) often being high for this category of product. The 25% proportion was therefore also considered adequate for beverages (EC, 1998). This assumes that a typical adult, weighing 60 kg, consumes daily 1.5 litres of beverages and 375 g of solid foods, containing the Quinoline Yellow. The theoretical maximum daily exposure for adults would therefore be:

\[(200 \times 0.1 \times 0.25) + (500 \times 0.025 \times 0.25) = 5 + 3.12 = 8.1 \text{ mg/kg bw/day}.\]

For children, the level of Quinoline Yellow considered in beverages was 100 mg/L (after exclusion of alcoholic drinks) and in solid food was 500 mg/kg. The proportion of 25% used, for beverages, was changed to 100% for children, in order to compensate the fact that the corresponding consumption rate of 375 mL/day could easily be exceeded by young children. This conclusion was derived from UK data on consumption of soft drinks by children aged under 5 years, where the 97.5\(^{th}\) percentile of consumption was between 70 and 80 mL/kg bw/day and a proportion factor of 100 % for beverages.
Re-evaluation of Quinoline Yellow (E 104) as a food additive

was recommended for children in the SCOOP Task 4.2 (EC, 1998). This assumes that a typical 3 year-old child, weighing 15 kg, consumes daily 1.5 litres of beverages and 94 g of solid foods containing Quinoline Yellow.

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

\[(100 \times 0.1 \times 1) + (500 \times 0.025 \times 0.25) = 10 + 3.12 = 13.1 \text{ mg/kg bw/day} \]

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

2.8.2.2. Refined estimates

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

Exposure estimates for children (1-10 years old) have been performed by the EXPOCHI consortium, based on detailed individual food consumption data from eight European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany) for Tier 2 and Tier 3. As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5-4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in Directive 94/36/EC on food colours (EC, 1994) from Table 2 (Tier 2 approach), and with the maximum reported use levels from Table 3 (Tier 3 approach).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and as estimates calculated from more refined adults food consumption data than those available to the Panel (e.g. EFSA Concise European Food Consumption Database, which gives access to aggregate food categories consumed by 15 European countries), the Panel decided to select the exposure estimate of the UK population as representative of the exposure of EU consumers to Quinoline Yellow for adults.

Estimates of Quinoline Yellow exposure from UK adult population (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 2000-2001) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC (EC, 1994) for the Tier 2 approach (Table 2), and with the maximum reported use levels for the Tier 3 approach (Table 3).

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

In the case of Quinoline Yellow, when considering MPLs of use (Tier 2), the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium ranged from 0.8 to 3.5 mg/kg bw/day, and ranged from 1.9 mg/kg bw/day to 9.6 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (13 to 41%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafer) (14 to 29%) and desserts (including flavoured milk products) (17 to 62%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, and piccalilli accounted for 10 to 50% of exposure in four countries. Confectionery accounted for 11% of exposure in one country.

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

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

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

When considering the maximum reported use levels from Table 3, mean dietary exposure to Quinoline Yellow for European children (aged 1-10 years and weighing 25-30 kg) considered by EXPOCHI consortium ranged from 0.45 to 2.0 mg/kg bw/day and from 1.1 to 4.1 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (10 to 39%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafer) (14 to 60%) and desserts (including flavoured milk products) (14 to 57%). Confectionery accounted for 13-18% of exposure (in two countries) and surimi, sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 15% of exposure in one country.

For UK children, aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 1.8 mg/kg bw/day and 4.3 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks (55%), confectionery (18%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers) and desserts (including flavoured milk products) (10-11%).

Estimates reported for the UK adult population give a mean dietary exposure to Quinoline Yellow of 0.5 mg/kg bw/day and of 1.2 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure to Quinoline Yellow (>10%) were soft drinks (30%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers), fruit wines, cider and perry (12%) and desserts (including flavoured milk products) (10%).

Table 4 Summary of anticipated exposure to Quinoline Yellow using the tiered approach (EC, 2001) in children and adult populations.
3. Biological and toxicological data

The present opinion briefly summarises the major studies on Quinoline Yellow evaluated by JECFA (1975a,b; 1978; 1984a,b), by the SCF (1984) and reviewed by TemaNord (2002). Quinoline Yellow has also been evaluated by the SCCNFP (2004), and the British Industrial Biological Research Association has issued a report on Quinoline Yellow (BIBRA, 1990). These latter evaluations contain studies not included in the evaluations of the SCF, JECFA, and TemaNord and the present opinion describes the new literature data in some more detail.

3.1. Absorption, distribution, metabolism and excretion

The 1984 JECFA evaluation (JECFA, 1984a) describes a series of toxicokinetic studies on Quinoline Yellow, carried out by the Laboratoire d'Etudes du Métabolisme des Médicaments, France (LEMM, 1978). The Panel has re-examined the report of these studies from LEMM and the results are summarised below.

Radioactivity derived from Quinoline Yellow was measured in the blood of groups of two male and two female rats at each of the following time periods: 15 and 30 minutes, 1, 2, 4, 8, 24 or 48 hours after an oral gavage dose of 4 mg/kg bw $[^{14}C]$-Quinoline Yellow (32.5 mCi/mMole), labelled on the phthalic moiety of the molecule. The peak of radioactivity occurred in plasma between 0.5 and 1 hour after dosing, with most of the radioactivity being bound to plasma proteins, as demonstrated by measurement in whole plasma and in the ultrafilterable fraction. According to the authors, the maximum concentration reached in the plasma was less than 0.00009 % of the dose of 4 mg/kg bw administered. The kinetics of the blood levels fitted a two-compartment model with the following parameters: $T_{1/2(\alpha)} = 0.6$ hours; $T_{1/2(\alpha)} = 12$ hours; and $T_{1/2(\beta)} = 70$ hours. Radioactivity from $[^{14}C]$-Quinoline Yellow was measured in the organs and in the carcass of all animals at 4, 8, 24 or 48 hours after dosing. Results showed that the small proportion of the dose that was absorbed from the gastrointestinal tract (estimated to be 3-4 %) was primarily associated with the liver (maximum 1 %), kidney (maximum 0.02 %), and bladder. Results expressed as concentration factors (radioactivity/gram tissue) showed that a selective concentration in the thyroid persisted up to 48 hours, and a relatively high concentration was found in the ovaries in the first 24 hours. Parallel investigations in the male rats confirmed that radioactivity was selectively concentrated in the thyroid (LEMM, 1978).

In a separate study carried out by the same authors in six male rats, faecal and urinary excretion of radioactivity from Quinoline Yellow, together with retained radioactivity in the carcass, was measured over the periods 0-4, 4-8, 8-24, 24-48, 48-72, 72-96 and 96-120 hours after oral gavage dosing with between 2 and 3 mg/kg bw $[^{14}C]$-Quinoline Yellow. Over the 120 hours period of the study, 95.6 % of the dose was measured in urine and faeces, with 93.6% being found in faeces, 2.06% in urine and 0.14% being retained in the carcass. Negligible amounts were found in expired air. Radioactivity in various organs was measured in five of the eight animals at termination, the results confirming the selective retention in the thyroid seen in the study described above and persisting up to 72 hours after dosing (LEMM, 1978).

