Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

on the presence of 1,2-Benzisothiazolin-3-one as an impurity in saccharin used as a food additive

Question n° EFSA-Q-2004-133

Adopted on 30 November 2006

SUMMARY

Following receipt of a report from The International Sweeteners Association (ISA), the Health and Consumer Protection Directorate-General asked EFSA to assess the health implications of the presence of 1,2-Benzisothiazolin-3-one (BIT) as an impurity in saccharin. Results from analyses have demonstrated the presence of BIT in some samples of commercial saccharin at concentrations in the range of 40-800 mg/kg, with an average value of 200 mg/kg. BIT is one of a number of trace impurities which may be present at varying concentrations depending on the synthetic route and the purification processes used to manufacture saccharin.

BIT is also a biocide used as an emulsion stabiliser in the preparation of food contact materials. The SCF established a t-TDI for BIT of 0.02 mg/kg bw in 1992. The Panel has estimated the intake of BIT from consumption of saccharin at the Acceptable Daily Intake (ADI) for sodium saccharin of 0-5 mg/kg bw and assuming this saccharin contained BIT at the highest reported concentration (800 mg/kg). Using these assumptions an intake of 0.004 mg BIT/kg bw would result from consumption of saccharin at its ADI. This is around 0.05% of (2000-fold lower than) the no-observed-adverse-effect-level (NOAEL) for BIT in a 90-day oral toxicity study in rats conducted since the SCF established the t-TDI.

The Panel accepted that the levels of BIT in commercially available saccharin are usually much lower than 800 mg/kg. The Panel considered that assuming consumption of saccharin at its ADI and containing the highest reported levels of BIT was conservative.

The Panel concluded that even the highest levels of BIT detected in these samples do not represent a safety concern.

KEYWORDS

Saccharin, E954, 1,2-Benzisothiazolin-3-one (BIT), CAS number: 002634-33-5.
BACKGROUND

Directive 94/35/EC on sweeteners for use in foodstuffs authorises the use of Saccharin (E954) only in certain foodstuffs. Directive 95/31/EC laying down specific criteria for purity concerning sweeteners for use in foodstuffs sets limits for the presence of a number of impurities among others for o-toluenesulphonamide and p-toluenesulphonamide (“not more than 10 mg/kg expressed on dry weight basis”). Directive 95/31/EC does not set any limit for the presence of 1,2-Benzisothiazolin-3-one (BIT) as an impurity.

The International Sweeteners Association (ISA) submitted to the Health and Consumer Protection Directorate-General a report with results from recent analyses demonstrating the presence of BIT in samples of commercial saccharin from China at concentrations in the range of 40-800 mg/kg with an average value of 200 mg/kg. The report submitted by ISA includes also an assessment of the potential health implications of the presence of BIT in saccharin.

BIT has been identified as an existing active substance\(^1\) in biocidal products and therefore been added in Annex I of Commission Regulation (EC) 2032/2003\(^2\) concerning the second phase of the 10-year work programme (review programme for the examination of existing active substances) under Directive 98/8/EC on biocidal products.

In the light of the EFSA’s evaluation, the Commission will consider if an amendment of the specifications for saccharin in Directive 95/31/EC is necessary.

TERMS OF REFERENCE

The Commission asks EFSA to provide a scientific opinion on the safety of the presence of 1,2-Benzisothiazolin-3-one (BIT) as an impurity in saccharin.

Chemistry

Name of the substance: 1,2-Benzisothiazolin-3-one

CAS number: 002634-33-5

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\(^{2}\) OJ L 307, 24.11.2003, p. 1
BIT is a biocide used as an emulsion stabiliser in the preparation of food contact materials.

**Previous and ongoing evaluations**

The SCF established a t-TDI of 0.02 mg/kg bw in 1992 (SCF, 1995). A dossier on a specific BIT formulation for use in Food Contact Materials was submitted to EFSA in early 2006.

