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Re-evaluation of stannous chloride (E 512) as food additive

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Abstract

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of stannous chloride and stannous chloride dihydrate (E 512) as food additives. The Panel considered that adequate exposure and toxicity data were available. Stannous chloride is only permitted as food additives in one food category and no reply on the actual use level of stannous chloride (E 512) as a food additive and on its concentration in food was provided by any interested party. According to the Mintel's Global New Products Database (GNPD), stannous chloride was not labelled on any products in the EU nor in Norway. The regulatory maximum level exposure assessment scenario is based on the maximum permitted levels (MPLs) for stannous chloride (E 512), which is 25 mg Sn/kg. The mean exposure to stannous chloride (E 512) from its use as a food additive was below 1.3 μg Sn/kg body weight (bw) per day for all age groups. The 95th percentile of exposure to stannous chloride (E 512) ranged from 0.0 μg Sn/kg bw per day in all groups to 11.2 μg Sn/kg bw per day in adults. Absorption of stannous chloride from the gastrointestinal tract is low there is no concern with respect to carcinogenicity and genotoxicity. Gastrointestinal irritation was reported in humans after ingestion of a bolus dose of 40 mg Sn. The Panel concluded that stannous chloride (E 512) is of no safety concern in this current authorised use and use levels.

Keywords: stannous chloride, stannous chloride dihydrate, E 512, Tin

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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) performed a refined exposure assessment of stannous chloride (E 512) when used as a food additive. The Panel was not provided with a newly submitted dossier and based this assessment on public available data.

Stannous chloride (E 512) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.

Stannous chloride was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970, 1972, 1982, 1988, 2001 and 2005 (JECFA, 1970, 1972, 1982, 1989, 2001, 2006a,b). In 1982, a Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 mg/kg body weight (bw) was established based on gastric irritancy with a threshold concentration of about 200 mg/kg in the food. In 1988, in its evaluation on tin levels as a contaminant from the use of canned foods (JECFA, 1989), the Committee allocated a Provisional Maximum Tolerable Weekly Intake (PTWI) of 14 mg/kg bw (converted from the PMTDI of 2 mg/kg bw) which was confirmed in the latest evaluation.

The EFSA NDA Panel (2006) evaluated the available data from human or animal studies and regarded them as insufficient to derive a tolerable upper intake level for tin.

In 2017, the European Food Safety Authority (EFSA) launched a public call for data aiming at collecting reported use levels from industry or analytical data on several food additives, including stannous chloride (E 512). In response to this call, no reply on the actual use level of stannous chloride (E 512) as a food additive and on its concentration in food was provided by any interested party. No information on the presence of food additives on the label of foods was retrieved from the Mintel's Global New Products Database (GNPD), an online database monitoring new introductions of packaged goods in the market worldwide. Consumption data were available through the EFSA Comprehensive Database.

As no use levels or concentration data were available for stannous chloride (E 512), refined exposure scenarios could not be carried out. The regulatory maximum level exposure assessment scenario is based on the maximum permitted levels (MPLs) for stannous chloride (E 512), which is 25 mg Sn/kg. The mean exposure to stannous chloride (E 512) from its use as a food additive was below 1.3 μg Sn/kg bw per day for all age groups. The 95th percentile of exposure to stannous chloride (E 512) ranged from 0.0 μg Sn/kg bw per day in all groups to 11.2 μg Sn/kg bw per day in adults.

Taking uncertainties into account, the Panel concluded that, the exposure to stannous chloride (E 512) would be an overestimation from its use as food additive according to Annex II.

After oral administration, the absorption of stannous chloride from the gastrointestinal tract of experimental animals is low.

Adequate toxicity data were available. A reduction in body weight gain and haemoglobin was observed with a no observed adverse effect level (NOAEL) of 63 mg/kg bw per day in rats in a 90-day study. There was no concern for genotoxicity and carcinogenicity.

Gastrointestinal irritation was reported in human after ingestion of a bolus dose of 40 mg Sn.

The Panel concluded that stannous chloride (E 512) is of no safety concern in this current authorised use and use level.

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1. Introduction

The present opinion document deals with the re-evaluation of stannous chloride (E 512) 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. 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 European Union before 20 January 2009 has been set up under the 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 Scientific Committee on Food (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 a 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 the European Food Safety Authority 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 the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.3. Interpretation of Terms of Reference (if relevant)

The EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) described its risk assessment paradigm in its Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012). This Guidance states that in carrying out its risk assessments the Panel sought to define a health-based guidance value, e.g. an acceptable daily intake (ADI) (IPCS, 2004) applicable to the general population.

It should be noted that organotin compounds, used for example as fungicides, stabilisers in plastics and as antifouling agents, are not assessed in this opinion.

[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.

1.2. Information on existing authorisations and evaluations

In the European Union (EU), stannous chloride (E 512) 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 the Commission Regulation (EU) No 231/2012[5].

In the EU, stannous chloride (E 512) was evaluated by the SCF in 1990 (SCF, 1991), when the Committee accepted the use of stannous chloride for stabilising the white colour of certain vegetable products because the contribution to the intake of tin from this source lies well below the Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 mg/kg body weight (bw) as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982. In an opinion on acute risks posed by inorganic tin in canned food, the SCF concurred with the JECFA conclusion that levels of 150 mg/kg in canned beverages or 250 mg/kg in other canned foods or higher may cause gastric irritation in some individuals (SCF, 2002).

Stannous chloride (INS 512) was evaluated by JECFA in 1970, 1972, 1982, 1988, 2001 and 2005 (JECFA, 1970, 1972, 1982, 1989, 2001, 2006a,b). In 1982, a PMTDI of 2 mg/kg bw was established based on gastric irritancy with a threshold concentration of about 200 mg/kg in the food. In 1988, in its evaluation on tin levels as a contaminant from the use of canned foods (JECFA, 1989), the Committee allocated a Provisional Maximum Tolerable Weekly Intake (PTWI) of 14 mg/kg bw (converted from the PMTDI of 2 mg/kg bw) which was confirmed in the latest evaluation. The PTWI, expressed as Sn, included tin from food additive uses. In 2001, the Committee concluded 'that insufficient data were available to establish an acute reference dose for inorganic tin. It noted that the gastric irritation that may occur after ingestion of a foodstuff containing tin may depend on the concentration and chemical form of the tin. It reiterated its opinion, expressed at its thirty-third meeting, that the limited human data available indicate that concentrations of 150 mg/kg in canned beverages or 250 mg/kg in other canned foods may produce acute manifestations of gastric irritation in certain individuals. In addition the Committee reiterated its advice, given at its thirty-third meeting that consumers should not store food in open tin-coated cans'. These conclusions were further emphasised in 2005, when 'The Committee concluded that the data available indicated that it is inappropriate to establish an ARfD for inorganic tin, since the occurrence of GI-irritation after ingestion of a food containing tin depends on the concentration and nature of tin in the product, rather than on the dose ingested on a body-weight basis. Therefore, short-term intake estimates were not relevant for the assessment'. In addition, the Committee noted that the basis for the PMTDI and PTWI established at its 26th and 33rd meetings was unclear and these values may have been derived from intakes associated with acute effects. The Committee concluded that it was desirable to (re)assess the toxicokinetics and effects of inorganic tin after chronic exposure to dietary doses of inorganic tin at concentrations that did not elicit acute effects. Stannous chloride (E 512) has also been reviewed by the Nordic Council of Ministers (TemaNord, 2002), who concluded that 'although the toxicological data are less than normally required, the very limited use of stannous chloride as a food additive is considered to pose no risk to food safety'. In addition, they recommended that new data concerning genotoxicity should be taken into consideration.