Biliary excretion was measured in two male rats over 30-36 hours after dosing by oral gavage with approximately 3 mg/kg bw $[^{14}C]$-Quinoline Yellow. The peak of biliary excretion occurred between 1.5 and 3 hours after dosing, with only 1 % of the total dose being excreted in the bile over the period of the study (LEMM, 1978).

These authors also demonstrated, using whole body autoradiography, that 1 hour after oral (gavage) administration of 10 – 12 mg/kg bw $[^{14}C]$-Quinoline Yellow to male rats, radioactivity was primarily associated with the gastrointestinal tract, kidneys and bladder (LEMM, 1978). After 24 hours only the large intestine and, to a minor degree, the cortical zone of the kidney displayed radioactivity.
In dogs, blood levels and excretion after intravenous and oral gavage administration of \( ^{14}\text{C}\)-Quinoline Yellow (0.2 and 0.44 mg/kg bw respectively) were examined. After intravenous administration, the disappearance of radioactivity corresponded to a two-compartment pharmacokinetic model with \( T_{1/2(\alpha)} = 4 \text{ hours} \) and \( T_{1/2(\beta)} = 43 \text{ hours} \). About 22% of the dose was excreted in the faeces. Intragastric administration showed that peak blood levels occurred at 1-4 hours after dosing. From 0-72 hours the urine contained 1-4% of the radio-label; 42-60% was excreted in the faeces within 72 hours. There was no indication of specific tissue accumulation, particularly in the thyroid after either route of administration (LEMM, 1978).

In rats given \([^{14}\text{C}]\)-Quinoline Yellow by the oral route, the studies carried out by LEMM (1978) indicated that the substance was metabolised to only a small extent, based on chromatographic examination of bile, urine, faeces and plasma. In the urine, between 10 and 15% of the radioactivity was associated with an unidentified metabolite which was more polar than the unchanged colour. The parallel study in Beagle dogs, in which urine, faeces and plasma were examined, indicated that Quinoline Yellow is also metabolised to only a small extent in this species.

Overall it can be concluded that there is limited absorption of Quinoline Yellow in rats and dogs (an estimated 3-4% of administered dose), and that most of an orally administered dose is excreted unchanged via the faeces. Accumulation of Quinoline Yellow in the rat thyroid is not substantiated by the dog study, indicating that the thyroid accumulation in male rats may be species-specific. These studies in rats and dogs provide little indication of metabolism of the dye although no qualitative or quantitative data are provided, other than the reported finding that in rats between 10 and 15% of the radio-activity was associated with an unidentified metabolite which was more polar than the parent colour. Quinoline Yellow showed biphasic elimination with initial and second phase half-lives of 12 and 70 hours respectively after intragastric administration in rats, and 4 and 43 hours respectively after intravenous administration in dogs (LEMM, 1978).

### 3.2. Toxicological data

#### 3.2.1. Acute oral toxicity

The JECFA evaluation of 1975 (JECFA, 1975a), the SCCNFP Opinion (2004) and the BIBRA report (1982) provide summary information on the acute toxicity of Quinoline Yellow. Acute oral toxicity studies in rats have provided LD\(_{50}\) values of greater than 2000 mg/kg bw (Lu and Lavallee, 1964; BIBRA, 1982, 1990) and greater than 5000 mg/kg bw (DFG, 1991). A recent \textit{in vivo} mouse micronucleus study showed that Quinoline Yellow was not toxic to mice when administered by gavage at a level of 2000 mg/kg (RCC, 2003). In a study in dogs the LD\(_{50}\) was found to be >1000 mg/kg bw (Hazleton Labs Inc, 1962).

The SCF concluded that “\textit{acute and short term toxicity tests in rats and dogs produced no obvious toxic effects}” (SCF, 1984).

Overall, it can be concluded that the acute oral toxicity of Quinoline Yellow is low.

#### 3.2.2. Short-term and subchronic toxicity

The JECFA evaluation of 1975 describes several short-term/subchronic studies on Quinoline Yellow (JECFA, 1975a), which are summarised below.

In an oral feeding study, rats (5 per sex) were fed diets containing 0, 0.25, 0.5, 1, 2, and 5% (equivalent to 0, 125, 250, 500, 1000, 2500 mg/kg bw/day) methylated Quinoline Yellow for 90 days. No treatment-related effects were observed on body weight, food intake, blood cell counts, and organ weights (Hansen \textit{et al.}, 1960).
Cats received doses of 100 mg/kg bw Quinoline Yellow per day for seven days (no details on route of administration provided). No increase in Heinz bodies in the blood was noted (Oettel et al., 1965).

An additional unpublished subchronic study, which was not included in the JECFA evaluation of 1975 (JECFA, 1975a), was reported in the BIBRA (1982, 1990) and the SCCNFP (2004) evaluations. In this study, oral administration of Quinoline Yellow to rats (20 per sex) at dietary levels of 3% (equivalent to 1500 mg/kg bw/day) for three months induced no adverse effects on growth, behaviour, appearance, blood and urinary parameters, or gross and microscopic appearance of an unspecified range of tissues (Hazleton, 1965). It is not clear whether this study was considered by the SCF.

The SCF concluded that “[acute and] short-term tests in rats and dogs did not produce obvious toxic effects.” Overall the Panel concurred that the short-term toxicity of Quinoline Yellow is low, with no obvious signs of toxicity at dose levels up to 2500 mg/kg bw/day in a 90 day oral feeding study in the rat.

### 3.2.3. Genotoxicity and mutagenicity

The JECFA evaluation (JECFA, 1975a) reported that methylated Quinoline Yellow at concentrations of 5000 and 10000 µg/mL in tests with cultures of Escherichia coli (assays not further specified) induced no mutagenic effect (Lück and Rickerl, 1960).

BIBRA reported two additional mutagenicity studies (BIBRA, 1982, 1990). In the first, no mutagenic activity was observed in the Ames Salmonella typhimurium test, using strains TA 98, TA 100, TA 1535 and TA 1538 at dose levels of 2, 20, 500 or 1000 µg/plate with or without metabolic activation (Viola and Noscoiti, 1978). Similar results were reported by Hollstein et al. (1978) using dose levels of 1000 µg/plate and strains TA 98, TA 100, TA 1535 and TA 1537 with or without metabolic activation. A negative result was also reported by Blevins and Taylor in a Salmonella typhimurium spot test (Blevins and Taylor, 1982). A rec assay in Bacillus subtilis was reported to be negative with or without metabolic activation (Fujita et al., 1976), but an inconclusive result was obtained in another study of the same type (NIOSH, 1985). No further experimental details were provided for these studies.

The SCF (1984) and TemaNord (2002) concluded on the basis of these evaluations and studies that Quinoline Yellow has no genotoxic potential.

