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# Re-evaluation of sucrose acetate isobutyrate (E 444) as a food additive

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

## Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of sucrose acetate isobutyrate (SAIB, E 444) as a food additive. The SCF allocated an acceptable daily intake (ADI) of 10 mg/kg body weight (bw) per day in 1994. JECFA established an ADI of 20 mg/kg bw per day in 1997. Both the JECFA and the SCF concluded that the hepatic effects observed in the dog have little relevance to the safety evaluation in humans. The Panel agreed with this conclusion. Oral absorption is approximately 70% in rats, about 50% in dogs and $\geq$ 88% in humans. The Panel concluded that SAIB does not raise concern for genotoxicity. No treatment-related effects have been observed on clinical signs, haematology and clinical chemistry in humans at a dose of 20 mg/kg bw per day for 14 days. The Panel identified a no observed adverse effect level (NOAEL) of 2,000 mg/kg bw per day, the highest dose tested, from chronic toxicity, carcinogenicity and reproductive and developmental studies in rats. Applying an uncertainty factor of 100, an ADI of 20 mg/kg bw per day for SAIB can be established. Overall, the Panel concluded that the present data set on absorption, distribution, metabolism and excretion, genotoxicity, subchronic, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw per day allocated by the SCF in 1994 to 20 mg/kg bw per day. Considering that the ADI is not exceeded in any population group, the Panel also concluded that the use of SAIB (E 444) as a food additive at the permitted or reported use and use levels would not be of safety concern.



**Keywords:** sucrose acetate isobutyrate, food additive, E 444, SAIB, CAS Registry Number 126-13-6, EINECS number 204-771-6

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## Summary

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of sucrose acetate isobutyrate (SAIB, E 444) when used as a food additive.

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

SAIB (E 444) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated SAIB in 1993 and 1997. An acceptable daily intake (ADI) of 0–20 mg/kg body weight (bw) per day was established in 1997 on the basis of the no observed adverse effect level (NOAEL) of 2,000 mg/kg bw per day in a chronic toxicity and carcinogenicity feeding studies in rats and applying an uncertainty factor of 100. The JECFA concluded that the effects on the liver in the dog were not relevant to humans.

The Scientific Committee on Food (SCF) evaluated SAIB in 1994 using the same database as JECFA and considered that the hepatic effects in the dog have little relevance to the evaluation of the safety in humans. The SCF concluded that the NOAEL in a chronic toxicity and carcinogenicity feeding study in rats was 1,000 mg/kg bw per day and allocated an ADI of 10 mg/kg bw applying an uncertainty factor of 100.

An additional evaluation on SAIB as a food additive performed in 2002 by TemaNord resulted in the conclusion that when excluding the dog, which is considered an inappropriate species for evaluating the risks to humans for this substance, the available studies did not show any treatment-related effects.

The available studies with $^{14}$C-SAIB labelled at the sucrose moiety revealed oral absorption of approximately 70% in rats, about 50% in dogs and of $\geq$ 88% in humans. Absorption was rapid in all three species. In general, SAIB is metabolised via de-esterification by non-specific esterases in the gastrointestinal tract, mainly in the small intestine to sucrose and partially acylated sucrose. Both metabolites appeared to be readily absorbed from the gut. A significant portion of ingested SAIB and partially de-esterified SAIB was excreted via the faeces, particularly in dogs. The absorbed radioactivity was eliminated in the urine with a minor proportion excreted in the bile as demonstrated in dogs. In humans receiving $^{14}$C-SAIB at doses of 0.1–2 mg/kg bw, the absorbed radioactivity, after further metabolism, was mainly excreted as carbon dioxide in exhaled air (44–66% of the dose). Urinary and faecal excretions accounted respectively for 15–21% and 10%. The Panel noted that no toxicokinetic data were available with SAIB labelled at the isobutyrate moiety.

The Panel noted that SAIB has a low acute toxicity. No mortality occurred in rats and mice at high dose levels.

The effects of SAIB on the hepatobiliary function in the dog appear to be the major factor to be considered in assessing the safety of this food additive for humans. These effects of SAIB in the dog appear to be unique to this species, as they do not occur in mice, rats, monkeys or humans. A probable explanation is the difference in metabolism between the dog on the one hand, and rodents and primates on the other. The species differences of experimental animals after exposure to SAIB and the lack of any untoward effect in clinical studies with volunteers renders it unlikely that the use of SAIB as a food additive would have adverse effects in humans.

On the basis of available genotoxicity studies *in vitro* and *in vivo*, the Panel considered that SAIB did not raise concern for genotoxicity.

Rats and mice as well as monkeys tolerate large doses of SAIB over prolonged periods without evidence of significant toxicity. The NOAEL for rats was 2,000 mg/kg bw per day, for mouse 2,500 mg/kg bw per day and for monkeys 2,400 mg/kg bw per day. Effects on liver function were reported in dogs but not in monkeys at single oral exposure $\geq$ 20 mg/kg bw. The Panel considered that the hepatic effects observed in dogs, have little, if any, relevance to the safety evaluation of SAIB for humans.

The Panel considered that the less than 10% decrease in body weight accompanied by decreased feed intake, observed in a 52-week study in rats, was most probably due to the palatability of the feed and, therefore, not adverse. Therefore, the Panel considered the 2,000 mg/kg bw per day the NOAEL in this study.

Moreover, no treatment-related effects were observed on clinical signs, haematology and clinical chemistry in humans at a dose of 20 mg/kg bw per day and a treatment period of 14 days. SAIB did not affect the hepatobiliary function in humans.

From the available reproductive and developmental toxicity studies, no effects on fertility, reproduction, maternal toxicity or development were reported at doses amounting up to 2,000 mg/kg bw per day for rats and 1,200 mg/kg bw per day for rabbits.

The Panel identified a NOAEL of 2,000 mg/kg bw per day, the highest dose tested, from chronic toxicity, carcinogenicity and reproductive and developmental studies in rats. Applying an uncertainty factor of 100, an ADI of 20 mg/kg bw per day can be established.

The Panel concluded that the present data set on the absorption, distribution, metabolism and excretion, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw per day allocated by SCF in 1994 to 20 mg/kg bw per day.

The dietary exposure to SAIB (E 444) from its use as a food additive was calculated based on (1) maximum levels set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage data obtained from the industry (defined as the *refined exposure assessment scenario*). No data on actual analysed levels were available to EFSA for use in the refined exposure scenario. In both exposure scenarios, the exposure estimates were below the ADI of 20 mg/kg bw per day. Therefore, considering that the ADI is not exceeded by any population group, the Panel also concluded that the use of SAIB (E 444) as a food additive at the permitted or reported use and use levels would not be of safety concern.

# Table of contents

# 1. Introduction

The present opinion deals with the re-evaluation of the safety of sucrose acetate isobutyrate (SAIB, E 444) when used as a food additive.

## 1.1. Background and Terms of Reference as provided by the European Commission

### 1.1.1. Background

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the SCF or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report *Food additives in Europe 2000*,[4] submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010, the 2003 Terms of References are replaced by those below.

### 1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

## 1.2. Information on existing authorisations and evaluations

SAIB (E 444) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in Commission Regulation (EU) No 231/2012[5].

The SCF (1994) evaluated SAIB and concluded that the no observed adverse effect level (NOAEL) from a chronic toxicity and carcinogenicity feeding study in rats was 1,000 mg/kg bw per day, allocating an acceptable daily intake (ADI) of 10 mg/kg bw per day applying an uncertainty factor of 100. Short- and long-term studies in mice and rats as well as a 1-year study in monkeys produced no adverse hepatic effects and showed no evidence of interference with hepatobiliary function. Studies in human volunteers similarly produced no indications of any adverse effects on biliary function. On the

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[3] COM(2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.
[5] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

basis of these observations, the SCF also concluded that the hepatic effects in the dog have little relevance to the evaluation of the safety of SAIB in humans. They based the NOAEL on the results of the chronic toxicity and carcinogenicity rat study which have shown that at 2,000 mg/kg bw per day there is some evidence of an effect on body weight and food consumption.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated SAIB in 1993 and 1997 (JECFA, 1993, 1997). An ADI of 0–20 mg/kg bw per day was established in 1997 on the basis of the NOAEL of 2,000 mg/kg bw per day in a chronic toxicity and carcinogenicity feeding studies in rats and applying an uncertainty factor of 100. The JECFA also concluded that the effects on the liver in the dog were not relevant to humans.

TemaNord reviewed SAIB and considered the liver to be the target organ in the dog only (TemaNord, 2002). The changes seen in the liver of dogs were reversible within 3 weeks after removal of SAIB from the diet. It was concluded that the effects observed in dogs represented a functional rather than a toxic effect. Therefore, they concluded that when excluding the dog, which is considered an inappropriate species for evaluating risk to humans for this substance, the available studies did not show any treatment-related effects.

# 2. Data and methodologies

## 2.1. Data

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier. EFSA launched public calls for data[6,7,8] to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to January 2016. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however not always were these available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[9]) was used to estimate the dietary exposure.

## 2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of SAIB (E 444) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001).

When the test substance was administered in the feed or in drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases the daily intake is expressed as equivalent.

Dietary exposure to SAIB (E 444) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels (MPLs) and/or reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

[6] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 23 November 2009 and modified on 5 February 2010. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[7] Call for food additives usages level and/or concentration data in food and beverages intended to human consumption. Published: 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

[8] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

[9] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

# 3. Assessment

## 3.1. Technical data

### 3.1.1. Identity of the substance

SAIB (E 444) is a mixture of reaction products formed by the esterification of food grade sucrose with acetic anhydride and isobutyric anhydride, following distillation (Commission Regulation (EU) No 231/2012).

According to Reynolds and Chappel (1998), the mixture can contain all 256 possible esters, with the major components being sucrose $(acetate)_2$ $(isobutyrate)_6$, while sucrose $(acetate)_6$ $(isobutyrate)_2$ can be present only in minor amounts.

The molecular formula assigned by Commission Regulation (EU) No 231/2012 is $C_{40}H_{62}O_{19}$ and the molecular weight is 832–856 g/mol (approximate); the molecular formula $C_{40}H_{62}O_{19}$ corresponding to 846.9 g/mol with a ratio of acetate:isobutyrate of 2:6. The European Inventory of Existing Commercial chemical Substances (EINECS) number is 204-771-6.

The Chemical Abstracts Service (CAS) Registry Number assigned to E 444 is 126-13-6 and Figure 1 shows the most representative structural formula (SciFinder[10], software) with a ratio of acetate: isobutyrate of 2:6.

According to JECFA specifications (JECFA, 2006), there are, in addition to CAS Registry Number 126-13-6, other two CAS Registry Numbers, 27216-37-1 and 137204-24-1, indicated for SAIB. According to CAS, Registry Number 27216-37-1 refers to an incompletely defined substance with the same molecular formula as CAS Registry Number 126-13-6 and EINECS 204-771-6. The CAS Registry Number 137204-24-1 refers to a sucrose (CAS Registry Number 57-50-1), ester of acetic acid (CAS Registry Number 64-19-7) and isobutyric acid (CAS Registry Number 79-31-2) with unspecified molecular ratios of the two acids (SciFinder, software).

SAIB is also known by the synonyms α-D-glucopyranoside, 6-*O*-acetyl-1,3,4-tris-*O*-(2-methyl-1-oxopropyl)-β-D-fructofuranosyl, 6-acetate 2,3,4-tris(2-methylpropanoate); saccharose acetate isobutyrate; SAIB; SAIB 100, SAIB 100S, SAIB FG; sucrose diacetate hexaisobutyrate; sucrose acetate isobutanoate and Sustane SAIB MCT (SciFinder, software).