**Manufacturing process of saccharin**

Saccharin (1,2-benzisothiazolin-3-one 1,1-dioxide) is manufactured using a variety of synthetic routes followed by various purification processes. A number of impurities may be present at very low levels, including ortho-toluene sulphonamide (o-TSA) and para-toluene sulphonamide (p-TSA) which arise from incomplete oxidation to the corresponding carboxylic acids, benzoic acid sulphonamide (ortho- and para-sulphamoyl benzoic acids) arising from incomplete cyclisation to saccharin. A number of other trace impurities may also be present at varying concentrations depending on the synthetic route and the purification processes used to manufacture saccharin.

**Information provided by ISA**

*Analytical data provided by ISA*

ISA reported that during 2003 one of ISA’s European members had detected the presence of a hitherto non-identified impurity in some saccharin samples using gas chromatography separation methods. These samples, which were intended for food use, were obtained from other commercial producers.

This impurity was subsequently characterised and identified by gas chromatography-mass spectrometry (GCMS) as BIT. BIT was detected in all of the saccharin samples analysed, with concentrations in the range of approximately 40-800 mg/kg with an average value of 200 mg/kg. In a sample of saccharin synthesised by an ISA member subsequently analysed, BIT could not be detected and ISA reported that the levels of BIT that may be found in saccharin synthesised by this member are much lower than 10 mg/kg.

*Toxicological and safety data provided by ISA*

A comprehensive literature search on BIT had been undertaken by the ISA to address any possible safety implications of this finding. The results of the search are summarised below.

The only study that ISA identified as suitable for the risk assessment of BIT present in food was identified from the United States Environmental Protection Agency, and a copy obtained under freedom of information. The information is available in the form of a 12 page review of a submitted 90-day feeding study in rats (Fricke, 1993). In that study groups of 12 male and 12 female adult rats were fed diets containing 0, 200, 900 or 4000 mg/kg of BIT *ad libitum*.
for 90-days in a study performed in 1990, that complied with good laboratory practice. Toxicity was assessed by the usual battery of tests, including body weight, food intake, ophthalmological observations, haematology, clinical chemistry, organ weights, gross pathology and histopathology. The only toxicologically significant effects were lower body weights throughout the study in animals given the highest dose, and on some occasions in males given the mid-dose (900 mg/kg diet), and hyperplasia of the forestomach limiting ridge in 11 of 12 males and 11 of 12 females given the highest dose level. Hyperplasia of the forestomach was not reported in animals given the mid-dose level, but gross pathology indicated thickening of the limited ridge in 1 male and 1 female, with no effects found at the lowest dose level. The review by the EPA concluded that the NOEL (no-observed-effect-level) for BIT was 200 mg/kg diet/day in males and 900 mg/kg diet/day in females. These dietary levels resulted in daily intakes of 15.3 and 78 mg/kg bw respectively.

**Risk assessment provided by ISA**

In the case of BIT ISA suggests that its TDI should be estimated as 0.015 mg/kg bw (based on dividing the NOEL in rats by an 1000-fold uncertainty factor to account for inter- and intra-species variability and incorporating an extra uncertainty factor since there are no data from a chronic animal toxicity study). ISA also noted that the observed effects on the limiting ridge of the forestomach were of questionable relevance to humans, who do not have a forestomach, and were reported at doses which also resulted in lower body weights. ISA suggests that using the NOEL from this study would therefore represent a precautionary approach.

Recent analyses have reported up to 800 mg/kg of BIT in some commercial samples of saccharin. Such an impurity level would result in a daily intake of 0.004 mg/kg bw by an individual who consumed saccharin at the ADI established by the SCF (5 mg/kg bw) (SCF, 1995). This would be equivalent to daily intake of about 0.3 mg of BIT per person per day. Even if BIT were present in all samples of saccharin at the highest reported impurity level of 800 mg/kg, the resulting daily intake of BIT from the ingestion of saccharin at the ADI would be only about 25% of the TDI for BIT estimated by ISA. The levels of BIT in commercial saccharin are usually much lower than 800 mg/kg, so that the average long-term daily intake would be considerably lower than 0.004 mg/kg. ISA concludes that the high levels of BIT in saccharin do not represent a health concern.