The SCCNFP Opinion provides details of some recent studies on the genotoxic potential of Quinoline Yellow (SCCNFP, 2004), conducted in accordance with OECD Test Guidelines, which were made available to EFSA for evaluation. The Panel noted that the relevance of these studies for the assessment of food-grade Quinoline Yellow is unclear as these studies have been carried out with a test substance (Quinoline Yellow) containing a high proportion of the monosulphonate component (85-91%) (SCCNFP, 2004) while the specifications for food-grade Quinoline Yellow indicate that disulphonate is the main component (>80%) and the monosulphonate only covers 15% (EC, 2008). The results of these studies are summarised below.

A bacterial reverse mutation assay has been conducted on Quinoline Yellow (certified total colour content 90 %) in accordance with the Organization for Economic Cooperation and Development (OECD) TG 471, using S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and E.coli WP2 uvr A and test concentrations of 0, 33, 100, 333, 1000, 2500 and 5000 µg Quinoline Yellow/plate in the presence and absence of metabolic activation (S9 mix) (RCC, 1999). Slight cytotoxicity was observed at higher doses of Quinoline Yellow, but there was no increase in the number of revertants in any of the strains tested (SCCNFP, 2004).

In the in vitro mouse lymphoma L5178Y (TK locus) gene mutation test, carried out in accordance with OECD TG 476, Quinoline Yellow (certified total colour content 90 %) did not induce a significant increase in the number of small or large mutant colonies either in the presence or in the
absence of metabolic activation (S9 mix) (RCC, 2000). Test concentrations used were 119, 238, 475, 950, 1900 or 3800 μg Quinoline Yellow/mL and no toxicity was observed until the dose of 3800 μg/mL (SCCNPFP, 2004).

In an in vivo mouse micronucleus test conducted in NMRI mice in accordance with OECD TG 474, Quinoline Yellow (certified total colour content 87 %) did not induce micronuclei in erythrocytes at a frequency higher than the vehicle-treated animals (RCC, 2003). Five males and five females per group were given single oral gavage doses of 500, 1000 or 2000 mg/kg bw Quinoline Yellow and bone marrow Polychromatic Erythrocytes (PCEs) were examined 24 hours after dosing for all doses and also at 48 hours for the highest dose level of 2000 mg/kg. No evidence of toxicity was seen even at the highest dose level of 2000 mg/kg, and there was no evidence that the test substance reached the bone marrow cells, as evidenced by the absence of any alteration in the PCE to NCE (Normochromatic Erythrocytes) ratio. The relevance of the results from this study can thus be questioned, given the lack of target tissue toxicity (SCCNFP, 2004).

Macioszek and Kononowicz (2004) reported the results of micronucleus and Comet assays carried out in vitro to investigate the genotoxicity of Quinoline Yellow in human lymphocytes and Allium cepa root cells. In both cell systems, Quinoline Yellow was tested at concentrations of 8.67, 86.7 or 867 μg/mL, only in the absence of metabolic activation. In the in vitro micronucleus assays, treatment with Quinoline Yellow induced a significant and concentration-dependent increase in the number of micronuclei in both cell systems. In these experiments, Quinoline Yellow also induced dose-related toxicity, as shown by the decrease in the nuclear division index of lymphocyte cultures and of the mitotic index of plant cells. In the Comet assays, a dose-related increase of damaged nuclei was observed in both cell types. In cultured lymphocytes, the increase of tail moment value compared to negative control attained statistical significance only at the highest dose; in Allium root cells the effect of exposure to Quinoline Yellow on tail moment was significant at all doses.

The Panel concluded that none of the studies described in previous evaluations provided any evidence of a genotoxic potential of Quinoline Yellow. However, the recent research of Macioszek and Kononowicz (2004) indicates that Quinoline Yellow may have some genotoxic properties in vitro, although the biological significance of this finding is unclear.

3.2.4. Chronic toxicity and carcinogenicity

Two long-term rat studies by the oral route are described in the 1975 JECFA evaluation (JECFA, 1975a). The Panel noted that these studies were all performed before OECD guidelines and GLP were established.

Groups of rats (20 per sex) were fed daily diets containing 0 or 1 % (equivalent to 0 and 500 mg/kg bw/day) methylated Quinoline Yellow for two years. Gross and microscopic examination disclosed that no colour-related anomalies occurred. No significant difference in tumour incidence was observed compared to controls (Oettel et al., 1965).

In another long-term rat study, groups of animals (25 per sex) received daily diets containing 0, 0.1, or 0.2 % (equivalent to 0, 50, and 100 mg/kg bw/day) of Quinoline Yellow in their diet for two years. No treatment-related effects on body weight, food intake, survival, haematology, urinalysis, organ weights, or gross and microscopic pathology were reported (Hazleton, 1967b).

In addition, as also described in the 1975 JECFA evaluation (JECFA, 1975a), rats (10 per sex) were administered Quinoline Yellow subcutaneously. The rats received 55 injections (1 mL of a 2 % aqueous solution) in seven months and were observed for 32 months. No significant treatment-related effects regarding behaviour, growth, mortality, or microscopic appearance of principal organs were noted (no further details provided) and no tumours appeared at the site of injection (Oettel et al., 1965).
Dogs (3 per sex) were fed diets containing 0.03, and 0.2 \% of the methylated form of the colour (stated to be equivalent to 7.5 and 50 mg/kg bw/day) for two years. No treatment-related effects were observed in terms of body weight, food consumption, or gross and microscopic pathology (Hazleton, 1967a).

In a long-term study carried out by Coquet and co-workers in the OFI mouse, also described by JECFA in its 1984 evaluation (JECFA, 1984a), involving \textit{in utero} exposure, four groups of mice (65 per sex in the exposed groups; 105 per sex in the control group) were fed daily diets containing 0, 0.1, 0.3, or 1 \% Quinoline Yellow for nine weeks prior to mating and throughout gestation and lactation (Coquet et al., 1981). These dietary levels were reported by the authors to be equivalent to mean intakes of 0, 150, 400, or 1500 mg/kg bw/day Quinoline Yellow. The authors also indicated that intake of the test substance varied over the period of the study, since it is proportional to food consumption. They reported that the ranges of intakes (in mg/kg bw/day) were 300 down to 100 for the 150 mg/kg bw/day group, 1100 down to 300 for the 450 mg/kg bw/day group and 3300 down to 1000 for the 1500 mg/kg bw/day group. On day 21 after parturition, animals were selected from the litters of the appropriate treatment groups to provide groups of 100 animals of each sex (controls) or 50 animals of each sex (test groups). These animals, comprising the F1 generation, received the same diet for 21 months (males) or 23 months (females).