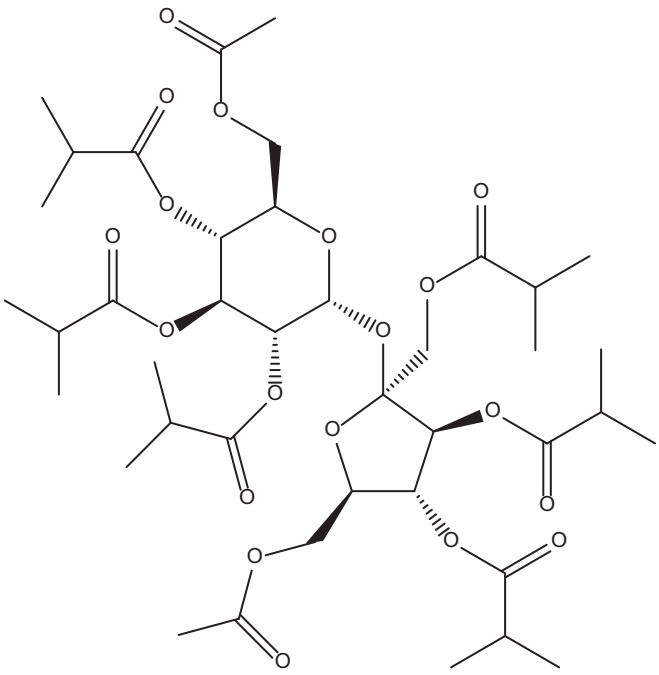

**Figure 1:** Structure of sucrose acetate isobutyrate (Chemical Abstracts Service Registry number 126-13-6) (SciFinder, software)

[10] SciFinder® (The Choice for Chemistry Research).

SAIB is a pale straw-coloured liquid, clear and free of sediment and having a bland odour, insoluble in water and soluble in most organic solvents (Commission Regulation (EU) No 231/2012). Reynolds and Chappel (1998) describe SAIB as a very viscous liquid at room temperature. According to industry, the log $P_{ow}$ ranges from 3.4 to 7 [Doc. provided to EFSA n. 10].

### 3.1.2. Specifications

Specifications for SAIB (E 444) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006) (Table 1). Besides the assay method and identification parameters, the specification includes purity criteria on acid value, saponification value and amount of triacetin.

**Table 1:** Specifications for SAIB (E 444) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

| | **Commission Regulation (EU) No 231/2012** | **JECFA (2006)** |
|---|---|---|
| Description | Pale straw-coloured liquid, clear and free of sediment and having a bland odour | Pale straw-coloured liquid, clear and free of sediment and having a bland odour |
| Assay | Content not less than 98.8% and not more than 101.9% of $C_{40}H_{62}O_{19}$ | Not less than 98.8% and not more than 101.9% of $C_{40}H_{62}O_{19}$ |
| Identification | | |
| Solubility | Insoluble in water, soluble in most organic solvents | Insoluble in water, soluble in most organic solvents |
| Refractive index | $[n]^{40}_{D}$: 1.4492–1.4504 | $[n]^{40}_{D}$: 1.4492–1.4504 |
| Specific gravity | $[d]^{25}_{D}$: 1.141–1.151 | $[d]^{25}_{D}$: 1.141–1.151 |
| Infrared absorption | – | The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum |
| Purity | | |
| Triacetin | Not more than 0.1% | Not more than 0.1% |
| Acid value | Not more than 0.2 | Not more than 0.2 |
| Saponification value | Not less than 524 and not more than 540 | Between 524 and 540 |
| Arsenic | Not more than 3 mg/kg | – |
| Lead | Not more than 2 mg/kg | Not more than 2 mg/kg |
| Mercury | Not more than 1 mg/kg | – |
| Cadmium | Not more than 1 mg/kg | – |

SAIB: sucrose acetate isobutyrate; JECFA: Joint FAO/WHO Expert Committee on Food Additives.

The Panel noted that, according to the EU specifications for SAIB (E 444), impurities of the toxic elements arsenic, lead, cadmium and mercury are accepted up to concentrations of 3, 2, 1 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012).

### 3.1.3. Manufacturing process

SAIB is manufactured by controlled esterification of sucrose with acetic and isobutyric anhydrides (Lewis, 1993). According to Commission Regulation (EU) No 231/201 and JECFA (2006), SAIB is produced by esterification of food grade sucrose with acetic anhydride and isobutyric anhydride following distillation; the molar ratio of acetate to butyrate is about 2:6.

Reynolds and Chappel (1998) reported the production of SAIB by reaction of sucrose and acetic and isobutyric anhydride in the presence of a catalyst; the co-products acetic and isobutyric acid are removed and the mixture is treated with activated carbon before distillation. A similar description of the manufacturing process has been provided by industry [Doc. provided to EFSA n. 11].

### 3.1.4. Methods of analysis in food

Different analytical methods for the determination of SAIB in soft drinks and in food additive premixes have been published.

A rapid screening procedure for the estimation of SAIB in soft drinks was presented by Conacher and Chadha (1972). The test substance was extracted with diethyl ether, followed by gas chromatography (GC) analysis using trimyristin as internal standard. Although SAIB gives a complex array of peaks in the chromatogram, the total area has a constant response factor relative to the standard, with recoveries of SAIB ranging between 94% and 98% and calculated limit of quantification (LOQ) 1.6 mg/L.

In a later publication, SAIB was analysed in soft drinks by GC after transesterification of the sucrose esters with decanol and estimation of the formed dodecyl acetate and dodecyl isobutyrate (Conacher et al., 1973). Recoveries for SAIB at levels close to the MPLs were estimated to range between 95.5% and 98% based on the acetic and isobutyric acid respectively and measured amounts in commercial samples were at levels lower than 24 mg/L.

A method for the determination of SAIB in food additive premixes and in food was published by Uematsu et al. (2001), where SAIB was analysed by GC with a flame ionisation detector and GC combined with mass spectrometry without derivatisation after enrichment by solid-phase extraction. The limit of detection (LOD) was reported to be 100 mg/L. The Panel noted that this method is not adequate to check compliance with current EU legislation as the LOQ is identical to the current MPL (300 mg/L).

### 3.1.5. Reaction and fate in food

No information on reaction in food was available. Nevertheless, when it is added to vegetable oil (a use which is currently not authorised according to Annex II to Regulation (EC) No 1333/2008) and then heated at temperature between 180 and 200°C, it remains stable (Reda, 2011).

## 3.2. Authorised uses and use levels

Maximum levels of SAIB (E 444) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are named MPLs.

Currently, SAIB (E 444) is an authorised food additive in the EU at the levels of 300 mg/L in two food categories.

Table 2 summarises the foods that are permitted to contain SAIB (E 444) and the corresponding MPLs as defined in Annex II to Regulation (EC) No 1333/2008.

**Table 2:** MPLs of SAIB (E 444) in foods according to Annex II to Regulation (EC) No 1333/2008

| Food category number | Food category name | Restrictions/exceptions | MPL (mg/L or mg/kg as appropriate) |
|---|---|---|---|
| 14.1.4 | Flavoured drinks | Only cloudy drinks | 300 |
| 14.2.8 | Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol | Only flavoured cloudy alcoholic drinks containing less than 15% of alcohol | 300 |

MPL: maximum permitted level; SAIB: sucrose acetate isobutyrate.

## 3.3. Exposure data

### 3.3.1. Reported use levels or data on analytical levels on SAIB (E 444)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to *quantum satis*.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[11] for occurrence data (usage level and/or concentration data) on SAIB (E 444). In response to this public call, updated information on the actual use levels of SAIB (E 444) in foods was made available to EFSA by industry. No analytical data on the concentration of SAIB (E 444) in foods were made available by the Member States.

#### 3.3.1.1. Summarised data on reported use levels in foods provided by industry

FoodDrinkEurope (FDE) provided EFSA with data on use levels (n = 13) of SAIB (E 444) in flavoured drinks (FCS 14.1.4). No use levels were reported in the food category 'Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol' (FCS 14.2.8).

Appendix A provides data on the use levels of SAIB (E 444) in foods as reported by industry.

### 3.3.2. Summarised data extracted from the Mintel GNPD database

Mintel's Global New Products Database (GNPD) is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 800,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries presented in the GNPD.[12]

For the purpose of this Scientific Opinion, GNPD[13] was used for checking the labelling of products containing SAIB (E 444) within the EU's food products as GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel GNPD, SAIB (E 444) was labelled on more than 300 products (n = 310) since 1996. Between 2011 and 2015, 180 drinks (carbonated soft drinks, flavoured alcoholic beverages, energy drinks, etc.) were labelled with SAIB.

Table 3 presents the percentage of the food products labelled with SAIB (E 444) between 2011 and 2015, out of the total number of food products per food sub-categories according to Mintel food categorisation.

**Table 3:** Number and percentage of food products labeled with SAIB (E 444) out of the total number of food products present in Mintel GNPD per food sub-category between 2011 and 2015

| Food sub-category[(a)] | Total number of products | Number of food products labelled with SAIB (E 444) | % of food products labelled with SAIB (E 444) |
|---|---|---|---|
| Carbonated soft drinks | 4,879 | 58 | 1.2 |
| Flavoured alcoholic beverages | 1,800 | 43 | 2.4 |
| Energy drinks | 1,484 | 34 | 2.3 |
| Sports drinks | 705 | 19 | 2.7 |
| Beverage concentrates | 2,097 | 14 | 0.7 |
| Fruit/flavoured still drinks | 2,590 | 9 | 0.3 |
| Flavoured water | 1,164 | 3 | 0.3 |

SAIB: sucrose acetate isobutyrate; GNPD: Global New Products Database.
(a): According to Mintel food categorisation.

### 3.3.3. Food consumption data used for exposure assessment

#### 3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the *Use of the EFSA Comprehensive European Food Consumption Database in Exposure*

[11] http://www.efsa.europa.eu/sites/default/files/consultation/130327%2C0.pdf
[12] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[13] http://www.gnpd.com/sinatra/home/ [Accessed: 18 April 2016].

*Assessment* (EFSA, 2011a). New consumption surveys recently[14] added in the Comprehensive database were also taken into account in this assessment.[9]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups were used for the exposure assessment: infants, toddlers, children, adolescents, adults and the elderly. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 4).

**Table 4:** Population groups considered for the exposure estimates of SAIB (E 444)

| Population | Age range | Countries with food consumption surveys covering more than 1 day |
|---|---|---|
| Infants | From more than 12 weeks up to and including 11 months of age | Bulgaria, Denmark, Finland, Germany, Italy, UK |
| Toddlers | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK |
| Children[(a)] | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK |
| Adolescents | From 10 years up to and including 17 years of age | Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK |
| Adults | From 18 years up to and including 64 years of age | Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK |
| The elderly[(a)] | From 65 years of age and older | Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK |

SAIB: sucrose acetate isobutyrate.

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform the exposure calculations.

#### 3.3.3.2. Food categories considered for the exposure assessment of SAIB (E 444)

The two food categories in which the use of SAIB (E 444) is authorised were selected from the FoodEx nomenclature of the EFSA Comprehensive Database at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011a).

For the non-alcoholic drinks (FCS 14.1.4), all the fruit soft drinks, flavoured soft drinks, energy drinks for sports people were selected, except cola drinks and milk-based drinks.

For the alcoholic drinks (FCS 14.2.8), all the mixed drinks and cocktails were selected.

In both food categories, the exclusion criteria that 'only cloudy drinks' (FCS 14.1.4) and 'only flavoured cloudy alcoholic drinks' (FCS 14.2.8) could not be addressed, resulting in a possible overestimation of the exposure.

For the refined scenario, the food category alcoholic drinks (FCS 14.2.8) was not taken into account because no use levels were provided to EFSA for this category (Appendix B).

[14] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

## 3.4. Exposure estimate to SAIB (E 444) from its use as a food additive

Dietary exposure to SAIB (E 444) was calculated by multiplying SAIB (E 444) MPLs for each food category (Appendix B) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The dietary exposure was assessed for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 4). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey for the total population and per population group. 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to SAIB (E 444) was carried out by the ANS Panel based on (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) use levels as provided by the industry (defined as the *refined exposure assessment scenario*). Both scenarios are discussed in detail below.

### 3.4.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 2.

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the consumer will be continuously (over a lifetime) exposed to SAIB (E 444) in food at MPL.

### 3.4.2. Refined exposure assessment scenario

For SAIB (E 444), the refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix B summarises the concentration levels of SAIB (E 444) used in this exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- *The brand-loyal consumer scenario*: In this scenario, it is assumed that a consumer is exposed long term to a food additive present at the maximum reported use level for one food category (main contributor to the exposure) and at the mean level for the other food categories. As for SAIB (E 444) only use levels were available for flavoured drinks (FCS 14.1.4; Appendix B), the consumption level of this food category at the individual level was combined with the maximum reported use level in flavoured drinks.
- *The non-brand-loyal consumer scenario*: In this scenario, it is assumed that a consumer is exposed long term to a food additive present at the mean reported use levels in food. For SAIB (E 444), this exposure estimate was calculated by combining the consumption level of flavoured drinks (FCS 14.1.4) at the individual level with the mean of the typical reported use levels for this food category.