**Evaluation of the dossier submitted to the Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers (SCCNFP)**

The Panel was provided with the dossier submitted to the Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers (SCCNFP) for the use of benzothiazolinone as a preservative in cosmetics. This provided additional toxicological data to that published in the open literature and included in the ISA submission. The relevant studies for this assessment were 28 and 90 day gavage studies in the rat and in vitro and in vivo genotoxicity studies which were conducted in 2002 (Toxicology Department Rallis Research Centre, 2002a,b). In addition, data on dermal and ocular irritation and skin sensitisation in animals and man were provided.

The 28- and 90- day studies involved dosage of a specific benzothiazolinone product containing 84.29 % BIT (99.02 % pure on a dry weight basis) and 15 % water to groups of 6 male and 6 female Wistar rats at doses of 0, 15, 45 and 135 mg/kg bw/day (12.63, 37.89 and 113.67 mg BIT/kg bw/day) and 0, 10, 30 and 75 mg/kg bw/day (8.42, 25.26 and 63.15 mg BIT/kg bw/day) respectively as a suspension in 0.5 % carboxy methyl cellulose (CMC).
recovery group was included at the highest dose. At 30 mg/kg bw/day (25.26 mg BIT/kg bw/day) in the 90-day study there were some macroscopic and histological changes observed in the non-glandular stomach region, which were considered treatment related but were reversible. These effects may have been due to the irritant nature to the test substance and the dosing regimen. The Panel noted that the NOAEL in these 28- and 90- day studies were 15 mg/kg bw/day (12.63 mg BIT/kg bw/day) and 10 mg/kg bw/day (8.42 mg BIT./kg bw/day) respectively. Although the effects at 30 mg/kg bw/day were reversible and might be related to irritancy associated with gavage administration, the Panel considers that because of the limited duration of the study it would be prudent to regard 10 mg/kg bw/day as the NOAEL.

The Panel considered three **in vitro** (bacterial reverse mutation assay, **in vitro** mammalian cell gene mutation assay, **in vitro** mammalian chromosome aberration test) and two **in vivo** (Mouse Micronucleus Test, Unscheduled DNA Synthesis (UDS) assay in rat liver) genotoxicity tests (Toxicology Department Rallis Research Centre, 2002c, d, e, f; RCC Cytotest Cell Research, 2002). A micronucleus test of BIT at doses of 63.15; 126.3; 210.5 mg a.i./kg bw was carried out in Swiss albino mice-HsdOla: MF1 strain according to OECD Guideline 474. There was a reduction of the ratio PCE/NCE in all treated mice indicating that the test item reached the target cells. BIT did not induce an increase in the number of micronuclei compared to untreated animals (Toxicology Department Rallis Research Centre, 2002f).

An Unscheduled DNA Synthesis (UDS) test at doses of 375 and 750 mg a.i./kg bw was carried out in Wistar Hanlbm: WIST (SPF) rat liver in vivo according to OECD Guideline 486 with sampling times of 2 and 16 hours (RCC Cytotest Cell Research, 2002). There was no indication of induction of UDS by BIT detected following autoradiography on at least three cultures of hepatocytes per animals. The Panel considered the bacterial reverse mutation assay study (Toxicology Department Rallis Research Centre, 2002c) could not be used for evaluation of mutagenicity due to the high toxicity of BIT towards the bacterial cells. The Panel noted that this toxicity was predictable since BIT is a preservative with antimicrobial activity. In the mammalian cell gene mutation study (Toxicology Department Rallis Research Centre, 2002d), the Panel considered that BIT was not mutagenic under the conditions of the test. BIT induced chromosome aberrations at the maximum tested dose (6.4 µg/ml) in the presence of a metabolic activation and at all concentrations in the absence of a metabolic activation system (Toxicology Department Rallis Research Centre, 2002e).

BIT is moderately irritating to the skin, severely irritating to the rabbit eye and is a moderate contact sensitizer. The SCCNFP (2004) noted that there were case reports of allergic contact dermatitis to BIT however its potency appeared lower than other cosmetic preservatives and its irritancy made testing difficult.