Growth rates and mortality rates showed no significant dose-related effects, other than a very slight increase in mortality in males at the highest dose level of 1500 mg/kg bw/day, of doubtful biological significance. Haematological examinations were also performed at 3, 6, 12, and 18 months of treatment for control and highest dose and at termination for all animals. After 3, 6, 9, 12, 18 months of treatment, there were no biologically significant differences observed between control animals and those treated at the highest dose (1500 mg/kg bw/day). At the end of the study (21 months for males and 23 months for females) white blood cell counts were slightly decreased in treated animals, 16 \%, 17 \% and 19 \% in males and 16\%, 2\% and 22 \% in females from the lowest to the highest dose respectively. This effect was statistically significant only for females at the highest dose. No examination of bone marrow aspirates was carried out.

As no treatment-related changes in white blood cell counts in females were observed throughout the study (at 3, 6, 9, 12, 18 months) at the highest dose before the terminal kill at 23 months, the decrease observed at 23 months was limited (22 \%), and no change in the ratio of the different cell types was noted, the Panel did not consider this effect as an adverse effect.

The number of animals bearing palpable masses remained low (approximately 10 \%) and the incidence and time of onset was similar in all groups. No treatment-related effects were seen on organ weights. Histological observation of a wide range of tissues (including the thyroid) at termination or when aberrant tissues were suspected showed no treatment-related toxic effects. There was no marked difference in the tumour types observed between groups, or in the overall incidence of the tumours. JECFA (1984a,b) indicated that the dose level having no toxicological effect was 1500 mg/kg bw/day.

BIBRA (1982, 1990) and the SCCNFP (2004), in reporting the results of the same study, indicated that the dose range used in the study was 0, 0.3 \%, 1 \% or 3 \% Quinoline Yellow in the diet. The Panel concluded that this divergence from the values given in the study report (Coquet et al., 1981) and by JECFA (JECFA, 1984b) was due to the interpretation by these bodies of the range of intakes over the period of the study reported by the authors, as indicated above. These reports also conclude that no carcinogenic activity of Quinoline Yellow was evident in this study, and the SCCNFP concluded that 300-1000 mg/kg bw/day (the range of intakes reported for the middle dose group in the study, although it is noted that the study authors themselves provide a range of 300-1100 mg/kg bw/day) represents a No-Observed-Adverse-Effect-Level (NOAEL) in this study on the basis of the effect on white blood cells seen in top dose females. The ANS Panel concurred with the NOAEL of 1500 mg/kg bw/day Quinoline Yellow reported by JECFA (JECFA, 1984a,b), noting that the haematological effect was limited (22 \%), occurred only at the end of the study in females at the highest dose, and no change in the ratio of the different cell types was reported.
Re-evaluation of Quinoline Yellow (E 104) as a food additive

Two additional unpublished long-term chronic toxicity and carcinogenicity studies with a reproductive toxicity phase, carried out in the rat and in the mouse, which were not included in the JECFA evaluations were reported by the International Life Sciences Institute (ILSI, 1986), BIBRA (1990) and the SCCNFP (2004). It is not known whether these studies were carried out on Quinoline Yellow matching the current specifications laid down in Commission Directive 2008/128/EC (EC, 2008) and by JECFA (1984a, 2006).

In a study (or studies) carried out by Biodynamics in rats (70 per sex), the F₀ generation was administered Quinoline Yellow in the diet at levels of 0, 0.03, 0.1, 0.5, 2 or 5 % of the colour for two months before mating. After parturition and weaning the F₁ animals were maintained on diets containing the same levels of the colour as the parental generation (equivalent to anticipated intakes of 0, 15, 50, 250, 1000 or 2500 mg/kg bw/day) for up to 30 months (Biodynamics, 1980; 1981). The Panel noted that the reports of this study or studies by BIBRA, the International Life Sciences Institute (ILSI) and the SCCNFP indicate that there appeared to be 2 sequential studies carried out by Biodynamics, one at dietary levels of 0, 0.03, 0.1 and 0.5 % and the other (later) at 2 % and 5 % in the diet. It is possible that the latter study was initiated due to indications that a dose resulting in toxicity (a Maximum Tolerated Dose, MTD) had not been achieved in the initial study employing dietary levels of up to 0.5 %. The original study reports are not available, and the Panel has not been able to confirm the described findings. The reproductive phase of this study is reported in section 3.6 below.

The study provided no evidence of a carcinogenic effect of Quinoline Yellow. Mortality was slightly higher in treated F₁ females at dietary levels of 2 and 5 % Quinoline Yellow compared with controls; these dietary levels were reported by BIBRA to be equivalent to 1000 or 2500 mg/kg bw/day. Decreased bodyweights compared with controls were observed in treated F₁ males, but not in females, at dietary levels of 2 and 5 % Quinoline Yellow. Weights of the kidneys, adrenals, spleen, thyroid, uterus and ovaries were changed without evidence of tissue damage in these organs (no further details were provided). No treatment-related effects were described at dietary levels of 0.5 % Quinoline Yellow, equivalent to 250 mg/kg bw/day although the extent of tissue examination is not clear from the summaries provided by the ILSI (ILSI, 1986), the SCCNFP (2004) and BIBRA (1990). According to the SCCNFP (2004), the FDA derived a NOAEL of 1000 mg/kg bw/day from this study, while DFG (1991) considered the NOAEL to be 250 mg/kg bw/day.

In a parallel study in mice, groups of animals (60 per sex) were fed dietary levels of up to 5 % Quinoline Yellow (equivalent to approximately 7500 mg/kg bw/day) for 23-24 months (Biodynamics, 1980). No adverse toxic effects or evidence of carcinogenicity were noted.

The SCF (1984) and TemaNord (2002) evaluations do not refer to any additional studies regarding chronic toxicity or carcinogenicity. Both groups concluded that long-term studies in mice and rats with Quinoline Yellow revealed no carcinogenic potential. The Panel also concluded that none of the studies described in the previous evaluations provide any evidence of a carcinogenic potential of Quinoline Yellow.

Conclusion on NOAELs for chronic toxicity

Limited evidence of chronic toxicity following the administration of Quinoline Yellow is provided by the long-term study in the OFI mouse (Coquet et al., 1981), and the long-term study or studies carried out in rats by Biodynamics (Biodynamics, 1980; 1981) as reported by the ILSI (ILSI, 1986), BIBRA (1990) and the SCCNFP (2004). JECFA (JECFA, 1984a) used the long-term study in the OFI mouse, involving in utero exposure to conclude that the dose level having no toxicological effect in this study was 1500 mg/kg bw/day Quinoline Yellow. The Panel concurred with this conclusion. The SCF established an ADI of 0-10 mg/kg bw for Quinoline Yellow based on a long-term mouse study with a NOAEL of 1000 mg/kg bw/day. Uncertainty exists regarding which study the NOAEL was derived from. However it is likely that the chronic mouse study of Coquet and co-workers that was used by JECFA was also used by the SCF as the basis for the ADI. In the long-term study involving in utero
exposure carried out in rats by Biodynamics (1980; 1981), decreases in bodyweight in males and changes in organ weights relative to controls were reported in the adults of the F1 generation at intakes of 1000 and 2500 mg/kg bw/day Quinoline Yellow. Consequently, the Panel considered that the No-Effect-Level was 250 mg/kg bw/day Quinoline Yellow in the adults of the F1 generation, as cited by DFG (1991).