### 3.4.3. Dietary exposure to SAIB (E 444)

Table 5 summarises the estimated exposure to SAIB (E 444) from its use as a food additive in six population groups (Table 4) according to the different exposure scenarios (Section 3.1.1). Detailed results per population group and survey are presented in Appendix C.

**Table 5:** Summary of dietary exposure to SAIB (E 444) from its use as a food additive in the maximum level exposure assessment scenario and in the two refined exposure scenarios in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

| | Infants (4–11 months) | Toddlers (12–35 months) | Children (3–9 years) | Adolescents (10–17 years) | Adults (18–64 years) | The elderly (≥ 65 years) |
|---|---|---|---|---|---|---|
| **Regulatory maximum level exposure assessment scenario** | | | | | | |
| Mean | < 0.01–0.63 | < 0.01–3.3 | 0.09–3.2 | 0.09–1.9 | 0.04–0.62 | 0.02–0.23 |
| 95th percentile | < 0.01–4.2 | < 0.01–12.9 | 0.67–10.2 | 0.63–5.6 | 0.25–2.7 | < 0.01–1.4 |
| **Refined estimated exposure assessment scenario** | | | | | | |
| *Brand-loyal scenario* | | | | | | |
| Mean | < 0.01–0.62 | < 0.01–3.3 | 0.09–3.1 | 0.09–1.9 | 0.04–0.61 | 0.02–0.22 |
| 95th percentile | < 0.01–4.1 | < 0.01–12.7 | 0.66–10.0 | 0.61–5.5 | 0.24–2.7 | < 0.01–1.4 |
| *Non-brand-loyal scenario* | | | | | | |
| Mean | < 0.01–0.11 | < 0.01–0.58 | 0.02–0.55 | 0.02–0.33 | 0.01–0.11 | < 0.01–0.04 |
| 95th percentile | < 0.01–0.73 | < 0.01–2.24 | 0.12–1.77 | 0.11–0.98 | 0.04–0.47 | < 0.01–0.25 |

SAIB: sucrose acetate isobutyrate.

Using the *regulatory maximum level exposure assessment scenario*, mean exposure to SAIB (E 444) from its use as a food additive ranged from < 0.01 to 3.3 mg/kg bw per day in six population groups. The high exposure to SAIB (E 444) using this scenario ranged from < 0.01 to 12.9 mg/kg bw per day.

The refined exposure to SAIB (E 444) using the *brand-loyal* exposure scenario was similar to the *regulatory maximum level exposure assessment scenario.* For the non-*brand-loyal* exposure scenario, the exposure to SAIB (E 444) was up to 0.58 mg/kg bw per day in toddlers for the mean and 2.24 mg/kg bw per day in toddlers for the 95th percentile.

#### 3.4.3.1. Main food categories contributing to exposure to SAIB (E 444) using the maximum level exposure assessment scenario (Table 6)

**Table 6:** Main food categories contributing to exposure to SAIB (E 444) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

| Food category number | Food category name | Infants | Toddlers | Children | Adolescents | Adults | The elderly |
|---|---|---|---|---|---|---|---|
| | | Range of % contribution to the total exposure (number of surveys)[a] | | | | | |
| 14.1.4 | Flavoured drinks | 100 (4) | 100 (8) | 99.2–100 (18) | 97.1–100 (17) | 80.1–100 (17) | 68.6–100 (14) |
| 14.2 | Alcoholic beverages | – | – | – | – | 5.4–19.9 (3) | 6.3–31.4 (4) |

SAIB: sucrose acetate isobutyrate.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted consumption data of more than one survey for a specific population.

#### 3.4.3.2. Main food categories contributing to exposure to SAIB (E 444) using the refined exposure assessment scenario

Considering that no data were received on the levels of SAIB (E 444) in alcoholic drinks, exposure to SAIB (E 444) was completely due to flavoured drinks (FCS 14.1.4).

#### 3.4.3.3. Uncertainty analysis

Uncertainties in the exposure assessment of SAIB (E 444) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 7.

**Table 7:** Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

| Sources of uncertainties | Direction[(a)] |
|---|---|
| Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard | +/– |
| Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile) | + |
| Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to | +/– |
| Regulatory maximum level exposure assessment scenario: food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008 | + |
| Refined exposure scenario: no use levels in food category alcoholic drinks (FCS 14.2.8) | – |
| Exclusion criteria of the use of SAIB (E 444) in flavoured drinks (FCS 14.1.4) and alcoholic drinks (FCS 14.2.8) could not be considered using FoodEx in both exposure scenarios | + |
| Uncertainty in possible national differences in use levels of food categories | +/– |

FCS: food categorisation system; MPL: maximum permitted level; SAIB: sucrose acetate isobutyrate.
(a): +: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of exposure to SAIB (E 444) as a food additive in European countries for the regulatory maximum level exposure scenario and for the brand-loyal refined exposure scenario, and in an underestimation for the non-brand-loyal refined exposure scenario.

## 3.5. Biological and toxicological data

### 3.5.1. Absorption, distribution, metabolism and excretion

Studies on metabolic fate and elimination of SAIB were performed by Reynolds et al. (1974) and Phillips et al. (1976). In both studies, SAIB was $^{14}$C-labelled at the sucrose moiety. An overview of the elimination of the radioactivity following oral dosing with $^{14}$C-SAIB in experimental animals and humans is presented in Table 8. The overview gives information about the absorption of different dose levels and in different species.

**Table 8:** Summary of data on elimination of radioactivity in per cent of administered dose in rats, dogs and human volunteers after oral exposure to $^{14}$C-SAIB

| Species (no. subjects) | Dose (vehicle) (mg/kg bw) | Observation period (days) | Elimination in % of the applied dose | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ | Urine | Faeces | Carcass | Total | |
| Rat (2)[(a)] | 89, 98 (CO) | 4 | 25, 27 | 13, 13 | 50, 55 | nd | 90–93 | Reynolds et al. (1974) |
| Rat (2)[(a)] | 26, 28 (CO) | 4 | 47, 55 | 18, 19 | 18, 25 | nd | 91–92 | Reynolds et al. (1974) |
| Rat (1) | 11.2 (AE) | 3 | 52 | 13 | 27 | 6 | 99 | Reynolds et al. (1974) |
| Rat (1) | 5.8 (AE) | 3 | 59 | 11 | 23 | 6 | 99 | Reynolds et al. (1974) |
| Rat (2) | 50 (CO) | 0.25 | 2.4 | 4.9 | – | 92.6 | 100 | Phillips et al. (1976) |

| Species (no. subjects) | Dose (vehicle) (mg/kg bw) | Observation period (days) | Elimination in % of the applied dose | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ | Urine | Faeces | Carcass | Total | |
| Rat (2) | 50 (CO) | 1 | 45.1 | 12.0 | 33.3 | 9.6 | 100 | Phillips et al. (1976) |
| Dog (1) | 4.8 (AE) | 7 | 27 | 7 | 46 | nd | 80 | Reynolds et al. (1974) |
| Dog (1) | 3.0 (AE) | 7 | 28 | 6 | 53 | nd | 86 | Reynolds et al. (1974) |
| Human (1) | 0.2 (AE) | 25–30(b) | 66 | 18 | 10 | nd | 95 | Reynolds et al. (1974) |
| Human (6) | 1–1.2 (AE) | 25–30(b) | 41–64 | 15–21 | 10 | nd | 71–90(c) | Reynolds et al. (1974) |

SAIB: sucrose acetate isobutyrate; CO: gavage in corn oil; AE: gavage in aqueous emulsion; nd: no data.
(a): Two different doses, one rat for each dose; no further details available than those presented in the table.
(b): Faeces collected for 5 days and other parameters measured for 25–30 days.
(c): Range of six subjects; total excretion of radioactivity via urine, faeces and exhaled air.

#### 3.5.1.1. Absorption

The studies presented in Table 8 indicate that rats, dogs and humans partially absorbed the radioactivity from the gut after oral exposure to $^{14}$C-SAIB. Reynolds et al. (1974) measured the elimination of $^{14}$C-SAIB in all three species. Assuming no or low elimination via the bile, the absorption rate in rats was 71% at a dose of 11.2 mg/kg bw and 76% at 5.8 mg/kg bw (sum of excretion via exhaled air and urine plus radioactivity in carcass). At dose levels of 26 or 28 mg/kg bw, a similar absorption rate of approximately 70% is found (sum of radioactivity excreted via $CO_2$ and urine), although data on carcass radioactivity are missing. Lower absorption rates are suggested when $^{14}$C-SAIB was applied with higher dose levels (89 and 98 mg/kg bw) in corn oil: about 40% absorption at 89 or 98 mg/kg bw (sum of radioactivity excreted via $CO_2$ and urine) (Reynolds et al., 1974). Elimination data on rats presented by Phillips et al. (1976) supported these results. After a dose of 50 mg/kg bw, 67% of the radioactivity was absorbed after an observation period of 1 day. Measurements after 6 h revealed that most radioactivity was found in the carcass (including content of gastrointestinal tract) indicating incomplete absorption/elimination after this short exposure period.

In dogs, about 50% of the applied dose was eliminated in the faeces (at a dose of 3–5 mg/kg bw). However, at these dose levels, radioactivity was also excreted via the bile (in dogs 6% of applied dose of 4 mg/kg bw) (Reynolds et al., 1974) suggesting absorption rates slightly higher than 50%.

On the basis of experiments in humans (dose levels of 0.2 or 1–1.2 mg/kg bw), Reynolds et al. (1974) concluded that $\geq$ 88% of the dose was absorbed. However, documented data were restricted to those presented in Table 8 with no further details or explanations given.

Phillips et al. (1976) studied the fate *in vitro* with rat tissues and gut microflora. SAIB was substantially hydrolysed by the content of the small intestine (ligated alimentary tract) under anaerobic conditions; approximately 75% of SAIB was hydrolysed within 6 h. Similar results were obtained with homogenates of the intestinal mucosa under aerobic conditions. Hydrolysis was assigned to the action of non-specific esterases in the intestinal mucosa and was not considered to be dependent on gut microflora. Incubation of $^{14}$C-SAIB with human faecal homogenates (1 mg SAIB/mL) resulted in 40% hydrolysis by 16 h. Further *in vitro* experiments with liver homogenate have shown only little hydrolysis.

In summary, studies in rats exposed to SAIB, which was $^{14}$C-labelled at the sucrose moiety, revealed absorption of radioactivity from the gastrointestinal tract; the calculated absorption was approximately 70%. The major site of intestinal metabolism and absorption of SAIB is in the lower part of the digestive tract although metabolism starts and is quite extensive in the duodenum and proximal jejunum (Reynolds, 1961 [Doc. provided to EFSA n. 45]). The degradation products of SAIB in the gut include partially de-esterified SAIB, acetate, isobutyrate, sucrose, fructose, glucose and unchanged SAIB. Acetate, isobutyrate, fructose and glucose formed from SAIB in the gut or intestinal mucosa will be rapidly absorbed. Absorption decreased with increasing dose levels. In humans, an

absorption rate of ≥ 88% was reported using the same labelling; lower rates of about 50% were measured in dogs.

#### 3.5.1.2. Distribution

A low level of radioactivity (uniformly distributed in the body as it was measured in separate studies in different organs by Reynolds et al., 1974) remained in the carcass of rats 3 days after $^{14}$C-SAIB after single oral doses. This effect may arise from the incorporation of $^{14}$C into the physiological constituents. The detected level in the carcass represents 6% of the applied dose and about 10% of the absorbed dose which was not eliminated. Similar results have been shown in additional experiments with $^{14}$C-sucrose (Reynolds et al., 1974). In comparison, rats fed pure $^{14}$C-sucrose incorporated about 11% of the applied dose. Thus, the residual radioactivity in rats exposed to $^{14}$C-SAIB was probably incorporation of the radiolabel from sucrose into cellular constituents. However, the peak of $^{14}$C-SAIB catabolism to $^{14}CO_2$ occurred later than the peak of $^{14}$C-sucrose catabolism to $^{14}CO_2$ suggesting that hydrolysis is required before the ester can be absorbed.

#### 3.5.1.3. Metabolism

*In vivo* experiments with rats (Phillips et al., 1976; see also Table 8) gave evidence for considerable hydrolysis 24 h after oral application of $^{14}$C-SAIB. Metabolism to $^{14}CO_2$ accounted for 45% of the applied radioactivity indicating complete hydrolysis and utilisation in physiological processes. Thirty-three per cent of the applied radioactivity was detected in faeces and approximately two out of three of this excreted radioactivity showed the chromatographic properties of SAIB. The extent of hydrolysis to sucrose and other metabolites of low chromatographic mobility was the highest in the caecum.