The EFSA NDA Panel (2006) evaluated the available data from human or animal studies and regarded them as insufficient to derive a tolerable upper intake level for tin. Concerning the indications of reduced zinc absorption after high intake of tin in short-term human studies, they concluded that the current daily intake of tin in the EU (e.g. ranging up to about 6 mg/day in the UK) appears to be well below the lowest intakes reported to cause adverse effects on zinc absorption Furthermore, the NDA Panel considered that regulatory limits of 200 and 100 mg/kg for the concentration of tin in canned food and beverages, respectively, have been established to protect against the occurrence of acute gastrointestinal effects of tin.

According to Commission Regulation (EC) No 1881/2006, maximum permitted residue levels of inorganic tin (mg/kg wet weight) are 200 for canned foods other than beverages, 100 for canned beverages, 50 for canned baby foods, canned infant formula and follow-up formula and canned dietary foods for special medical purposes.

Stannous chloride (CAS 7772-99-8) has been registered under the REACH Regulation 1907/2006 (ECHA, online). ECHA has no public registered data indicating whether or in which chemical products stannous chloride might be used. Stannous chloride has registered industrial uses.

[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.

2. Data and methodologies

2.1. Data

The ANS Panel ANS was not provided with a newly submitted dossier. EFSA launched public call for data[6] and, if relevant, contacted other risk assessment bodies to collect relevant 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 24 April 2018. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however, these were not always available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[7]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of stannous Chloride (E 512) in food products.

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 stannous chloride as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the 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. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to stannous chloride (E 512) 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 levels according to Annex II to Regulation (EC) No 1333/2008[8] and/or reported use levels and analytical data submitted to EFSA following a call for data. Regulatory maximum level exposure assessment scenario was used to calculate exposure (see Section 3.3.4). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[9]), stannous chloride (E 512) is identified by the chemical name stannous chloride dihydrate, has molecular formula $SnCl_2 \cdot 2H_2O$, molecular weight 225.63 g/mol and EINECS (EC) No 231-868-0.

[6] Call for Call for technical and toxicological data on miscellaneous food additives to be re-evaluated under the Regulation (EU) No 257/2010. Published: 11 August 2017. Available from: https://www.efsa.europa.eu/en/consultations/call/170811

[7] Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

[8] Commission 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.

[9] 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.

In marketed products, the hydrous stannous chloride can be found identified by CAS Registry No 10025-69-1.

The anhydrous form ($SnCl_2$) is identified by CAS Registry No 7772-99-8: this identifier is present in ECHA Inventory together with the aforementioned EINECS (EC) number and is reported by JECFA (2006a,b) in the data sheet of stannous chloride used as a food additive (INS No 512), with molecular formula $SnCl_2 \cdot 2H_2O$.

In Commission Regulation (EU) No 231/2012, stannous chloride dihydrate is reported to be very soluble in water, but forming an insoluble basic salt with excess water; the substance is also described as being soluble in ethanol. Stannous chloride dihydrate is a white crystalline solid with an apparent melting point of 37.7°C caused by the loss of crystal water; at 25°C, its density is 2.63 g/cm^3 (IPCS INCHEM, 2004; Gitlitz and Moran, 2007). Stannous chloride dihydrate is also soluble in methanol, ethyl acetate, glacial acetic acid, sodium hydroxide solution and hydrochloric acid; it is insoluble in mineral spirits, petrol naphtha and xylene (Gitlitz and Moran, 2007; Merck, 1996).

Stannous chloride (hydrous and anhydrous) is also known by the synonyms: tin(II) chloride, tin dichloride, tin(II) chloride (1:2), dichlorotin, tin crystals, tin salt, tin protochloride, stannosi chloridum dihydricum (Sax and Lewis, 1987; Gitlitz and Moran, 2007; EDQM, 2016).

3.1.2. Specifications

The specifications for stannous chloride (E 512) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006a,b) are listed in Table 1.

Table 1: Specifications for stannous chloride (E 512) according to Commission Regulation (EU) No 231/2012 and JECFA (2006a,b)

	Commission Regulation (EU) No 231/2012	JECFA (2006a,b)
Definition	EINECS (EC) No: 231-868-0	CAS Registry No: 7772-99-8
	Chemical name: stannous chloride dihydrate	Chemical names: tin(II) chloride, stannous chloride dihydrate
	Chemical formula: $SnCl_2 \cdot 2H_2O$	Chemical formula: $SnCl_2 \cdot 2H_2O$
	Molecular weight: 225.63	Formula weight: 225.63
	Assay: content not less than 98.0%	Assay: not less than 98.0% and not more than 102.0%[(a)]
Description	Colourless or white crystals. May have a slight odour of hydrochloric acid	Colourless or white crystals, odourless or having slight odour of hydrochloric acid
Functional uses	—	Reducer or antioxidant in some bottled or lacquered canned vegetables
Identification	Test for tin(II): passes test	Test for stannous ion: passes test[(a)]
	Test for chloride: passes test	Test for chloride: passes test
	Solubility: soluble in water in less than its own weight of water, but it forms an insoluble basic salt with excess water; soluble in ethanol	Solubility: soluble in water in less than its own weight of water, but it forms an insoluble basic salt with excess water; soluble in ethanol
Purity	Sulfate: not more than 30 mg/kg	Sulfate: not more than 30 mg/kg[(a)]
	Arsenic: not more than 2 mg/kg	—
	Mercury: not more than 1 mg/kg	—
	Lead: not more than 2 mg/kg	Lead: not more than 2 mg/kg[(a)]
	—	Hydrochloric acid insoluble matter[(a)]

(a): In JECFA (2006a,b), a specific test is directly available from the data sheet.

The Panel noted that EINECS (EC) No 231-868-0 is also found as an identifier of the anhydrous form $SnCl_2$ and that CAS Registry No 7772-99-8, identifying the anhydrous form, occurs in JECFA (2006a,b) data sheet for $SnCl_2 \cdot 2H_2O$.