**ASSESSMENT**

The Panel concluded that while BIT was clastogenic to CHO mammalian cells in vitro, BIT was not clastogenic following **in vivo** oral administration in mice. The Panel concluded that BIT did not induce UDS in rat hepatocytes following oral administration **in vivo**. The study on the induction of gene mutations on bacterial cells was inadequate due to the (predictable) toxicity of 1,2–benzisothiazolin-3-one to bacterial cells. 1,2–benzisothiazolin-3-one was clastogenic in mammalian cells **in vitro** but was neither mutagenic, clastogenic nor DNA damaging **in vivo**. Therefore, although BIT shows clastogenic activity **in vitro**, two adequately
performed in vivo tests in two different tissues provide no evidence for a genotoxic potential of BIT in vivo.

The Panel considers that low dose BIT exposure via the oral route would be extremely unlikely to cause sensitisation given the rarity of skin allergy to BIT and the limited ability of the systemic route to elicit reactions. The Panel concludes dermal sensitisation is not relevant to the assessment of consumer exposure to BIT present in food as an impurity of saccharin.

The Panel has estimated the intake of BIT from consumption of saccharin at the Acceptable Daily Intake (ADI) for sodium saccharin of 0-5 mg/kg bw and assuming this saccharin contained BIT at the highest reported concentration (800 mg/kg). Using these assumptions an intake of 0.004 mg BIT/kg bw would result from consumption of saccharin at its ADI. This is around 0.05% of (2000-fold lower than) the NOAEL for BIT in a 90-day oral toxicity study in rats.

CONCLUSION

The Panel accepted that the BIT levels in commercially available saccharin are usually much lower than 800 mg/kg. The Panel considered that assuming consumption of saccharin at its ADI and containing the highest reported levels of BIT was conservative.

The Panel considered that consumption at the ADI of saccharin containing the highest reported levels of BIT (800 mg/kg) would result in BIT intakes 2000 times below the NOAEL identified by the Panel from a 90-day oral toxicity study in rats. The Panel concluded that even the highest levels of BIT detected in some samples of saccharin do not represent a safety concern.

DOCUMENTS PROVIDED TO EFSA

Report on the presence of 1,2-benzisothiazolin-3(2H)-one in saccharin and assessment of any possible safety implications submitted by The International Sweeteners Association (AG Renwick, 2004).
Report on 1,2-benzisothiazolin-3(2H)-one (Erik Soderlund, 1994).
Dossier on Promex BIT in food contact materials submitted to EFSA.

REFERENCES


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SCF (1995) Reports of the Scientific Committee for Food (33rd series) First report of the Scientific Committee for Food on certain additives used in the manufacture of plastic
materials intended to come into contact with foodstuffs (opinions expressed until 3 May 1992).
http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_33.pdf


Toxicology Department Rallis Research Centre (2002a), Study No. 3286/01 "Repeated Dose (28-Day) Oral Toxicity Study by Gavage with Promex BIT in Wistar Rats", 2002

Toxicology Department Rallis Research Centre (2002b), Study No. 3287/01 "Repeated Dose (90-Day) Oral Toxicity Study by Gavage with Promex BIT in Wistar Rats", 2002

Toxicology Department Rallis Research Centre (2002c), Study No. 3288/01 "Bacterial Reverse Mutation Test with Promex BIT", 2002

Toxicology Department Rallis Research Centre (2002d), Study No. 3291/01 "In vitro Mammalian Cell Gene Mutation test with Promex BIT", 2002

Toxicology Department Rallis Research Centre (2002e), Study No. 3289/01 "In vitro Mammalian Chromosome Aberration test with Promex BIT", 2002

Toxicology Department Rallis Research Centre (2002f), Study No. 3290/01 "Mutagenicity Study -Micronucleus Test in Swiss Albino Mice with Promex BIT", 2002

SCIENTIFIC PANEL MEMBERS

Fernando Aguilar, Herman Autrup, Sue Barlow, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Natalie Gontard, David Gott, Sandro Grilli, Rainer Gürtler, John Chr. Larsen, Catherine Leclercq, Jean-Charles Leblanc, F. Xavier Malcata, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Rietjens, Paul Tobback, Fidel Toldrá.