Reynolds (1961 [Doc. provided to EFSA n. 45]) exposed rats to a single dose of unlabelled and $^{14}$C-sucrose-labelled SAIB and followed the elimination of metabolites by chemical and radiochemical techniques. The results of studies with unlabelled SAIB were inconclusive. Studies with $^{14}$C-SAIB showed that the rat rapidly disposes SAIB by excretion and metabolism, with excretion in the faeces being the main route at low dose levels, and metabolism to $CO_2$ is twice as effective as urinary excretion in disposing absorbed SAIB or its metabolites. At a dose level of 100 mg/kg bw, SAIB is rapidly eliminated by the rat. Elimination is nearly complete (90–93% of the dose) in 4 days. About 50% of the dose is eliminated unchanged in the faeces, but metabolites are also excreted. The portion of the sucrose moiety of SAIB that is absorbed is rapidly metabolised and excreted as $CO_2$ (26% of the dose) or as sucrose or sucrose esters in the urine (33% of the dose). This indicates that SAIB is not absorbed as such but first degraded in the intestines. The greater part of the radioactivity excreted in the urine appears as sucrose with little or no glucose.

In a study of Reynolds and Zeigler (1977 [Doc. provided to EFSA n. 48]), dogs, rats and humans received a single dose of $^{14}$C-SAIB (no further details about labelling or dose). Chromatography of urine showed that $^{14}$C-labelled molecules larger than sucrose were absent (no further details on metabolites).

In another trial, a male subject ingested a single dose of 1.18 mg $^{14}$C-SAIB/kg bw. Urine was collected before dosing and 6.2 h after dosing, and subjected to chromatography (Reynolds et al., 1971 [Doc. provided to EFSA n. 46]). Two unidentified peaks were considered to be the main metabolites of SAIB. Glucose, fructose and their esters were not detected. Comparison between rats, dogs and humans showed the greater resemblance in disposition and urinary excretion products to exist between humans and rats.

Sucrose or sucrose esters were not detected in the urine of human volunteers after ingestion of a single dose or seven repeated daily doses of unlabelled SAIB at levels of 0.2 or 1 g (Phillips et al., 1976). On the other hand, neither unchanged SAIB nor its metabolites were detected in the faeces of one volunteer who ingested 0.1 g/day of unlabelled SAIB for 7 days.

#### 3.5.1.4. Excretion

Reynolds et al. (1974) studied the excretion of SAIB in humans, dogs and rats. Generally, elimination data suggested rapid absorption in all three species; however, dogs eliminated a much higher proportion of radioactivity via faeces than humans or rats at dose levels ≤ 28 mg/kg bw (see Table 8). More than 50% of the absorbed dose (see preceding paragraphs) was eliminated as $^{14}CO_2$ by all species. The proportion of applied radioactivity eliminated in breath (via $^{14}CO_2$) and urine is similar in rats and humans; lower amounts were eliminated in the urine of dogs. The elimination of radioactivity in humans was not influenced by further exposure to unlabelled SAIB for 7 days or by

food intake. Studies in rats have shown that the proportion of exhaled $^{14}CO_2$ decreased, and elimination in the urine increased slightly with increasing doses. Comparing data in Table 8, rats and humans eliminated 11–21% of the applied dose in the urine whereas dogs excreted only 6–7% of the applied radioactivity in urine. The elimination of radioactivity in the urine was rapid in all species; peak levels were measured immediately after exposure and excretion was nearly complete after 15 h. Also the elimination as $^{14}CO_2$ started rapidly in all species; the peak elimination was reached 7–8 h after application in rats and 8–16 h in humans and dogs. The rates then fell off sharply and minor amounts < 0.5% of the applied radioactivity were eliminated 30 h after treatment in all three species.

Overall, the available studies with $^{14}$C-SAIB labelled at the sucrose moiety revealed oral absorption of approximately 70% in rats, about 50% in dogs and of ≥ 88% in humans. Absorption was rapid in all three species. In general, SAIB is metabolised via de-esterification by non-specific esterases in the gastrointestinal tract, mainly in the small intestine to sucrose and partially acylated sucrose. Both metabolites appeared to be readily absorbed from the gut. A significant portion of ingested SAIB and partially de-esterified SAIB was excreted via the faeces, particularly in dogs. The absorbed radioactivity was eliminated in the urine with a minor proportion excreted in the bile as demonstrated in dogs. In humans receiving $^{14}$C-SAIB at doses of 0.1–2 mg/kg bw, the absorbed radioactivity, after further metabolism, was mainly excreted as carbon dioxide in exhaled air (44–66% of the dose). Urinary and faecal excretions accounted respectively for 15–21% and 10%. The Panel noted that no toxicokinetic data were available with SAIB labelled at the isobutyrate moiety.

### 3.5.2. Acute oral toxicity

The $LD_{50}$ value of SAIB in rats was above 5,000 mg/kg bw (Fassett and Reynolds, 1962 [Doc. provided to EFSA n. 12]; Reynolds, 1972; as referred to by JECFA, 1993).

In a study performed by Biodynamics (1979b [Doc. provided to EFSA n. 4]) using a stepwise protocol, two male and two female cynomolgus monkeys were gavaged with single doses of SAIB in orange juice at dose levels of 1.25, 2.5, 5, 10 and 20 g SAIB/kg bw. The doses were separated by intervals of 72 h. The monkeys were sacrificed 48 h after the last dose. Emesis (yellow watery vomit) and diarrhoea occurred after first applications using lower dose levels; both effects were attributed to the orange juice. No mortality was reported, and necropsy did not reveal any treatment-related effects.

Single doses of 400–25,600 mg/kg bw of SAIB in corn oil, administered via gavage to rats and mice, did not cause mortality over a 2-week observation period (Hewing, 1958 as referred to by Reynolds and Chappel, 1998).

Overall, only minor clinical effects occurred in monkeys at high doses. No mortality occurred in rats and mice at high dose levels. The results suggested low acute oral toxicity.

### 3.5.3. Short-term and subchronic toxicity

The Panel considered the available studies summarised in Table 9 relevant to the re-evaluation of SAIB as a food additive.

**Table 9:** Subacute and subchronic toxicity of SAIB in experimental animals

| Species, strain, sex[(a)], n[(b)] | Duration | Dosing information | NOAEL | Investigated parameters/observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| **Studies in mice** | | | | | |
| Mouse, B6C3F1, M & F, 10 | 4 weeks | Via the diet: 0, 0.625, 1.25, 2.5 or 5.0 g/kg bw per day | 5,000 mg/kg bw per day | ≤ **5 g/kg bw per day**: no effects on body weights, food consumption, physical examinations and in gross pathology | Mackenzie (1987) (as referred to by JECFA, 1993) |

| Species, strain, sex[(a)], n[(b)] | Duration | Dosing information | NOAEL | Investigated parameters/observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| **Studies in rats** | | | | | |
| Rat, Sprague-Dawley, M, 5 | 7 days | 0% or 4% in the diet | 4% (4,800 mg/kg bw per day) | **4%:** no effects on plasma BSP clearance 0, 24, 48 h after the treatment period | Procter et al. (1973) |
| Rat, Sprague-Dawley, M & F, 5 | 1, 2 or 3 weeks | 0%, 0.5% or 5% in the diet | 5% (6,000 mg/kg bw per day) | ≤ **5%:** no treatment related clinical signs or effects on body weight, food consumption, liver weight or at necropsy | Procter and Chappel (1971) [Doc. provided to EFSA n. 41] |
| Rat, Sprague-Dawley, M, 2 | Up to 36 days | 0% or 4% in the diet, SAIB supplemented with 5% corn oil | 4% (4,800 mg/kg bw per day) | **4%:** no effects on indocyanine green clearance rates 1, 3, 5, 8, 10, 22, 26 and 36 days after i.v. injection | Krasavage and Terhaar (1972) [Doc. provided to EFSA n. 26] |
| Rat, Sprague-Dawley, M & F, 10 | 28 or 56 days[(c)] | 0, 1, 2, or 4% in the diet, SAIB supplemented with 5% corn oil | 2% (2,400 mg/kg bw per day) | ≤ **4%:** no effects on mortality, body weight, feed consumption, clinical chemistry[(d)], liver weight, and in gross pathological examination. **4%**: liver glucose-6-phosphatase↑[(e)] | Krasavage et al. (1973) |
| Rat, Sprague-Dawley, M & F, 20 | 6 or 12 weeks | 0, 2.5, 5 or 10% in the diet, SAIB dissolved in acetone | 10% (9,000 mg/kg bw per day) | ≤ **10%:** no clinical signs, no effects of toxicological relevance on body weight, food consumption, organ weights and in gross and microscopic pathology[(f)] or clinical chemistry[(g)] **10%:** liver glycogen ↑ due to increased metabolism | Procter and Chappel, (1970a) [Doc. provided to EFSA n. 39]; Procter et al. (1973); Procter and Chappel (1998) |
| Rat, Sprague-Dawley, M & F, 25 | 95 days | 0, 1, or 5% in the diet, SAIB solubilised with acetone | 5% (4,500 mg/kg bw per day) | ≤ **5%:** no effects on body weight, haemoglobin & haematocrit, white blood cell counts, liver & kidney weights; histopathology negative | Krasavage et al. (1973) |
| Rat, Sprague-Dawley, M & F, 10 | 13 weeks | 0, 0.3, 1.8 and 9.1% in the diet, SAIB dissolved in vegetable oil | 1.8% (1,620 mg/kg bw per day) | ≤ **9.1%:** no effects on body weight, haemoglobin, white blood cell counts, organ weights; histopathology negative **9.1%:** diarrhoea | Hint (1964) (as referred to by JECFA, 1993) |
| **Studies in dogs** | | | | | |
| Beagle, M, 6 | 28 days [(h)] | 5% in the diet (equivalent to 2,250 mg/kg bw per day) | No NOAEL | **5%:** indocyanine green clearance rates ↓ and serum alkaline phosphatase (SAP) ↑ reversibility during non-treatment period, no effects on other parameters[(h)] | Krasavage et al. (1973) |

| Species, strain, sex[a], n[b] | Duration | Dosing information | NOAEL | Investigated parameters/observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| Beagle, M, 4 or 5 (control) | 91 days | 0 or 5% in the diet, vehicle corn oil (equivalent to 2,250 mg/kg bw per day) | No NOAEL | **5%:** indocyanine green clearance ↓ and SAP ↑ (related to liver isoenzymes), liver weight ↑; liver glycogen & phosphor-lipid ↑; liver & SAP ↑; no effects on other parameters[i] | Krasavage and Terhaar (1971) [Doc. provided to EFSA n. 25] |
| Beagle, M & F, 4 (control 6) | 90 days | 0, 0.2, 0.6 or 2.0% in the diet, SAIB dissolved in cottonseed oil (equivalent to 90; 270 and 900 mg/kg bw per day) | 0.2% (90 mg/kg bw per day) | ≤ **2%:** no clinical signs; no effects on body weight, food consumption, reflexes, haematology, clinical chemistry, urinalysis, organ weights and in gross and microscopical pathology[j]<br>≥ **0.6%:** in M & F liver weight ↑<br>**2%:** in M & F SAP ↑ | FDRL (1965) [Doc. provided to EFSA n. 17] |
| Beagle, M & F, 6 (4 in additional post-exposure group) | 90 days (at 2% post-exposure period 14 days) | 0, 0.5, 1.0, 2.0 or 4.0% in the diet, SAIB dissolved in acetone, (equivalent to 225, 450, 900 and 1,800 mg/kg bw per day) | No NOAEL | ≤ **4%:** no clinical signs, no effect on body weight (but food consumption ↓) or in ophthalmology, haematology, urinalysis, clinical chemistry, histopathology[k]<br>≥ **0.5%:** SAP & BSP retention ↑ in M & F; liver weight in m ↑; glucose-6-phosphate dehydrogenase, alkaline phosphatase and adenosine triphosphatase in bile capillaries ↑; liver carboxylesterase activity ↑;<br>**4%:** liver weight in F ↑<br>**Remark:** all effects reversible after 14 days post-exposure period | Procter et al. (1970) [Doc. provided to EFSA n. 42] |
| **Studies in monkeys** | | | | | |
| Cynomolgus, M & F, 1 | 15 days | Gavage: 0, 50, 1,000, 2,000, 5,000 or 10,000 mg/kg bw per day in orange juice | 10,000 mg/kg bw per day | ≤ **10 g/kg bw per day:** no effects on body weight, food consumption; no gross or microscopic (liver, adrenals, heart, kidneys) or ultrastructural (liver) pathological changes<br>**Remarks:** alterations of stool consistency not related to a specific dose | Tierney and Rinehart (1980) [Doc. provided to EFSA n. 49] |
| Cynomolgus, M & F, 1 | 15 days | Gavage: 0, 2,000, 5,000 and 10,000 mg/kg bw per day in orange juice | 10,000 mg/kg bw per day | ≤ **10 g/kg bw per day:** no effects on body weight, food consumption, or clinical chemistry parameters[l]<br>**Remarks**: 2 monkeys died due to mis-dosing; replaced by controls | Tierney and Rinehart (1980) [Doc. provided to EFSA n. 49] |

| Species, strain, sex[(a)], n[(b)] | Duration | Dosing information | NOAEL | Investigated parameters/observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| Cynomolgus, M & F, 1 | 4 weeks | Gavage: 0, 0.5, 1.45 and 2.4 g/kg bw per day; vehicle corn oil | 1,450 mg/kg bw per day | ≤ **2,400 mg/kg bw per day:** no clinical signs and no effects in haematology, gross pathology and clinical chemistry[(m)]; **2,400 mg/kg bw per day:** 12% of body weight loss in females | Blair and Chappel (1998) |