3.1.3. Manufacturing process

Preparation of the anhydrous form ($SnCl_2$) may be carried out by direct reaction of chlorine on molten tin, by heating tin in a hydrogen chloride atmosphere or by reducing a stannic chloride

($Sn^{IV}Cl_4$) solution with tin metal followed by dehydration. Stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) is prepared either by treatment of granulated tin with hydrochloric acid followed by evaporation and crystallisation, or by reduction of a stannic chloride solution with a cathode or tin metal followed by crystallisation (Gitlitz and Moran, 2007).

3.1.4. Methods of analysis in food

For the analysis of tin, reference can be made to the AOAC Official Methods (OM) 980.19 ('Tin in Food') and 985.16 ('Tin in Canned Foods') of the Association of Official Analytical Chemists International: these methods are recommended by the Codex Alimentarius Commission (Codex Alimentarius, 2017) for the determination of tin in processed fruits and vegetables (OM 980.19) and in a variety of beef, poultry, hog, and luncheon meats (OM 985.16). According to OM 980.19, samples are digested with a mixture of concentrated nitric and sulfuric acid (3:1 by volume) at boiling temperature. When digestion is finished, the concentrated clear solution is allowed to cool, added with aqueous ammonium chloride, and taken up to final volume with methyl alcohol and water. As to OM 985.16, samples are digested with concentrated nitric acid and then concentrated hydrochloric acid, and are diluted. Aqueous potassium chloride is added to samples and standards to reduce positive instrument interference. In both methods, tin is determined by atomic absorption spectrophotometry (AAS) at 235.5 nm with oxidising nitrous oxide-acetylene (N_2O-C_2H_2) flame.

Guideline EN 15764 ('Foodstuffs — Determination of Trace Elements — Determination of Tin by Flame and Graphite Furnace Atomic Absorption Spectrometry (FAAS and GFAAS) after Pressure Digestion'), prepared by the Technical Committee CEN/TC 275 'Food Analysis — Horizontal Methods' of the European Committee for Standardization, is a standardised method for the determination of tin in several foods such as carrot puree, tomato puree, pineapple, mixed fruit, white wine, peach powder, tomato powder, powdered beans, powdered fruit yoghurt, and fish powder. Samples are mineralised through pressurised digestion with nitric acid and hydrochloric acid in accordance with Guideline EN 13805 ('Foodstuffs — Determination of Trace Elements — Pressure Digestion'). In the resulting digestion solution, tin is quantified by FAAS or by GFAAS, when present at levels of 43–260 mg/kg and 2.5–269 mg/kg, respectively.

Examples of tin determination by means of different instrumental setups are also available, as per the following examples. Sumitani et al. (1993) analysed a variety of canned food samples comprising fruits, apple juice, vegetables, evaporated milk, clam, meat and salmon. Samples were digested sequentially with nitric acid and hydrochloric acid, diluted to 100 mL, and filtered; tin was then quantified by inductively coupled plasma atomic emission spectrometry (ICP-AES). The standard addition method was used to determine tin concentration. Accuracy of the method was successfully tested by analysing analytical standards containing tin at 50 and 250 mg/kg; the repeatability coefficients of variation were 4.0% and 3.8%, respectively. Recovery of tin from 13 canned foods spiked at 50 and 250 mg/kg was substantially quantitative. The quantification limit for tin standard solution was about 0.5 mg/kg. Mino (2006) described a method using wavelength-dispersive X-ray fluorescence spectrometry following a simple pretreatment to determine levels of dissolved tin in canned foods. Sample syrup or a homogenate solution of fruit (meat) was freeze-dried and diluted with the same weight of cellulose powder; the mixed powder was then quickly formed into a pellet for X-ray measurements. This analytical method had a detection limit of 5 mg/kg and was used to determine levels of tin in several kinds of marketed canned foods. The analytical results indicated that high concentrations (100–300 mg/kg) of tin were present in cans of many kinds of fruit; a relationship was observed between the concentration and the length of time after manufacture. After a can was opened, a rapid increase of dissolved tin amount was observed.

3.1.5. Stability of the substance, and reaction and fate in food

The hydrous and anhydrous forms of stannous chloride are sometimes used interchangeably; however, the latter is in general preferred where stability, concentration, and adaptability are relevant. Tin(II) solutions are readily oxidised by oxygen and, unless carefully protected from air, normally contain some tin(IV). Tin chloride solutions are often used as mild reducing agents as, under specific conditions, the following redox reaction can occur:

$$Sn^{IV}Cl_6^{2-} + 2e^- = Sn^{II}Cl_3^- + 3Cl^-$$

Aqueous solutions of stannous chloride tend to hydrolyse; however, hydrolysis can be prevented by addition of dilute hydrochloric acid as, in solutions containing excess chloride anions, $SnCl_3^-$ ions are formed (see above). Treatment of stannous chloride solutions with alkali hydroxides causes stannous oxide or its metastable hydrate to precipitate; the hydrolysis of Sn^{II} in aqueous media can yield various products, such as $[Sn(OH)]^+$, $[Sn_2(OH)_2]^{2+}$, $[Sn_3(OH)_4]^{2+}$ and finally $Sn_3O_2(OH)_2$, the hydrous tin(II) oxide (Cotton et al., 1999; Gitlitz and Moran, 2007).

3.2. Authorised uses and use levels

Maximum levels of stannous chloride (E 512) have been defined in Annex II to Regulation (EC) No 1333/2008[10] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, stannous chloride (E 512) is an authorised food additive in the EU at 25 mg Sn/kg in only one category listed in Table 2.

Table 2: MPLs of stannous chloride (E 512) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
04.2.3	Canned or bottled fruits and vegetables	E 512	Only white asparagus	25 (expressed as Sn)

MPL: maximum permitted level.

Stannous chloride (E 512) is not authorised according to Annex III of Regulation (EC) No 1333/2008.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of stannous chloride (E 512)

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* (*QS*).

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 stannous chloride (E 512).

In response to this call, no reply on the actual use level of stannous chloride (E 512) as a food additive and on its concentration in food was provided by any interested party.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.5 million food and beverage products of which more than 900,000 are or have been available on the European food market. The Mintel GNPD[12] started covering EU's food markets in 1996; currently, it contains data from 20 out of its 28 Member-States and Norway.

For the purpose of this Scientific Opinion, the Mintel's GNPD[13] was used for checking the labelling of food and beverages products and food supplements for stannous chloride (E 512) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to the Mintel's GNPD, stannous chloride (E 512) was not labelled on any products in the EU nor in Norway, between January 2013 and April 2018.

[10] 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.
[11] https://www.efsa.europa.eu/en/data/call/170223
[12] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[13] http://www.gnpd.com/sinatra/home/ accessed on 28/4/2018.

3.3.3. Food consumption data used for exposure assessment

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 Assessment' (EFSA, 2011a). Consumption surveys added in the Comprehensive database in 2015 were also taken into account in this assessment.[14]

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 includes the currently best available food consumption data across Europe.

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 3).

Table 3: Population groups considered for the exposure estimates of stannous chloride (E 512)

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[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(b)]	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, Netherlands, 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[(b)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Netherlands, Sweden, UK

(a): The term 'toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): 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 exposure estimates. In practice, the FoodEx food codes were matched to the FCS food categories.