3.2.5. Reproductive and developmental toxicity

The 1975 JECFA evaluation describes two studies on developmental toxicity of Quinoline Yellow (JECFA, 1975a). In a developmental toxicity in rats, groups of 20-24 pregnant females were administered doses of 0, 15, 50, or 150 mg/kg bw/day Quinoline Yellow by gavage from gestational day 6 to 15 (Biodynamics, 1972a). At sacrifice on day 20 no signs of foetal toxicity or anomalies were reported that could be attributed to administration of Quinoline Yellow. No details were provided in the JECFA report on the parameters studied.

In a developmental toxicity study in rabbits, groups of 15 pregnant females received 0, 15, 50, or 150 mg/kg bw/day of Quinoline Yellow from gestation days 6 to 18 (Biodynamics, 1972b). No significant maternal or foetal abnormalities were reported that could be attributed to administration of Quinoline Yellow. No details on the parameters studied were provided in the JECFA report (JECFA, 1975a).

The JECFA evaluation of 1978 reports an additional long-term study on reproductive and developmental toxicity that was not yet available at the time of the 1975 evaluation. In this study groups of rats were fed 0, 0.5, 5.0, 15, and 50 mg/kg bw/day Quinoline Yellow in their diet through three successive generations. Offspring of the various matings were autopsied at weaning or selected for further breeding. No compound-related effects were observed with regard to parental mortality, body weight, food consumption, mating, pregnancy, fertility rates, numbers of embryos, corpora luteae, resorptions or necropsy findings. In pups, no anomalies were observed in terms of survival, body weight, or gross and histological analysis (Smith, 1973; Biodynamics 1973). JECFA did not derive a NOAEL from this study, but the Panel concluded that it could be 50 mg/kg bw/day, the highest dose tested.

In the long-term study in the OFI mouse (already reported in section 3.2.4), involving in utero exposure, four groups of mice (65 per sex in the exposed groups; 105 per sex in the control group) were fed daily diets containing 0, 0.1, 0.3, or 1 % of Quinoline Yellow for nine weeks prior to mating and throughout gestation and lactation (Coquet et al., 1981). No treatment-related effects were seen on fertility, pregnancy rate and numbers of live and dead pups (Coquet et al., 1981).

A long-term chronic toxicity/carcinogenicity study with a reproductive toxicity phase already summarised in section 3.2.4 (Biodynamics, 1980; 1981) was reported by ILSI (ILSI, 1986), BIBRA (1990) and the SCCNFP (2004). In this study, groups of rats (60 per sex) were exposed to 0, 0.03, 0.1, 0.5, 2, or 5 % Quinoline Yellow in the diet (equivalent to 0, 15, 50, 250, 1000, or 2500 mg/kg bw/day Quinoline Yellow) two months prior to mating and continuously throughout pregnancy and lactation. The pups of the F0 dams were reported to display reduced viability and lower weight gains during lactation at dose levels of 0.5 % Quinoline Yellow in the diet (equivalent to 250 mg/kg bw/day) and above, although no other treatment-related effects on reproductive parameters were noted (ILSI, 1986; BIBRA, 1990; SCCNFP, 2004). The Panel has not been able to obtain the study report in order to verify the findings independently.

The Panel concluded that the reduced viability and lower weight gains in pups during lactation at dose levels of 250, 1000 and 2500 mg/kg bw/day Quinoline Yellow reported to have been seen in the chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats carried out by Biodynamics (1980, 1981) were indicative of a treatment-related effect, and that a NOAEL of 50 mg/kg bw/day, based on effects in the offspring of the F0 dams, is derived from this study. Regarding this study, the SCCNFP has also stated, that “From these findings, a NOAEL of 50 mg/kg bw/day
could be deduced.” (SCCNFP, 2004). The No-Effect-Level in the adult rats of the F1 generation was 250 mg/kg bw/day Quinoline Yellow (see section 3.2.4 above).

3.2.6. Allergenicity, hypersensitivity and intolerance

In a clinical study in which 330 patients with recurrent urticaria were investigated for possible triggering factors, 30 % of individuals indicated that their condition was worsened by consumption of certain foods, while 18 % mentioned drinks as possible triggering factors (Juulin, 1981). Quinoline Yellow was one of an extensive number of food additives investigated in provocation tests in a number of patients, others included azo dyes, benzoates, antioxidants, sorbic acid, carotene, canthaxanthin, annatto and nitrite. The additives were given orally by capsule, Quinoline Yellow being administered at levels of 1, 5 or 10 mg per individual to 91 patients. The dosing regime was over several hours and it is assumed that increasing doses were administered sequentially. Of the 91 patients tested, 13 % showed positive evidence of sensitivity reactions, as manifest by a flare-up of urticaria, while 15 % showed uncertain reactions and 72 % were negative. The incidence of positive reactions to Quinoline Yellow was comparable to that found for a number of other food additives tested (Juulin, 1981).

Five of 62 children diagnosed as atopic developed itching and/or reddening of the skin or urticarial rashes following oral challenge with mixtures of food colours including 1 or 10 mg Quinoline Yellow (Ostergaard, 1986). Other colours tested included Annatto, Erythrosine, Ponceau 4R, Tartrazine, Sunset Yellow, Patent Blue V, Curcumin, Betanin, and the dyes were tested in combination rather than individually. The author concluded that intolerance phenomena to synthetic colouring agents are relatively rare (Ostergaard, 1986).

The BIBRA toxicity profile (1990) describes a study in which 81 subjects, drawn from a larger population reporting symptoms of food-related intolerance, were administered capsules containing either a food dye mixture including 2.5 mg Quinoline Yellow or a lactose placebo. Analysis of symptoms recorded by the patients did not demonstrate an increased reactivity to the dye mixture compared with the placebo (Young et al., 1987).

The TemaNord (2002) assessment very briefly describes one additional clinical study in which a small subgroup of patients with atopic dermatitis responded to oral provocation with a mixture of food additives including Quinoline Yellow. Based on the abstract of this study (Worm et al., 2000) it appears that there is no discrimination between the different substances investigated.

The topic of allergenicity/hypersensitivity was not addressed by the SCF Opinion (1984).

The results of a number of skin sensitisation studies conducted in human volunteers and in animals are described in the BIBRA toxicity profile (1990) and in JECFA (1975), but these are considered of little importance in the context of food consumption and therefore not discussed in this evaluation.

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

3.2.7. Other studies

A study by McCann et al. (2007) has concluded that exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet result in increased hyperactivity in 3-year old
and 8- to 9-year old children in the general population. In an earlier study by the same research team there was some evidence for adverse behavioural effects of a mixture of four synthetic colours and sodium benzoate in 3-year old children on the Isle of Wight (Bateman et al., 2004). In this recent study the effects of two combinations of Tartrazine (E 102), Quinoline Yellow (E 104), Sunset Yellow FCF (E 110), Ponceau 4R (E 124), Allura Red AC (E 129), Carmoisine (E 122) and sodium benzoate (E 211) on children’s behaviour were studied.