The NOAEL is defined on the information available in the corresponding reference. i.v.: intravenous.
SAIB: sucrose acetate isobutyrate; NOAEL: no observed adverse effect level.
(a): M: male; F: female.
(b): Number of animals/sex/dose.
(c): Totally 14 groups: 28 or 56 days continuously or for 28 days followed or preceded by 28 days on control diet.
(d): SAP, ornithine carbamyl transferase (OCT), blood urea nitrogen, triglyceride, cholesterol and glucose; liver: *p*-nitroanisole demethylase, glucose-6-phosphatase and bilirubin-β-D-glucuronyl transferase.
(e): ↑ indicates an increase.
(f): Organ weights of the liver, heart, kidneys and adrenal glands; histopathology in controls and high dose rats of cerebrum, cerebellum, eye and optic nerve, salivary gland, tongue, oesophagus, stomach, duodenum, jejunum, ileum, colon, pancreas, heart, aorta, trachea, lung, kidneys, liver, spleen, mesenteric lymph nodes, skin, skeletal muscle, sciatic nerve, adrenals, parathyroids, thyroid, thymus, pituitary, urinary bladder, prostate, seminal vesicles, testes, ovaries, mammary gland, vagina and uterus.
(g): After 12 weeks: serum OCT, serum alanine aminotransferase (ALAT), SAP, and bilirubin; protein, glycogen, carboxylesterase activity, lipid and water of the liver.
(h): Prior to SAIB treatment 5% corn oil for 3 weeks; after 28 days' oral exposure, dogs were given again control diet for 57 days followed by 5% SAIB in the diet (n = 4) and 24 and 48 h later plasma indocyanine green clearance rates and SAP were determined. Further parameters: haematocrit, haemoglobin, white cell and differential counts, aspartate aminotransferase (ASAT), blood glucose, blood urea nitrogen (BUN), serum protein, SAP, and OCT, triglyceride; physical appearance, behaviour, food consumption, and body weight. Histopathology of 'all organ systems'.
(i): Same parameters than in (h) plus serum bilirubin, liver glycogen content, liver samples processed for microsomal enzymes [*p*-nitroanisole demethylase, glucose-6-phosphatase, and bilirubin-P-D-glucuronyl transferase]; liver, kidney, bone, bile and scrapings of the intestinal mucosa analysed for alkaline phosphatase activity.
(j): Haemoglobin, haematocrit, total and differential white blood cell count, blood glucose, BUN, SAP and lactate dehydrogenase (LDH) measured; standard urinalysis; organ weights of liver, kidneys, spleen, gonads, adrenals, pituitary and brain; histopathology of liver and kidney in all groups, other 21 tissues/organs in control and high-dose group.
(k): Haematology: total and differential leucocyte count, prothrombin time, clotting time, red blood cell count, haemoglobin concentration, haematocrit and sedimentation rate; clinical chemistry: urea nitrogen, ALAT, ASAT, SAP, lactic dehydrogenase, serum bilirubin, glucose, sodium, potassium, total proteins, albumin and globulin, bromosulfophthalein (BSP) retention; urinalysis: occult blood, protein, glucose, pH, bilirubin and ketone bodies, colour, pH and specific gravity; levels of liver lipid, protein, glycogen and carboxylesterase activity determined; organ weights of the brain, heart, liver, lung, kidneys, adrenals, gonads, prostate, uterus, pituitary, spleen and thyroid; liver histochemistry for glycogen, phosphorylase, succinate dehydrogenase, adenosine triphosphatase, glucose-6-phosphate dehydrogenase and acid and alkaline phosphatases; histopathology of heart, liver, kidneys, adrenals, stomach, and small and large intestine; liver electron microscopy.
(l): ASAT, ALAT, SAP, BUN, total protein, albumin, globulin, creatinine, total bilirubin and BSP retention.
(m): Gamma-glutamyl transpeptidase, SAP, OCT and 30-min BSP clearance.

The data presented in Table 9 indicate that dogs differ from other species in their response after oral exposure to SAIB. In subchronic feeding studies, dogs revealed effects on liver weight and liver function (BSP retention increased) at dose levels ≥ 0.5% in the diet (corresponding to 225 mg/kg bw per day) as well as the increased activity of some enzymes in serum (alkaline phosphatase), bile capillaries or liver. However, no effects were detected in any other parameter investigated (e.g. serum proteins, enzymes such as ALAT or ASAT, serum bilirubin). Additionally, no changes in histopathology of the liver were detected, even at a dose of 1,800 mg/kg bw per day.

The studies in rats have shown the absence of any untoward effects. In a study performed by Procter and Chappel (1998), the highest dose of 10% in the diet did not induce effects of toxicological relevance. The decrease in body weight gain in males was not dose dependent and was found in all treatment groups and might be related to palatability. The effects on food consumption were inconsistent. Additional to toxicological parameters, the authors measured urinary ascorbic acid excretion and reduced zoxazolamine hypnotic effects which are discussed as indication of liver microsomal metabolic enzyme induction; the results were negative. The highest dietary level of SAIB fed in this study (10%) was considered by the authors to be the NOAEL corresponding to 9,000 mg/kg bw per day.

In accordance with the findings in rats, subacute gavage studies in monkeys have shown no toxicological effects at doses up to 10,000 mg/kg bw per day. The Panel noted that effects on hepatobiliary function were observed in dogs at a dose of 225 mg/kg bw per day but neither in rats

nor in mice or monkeys. Rats, mice and monkeys tolerated much higher dose levels (up to 10,000 mg/kg bw per day in monkeys) without any adverse effects.

### 3.5.4. Genotoxicity

#### 3.5.4.1. *In vitro* studies

The available *in vitro* genotoxicity studies are summarised in Table 10.

**Table 10:** Genotoxicity *in vitro*

| Endpoint and test system | Tested organisms | Tested concentrations vehicle | Cytotoxic concentration | Results −MA[a] | Results +MA | Reference |
|---|---|---|---|---|---|---|
| **Gene mutation** | | | | | | |
| Ames test | *S. typhimurium* TA98, TA100 | 0.1–10 mg/plate DMSO[b] | No cytotoxic effects | (-ve) | (-ve) | Bonin and Baker (1980) |
| Ames test | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 0.333–10 mg/plate DMSO | No cytotoxic effects | (-ve) | (-ve) | Lawlor and Valentine (1989) [Doc. provided to EFSA n. 27] |
| Ames test | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 0.333–10 mg/plate DMSO | No cytotoxic effects but precipitation at ≥ 3.3 mg/plate | (-ve) | (-ve) | Litton Bionetic (1978) [Doc. provided to EFSA n. 28] |
| HPRT[c] assay | Chinese hamster ovary cells | 0.025–1 mg/mL DMSO | Tested up to cytotoxic dose levels | (-ve) | (-ve) | Litton Bionetics (1985c) [Doc. provided to EFSA n. 31] |
| **Chromosome mutation** | | | | | | |
| Chromosome aberration test[d] | Chinese hamster ovary cells | 0.4–2 mg/mL DMSO | Precipitation at ≥ 1.2 mg/mL cytotoxic effects at 2 mg/mL | (-ve) | (-ve) | Litton Bionetics, (1985b) [Doc. provided to EFSA n. 30] |
| **DNA damage** | | | | | | |
| Unscheduled DNA synthesis[e] | Rat hepatocytes | 0.25–1,000 μg/mL acetone | No cytotoxic effects but precipitation at 1 mg/mL | (-ve) | Not applicable | Litton Bionetics (1985a) [Doc. provided to EFSA n. 29] |

(a): Metabolic activation system.
(b): Dimethylsulfoxide (DMSO).
(c): Forward mutation at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus; treatment: 4 h ± S9.
(d): Treatment/harvest time: 2/10 h (+S9), 7.5/10 h (−S9).
(e): Treatment/harvest time: 18/21 h.

SAIB was not mutagenic in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 or TA1538 with or without S9 metabolic activation in two independent trials. SAIB was not tested in TA102 or *Escherichia coli* WP2; however, oxidising or cross-linking properties are not expected. The test substance induced no forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells in the presence or absence of metabolic activation. Chromosomal aberrations were not induced in CHO cells with or without metabolic activation. Rat hepatocytes exposed to SAIB did not show induction of unscheduled DNA synthesis suggesting that no DNA damage was induced (Table 10).

The Panel noted that the genetic endpoint numerical chromosomal aberrations (aneugenicity) was not fully covered with the available genotoxicity tests as an *in vitro* micronucleus assay is not available and chromosomal aberration assays are primarily used to detect structural chromosomal aberrations. However, as the *in vitro* chromosomal aberration assay did not provide an indication for disturbance of mitosis, the lack of an *in vitro* micronucleus assay could be considered to be of no major relevance in this case.

#### 3.5.4.2. *In vivo* studies

SAIB was assessed for its genotoxic properties in the rat dominant lethal assay in which 20 male Sprague–Dawley rats per group were dosed once with SAIB at dose levels of 20, 200 or 2,000 mg/kg bw (Krasavage, 1973 [Doc. provided to EFSA n. 24]). Solvent-treated animal group was also included. All treatments were administered by oral gavage in corn oil.

Following treatments, the males were sequentially mated to two untreated virgin females for 5 days a week for 7 weeks. At the end of 5 days, females were removed from the males and housed separately until sacrifice. Females were sacrificed at 14 days after separation from males and at necropsy the uteri were analysed for early deaths, late fetal deaths and total implantations. Results showed that pregnancy rate, early deaths per pregnancy and mean total implantations in the SAIB treatment groups did not differ significantly from the solvent treatment group values. The positive control group showed clear positive outcomes. The author concluded that, under the reported experimental conditions, SAIB did not cause dominant lethal mutations in rats. The Panel agreed with this conclusion.

In addition, from this study the analysis of induction of chromosomal aberrations in lymphocytes from peripheral blood collected from the tail vein 72 h from the end of treatment was also performed. The results obtained, although of limited value, due to the low number of metaphases scored (10 metaphases per animal and 50 metaphases per group) indicated the absence of clastogenicity by SAIB.

Overall, the Panel considered that SAIB did not raise concern for genotoxicity.

### 3.5.5. Chronic toxicity and carcinogenicity

A summary of the available chronic toxicity data is given in Table 11.