Food categories considered for the exposure assessment of stannous chloride (E 512)

The food category in which the use of stannous chloride (E 512) is authorised is 'Canned or bottled fruit and vegetables' with the restriction 'only white asparagus'. While 'asparagus' is included in the nomenclature of the EFSA Comprehensive Database (FoodEx classification system, EFSA, 2011b), the restrictions 'canned/bottled' and 'white' are not referenced. For this reason, the entire food category

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

'asparagus' was considered for the exposure assessment and this might have generated an overestimation of the exposure.

3.3.4. Exposure to E 512 from its use as a food additive

The Panel estimated chronic exposure to stannous chloride (E 512) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to stannous chloride (E 512) was calculated by multiplying MPL of stannous chloride (E 512) expressed as Sn for the food item 'asparagus' with its respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per consumption event 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.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 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, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to stannous chloride (E 512) was carried out by the ANS Panel based on MPL as set down in the EU legislation, defined as the *regulatory maximum level exposure assessment scenario*.

As no use levels or concentration data were available for stannous chloride (E 512), refined exposure scenarios could not be carried out.

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. For stannous chloride (E 512), the MPL used in the assessment was 25 mg Sn/kg.

The Panel considers the exposure estimates derived following this scenario as the most conservative since it is assumed that that the population will be exposed to the food additive present in food at the MPL over a longer period of time.

Dietary exposure to stannous chloride (E 512)

Table 4 summarises the estimated exposure to stannous chloride (E 512) from its use as a food additive in six population groups according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix A.

Table 4: Summary of dietary exposure to stannous chloride (E 512) expressed as Sn from its use as a food additive in the maximum level exposure assessment scenario, in six population groups (minimum–maximum across the dietary surveys in μg Sn/kg bw per day)

	Infants (12 weeks–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	0.0–0.2	0.0–0.5	0.0–1.0	0.0–0.8	0.0–1.2	0.0–1.3
95th percentile	0	0.0–1.8	0.0–4.2	0.0–3.3	0.0–11.2	0.0–4.9

bw: body weight.

From the *regulatory maximum level exposure assessment scenario*, the mean exposure to stannous chloride (E 512) from its use as a food additive was below 1.3 μg Sn/kg bw per day for all age groups. The 95th percentile of exposure to stannous chloride (E 512) ranged from 0.0 μg Sn/kg bw per day in all groups to 11.2 μg Sn/kg bw per day in adults.

Uncertainty analysis

Uncertainties in the exposure assessment of stannous chloride (E 512) have been discussed above. In accordance with the guidance provided by EFSA related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 5.

Table 5: 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)	+
Food categories selected for the exposure assessment: inclusion of food category without considering accurately the restriction/exception	+
Maximum level exposure assessment scenario: • exposure calculations based on the MPL	+

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

The additive stannous chloride (E 512) is only authorised in one very specific food (canned/bottled white asparagus). The restrictions related to the authorisation 'white' and 'canned/bottled' were not taken into account as not referenced in the Comprehensive Database. Mintel data indicated that the additive is not used in the EU.

As no use levels or concentration data were available for stannous chloride (E 512), only the MPL (expressed as Sn) exposure scenario could be carried out.

Overall, the Panel considered that the uncertainties identified would, result in an overestimation of the exposure to stannous chloride (E 512) as a food additive in European countries considered in the EFSA Comprehensive database.

3.3.5. Exposure to tin via the regular diet and other sources

Dietary intake of tin was reviewed by the NDA Panel (2005). A mean daily intake of tin in the Netherlands was estimated to be 0.65 mg, which was lower than the intake of 1.7 mg, estimated from a study eight years earlier (van Dokkum et al., 1989).

The average intake of tin in the UK was estimated in a total diet study to be 1.8 mg/day, with an upper 97.5th percentile of 6.3 mg/day (EGVM, 2002). This was decreased from an average intake of 2.4 mg/day estimated three years earlier. Canned food products were reported to be the main contributor to the intake of food in the UK.

Tin dietary intake in France was reported to be 2.7 mg/day (Biégo et al., 1999). The contribution from food stored in tin cans was 98%, which according to the study, represents 5.6% of the total daily consumption of food by French citizens.

3.4. Biological and Toxicological data

Stannous chloride has been reviewed by JECFA (1970, 1972, 1982, 1989, 2001, 2006a,b), WHO (1980, 2004), SCF (1991, 2002), TemaNord (2002), Blunden and Wallace (2003), ATSDR (2005), Ausschuss für Gefahrstoffe AGS (2008) and Ostrakhovitch, 2015. Some of the studies cited in these reviews are unpublished or not available. Information from these studies is only described and cannot be validated. In the present opinion, mainly human data are used for the risk assessment of the absorption, distribution, metabolism and excretion (ADME) and gastrointestinal effects, while data from animal studies are used as source of information on ADME, systemic effects including genotoxicity, reproductive and developmental toxicity.

3.4.1. Absorption, distribution, metabolism and excretion

Human study

Eight adult males received mixed diets, either control diet with 0.11 mg Sn daily or a test diet with 49.7 mg Sn daily (added as stannous chloride to juices) for 20 days in a cross-over design. The

apparent retention (intake – faecal loss – urinary loss) was 0.03 ± 0.03 mg/day and 1.3 ± 1.5 mg/day from the control and test diet, respectively. The authors concluded that the percentage apparent absorption was higher at the low exposure, –4% to 71% (average 50%), than at the higher exposure, –7% to 9% (average 3%), and suggested that the gut appeared to be an effective barrier against tin absorption (Johnson and Greger, 1982).

Animal studies

Absorption

Whole body and tissue retention kinetics have been measured in RF mice, rhesus monkeys (*Macaca mulatta*), Sprague–Dawley rats, African white-tailed rats (*Mystromys albicaudatus*) and beagle dogs after one oral dose of stannous chloride by gavage. In all four species, the elimination kinetics of absorbed stannous chloride were similar and could be described with 1- or 2-compartment models in which 96–100% of the initially applied substance is eliminated with a half-life of 0.2–0.4 days (Furchner, 1976).

When radiolabelled stannous chloride was administered by gavage to rats, 90–99% of the administered radioactivity was excreted in the faeces within 48 h. The mean percentage detected in the urine was less than 1.1% and in organs or tissues examined less than 0.005% was detected (Fritsch et al., 1977).

Male Wistar rats were given stannous chloride dihydrate in drinking water for 1–18 weeks. No increase in blood levels of tin were seen at doses up to 250 mg stannous chloride/L (equivalent to 11.8–15.8 mg Sn/kg bw per day). At doses of 500 mg/L (equivalent to 23.6–31.6 mg Sn/kg bw per day), tin concentrations in blood increased in the first week of application and remained at 2–7 μg/L (2–5 times of control values) for the rest of the study. The authors concluded that the mucosal barriers are effective in preventing absorption of low tin doses but are overcome at higher doses (Savolainen and Valkonen, 1986).