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

Mix B in this study contained Quinoline Yellow and in addition Sunset Yellow FCF, Carmoisine, Allura Red AC and sodium benzoate. Mix B had no effect on GHA scores in 3-year old children as compared to the placebo control GHA scores (effect size 0.17 [CI -0.03 to 0.36]). This result persisted when analysis was restricted to 3-year old children who consumed more than 85 % of juice and had no missing data (complete case group); in this analysis for Mix B no significant effect on GHA scores was observed (effect size 0.21 [CI -0.06 to 0.48]). For the 8- to 9-year old children Mix B was reported to have a significant effect on GHA scores (effect size 0.12 [CI 0.03 to 0.22] p<0.05). The clinical significance of the observed effects for normal functioning of the exposed children remains unclear (EFSA, 2008a).

The effects of Quinoline Yellow have been tested on neurite outgrowths of a differentiated mouse NB2a neuroblastoma cell line in vitro, a test system which the authors (Lau et al., 2006) described as a developmental neurotoxicity test. The authors reported that over a 24 hour period, Quinoline Yellow (10 μM) in combination with aspartame (8.06 μM) synergistically reduced neurite outgrowth length by 50 % in the presence of appreciable cell death (approximately 60%), although Quinoline Yellow alone was a relatively weak inhibitor of neurite outgrowth (IC50= 106 μM) (Lau et al., 2006). According to the authors, the maximum tested concentrations were theoretically achievable in plasma of children consuming typical Quinoline Yellow and aspartame-containing foods and beverages (Lau et al., 2006). However, this does not necessarily reflect the concentrations that would reach the nervous system due to the presence of the blood-brain-barrier.

The Panel considered that the experimental model had a number of limitations including the following: (i) neuroblastoma cells may not differentiate normally, (ii) neurites do not represent axons or dendrites, (iii) cell-to-cell interaction is absent, and (iv) genetic instability increases at higher passages (Bal-Price et al., 2008). In addition, a review by Radio and Mundy (2008) demonstrates effects of a wide range of chemicals on neurite outgrowth in a variety of cell lines and primary cultures (Radio and Mundy, 2008). The Panel concluded that interpretation of the study of Lau et al. (2006) is hampered by the very high toxicity as evidenced by cell death seen in the study and that no conclusion can be reached on the possible risks of Quinoline Yellow for human health based on these results.

4. Discussion

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

Quinoline Yellow (E 104) is a quinophthalone dye that has been previously evaluated by JECFA and the SCF, and also by the SCCNFP. Both JECFA and SCF established an ADI of 0-10 mg/kg bw for
Quinoline Yellow. The JECFA’s ADI was based on the long-term dietary study in the mouse carried out by Coquet et al., in which no compound-related effects were observed at the highest concentration of 1 % Quinoline Yellow in the diet (reported by the authors to be equivalent to a dose of 1500 mg/kg bw/day). It appears that in determining the ADI an uncertainty factor of 150 was applied by JECFA, the rationale for which was unclear to the Panel. The SCF established an ADI of 0-10 mg/kg bw based on a long-term mouse study with a NOAEL of 1000 mg/kg bw/day and application of an uncertainty factor of 100. The study used by the SCF may also have been the study carried out by Coquet and co-workers.

The Panel noted that toxicokinetic considerations indicate that there is limited absorption (an estimated 3-4 % of the administered dose) of Quinoline Yellow in rats and dogs, and that most of an orally administered dose is excreted unchanged via the faeces.

The Panel noted the reported absence of toxicity at dose levels up to 2500 mg/kg bw/day Quinoline Yellow for 90 days in the rat. Longer term toxicity studies also provided little evidence of Quinoline Yellow toxicity, no treatment-related effects being seen in a 2-year study in the dog at dose levels up to 50 mg/kg bw/day. In the long-term study (approximately 24 months) of Coquet et al. in mice, on which the JECFA ADI was based, the only reported treatment-related effect was a decrease in white blood cell counts in female mice at the termination of the study, at the highest dose level of 1500 mg/kg bw/day Quinoline Yellow; a finding which the Panel did not consider as an adverse effect. The Panel considered that the haematological modifications observed in the Coquet et al. study were of limited biological significance, in the light of the limited effect (22 %), occurring only at the end of the study while no such changes were observed throughout the study (at 3, 6, 9, 12, 18 months) and no change in the ratio of the different cell types was noted.

An oral chronic toxicity and carcinogenicity study in the rat with a reproductive toxicity phase, which was not included in the JECFA evaluations, used dose levels of up to 5 % Quinoline Yellow in the diet. Decreased body weights compared with controls were observed in treated F1 males, but not in females, at dose levels of 2 and 5 % Quinoline Yellow in the diet (reported to be equivalent to 1000 or 2500 mg/kg bw/day). The NOAEL in the adults of the F1 generation was considered by the Panel to be 250 mg/kg bw/day Quinoline Yellow (0.5 % in the diet). The pups of the F0 dams were however reported to display a slightly reduced viability and slightly lower body weight gains during lactation at dose levels of 0.5 % Quinoline Yellow in the diet (reported to be equivalent to 250 mg/kg bw/day). The Panel considered therefore that the NOAEL for the reproductive phase of this study was 50 mg/kg bw/day, based on the reported effects in the F1 pups.

This study, together with several further oral long-term carcinogenicity studies at dose levels up to 2500 mg/kg bw/day Quinoline Yellow in the rat and 7500 mg/kg bw/day Quinoline Yellow in mice provided no evidence of a carcinogenic effect of Quinoline Yellow, and a study involving subcutaneous injection of Quinoline Yellow in the rat also provided no evidence of carcinogenic potential.

The SCF, JECFA evaluations concluded, based on studies available at that time, that Quinoline Yellow did not show any genotoxic activity. A lack of genotoxic potential has been confirmed by more recent studies on Quinoline Yellow, comprising a bacterial reverse mutation assay, an in vitro mouse lymphoma L5178Y (TK locus) gene mutation test and an in vivo mouse micronucleus test conducted in NMRI mice. However the Panel noted that the relevance of these recent studies for the assessment of food-grade Quinoline Yellow is unclear as these studies have been carried out with a test substance (Quinoline Yellow) containing a high proportion of the monosulphonate component (85-91%) while the specifications for food-grade Quinoline Yellow indicate that disulphonate is the main component (>80%) and the monosulphonate only covers 15%.