**Table 11:** Chronic toxicity of SAIB in experimental animals

| Species, strain, sex[(a)], n[(b)] | Duration | Dosing information | NOAEL | Investigated parameters/ observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| **Studies in mice** | | | | | |
| Mouse, B6C3F1, M & F, 50 | 105 weeks | 0, 1,250, 2,500 or 5,000 mg SAIB/kg bw per day via the diet (dissolved in acetone which evaporated prior to feeding); 2 control groups | 2,500 mg/kg bw per day[(c)] | ≤ **5,000 mg/kg bw per day**: no effect on survival; no consistent effect on body weight (decrease < 10%) and food consumption; no treatment-related effects in haematology, clinical chemistry, necropsy and histopathology; organ weights except kidney[(d)] **5,000 mg/kg bw per day**: kidney weight ↑[(e)] in F **Remark:** no data on clinical signs | MacKenzie (1990c) [Doc. provided to EFSA n. 34] |
| **Studies in rats** | | | | | |
| Rat, F344, M & F, 20 | 52 weeks | 0, 500, 1,000 or 2,000 mg SAIB/kg bw per day via the diet (dissolved in acetone which evaporated prior to feeding) | 2,000 mg/kg bw per day | ≤ **2,000 mg/kg bw per day:** no clinical signs; no effects on survival and body weights (↓ in high-dose F but related to reduced food consumption, ↓[(e)] palatability); no effects on general health, organ weights, and ophthalmology, haematology, clinical chemistry and BSP, urine analysis, histopathology, electron microscopy of the liver[(d)] | MacKenzie, (1990a) [Doc. provided to EFSA n. 32] |

| Species, strain, sex[(a)], n[(b)] | Duration | Dosing information | NOAEL | Investigated parameters/ observed effects at particular doses (in bold) | Reference |
|---|---|---|---|---|---|
| Rat, F344, M & F, 50 | 104 weeks | 0, 500, 1,000 or 2,000 mg SAIB/kg bw per day via the diet (dissolved in acetone which evaporated prior to feeding); 2 control groups | 2,000 mg/kg bw per day | ≤ **2,000 mg/kg bw per day:** no effects on general health, survival, body weight, food consumption; no treatment-related effects in haematology and gross and microscopical pathology[(d)] | MacKenzie, (1990b) [Doc. provided to EFSA n. 33] |
| **Studies in monkeys** | | | | | |
| Cynomolgus monkey, M & F, 4 | 52 weeks | 0, 500, 1,450 or 2,400 mg SAIB/kg bw per day by corn oil gavage | 2,400 mg/kg bw per day | ≤ **2,400 mg/kg bw per day:** no difference to control with respect to clinical signs, body weight, ophthalmology, clinical chemistry and haematology and organ weights (effects not consistent), necropsy and histopathology (no treatment-related effects) or liver electron microscopy[(f)] | Blair and Chappel (1998) |

The NOAEL is defined by the authors of this evaluation based on the information available in the corresponding reference.
SAIB: sucrose acetate isobutyrate; NOAEL: no observed adverse effect level.
(a): M: male; F: female.
(b): Number of animals/sex/dose.
(c): The chronic feeding study in mice revealed increased absolute and relative kidney weights in high-dose females. The authors suggested a NOAEL of 2,500 mg/kg bw per day due to these organ weight effects. Urinalysis was not performed in the mouse study. However, histopathology of the kidney was negative and no treatment-related effects were detected in clinical chemistry. Thus, increased organ weight might be related to an adaptive process and therefore of limited toxicological relevance.
(d): Clinical signs recorded twice daily and physical examination once weekly; body weight and food consumption measured once weekly; ophthalmology at initiation and after 6 and 12 months (only in 1-year rat study); standard haematology and clinical chemistry performed after 6 and 12 months in the 1-year rat study or at week 28, 53, 79 and 105 in the 2-year mouse study or at termination in the 2-year rat study (only haematology); urinalysis only in 1-year rat study after 6 and 12 months; haematology: erythrocyte count, reticulocyte count, haemoglobin, total and differential white blood cell count, haematocrit, prothrombin time, platelet count; clinical chemistry: glucose, bilirubin, cholesterol, ASAT, ALAT, SAP, gamma glutamyl transferase, urea nitrogen, creatinine, BSP retention (10 min after 20 mg BSP/kg bw), calcium, chloride, inorganic phosphorus, sodium, potassium, total protein, albumin, globulin; urinalysis: volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, appearance, sediment examination; necropsy performed; organ weights of heart, kidneys, liver, gonads and brain; histopathology: kidney, liver, lung and gross lesions in all groups (52-week rat study only) and the following tissues of controls and high-dose rats: adrenals, aorta, brain (three sections), caecum, cervix, colon, common bile duct, duodenum, oesophagus, eyes, femur (and bone marrow), gross lesions, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical, thoracic and mesenteric), muscle, nasal turbinates, ovaries, pancreas, pituitary, prostate, rectum, rib, salivary gland (submandibular), sciatic nerve, seminal vesicles, skin (with mammary gland), skull, spinal column (three sections), spleen, sternum, stomach, testes (with epididymides), thymus, thyroid (with parathyroids), tongue, trachea, vagina, zymbal gland; ultrastructure of the liver including bile canaliculi from three control and three high-dose in the 1-year rat study.
(e): ↑ indicates an increase, ↓ indicates a decrease**.**
(f): Clinical signs recorded twice daily and physical examination plus ophthalmology, standard haematology, clinical chemistry including BSP retention (30 min after i.v. 5 mg/kg bw BSP), SAP, gamma-glutamyl transpeptidase, OCT and serum bile acids after 3, 6, 9 and 12 months (no further details); body weight measured once weekly; necropsy at termination, organ weights determined; histopathology of 40 tissues/organs; electron microscopy of the liver of controls and high-dose monkeys.

Neither in rodents (i.e. mouse and rats) nor in monkeys were any effects on the hepatobiliary function detected even at the highest dose level tested. In comparison, these effects were apparent in dogs in a subchronic study at a dose of 225 mg/kg bw per day (Procter et al., 1970 [Doc. provided to EFSA n. 42]), 10-fold below the NOAEL for the other species.

Rats and mice as well as monkeys tolerate large doses of SAIB over prolonged periods without evidence of significant toxicity. The NOAELs identified from the available chronic and carcinogenicity toxicity studies were 2,000 mg/kg bw per day for rats, 2,500 mg/kg bw per day for mice and 2,400 mg/kg bw per day for monkeys. In rats and monkey, these NOAELs were the highest doses tested.

### 3.5.6. Reproductive and developmental toxicity

#### 3.5.6.1. Reproductive toxicity

In a three-generation reproductive toxicity study presented by MacKenzie (1990d [Doc. provided to EFSA n. 35]), groups of male and female F344 rats (30/sex) were given a diet containing SAIB (dissolved in acetone) to achieve dose levels of 0, 500, 1,000 or 2,000 mg/kg bw per day (diets adjusted weekly). $F_0$ males were exposed for 10 weeks and $F_0$ females for 2 weeks prior to mating.

Exposure was continued in $F_0$, $F_1$ and $F_{2a}$ generations up to sacrifice. $F_0$ rats were killed after weaning of $F_1$ generation. The $F_1$ rats were mated twice for $F_{2a}$ and $F_{2b}$ generation. $F_1$ males were sacrificed after the second mating and $F_1$ females at the time of caesarean sections to deliver the $F_{2b}$ litter (gestation day (GD) 20). The $F_{2b}$ litters were used for the teratology study (see below). $F_{2a}$ rats were mated to study fertility indices for the $F_3$ pregnancy. The $F_{2a}$ males were sacrificed after mating and $F_{2a}$ females at GD 14. Reproduction indices were determined in each generation after mating groups of 22–30 females and 19–30 males (no further details on matings). Rats of all generation were checked once daily for clinical signs and females for abortion, premature delivery or difficult parturition. Body weights and food consumption were recorded weekly. After birth, data on litter size and numbers of live and dead pups were collected. Dead pups and pups of culled litters (see below) were examined for visceral abnormalities. At day 4 and day 28, postnatal pups were weighed, sexed and examined for abnormalities. Litters with more than 10 pups were culled at day 4 postnatal and at day 28 pups not selected as future parents were discarded. Necropsy was performed for all $F_0$ and $F_1$ rats. No clinical signs occurred in $F_0$, $F_1$ and $F_2$ rats. No consistent effects on food consumption and body weight were found. There were no differences between control groups and SAIB-treated groups concerning reproduction parameters. The only significant adverse effect noted was a slight decrease in the fertility index of the high-dose $F_1$ female rats mated to produce the $F_{2a}$ litters. No effects on fertility index were detected in the following mating to produce $F_{2b}$ litters or in any other mating of $F_0$ or $F_2$ dams. According to the author, the reduced female fertility in $F_1$ rats at 2,000 mg/kg bw per day was considered to be not treatment related and identified a NOAEL of 2,000 mg/kg bw per day. The Panel agreed with this conclusion.

In another study, three groups of 10 male and 10 female Sprague–Dawley rats were administered SAIB in the diet at levels of 0%, 0.4% and 9.4% (equivalent to 200 and 4,200 mg/kg bw per day, respectively) during breeding cycles at week 10–36 of a 104-week study (Harper et al., 1966; as referred to by JECFA, 1993). No differences were seen between the treatment groups and controls with respect to the mean numbers of pups per litter or in growth rates of the pups. No abnormalities were seen in any of the offspring. However, the conception rate and the percentage of pups born or reared to weaning were lower in the high-dose group. According to JECFA (1993), the reported effects might be related to the compromised nutritive value of the feed at the high dose level. The Panel agreed with the JECFA evaluation.

#### 3.5.6.2. Developmental toxicity

*Rats*

The $F_{2b}$ litters of the above-mentioned three-generation reproductive toxicity study with SAIB were used for the assessment of developmental toxicity (MacKenzie, 1990d [Doc. provided to EFSA n. 35]). Groups of 26–27 $F_1$ dams were exposed to 0, 500, 1,000 or 2,000 mg/kg bw per day at GD 0–20. Dams were checked daily for clinical signs, abortion and premature delivery. Body weights and food consumption were recorded weekly. Caesarean section and necropsy of dams were performed at GD 20. The fertility index was measured. The number and distribution of fetuses in each uterine horn, the number of implantations, early and late resorptions, the number of corpora lutea and fetal viability were recorded. Fetuses were weighed, had their sex determined and examined for external abnormalities. One-half of the fetuses were examined for soft tissue abnormalities and the remaining animals were stained with Alizarin Red and examined for skeletal abnormalities. No maternal toxicity was reported. The external, skeletal or visceral examination of the fetuses did not reveal a developmental effect. The Panel identified a NOAEL of 2,000 mg SAIB/kg bw per day, the highest dose tested, based on the fetopathological evaluations of the $F_{2b}$ litters.

*Rabbits*

In a developmental toxicity study in New Zealand White rabbits (ILSI, 1988 [Doc. provided to EFSA n. 23]), groups of 16 artificially inseminated rabbits were administered by gavage with SAIB in corn oil at dose levels of 0, 500, 850 or 1,200 mg SAIB/kg bw per day at GD 7–19. Pregnant rabbits were observed for clinical signs twice daily and body weights and food consumption were recorded at GD 0, 7, 13, 20 and 29. Caesarean section was performed at GD 29. The following parameters were recorded: number of corpora lutea, total implantations, post-implantation loss, viable fetuses, mean fetal weights and fetal sex distribution. Each fetus was dissected and examined for visceral malformations. The eviscerated, skinned fetuses were fixed and stained for skeletal examination. No treatment-related maternal toxicity was reported. No malformations or developmental variations

indicative of a treatment-related effect were observed. The authors identified a NOAEL of 1,200 mg SAIB/kg bw per day, the highest dose tested. The Panel agreed with this NOAEL.

Overall, in a three-generation reproductive toxicity study in rats, no effects on fertility and reproduction were detected after oral exposure via the diet at dose levels up to 2,000 mg SAIB/kg bw per day. In oral developmental toxicity studies in rats and rabbits, no maternal toxicity and no developmental effects were reported even at the highest dose level tested. The Panel identified NOAELs in rats at 2,000 mg SAIB/kg bw per day and in rabbits at 1,200 mg SAIB/kg bw per day, the highest doses tested.

### 3.5.7. Other studies

#### 3.5.7.1. Effects on liver function after acute oral exposure

The BSP elimination rate was measured in two male and two female Beagle dogs prior to treatment, and 24 and 48 h after treatment with 450, 1,350, and 2,250 mg SAIB/animal via the diet (1 week interval between each trial) (Procter and Chappel, 1971 [Doc. provided to EFSA n. 41]). The BSP elimination rate was significantly reduced at a dose ≥ 1,350 mg SAIB/dog, and the effect was reversible within 48 h.

In a study by Dickie et al. (1980a [Doc. provided to EFSA n. 8]), dogs (n = 2–3; dogs used repeatedly) were fed a single dose of 2,000 mg SAIB/kg bw. The BSP elimination rate was measured at different time points and the maximum effect was found 4–6 h after treatment. The BSP elimination rate, measured 5 h after application of 20–2,000 mg SAIB/kg bw, was decreased at doses ≥ 20 mg/kg bw; no effects were detected in two of three dogs treated with 5 mg SAIB/kg bw. The authors reported no effects on SAP.