Distribution

Charles-River CD mice were administered stannous chloride in drinking water, 5 mg Sn/L, for their lifespan. The exposure is equivalent to 0.45 mg Sn/kg bw per day. Increased tin levels (1.2–4.4 mg/kg wt tissue) were found in the kidneys, liver, heart, lungs, spleen, and thyroid compared to controls (0.5 mg/kg wet tissue) (Schroeder and Balassa, 1967).

After lifelong exposure of Long-Evans rats to stannous chloride in the drinking water, 5 mg Sn/L (equivalent to 0.25 mg/kg bw per day), the mean tin concentrations in the liver, heart, lungs and spleen (bone was not examined) were increased approximately two- to threefold, however, not statistically significantly different from the controls (Schroeder et al., 1968).

Administration of anhydrous stannous chloride in the diet to rats (1,000 and 2,000 mg/kg diet, equivalent to 31.3 and 62.6 mg Sn/kg bw per day) for 2 years resulted in a 10-fold higher concentration of tin in the bone and kidney than in the liver. Similar exposure to mice, equivalent to 93.4 and 186.8 mg Sn/kg bw per day, produced a 10-fold higher concentration of tin in the bone than in the kidney and liver (NTP, 1982).

In rats fed diets containing stannous chloride (100–500 mg Sn/kg diet, equivalent to 12–60 mg Sn/kg bw per day) for 3 weeks, tin accumulated in the tibia and the kidneys in a dose-dependent manner, and it was also found in the liver (Johnson and Greger, 1985).

Wistar rats were given stannous chloride dihydrate in drinking water at concentrations of 100, 250 and 500 mg stannous chloride dehydrate/L for 18 weeks. At a dose of 100 mg/L (equivalent to 4.7 mg Sn/kg bw per day), the tin concentration in the brain of treated rats was 0.8–2.3 μg Sn/kg wet weight) and not statistically significantly different from that of controls (0.6–1.2 μg Sn/kg wet weight); at 250 mg/L (equivalent to 11.8 mg Sn/kg bw per day), the tin concentration in the brain was increased after 15 weeks to 2.3 μg Sn/kg wet weight and after 18 weeks to 4.5 μg Sn/kg wet weight; and at 500 mg/L (equivalent to 23.6 mg Sn/kg bw per day), the tin concentration in the brain increased throughout the 18 weeks exposure to 9.5 μg Sn/kg wet weight (Savolainen and Valkonen, 1986).

Overall, after oral administration the absorption of stannous chloride from the gastrointestinal tract of experimental animals is low (< 5%). Unabsorbed ingested stannous chloride is mainly (90–99%) excreted with the faeces within 48 h, while absorbed tin distributes mainly to the bone, but also to the liver, kidneys and brain. In humans, the apparent percentage absorption is around 3% at moderate levels of tin in the diet and higher at low background levels in the diet.

3.4.2. Acute toxicity

The acute oral toxicity of stannous chloride was tested in various species and reported LD_{50} values varied widely (Eckhardt, 1909; Calvery, 1942; Le Breton, 1962, cited in JECFA, 1982; Pelikan et al., 1968, cited in JECFA, 1982; Halacka, 1970; Conine et al., 1975).

In an NTP study, groups of five males and five females F344/N rats and B6C3F1/N mice were given a single dose of anhydrous stannous chloride by gavage (rats: 93.75–1,500 mg/kg bw, equivalent to 59.7–939 mg Sn/kg bw; mice: 150–2,400 mg/kg bw, equivalent to 93.9–1,503 mg Sn/kg bw). The animals were observed for 16 days. In rats, all males survived, deaths occurred in one female rat in each of the two highest dose groups. All mice in the highest dose group died, and there were single deaths in the dose groups receiving 376 and 752 mg Sn/kg bw (NTP 1982).

Overall, the Panel noted great variations in acute oral toxicity of stannous chloride between species and between studies.

3.4.3. Short-term and subchronic toxicity

Several short-term and subchronic studies have been performed in mice and rats (Table 6). The main adverse effects reported were reduced body weight gain and reduced haemoglobin levels. The lowest no observed adverse effect level (NOAEL) in a 90-day study in rats was 63 mg Sn/kg bw per day, while a much higher NOAEL, 1,878 mg Sn/kg bw per day, was reported in mice (NTP, 1982 (Table 6)).

Table 6: Short-term and subchronic oral toxicity of stannous chloride

Species; strain; sex; n/group	Duration [days]	Dose	NOAEL (in studies ≥ 28 days)	Effects/remarks	Reference
Mouse; B6C3F1/N; m, f; 5 m, 5 f	14	0; 1,900; 3,800; 7,500; 15,000; 30,000 mg anhydrous stannous chloride/kg diet		Minor effect on body weight in females in highest dose groups	NTP (1982)
Rat; F344/N; m, f; 5 m, 5 f	14	0; 1,900; 3,800; 7,500; 15,000; 30,000 mg anhydrous stannous chloride/kg diet		Weight loss in the highest dose group	NTP (1982)
Rat; Wistar; m, f; 10 m, 10 f	28	0; 50; 150; 500 mg Sn/kg diet	150 = 13.5 mg Sn/kg bw per day	Reduced body weight gain, reduced levels of haemoglobin	De Groot (1973)
Rat; Wistar; m, f; 10 m, 10 f	28	0; 0.03; 0.1; 0.3; 1.0% stannous chloride dehydrate/kg diet	0.1% = 63 mg Sn/kg bw per day	Reduced body weight gain; reduced haemoglobin ≥ 0.3%	DeGroot et al. (1973)
Rat; Wistar; m; 10	28	0; 250; 500 mg Sn/kg diet	250 = 30 mg Sn/kg bw per day	Reduced body weight gain, reduced haemoglobin levels	Janssen et al. (1985)
Rat; Wistar; m; 7	28	0; 10; 50; 100; 200 mg Sn/kg diet	No NOEL was identified	Dose-dependent decrease in haemoglobin and plasma levels of Cu, Fe and Zn	Pekelharing et al. (1994)
Mouse; B6C3F1/N; m, f; 10 m, 10 f	90	0; 1,900; 3,800; 7,500; 15,000; 30,000 mg anhydrous stannous chloride/kg diet	15.000 = 1.878 mg Sn/kg bw per day	Reduced body weight gain in the highest dose group; Gross distension of the caecum ≥ 3,800 mg/kg	NTP (1982)
Rat; F344/N; m, f; 10 m, 10 f	90	0; 500; 1,000; 1,900; 3,800; 7,500 mg anhydrous stannous chloride/kg diet	3.800 = 214 mg Sn/kg bw per day	Reduced body weight gain (> 10%) in highest dose group. Gross distension of the caecum and reddened gastric mucosa ≥ 3,800 mg/kg	NTP (1982)

Species; strain; sex; n/group	Duration [days]	Dose	NOAEL (in studies ≥ 28 days)	Effects/remarks	Reference
Rat; Wistar; m, f; 10 m, 10 f	90	0; 0.03; 0.1; 0.3; 1.0% in diet	0.1% = 63 mg Sn/kg bw per day	Reduced body weight gain, reduced levels of haemoglobin and hematocrit; homogeneous cytoplasm of hepatocytes and bile duct hyperplasia	DeGroot et al. (1973)
Rat; Wistar; m; 6	90	0; 0.3; 1.0; 3 mg Sn/kg bw per day by gavage	0.6 mg Sn/kg bw per day	Reduced calcium concentration in serum and femur	Yamaguchi et al. (1980)

NOAEL: no observed adverse effect level; NOEL: no-observed-effect-level; bw: body weight.