Results obtained by Macioszek and Kononowicz in 2004 indicated however that Quinoline Yellow may have clastogenic and/or aneugenic and DNA-damaging properties, based on positive results obtained in a micronucleus test and a Comet assay in vitro. However the Panel noted that several oral long-term carcinogenicity studies with Quinoline Yellow revealed no evidence of carcinogenicity and
that the SCF, JECFA, BIBRA, TemaNord and the SCCNFP have also concluded that there is no evidence for carcinogenicity of Quinoline Yellow. The Panel therefore considered that the results obtained by Macioszek and Kononowicz were of uncertain biological significance.

The Panel noted that the specifications for Quinoline Yellow allow for the presence of unsulphonated aromatic amines (e.g. aniline) and also 2-methylquinoline and/or 2,6-dimethylquinoline at relatively high concentrations. Theoretical concentrations in food could reach 50 μg/kg for the unsulphonated aromatic amines and 2.5 mg/kg for the methylquinonines, if these impurities were present in Quinoline Yellow at the maximum levels allowed by the specifications. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel notes that Quinoline Yellow was negative overall in in vitro genotoxicity as well as in long term carcinogenicity studies.

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

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

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

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

Additionally, the AFC Panel concluded that:

- since mixtures and not individual additives were tested in the study by McCann et al. it is not possible to ascribe the observed effects to any of the individual compounds, and;
- in the context of the overall weight-of-evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed, the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

The ANS Panel concurs with these conclusions.

Adverse reactions after oral intake of Quinoline Yellow, mostly taken as part of a mixture of other synthetic colours, have been reported for urticaria and rhinitis. Reports are often characterised by poorly controlled challenge procedures and recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon.

Therefore the Panel concluded that while some sensitivity reactions after Quinoline Yellow intake (urticaria, rhinitis and asthma) have been reported, no conclusion on the induction of sensitivity by
Quinoline Yellow could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The Panel has reviewed the ADI of 0-10 mg/kg bw established by both JECFA and SCF for Quinoline Yellow, based on the long-term dietary study in the mouse carried out by Coquet et al., in which no compound-related effects were observed at the highest concentration of 1 % Quinoline Yellow in the diet (reported by the authors to be equivalent to a dose of 1500 mg/kg bw/day). The Panel concurred with the conclusion of JECFA that 1500 mg/kg bw/day can be considered a NOAEL for this study.

The Panel has also noted, however, the results of a long-term chronic toxicity/carcinogenicity study in rats with a reproductive toxicity phase carried out by Biodynamics, as reported by BIBRA, ILSI and in the 2004 SCCNFP Opinion. This study does not appear to have been taken into consideration by JECFA and the SCF. However, it is not known whether the study was carried out on Quinoline Yellow matching the current specifications laid down in Commission Directive 2008/128/EC (EC, 2008) and by JECFA (1984a, 2006).

In this study, decreases in body weight in treated F1 males and changes in organ weights relative to controls were reported at intakes of 1000 and 2500 mg/kg bw/day Quinoline Yellow. The NOAEL in the adults of the F1 generation was considered by the Panel to be 250 mg/kg bw/day Quinoline Yellow (0.5 % in the diet). However, the Panel noted that in this study, the pups of the F0 dams were reported to display a slightly reduced viability and slightly lower body weight gains during lactation at dose levels of 0.5 % Quinoline Yellow in the diet (equivalent to 250 mg/kg bw/day). This led the Panel to conclude that the NOAEL in this study for effects on pup viability and development was 50 mg/kg bw/day, while 250 mg/kg bw/day represents a NOAEL for the adult animals. Regarding this study, the SCCNFP Opinion on Quinoline Yellow also states, that “From these findings, a NOAEL of 50 mg/kg bw/day could be deduced.”

Thus, in the opinion of the Panel, the available database on semi-chronic, reproductive, developmental and long-term toxicity of Quinoline Yellow, including a study apparently not taken into consideration by JECFA or the SCF, provides a basis for re-definition of the ADI. The Panel considered that the long-term chronic toxicity/carcinogenicity study with a reproductive toxicity phase carried out by Biodynamics in rats should be considered as the pivotal study on which to base an ADI. In this study the reported reduced viability and lower body weight gains in pups during lactation at a dose level of 250 mg/kg bw/day Quinoline Yellow are considered to be indicative of a treatment-related effect, and a NOAEL of 50 mg/kg bw/day is therefore derived from this study. Application of an uncertainty factor of 100 to this NOAEL would provide an ADI of 0.5 mg/kg bw/day.

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

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

For children (1- 10 years old), estimates have been calculated for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland, Germany). For the adult population, the Panel has selected the UK population as representative of EU consumers for Quinoline Yellow intake estimates.
When considering MPLs (Tier 2), the mean dietary exposure to Quinoline Yellow for European children (aged 1-10 years), ranged from 0.8 to 3.5 mg/kg bw/day and from 1.8 to 9.6 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (13 to 55%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafer) (14 to 29%) and desserts (including flavoured milk products) (12 to 62%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 10 to 50% of exposure in four countries. Confectionery accounted for 11-13% of exposure in two countries.

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

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Quinoline Yellow for European children (aged 1-10 years), ranged from 0.45 to 2.0 mg/kg bw/day, and from 1.1 to 4.1 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) at average level were soft drinks (10 to 55%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafer) (10 to 60%) and desserts (including flavoured milk products) (11 to 57%). Confectionery accounted for 13-18% of exposure (in two counties) and surimi, sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 15% of exposure in one country.

Estimates reported for the UK adult population give a mean dietary exposure to Quinoline Yellow of 0.5 mg/kg bw/day and of 1.2 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were soft drinks (30%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers), fruit wines, cider and perry (12%) and desserts (including flavoured milk products) (10%)

The Panel noted that the specifications of Quinoline Yellow need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components. The Panel also considered that further clarification on the proportion of methylated and unmethylated Quinoline Yellow may be required.

The Panel also notes that the specification for lead in Directive 2008/128/EC (≤10 mg/kg) appears to be high compared to the JECFA specification (≤2 mg/kg).

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

**CONCLUSIONS**

Quinoline Yellow (E 104) is a quinophthalone dye allowed to be used as a food additive in the EU and has been previously evaluated by JECFA and the SCF. Both committees have established an ADI of 0-10 mg/kg bw.

The Panel concludes that the currently available database on semi-chronic, reproductive, developmental and long-term toxicity of Quinoline Yellow, including a study in rats apparently not taken into consideration by JECFA or the SCF, provides a rationale for re-definition of the ADI. Using the NOAEL of 50 mg/kg bw/day provided by the chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats carried out by Biodynamics in rats and applying an uncertainty factor of 100 to this NOAEL, the Panel establishes an ADI of 0.5 mg/kg bw/day.

The Panel notes that at the maximum levels of use of Quinoline Yellow, refined (Tier 2 and Tier 3) intake estimates are generally well over the ADI of 0.5 mg/kg bw/day.
The Panel also concludes that while some sensitivity reactions after Quinoline Yellow intake (urticaria, rhinitis and asthma) have been reported, mostly when Quinoline Yellow is taken as part of a mixture with other synthetic colours, no conclusion on the induction of sensitivity by Quinoline Yellow could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The Panel further notes that the specifications of Quinoline Yellow need to be updated with respect to percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components. The Panel also considers that further clarification on the proportion of methylated and unmethylated Quinoline Yellow may be required.