Some authors studied the effect of SAIB on liver function, as shown by BSP elimination rates, using a crossover protocol in squirrel monkeys (Procter and Chappel, 1970b, 1971 [Doc. provided to EFSA n. 40 and 41]; Procter et al., 1973). Groups of three male monkeys (served as controls) were gavaged with 1 g SAIB per animal in cottonseed oil. BSP elimination rates were determined 24 h after treatment. No effect on the elimination of BSP was observed.

Dickie et al. (1980b [Doc. provided to EFSA n. 9]) studied the effects of SAIB ingestion on liver function in young adult male cynomolgus monkeys (n = 4; 2 controls) following a single dose of 5,000 mg SAIB/kg bw in corn oil. SAP levels and BSP elimination rates were determined 24 h before treatment and 5 h after treatment. No effect on SAP or BSP retention was reported.

Overall, effects on liver function were reported in dogs but not in monkeys at single oral exposure ≥ 20 mg SAIB/kg bw.

#### 3.5.7.2. Observations in humans

In a clinical study, 20 volunteers (10 males and 10 females; 18–22 years of age) ingested SAIB each morning at a daily dose of 10 mg SAIB/kg bw per day for a period of 14 days (Hensley, 1975; as referred to by JECFA, 1993). Blood samples were collected prior to treatment and at days 7 and 18 of the study. There was no difference in any of the following parameters in any volunteer: ASAT, ALAT, SAP, serum bilirubin, total protein, albumin, uric acid, BUN, erythrocyte sedimentation rate, sodium, potassium, phosphorous, total carbon dioxide, cholesterol and glucose.

In another clinical study, groups of four male and four female volunteers received SAIB via a soft drink in a dosage of 0, 7 or 20 mg SAIB/kg bw per day for 14 days (Orr et al., 1976 [Doc. provided to EFSA n. 37]). Possible alterations in normal hepatic function were evaluated. Subjects participating in this study were 21–42 years of age. Blood was collected for haematology (platelets, total and differential white blood cell count, erythrocyte sedimentation rate, haematocrit and haemoglobin), and clinical chemistry (total protein, albumin, albumin/globulin ratio, calcium, cholesterol, glucose, BUN, uric acid, total bilirubin, SAP, ASAT, ALAT and LDH) and standard urinalysis parameters (no details available) were recorded prior to testing and on study days 7 and 14. A 45-min BSP retention test (5 mg/kg bw BSP) was also conducted on all subjects prior to treatment and after completion of treatment. Oral exposure to SAIB did not affect any of these parameters for any individual.

In a valid, single-blind clinical study, healthy volunteers (13 men and 14 women) ingested SAIB once daily for 14 days in a gum arabic/water emulsion diluted with orange juice (see Chiang 1988 under 'Documentation provided to EFSA'). The dose of 20 mg/kg bw per day was given in a total volume of 1.16 mL/kg bw. In the 7 days prior to treatment (day −6 to 0), each subject acted as his/her own control by ingesting an orange juice beverage-placebo emulsion. Blood samples were collected from each subject on treatment days −6, 0, 7 and on the day after the final dose for

measurement of routine haematological (erythrocyte count, packed cell volume, haemoglobin, erythrocyte sedimentation rate, total and differential leucocyte count, reticulocyte count, prothrombin time) and clinical chemistry parameters (SAP, ALAT, ASAT, LDH, gamma-glutamyl transferase, bilirubin, total protein, albumin, creatinine, urea, urate, inorganic phosphate, sodium, potassium, cholesterol, glucose, bicarbonate, protein electrophoretogram, serum bile acids) including the specific indicators of hepatobiliary function. None of the subjects reported symptoms that could be related to ingestion of SAIB. No treatment-related changes were detected in haematology and clinical chemistry.

Overall, no treatment-related effects were observed on clinical signs, haematology and clinical chemistry in humans at a dose of 20 mg SAIB/kg bw per day and a treatment period of 14 days. SAIB did not affect hepatobiliary function in humans at this dose.

#### 3.5.7.3. Studies with other emulsifiers

In several recent studies, some other emulsifiers have been reported to alter the gut microbiota, to promote gut inflammation, obesity and to impair glycaemic control (Swidsinski et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015). The Panel noted that, even though some of these effects are not systematically studied in toxicity studies performed according to toxicity testing guidelines, they would be investigated on a case by case basis if indicated by the results of the general toxicity testing as recommended in the Guidance for submission of food additives (EFSA ANS Panel, 2012). The Panel considered that additional studies will be needed to show the relevance of the effects seen in mice for human health.

## 3.6. Discussion

Studies in three different species with SAIB, which was $^{14}$C-labelled at the sucrose moiety, revealed oral absorption of approximately 70% in rats, about 50% in dogs and of $\geq$ 88% in humans. Absorption was rapid in all three species. In general, SAIB is metabolised via de-esterification by non-specific esterases in the gastrointestinal tract, mainly in the small intestine to sucrose and partially acylated sucrose. Both metabolites appeared to be readily absorbed from the gut. A significant portion of ingested SAIB and partially de-esterified SAIB was excreted via the faeces, particularly in dogs. The absorbed radioactivity was eliminated in the urine with a minor proportion excreted in the bile as demonstrated in dogs. In humans receiving $^{14}$C-SAIB at doses of 0.1–2 mg/kg bw, the absorbed radioactivity, after further metabolism, was mainly excreted as carbon dioxide in exhaled air (44–66% of the dose). Urinary and faecal excretions accounted respectively for 15–21% and 10%. The Panel noted that no toxicokinetic data were available with SAIB labelled at the isobutyrate moiety.

The Panel noted that recent studies with other emulsifiers had demonstrated effects on the microbiota which might also be relevant to emulsifiers in general; however, there were no specific studies on SAIB itself.

The Panel noted that SAIB has a low acute toxicity.

Subchronic toxicity studies in mice, rats, dogs and monkeys were available. The Panel noted that effects on hepatobiliary function were observed in dogs at a dose of 225 mg/kg bw per day but neither in mice nor in rats or monkeys at higher doses of 5,000, 9,000 and 10,000 mg/kg bw per day, respectively.

On the basis of available genotoxicity studies, the Panel considered that SAIB did not raise concern for genotoxicity.

From the available chronic toxicity and carcinogenicity studies, the Panel identified a NOAEL of 2,500 mg/kg bw per day for mice and 2,000 mg/kg bw per day for rats (the highest dose tested). The Panel considered the less than 10% decrease in body weight accompanied by decreased feed intake, observed in a 52-week study in rats, was probably due to the palatability of the feed and, therefore, not considered to be adverse. Therefore, the Panel considered that the 2,000 mg/kg bw per day was the NOAEL in this rat study.

The SCF identified a NOAEL of 1,000 mg/kg bw per day from a chronic toxicity and carcinogenicity study based on the less than 10% decrease in body weight at the 2,000 mg/kg bw per day dose group. However, the Panel noted that this effect on body weight was observed in a 52-week study in rats but not in the chronic toxicity and carcinogenicity study.

Hepatobiliary function was affected in dogs at low dose levels in subchronic feeding studies. Even a single oral dose of 20 mg/kg bw resulted in effects on BSP retention; no such effects occurred at 5 mg/kg bw. In contrast, clinical studies in human volunteers have shown that a dose of 20 mg/kg bw per day ingested for a period of 14 days does not affect the hepatobiliary function in humans. Rodents

such as rats and mice as well as monkeys appear to tolerate large doses of SAIB in chronic studies without evidence of significant toxicity. No effects on hepatobiliary function were detected in these animals.

Concerning the effects of SAIB on the hepatobiliary function in the dog, these effects appear to be unique to this species, as they do not occur in mice, rats, monkeys or humans. The species differences in experimental animals after exposure to SAIB combined with the lack of any untoward effect in clinical studies with volunteers suggested that the effects on the hepatobiliary function in dogs are not relevant to humans.

From the available reproductive and developmental toxicity studies, no effects on fertility, reproduction, maternal toxicity or development were reported at doses amounting up to 2,000 mg/kg bw per day for rats and 1,200 mg/kg bw per day for rabbits.

The Panel identified a NOAEL of 2,000 mg/kg bw per day, the highest dose tested, from chronic toxicity, carcinogenicity and reproductive and developmental studies in rats. Applying an uncertainty factor of 100, an ADI of 20 mg/kg bw per day can be established.

The Panel considered that the present data set on ADME, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw per day allocated by SCF in 1994 to 20 mg/kg bw per day.

To assess the dietary exposure to SAIB (E 444) from its use as a food additive, the exposure was calculated based on (1) MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage data (defined as the *refined exposure assessment scenario*). Use levels of SAIB (E 444) were only provided for the food category flavoured drinks (FCS 14.1.4) and not for alcoholic drinks (FCS 14.2.8). Due to this, the exposure in the refined exposure scenarios was potentially underestimated (Section 3.1.1). However, it is very unlikely that the exposure will be higher than calculated in the *regulatory maximum level exposure assessment scenario*, in which it is assumed that consumers are exposed to SAIB (E 444) at the MPL over their lifetime. The Panel, therefore, noted that the exposure to SAIB (E 444) is below the ADI of 20 mg/kg bw per day.

## 4. Conclusions

The Panel concluded that based on the NOAEL of 2,000 mg/kg bw per day identified from chronic toxicity, carcinogenicity and reproductive and developmental studies in rats and applying an uncertainty factor of 100, an ADI of 20 mg/kg bw per day for SAIB (E 444) can be established.

Considering that the ADI is not exceeded in any population group, the Panel also concluded that the use of SAIB (E 444) as a food additive at the permitted or reported use and use levels would not be of safety concern.

## 5. Recommendation

The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, cadmium, lead and mercury) in the EU specifications for SAIB (E 444) should be revised in order to ensure that SAIB (E 444) as a food additive will not be a significant source of exposure to those toxic elements in food.

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## Abbreviations

| | |
|---|---|
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism and excretion |
| ALAT | alanine aminotransferase |
| ANS | EFSA Panel on Food Additives and Nutrient Sources added to Food |
| ASAT | aspartate aminotransferase |

| | |
|---|---|
| BSP | bromosulfophthalein |
| BUN | blood urea nitrogen |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CHO | Chinese hamster ovary |
| CONTAM | EFSA Panel on Contaminants in Food Chain |
| DNA | deoxyribonucleic acid |
| EC | European Commission |
| EINECS | European Inventory of Existing Commercial chemical Substances |
| FAO | Food and Agriculture Organization of the United Nations |
| FASEB | Federation of American Societies for Experimental Biology |
| FCS | food categorisation system |
| FDE | FoodDrinkEurope |
| FDRL | Food and Drug Research Laboratories |
| GC | gas chromatography |
| GD | gestation day |
| GNPD | Global New Products Database |
| HPRT | hypoxanthine-guanine phosphoribosyl transferase |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| $LD_{50}$ | median lethal dose |
| LDH | lactate dehydrogenase |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MPL | maximum permitted level |
| NOAEL | no observed adverse effect level |
| OCT | ornithine carbamyl transferase |
| OEDC | Organisation for Economic Co-operation and Development |
| SAIB | sucrose acetate isobutyrate |
| SAP | serum alkaline phosphatase |
| SCF | Scientific Committee on Food |
| TemaNord | is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion |
| WHO | World Health Organization |

## Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of SAIB (E 444) provided by industry

| Food category number | Food category name | MPL | Restrictions/ exceptions | Reported use levels | | | Information provided by |
|---|---|---|---|---|---|---|---|
| | | | | n | Typical mean (range) | Maximum level (range) | |
| 14.1.4 | Flavoured drinks | 300 | Only cloudy drinks | 13 | 52 (11–80) | 139 (50–295) | FDE [Doc. provided to EFSA n. 16] |

SAIB: sucrose acetate isobutyrate; MPL: maximum permitted level; FDE: FoodDrinkEurope; EFSA: European Food Safety Authority.

## Appendix B – Concentration levels of SAIB (E 444) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

| Food category number | Food category name | Restrictions/exceptions | MPL | Concentration levels used in the exposure assessment | | Data sources/comments |
|---|---|---|---|---|---|---|
| | | | | Mean | Maximum | |
| 14.1.4 | Flavoured drinks | Only cloudy drinks | 300 | 52 | 295 | |
| 14.2.8 | Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol | Only flavoured cloudy alcoholic drinks containing less than 15% of alcohol | 300 | – | – | Not taken into account in the refined exposure scenario (no concentration data available) |

SAIB: sucrose acetate isobutyrate; MPL: maximum permitted level.