Overall, repeated administration of stannous chloride reduced body weight gain and in haemoglobin levels. Gross distension of the caecum was observed in mice and rats. Also, effects on body status of copper, iron, zinc and calcium were reported (see Section 3.4.7). Rats were more sensitive than mice to the effects of stannous chloride on body weight gain and haemoglobin and the lowest NOAEL in rats in a 90-day study was 63 mg Sn/kg bw per day.

3.4.4. Genotoxicity

In vitro

Stannous chloride was not mutagenic in the *Salmonella*/microsome assay using TA98, TA100, TA1535, TA1537 and TA1538 up to 10 mg/plate with and without metabolic activation (Mortelmans et al., 1986; Prival et al., 1991). Also, in the umu test using *Salmonella* Typhimurium TA1535/psk1002 no induction of SOS response was reported with and without S9 (Yamamoto et al., 2002). In another reverse mutation assay, stannous chloride was negative for *S.* Typhimurium TA97, TA98 and TA100 but positive for TA102, a strain detecting oxidative and alkylating mutagens and reactive oxygen species (ROS) (Pungartnik et al., 2005). In the WP2-Mutoxitest with *Escherichia coli* IC188 (the WP2 uvrA/pKM101 OxyR+) and IC203 (WP2 uvrA/pKM101 OxyR-deficient in the synthesis of antioxidant enzymes and with enhanced sensitivity to ROS), positive results were obtained in a pre-incubation test, from 1.25 mM onwards (Pungartnik et al., 2005), while negative results were reported in a limited plate incorporation assay at the single dose tested of 1,000 μg/plate (Martinez et al., 2000).

In different *E. coli* DNA-repair mutants, stannous chloride induced SOS response (Bernardo-Filho et al., 1994) and forward mutations in a vector carrying the supF tRNA gene, largely attributed to 8-oxoG formation by the study authors (Cabral et al., 1998). Stannous chloride caused strand breaks in plasmid DNA *in vitro* and the authors suggested that in addition to ROS formation also direct binding to DNA is involved (De Mattos et al. 2000). The SOS chromotest showed a positive result in Escherichia coli PQ37 and PQ35, but a high degree of bacterial toxicity complicates the interpretation of the data (Olivier and Marzin, 1987). In the Rec-assay with *Bacillus subtilis* H17+ and M45–, stannous chloride did induced mutations (Nishioka, 1975, Kada et al., 1980).

In the experimental system with *Saccharomyces cerevisiae*, D7 stannous chloride did not induce mutations or gene conversions (Singh, 1983). In another study, in yeasts stannous chloride induced reversion of his1-798, his1-208, lys1-1 and hom3-10 mutant alleles and intragenic mitotic recombination (Pungartnik et al., 2005). Testing in five isogenic haploid strains of yeast differing from each other in a particular repair-deficiency demonstrated differential sensitivity depending on the repair deficiency (Pungartnik et al., 2005). Based on these findings, together with the results obtained with bacterial assays as quoted above, the authors proposed that stannous chloride induced genotoxicity occurs via error-prone repair of DNA damage that is produced by reactive oxygen species.

In Chinese hamster ovary (CHO) cells, stannous chloride induced dose-related DNA damage, detected by sucrose gradient analysis (Mclean et al., 1983b). Significant increases in sister chromatid exchanges and chromosomal aberrations were observed in CHO cells after treatment with stannous chloride (Gulati et al., 1989). The studies of DNA damage induction and inhibition of methyl methane sulfonate (MMS) induced DNA damage repair in Chinese hamster lung cells (V79) using comet assay showed that stannous chloride (anhydrous) induced DNA damage and inhibited MMS-induced DNA repair. The authors suggested that this substance can interfere with DNA repair systems and may thus contribute to increased mutation rate (Viau et al., 2009). Stannous chloride was readily taken up by

human white blood cells and caused dose-dependent increase of DNA strand breaks, measured by fluorimetric analysis of DNA unwinding (McLean et al., 1983a). In K562 human leukaemia cells, that are resistant to ROS, stannous chloride dihydrate induced significant ($p < 0.001$) dose-related loss of cell viability and DNA damage. Both actions appear to be correlated with ROS formation and direct binding to DNA (Dantas et al., 2002). Significant elevation of chromosome aberrations and sister chromatid exchanges were observed after treatment of human peripheral lymphocytes with stannous chloride (Ganguly, 1993). In another study, stannous (IV) chloride (CAS No. 7646-78-8; SnCl4; > 99.9% pure), tested without metabolic activation at concentrations up to 50 μM, did not induce micronuclei (MN) formation in isolated peripheral human lymphocytes (Damati et al., 2014) Finally, stannous chloride was not mutagenic in the forward mutation assay at Tk locus in mouse lymphoma cells (Myhr and Caspary, 1991).

In vivo

Stannous chloride induced a dose-dependent increase in the MN frequency in peripheral erythrocytes of adult zebrafish (*Danio rerio*) after 120 h exposure to doses ≥ 50 μM (Sisman, 2011).

Stannous chloride dihydrate did not induce MN in the bone marrow of mice after intraperitoneal (i.p.) injections of up to 210 mg/kg bw per day for 3 days and sampling 24 h after the final treatment (Shelby et al., 1993, Shelby and Witt, 1995). Increased incidence of chromosomal aberration were observed in mice bone marrow after single i.p. injection of up to 210 mg stannous chloride/kg bw, at sampling time of 36 h. However, the response was weak with no dose response and was significant only in two out of three experiments at 36 h sampling time and negative at 17 h sampling time (Shelby and Witt, 1995). Moreover, this result was based on the scoring of an inadequately low number of metaphases (50 per animal instead on 200 per animal as recommended in OECD TG 475). Overall, the Panel considered this study as inconclusive.