The Panel also notes that the specification for lead in Directive 2008/128/EC (≤ 10 mg/kg) appears to be high compared to the JECFA specification (≤ 2 mg/kg).

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

**DOCUMENTATION PROVIDED TO EFSA**


2. UNESDA (Union of European Beverage Associations), 2009. Comments to the CIAA/DG Sanco in response to a written request from DG Sanco to the CIAA, dated April 8 2009: ‘Use of certain colour additives in non-alcoholic beverages’ (May 26, 2009).

3. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: “EFSA re-evaluation of food colours” - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009).

4. ELC (Federation of European Food Additives, Food Enzymes and Food Culture Industries), 2009. ELC comments to EFSA in response to a written request from DG Sanco: “EFSA re-evaluation of food colours” – DG Sanco’s additional call for data dated 8 April 2009, letter to EFSA on 20 May 2009).


**REFERENCES**


BIBRA (The British Industrial Biological Research Association Toxicology), 1990. Toxicity profile of Quinoline Yellow.


EFSA (European Food Safety Authority), 2008a. Opinion of the Scientific Panel on food additives, flavourings, processing aids and material in contact with food (AFC) related to the Assessment of the results of the study by McCann et al (2007) on the effect of some colours and sodium benzoate on children and behaviour. The EFSA Journal 660, 1-54.


FSAI (Food Safety Authority of Ireland), 2009. A Surveillance Study on Levels of Artificial Colours and Sweeteners in Irish Retail Products. http://www.fsai.ie/assets/0/86/204/7f60074b-56eb-4fa2-ac6c-e013e20b9e7d.PDF


Macioscek VK and Kononowicz AK, 2004. The evaluation of the genotoxicity of two commonly used food colours: Quinoline Yellow (E 104) and Brilliant Black BN (E 151). Cellular and Molecular Biology Letters 9(1), 107-122.


Merck Index, 2006. Farbstoffkommission der DFG (Deutschen Forschungsgemeinschaft).


Re-evaluation of Quinoline Yellow (E 104) as a food additive


SCF (Scientific Committee on Food), 1984. Reports of the Scientific Committee for Food (14th series), opinion expressed 1983, 60-61.


ANNEX A

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

1. Decision rules taken to deal with QS authorisations:

   a. In the category ‘All other foodstuffs, the value of 500 mg/kg (the highest MPL) is used
   b. At the food category level: if a colour is authorised QS in a food category for one or more colours
      i. If a value is available for only one colour, this value is used for all the colours (except if this value is available only for annatto-cf point c)
      ii. If many values are available for more than one colour, the highest value is used
   c. At the colour level: if there is no available value or if there is just a single value for annatto, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

   Particular cases:

   - **Edible casings**: if available use the pork-based products use level; if not available, the highest MPL of 500 mg/kg is used.
   - **Edible cheese rinds**: 100 mg/kg (as the flavoured processed cheese category) is used, except for the E 120 (Cochineal) colour whose level is 125 mg/kg for red marbled cheese.

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

   a. If the identified maximum reported use level, adjusted for the highest current usage data or the highest analytical value, is lower than or equal to the actual MPL, then the actual MPL is used by default.
   b. If analytical and current use level data are available, priority is given to the use level data, even if analytical values are higher; the figure is rounded up to the nearest integer.
   c. If no use level data are available because no uses were reported (use level = 0) or industry was not asked, the choice is made between the highest analytical value or the MPL:
      i. If more than 10 analytical data are available, the highest value is used;
      ii. If less than 10 analytical data are available, the MPL is used.
   d. If no data were reported by the industry, the MPL is used by default.
   e. If the highest use level or the highest analytical data are higher than the proposed adjusted QS values, priority is given to the highest use level/analytical data

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

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

a. Tier 1: Estimates are based MPLs of use, as specified in the Directive 94/36/EC on food colours and the principles of the Budget method.

b. Tier 2: Estimates are based on MPLs of use, as specified in the Directive 94/36/EC on food colours, adjusted for quantum satis usages, and national individual food consumption data.

c. Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.
## GLOSSARY AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>Attention-Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake</td>
</tr>
<tr>
<td>AFC</td>
<td>Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food</td>
</tr>
<tr>
<td>AFSSA</td>
<td>Agence Française de Sécurité Sanitaire des Aliments</td>
</tr>
<tr>
<td>Aluminium lakes</td>
<td>Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product</td>
</tr>
<tr>
<td>ANS</td>
<td>Scientific Panel on Food Additives and Nutrient Sources added to Food</td>
</tr>
<tr>
<td>BIBRA</td>
<td>British Industrial Biological Research Association</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>CEPS</td>
<td>The European Spirits Organisation</td>
</tr>
<tr>
<td>CIAA</td>
<td>Confederation of the Food and Drink Industries of the EU</td>
</tr>
<tr>
<td>DG SANCO</td>
<td>The Directorate General for Health and Consumers</td>
</tr>
<tr>
<td>DFG</td>
<td>Farbstoffkommission der Deutschen Forschungsgemeinschaft</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>ELC</td>
<td>The Federation of European Food Additives, Food Enzymes and Food Culture Industries</td>
</tr>
<tr>
<td>EXPOCHI</td>
<td>Referes to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin Mononucleotide</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FSA</td>
<td>UK Food Standard Agency</td>
</tr>
<tr>
<td>FSAI</td>
<td>Food Safety Authority of Ireland</td>
</tr>
<tr>
<td>GHA</td>
<td>Global Hyperactivity Aggregate</td>
</tr>
<tr>
<td>HPLC-DAD</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>ILSI</td>
<td>International Life Sciences Institute</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>Lethal Dose, 50 % i.e. dose that causes death among 50 % of treated animals</td>
</tr>
<tr>
<td>LEMM</td>
<td>Laboratoire d'Etudes du Métabolisme des Médicaments</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit Of Detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification</td>
</tr>
<tr>
<td>MPL</td>
<td>Maximum Permitted Levels</td>
</tr>
<tr>
<td>MTD</td>
<td>Maximum Tolerated Dose</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No Observed Adverse Effect Level (NOAEL)</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PCE</td>
<td>Polychromatic Erythrocyte</td>
</tr>
<tr>
<td>RCC</td>
<td>Research and Consulting Company</td>
</tr>
<tr>
<td>SCF</td>
<td>Scientific Committee on Food</td>
</tr>
<tr>
<td>SCCNFP</td>
<td>Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers</td>
</tr>
<tr>
<td>SCOOP</td>
<td>A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety</td>
</tr>
<tr>
<td>UNESDA</td>
<td>Union of European Beverage Associations</td>
</tr>
<tr>
<td>WHO/FAO</td>
<td>World Health Organization/Food and Agriculture Organization</td>
</tr>
</tbody>
</table>