## Appendix C – Summary of total estimated exposure of SAIB (E 444) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

| – | Number of subjects | Maximum level scenario | | Brand-loyal scenario | | Non-brand-loyal scenario | |
|---|---|---|---|---|---|---|---|
| | | Mean | 95th percentile | Mean | 95th percentile | Mean | 95th percentile |
| **Infants** | | | | | | | |
| Bulgaria (NUTRICHILD) | 659 | 0.044 | 0.000 | 0.043 | 0.000 | 0.008 | 0.000 |
| Germany (VELS) | 159 | 0.632 | 4.211 | 0.621 | 4.140 | 0.110 | 0.730 |
| Denmark (IAT 2006–2007) | 826 | 0.025 | 0.000 | 0.025 | 0.000 | 0.004 | 0.000 |
| Finland (DIPP 2001–2009) | 500 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| United Kingdom (DNSIYC 2011) | 1,366 | 0.131 | 0.839 | 0.129 | 0.825 | 0.023 | 0.145 |
| Italy (INRAN SCAI 2005–2006) | 12 | 0.000 | | 0.000 | | 0.000 | |
| **Toddlers** | | | | | | | |
| Belgium (Regional Flanders) | 36 | 0.839 | | 0.825 | | 0.146 | |
| Bulgaria (NUTRICHILD) | 428 | 0.348 | 2.500 | 0.342 | 2.458 | 0.060 | 0.433 |
| Germany (VELS) | 348 | 3.333 | 12.942 | 3.278 | 12.726 | 0.578 | 2.243 |
| Denmark (IAT 2006–2007) | 917 | 0.497 | 2.293 | 0.489 | 2.255 | 0.086 | 0.397 |
| Spain (enKid) | 17 | 0.000 | | 0.000 | | 0.000 | |
| Finland (DIPP 2001–2009) | 500 | 0.010 | 0.000 | 0.010 | 0.000 | 0.002 | 0.000 |
| United Kingdom (NDNS-RollingProgrammeYears1–3) | 185 | 1.300 | 5.093 | 1.279 | 5.008 | 0.225 | 0.883 |
| United Kingdom (DNSIYC 2011) | 1,314 | 0.707 | 3.280 | 0.695 | 3.226 | 0.123 | 0.569 |
| Italy (INRAN SCAI 2005–2006) | 36 | 0.000 | | 0.000 | | 0.000 | |
| Netherlands (VCP kids) | 322 | 0.956 | 3.920 | 0.940 | 3.855 | 0.166 | 0.679 |
| **Children** | | | | | | | |
| Austria (ASNS Children) | 128 | 0.986 | 3.893 | 0.969 | 3.829 | 0.171 | 0.675 |
| Belgium (Regional Flanders) | 625 | 1.230 | 5.813 | 1.209 | 5.716 | 0.213 | 1.008 |
| Bulgaria (NUTRICHILD) | 433 | 0.626 | 3.750 | 0.616 | 3.688 | 0.109 | 0.650 |
| Czech Republic (SISP04) | 389 | 1.615 | 6.724 | 1.588 | 6.612 | 0.280 | 1.166 |
| Germany (EsKiMo) | 835 | 1.203 | 5.111 | 1.183 | 5.026 | 0.209 | 0.886 |
| Germany (VELS) | 293 | 3.067 | 10.216 | 3.016 | 10.046 | 0.532 | 1.771 |
| Denmark (DANSDA 2005–2008) | 298 | 1.105 | 3.391 | 1.086 | 3.334 | 0.191 | 0.588 |
| Spain (enKid) | 156 | 0.546 | 3.257 | 0.537 | 3.202 | 0.095 | 0.564 |
| Spain (NUT INK05) | 399 | 0.160 | 1.141 | 0.158 | 1.122 | 0.028 | 0.198 |
| Finland (DIPP 2001–2009) | 750 | 0.225 | 1.626 | 0.221 | 1.599 | 0.039 | 0.282 |
| France (INCA2) | 482 | 0.393 | 2.053 | 0.386 | 2.018 | 0.068 | 0.356 |
| United Kingdom (NDNS-RollingProgrammeYears1–3) | 651 | 1.453 | 4.433 | 1.429 | 4.360 | 0.252 | 0.768 |
| Greece (Regional Crete) | 838 | 0.108 | 1.042 | 0.106 | 1.024 | 0.019 | 0.181 |
| Italy (INRAN SCAI 2005–2006) | 193 | 0.092 | 0.667 | 0.091 | 0.656 | 0.016 | 0.116 |
| Latvia (EFSA TEST) | 187 | 0.733 | 3.120 | 0.715 | 3.068 | 0.126 | 0.541 |
| Netherlands (VCP kids) | 957 | 0.918 | 3.552 | 0.903 | 3.493 | 0.159 | 0.616 |

| – | Number of subjects | Maximum level scenario | | Brand-loyal scenario | | Non-brand-loyal scenario | |
|---|---|---|---|---|---|---|---|
| | | Mean | 95th percentile | Mean | 95th percentile | Mean | 95th percentile |
| Netherlands (VCPBasis AVL2007 2010) | 447 | 3.160 | 7.880 | 3.107 | 7.749 | 0.548 | 1.366 |
| Sweden (NFA) | 1,473 | 2.258 | 5.919 | 2.220 | 5.820 | 0.391 | 1.026 |
| **Adolescents** | | | | | | | |
| Austria (ASNS Children) | 237 | 0.892 | 3.563 | 0.876 | 3.504 | 0.154 | 0.618 |
| Belgium (Diet National 2004) | 576 | 0.875 | 3.823 | 0.848 | 3.759 | 0.149 | 0.663 |
| Cyprus (Childhealth) | 303 | 0.353 | 1.672 | 0.347 | 1.644 | 0.061 | 0.290 |
| Czech Republic (SISP04) | 298 | 1.593 | 5.385 | 1.566 | 5.295 | 0.276 | 0.933 |
| Germany (National Nutrition Survey II) | 1,011 | 0.528 | 2.746 | 0.504 | 2.700 | 0.089 | 0.476 |
| Germany (EsKiMo) | 393 | 1.103 | 4.444 | 1.085 | 4.370 | 0.191 | 0.770 |
| Denmark (DANSDA 2005–2008) | 377 | 0.792 | 2.504 | 0.779 | 2.462 | 0.137 | 0.434 |
| Spain (AESAN FIAB) | 86 | 0.093 | 0.625 | 0.092 | 0.615 | 0.016 | 0.108 |
| Spain (enKid) | 209 | 0.596 | 3.059 | 0.586 | 3.008 | 0.103 | 0.530 |
| Spain (NUT INK05) | 651 | 0.188 | 1.222 | 0.185 | 1.201 | 0.033 | 0.212 |
| Finland (NWSSP07 08) | 306 | 0.274 | 1.179 | 0.267 | 1.160 | 0.047 | 0.204 |
| France (INCA2) | 973 | 0.210 | 1.040 | 0.201 | 1.011 | 0.035 | 0.178 |
| United Kingdom (NDNS-RollingProgrammeYears1–3) | 666 | 1.066 | 3.194 | 1.036 | 3.121 | 0.183 | 0.550 |
| Italy (INRAN SCAI 2005–2006) | 247 | 0.088 | 0.673 | 0.087 | 0.662 | 0.015 | 0.117 |
| Latvia (EFSA TEST) | 453 | 0.499 | 2.065 | 0.491 | 2.031 | 0.086 | 0.358 |
| Netherlands (VCPBasis AVL 2007–2010) | 1,142 | 1.893 | 5.635 | 1.856 | 5.541 | 0.327 | 0.977 |
| Sweden (NFA) | 1,018 | 1.315 | 3.962 | 1.293 | 3.896 | 0.228 | 0.687 |
| **Adults** | | | | | | | |
| Austria (ASNS Adults) | 308 | 0.221 | 1.360 | 0.215 | 1.337 | 0.038 | 0.236 |
| Belgium (Diet National 2004) | 1292 | 0.474 | 2.408 | 0.448 | 2.347 | 0.079 | 0.414 |
| Czech Republic (SISP04) | 1,666 | 0.447 | 2.447 | 0.440 | 2.407 | 0.077 | 0.424 |
| Germany (National Nutrition Survey II) | 10,419 | 0.199 | 1.291 | 0.190 | 1.246 | 0.033 | 0.220 |
| Denmark (DANSDA 2005–2008) | 1,739 | 0.442 | 1.814 | 0.434 | 1.784 | 0.077 | 0.314 |
| Spain (AESAN) | 410 | 0.183 | 1.027 | 0.180 | 1.010 | 0.032 | 0.178 |
| Spain (AESAN FIAB) | 981 | 0.105 | 0.704 | 0.103 | 0.692 | 0.018 | 0.122 |
| Finland (FINDIET2012) | 1,295 | 0.174 | 1.242 | 0.137 | 1.001 | 0.024 | 0.176 |
| France (INCA2) | 2,276 | 0.112 | 0.581 | 0.090 | 0.527 | 0.016 | 0.093 |
| United Kingdom (NDNS-RollingProgrammeYears1–3) | 1,266 | 0.358 | 1.604 | 0.333 | 1.519 | 0.059 | 0.268 |
| Hungary (National Repr Surv) | 1,074 | 0.213 | 1.176 | 0.210 | 1.157 | 0.037 | 0.204 |
| Ireland (NANS 2012) | 1,274 | 0.279 | 1.365 | 0.262 | 1.294 | 0.046 | 0.228 |
| Italy (INRAN SCAI 2005–2006) | 2,313 | 0.039 | 0.246 | 0.039 | 0.242 | 0.007 | 0.043 |
| Latvia (EFSA TEST) | 1,271 | 0.162 | 0.880 | 0.159 | 0.865 | 0.028 | 0.153 |
| Netherlands (VCPBasis AVL 2007–2010) | 2,057 | 0.623 | 2.722 | 0.610 | 2.677 | 0.108 | 0.472 |
| Romania (Dieta Pilot Adults) | 1,254 | 0.316 | 1.039 | 0.311 | 1.022 | 0.055 | 0.180 |
| Sweden (Riksmaten 2010) | 1,430 | 0.286 | 1.419 | 0.268 | 1.324 | 0.047 | 0.233 |

| – | Number of subjects | Maximum level scenario | | Brand-loyal scenario | | Non-brand-loyal scenario | |
|---|---|---|---|---|---|---|---|
| | | Mean | 95th percentile | Mean | 95th percentile | Mean | 95th percentile |
| **The elderly** | | | | | | | |
| Austria (ASNS Adults) | 92 | 0.137 | 0.934 | 0.119 | 0.918 | 0.021 | 0.162 |
| Belgium (Diet National 2004) | 1,215 | 0.180 | 1.125 | 0.169 | 1.054 | 0.030 | 0.186 |
| Germany (National Nutrition Survey II) | 2,496 | 0.051 | 0.141 | 0.049 | 0.092 | 0.009 | 0.016 |
| Denmark (DANSDA 2005–2008) | 286 | 0.131 | 0.476 | 0.129 | 0.469 | 0.023 | 0.083 |
| Finland (FINDIET2012) | 413 | 0.056 | 0.381 | 0.043 | 0.000 | 0.008 | 0.000 |
| France (INCA2) | 348 | 0.025 | 0.125 | 0.017 | 0.071 | 0.003 | 0.013 |
| United Kingdom (NDNS-RollingProgrammeYears1–3) | 305 | 0.167 | 0.945 | 0.161 | 0.889 | 0.028 | 0.157 |
| Hungary (National Repr Surv) | 286 | 0.109 | 0.750 | 0.108 | 0.738 | 0.019 | 0.130 |
| Ireland (NANS 2012) | 226 | 0.065 | 0.474 | 0.063 | 0.466 | 0.011 | 0.082 |
| Italy (INRAN SCAI 2005–2006) | 518 | 0.023 | 0.000 | 0.023 | 0.000 | 0.004 | 0.000 |
| Netherlands (VCPBasis AVL 2007–2010) | 173 | 0.228 | 1.422 | 0.224 | 1.398 | 0.039 | 0.246 |
| Netherlands (VCP-Elderly) | 739 | 0.134 | 0.847 | 0.132 | 0.833 | 0.023 | 0.147 |
| Romania (Dieta Pilot Adults) | 128 | 0.221 | 0.657 | 0.217 | 0.647 | 0.038 | 0.114 |
| Sweden (Riksmaten 2010) | 367 | 0.149 | 0.667 | 0.137 | 0.644 | 0.024 | 0.113 |

SAIB: sucrose acetate isobutyrate.