In another test, stannous chloride did not induce micronuclei in Wistar rat bone marrow polychromatic erythrocytes when evaluated 36 h after an intravenous (i.v.) injection of 100 μL at a concentration of 500 μg/mL (approx. 180 μg/kg bw) (de Mattos et al., 2012). In the same experiment, the rat blood cells were additionally evaluated in a Comet assay 3 and 24 h after a single dose administration. After 3 h, stannous chloride induced a statistically significant increment in comet numbers in peripheral blood cells but after 24 h no increase compared to the control was observed. This result may indicate that DNA lesions induced by treatment and detected at the early time by comet assay are processed by error-free repair, preventing their fixation in permanent chromosomal damage (e.g. chromosome breaks visualised as MN). In this respect, the Panel also noted that in case of discrepant results mutation assays have greater relevance than indicator assays for genotoxic hazard identification (ECHA, 2014).

El-Makawy et al. (2008) performed a reproductive and teratogenicity study of stannous chloride (CAS No: $SnCl_2x2H_2O$; 10025-69-1; 99% pure) in Swiss mice (described in Section 3.3.5) in which they performed also the analyses of the induction of chromosomal aberrations (CA). During 3-week treatment with stannous chloride administered by gavage daily at doses 2, 10 and 20 mg/kg bw, the females were paired with males and the treatment was continued throughout gestation period (gestation day (GD) 0 was the day when vaginal plug was observed). Females were sacrificed at day 18 of gestation. Cytogenetic analysis was performed on bone marrow of dams and fetal tissues. Dams were injected intraperitoneally with colchicine 1.5 h prior to sacrifice. Significant dose-dependent increase of chromosomal aberrations was observed in dams exposed to 10 and 20 mg stannous chloride/kg bw per day. However, this study has a number of experimental and reporting deficiencies: insufficient details on the experimental procedure for the bone marrow preparation are given, the details on clinical signs of toxicity, gross necropsy observations or changes in body weight of the treated animals are completely lacking, which prohibits a correlation of toxicity with clastogenicity, the CA frequencies of the control animals are 6- to 20-fold above historical control CA frequencies observed in other laboratories. Moreover, extremely high fetal loss was observed in this study at low doses that is not in concordance with other developmental toxicity studies (see Section 3.4.6). Due to the significant deficiencies in reporting and uncertainties in reliability of the reported results this study cannot be used for genotoxicity assessment.

In summary, *in vitro* stannous chloride did not induce mutations in the *Salmonella*/microsome assay with exception of strain TA102, detecting oxidative mutagens and ROS. In B*. subtilis* stannous chloride was negative; genotoxic effects were observed in some, but not all, tests with stannous chloride in yeast (reversion and intragenic recombination) and *E. coli* (SOS response and forward mutation). The study authors attributed the observed effects to ROS generation. In mammalian cells cultures,

stannous chloride induced sister chromatid exchanges (SCE) and chromosomal aberrations (ABS) in hamster ovary cells, DNA damage in hamster lung fibroblasts and human leukaemia K562 cells. Also, in these systems, there is some evidence that the DNA damaging effect of stannous chloride may arise from a secondary mechanism involving reactive oxygen species. In CHO and peripheral lymphocytes, stannous chloride induced SCE and CA whereas induction of MN in human peripheral lymphocytes was not observed. Stannous chloride also did not induce mutations in mouse lymphoma cells. *In vivo*, no indication of genotoxicity was obtained in rats in a micronucleus assay by i.p. administration. In mice, the i.v. administration of stannous chloride induced a transient increase of DNA damage in erythropoietic cells which did not result in permanent chromosomal damage (chromosome breaks visualised as micronuclei).

Overall, the *in vitro* genotoxicity data on stannous chloride show a pattern of activity which suggest the involvement of ROS, even though the contribution by other mechanisms (inhibition of DNA repair, direct DNA binding) cannot be ruled out. *In vivo* no indication of permanent DNA damage was obtained in rats and mice in experimental condition associated with significant internal exposure (i.p. and i.v. administration). The Panel considered that stannous chloride used as a food additive does not represent a concern with regard to genotoxicity.

3.4.5. Chronic toxicity and carcinogenicity

Diets containing 1,000 and 2,000 mg anhydrous stannous chloride/kg were fed to groups of 50 F344/N rats and 50 B6C3F1/N mice of each sex for 105 weeks (NTP, 1982). In rats, the doses are equivalent to 31.3 and 62.6 mg Sn/kg bw per day, and in mice, the doses are equivalent to 93.9 and 187.8 mg Sn/kg bw per day. In total, 50 untreated rats and 50 untreated mice of each sex served as controls. In rats, no effects were reported on mean body weight gain and feed consumption compared to control rats; survival of the rats in the high-dose group was lower than that of controls or rats receiving the low dose (control: 37/50; 1,000 mg/kg diet: 39/50; 2,000 mg/kg diet: 30/50); C-cell adenomas and carcinomas of the thyroid and adenomas of the lung were observed in male rats (table 13). In mice, there was no effect on mean body weight gain and feed consumption compared to control mice; survival of control mice was less than in treated animals (control: 32/50, 1 000 mg/kg diet: 42/50, 2 000 mg/kg diet: 45/50), survival of the female mice appeared to be negatively correlated to the dose (control: 38/50, 1 000 mg/kg diet: 33/50, 2 000 mg/kg diet: 28/50); hepatocellular adenomas and carcinomas and histiocytic malignant lymphomas were observed in female mice (Table 7).

Table 7: Carcinogenicity of stannous chloride in rats and mice after exposure via the diet (NTP, 1982)

	Control	1,000 mg/kg diet	2,000 mg/kg diet	Historical controls*
Rats, male				
C-cell adenomas (thyroid)	2/50 (4%)	9/49 (18%)	5/50 (10%)	24/288 (8.3%)
C-cell adenomas and c-cell carcinomas (thyroid)	2/50 (4%)	13/49 (27%)	8/50 (16%)	32/288 (11.1%)
Adenomas (lung)	0/50 (0%)	0/50 (0%)	3/50 (6%)	6/289 (2.1%, range: 0–6%)
Mice, female				
Adenomas and carcinomas (liver)	3/49 (6%)	4/49 (8%)	8/49 (16%)	24/297 (8%, range: 4–18%)
Histiocytic lymphomas	0/50 (0%)	0/50 (0%)	4/50 (8%)	9/298 (3%, range: 0–6%)
Lymphomas and leukaemias	6/50 (12%)	10/49 (20%)	11/49 (22%)	67/298 (22%)

*: Reported in NTP (1982).

C-cell adenomas of the thyroid were statistically significantly increased in low-dose male rats, C-cell carcinomas of the thyroid in males did not occur at a statistically significant incidence, adenomas and carcinomas combined were seen in male rats with a positive trend and the incidence was statistically significantly (1,000 mg/kg diet, 2,000 mg/kg diet) higher than in the controls, but did not demonstrate a dose–response relationship. If the historical control rate is used as a basis of comparison, the effect at low dose remains significant, but not at the high dose. Adenomas of the lung were found in high-dose male rats, but incidence was not statistically significantly increased compared to the control group.

The increase in incidence of hepatocellular adenomas and carcinomas in female mice was statistically significant and dose-related, but the incidence in the high-dose group falls within the range of the historical control. Therefore, the increase was not considered by the authors to be substance related.

The incidence of histiocytic lymphomas was increased in high-dose female mice, but was within the range of historical controls. The incidence of lymphomas and leukaemias in the dosed groups was similar to the historical incidence for female control mice.

Under the conditions of this bioassay, the authors judged stannous chloride not to be carcinogenic to male or female F344/N rats or B6C3F1/N mice, although they did not ruled out whether the increase in incidence of C-cell tumours of the thyroid gland in male rats might have been associated with the administration of the test chemical. The Panel noted that these tumours were seen in male rats only, did not show a dose–effect relationship and may be related to the unusual low incidence of thyroid adenomas and carcinomas in the controls.

Groups of 60 Cpb-Wu rats (30 males and 30 females) were fed diets containing stannous chloride in doses of 0 (control), 200, 400 and 800 mg Sn/kg diet for 115 weeks. The doses are equivalent to 0, 10, 20 and 40 mg Sn/kg bw per day. No differences in mortality rates between the groups were reported, also no effects on growth or food intake was observed, but food efficiency was decreased in the highest dose group. A transient decrease in haemoglobin and haematocrit values was seen. No other compound-related effects were reported. Accumulation of tin was not observed in the organs examined with exception of bone after application of 800 mg Sn/kg diet (Sinkeldam et al., 1981; unpublished results, cited in JECFA, 1982).

Stannous chloride was applied for their lifespan in drinking water (5 mg Sn/L, equivalent to 0.25 mg Sn/kg bw per day) to 56 male and 56 female Long-Evans rats; 56 male and 76 female rats served as controls, for their whole lifespan. The survival of the treated females was significantly ($p < 0.005$) reduced. Animals exposed to tin showed increased incidences of fatty degeneration of the liver (67% vs 37% in controls) and of vacuolar changes in the proximal tubules of the kidneys (32% vs 18% in controls). No carcinogenic effect was observed (Schroeder et al., 1968). The Panel considered this study of limited value for risk assessment because of the low dose administered, lack of data on the tin levels in control diets, and the high background incidence of pathological changes.

Overall, studies on chronic toxicity and carcinogenicity performed with rats and mice did not indicate concern for carcinogenicity of stannous chloride.

3.4.6. Reproductive and developmental toxicity

Reproductive toxicity studies

In a multigeneration study, CPB:WU rats (randomly bred) were given stannous chloride ($SnCl_2.2H_2O$) in diet (0, 200, 400 and 800 tin mg/kg) for three generations (equivalent to 10, 20 or 40 mg tin/kg bw per day) (EFSA document: Sinkeldam et al., 1979, unpublished report). The iron content of the diets was increased for the F2 generations onwards (from initially 70 mg Fe/kg diet to 140 mg Fe/kg diet). Stannous chloride did not affect growth of the parents, fertility, number of offspring per litter or birth weight. The increased mortality of F2 offspring during the first half of lactation was corrected by increasing the iron content in the mothers' diet. At weaning, the pup weights were reduced in the high dose groups (all generations). In the other dose groups, a decrease in pup weight was observed, however, it should be noted that these litters were larger. Haemoglobin levels were reduced during lactation but not thereafter at any dose levels in any generation. The pathological examination of rats from the F3b and F3c generation revealed no microscopic changes in the liver and spleen of F3b and F3c pups at the age of 4 weeks after weaning. The authors considered the abnormalities observed in the pups to be attributed to 'sub-optimal' level of iron in the diet. The authors concluded that stannous chloride administered for three generations up to 800 mg Sn/kg diet (40 mg Sn/kg bw per day) did not cause reproductive effects. The Panel identified the highest dose given, 31.2 mg Sn/kg bw per day as the NOAEL for maternal and developmental toxicity.

Swiss albino mice (10 females and 5 males/group) were dosed daily by gavage with 0, 2 (1.1), 10 (5.3) or 20(10.5) mg stannous chloride ($SnCl_2.2H_2O$) (Sn)/kg bw per day (El-Makawy et al., 2008). During week 3 of dosing, females were paired with males (2:1). The treatment was continued throughout the gestation period. At necropsy on GD 18, a Caesarean section was performed. The number of implantations, number of live and dead fetuses, fetal weight and abnormal external, visceral and skeletal findings were recorded. No difference was observed in the number of implantations. The

number of live fetuses was decreased in the mid- and high-dose groups. In the latter group no live fetuses were observed. Fetal body weight was decreased in the low- and mid-dose groups compared to the control group (0.65 (low), 0.50 (mid) vs 1.17 g (control group). The skeletal examination revealed a decrease in ossification of the fetuses of the low-and mid-dose groups. The immune-response genes *GARP* and *SIMP* were upregulated in the liver of dams and their fetuses of the mid-dose group. In this study, effects were observed at a very low dose, in contrast to other prenatal developmental studies (FDRL 1972), where no effects were observed at approx. 30 times higher dose levels (the highest dose tested in these studies). No data on maternal effects were reported in the study by El-Makawy et al. (2008), and the effects on fetuses are indicative of maternal toxicity. However, in the 14-day NTP study in mice (NTP, 1982), there were no reported adverse effects even at the highest dose, equal to 3756 mg/kg bw per day administered via the diet (Table 7). Due to the uncertainties, the Panel did not consider this study for risk assessment.

Developmental toxicity

In all studies performed by the Food and Drug Res. Lab. (FDRL, 1972) with stannous chloride (FDA 71-33) described below, body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. The test substance was described as 'stannous chloride'. In the estimation of doses in mg Sn/kg bw per day, the Panel assumed that it is the anhydrous form of stannous chloride (MW 189.6). All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live fetuses were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities (one-third detailed visceral examination and two-third stained and examined for skeletal defects).

Mice

Groups of 20–21 pregnant CD-1 mice received on GD 6–15 daily doses of 0, 0.5 (0.3), 2.3 (1.4), 11 (6.9) or 50 (31.2) mg stannous chloride (Sn)/kg bw per day by gavage (vehicle was water; dose volume 1 mL/kg bw) (FDRL, 1972). Body weight was determined at GD 0, 6, 11, 15 and 17. All dams were subjected to caesarean section at GD 17. No maternal or developmental toxicity was detected at any dose. The Panel identified the highest dose tested of 31.2 Sn/kg bw as the NOAEL for maternal and developmental toxicity.

Rats

Groups of 20–24 pregnant Wistar rats received on GD 6–15 daily doses of 0, 0.5 (0.3), 2.3 (1.4), 11 (6.9) or 50 (31.2) mg stannous chloride (Sn)/kg bw per day by gavage (vehicle was water; dose volume 1 mL/kg bw) (FDRL, 1972). Body weight was determined at GD 0, 6, 11, 15 and 20. On GD 20, a Caesarean section was carried out. No treatment-related maternal or developmental toxicity was detected. The Panel identified the highest dose tested of 31.2 mg Sn/kg bw as the NOAEL for maternal and developmental toxicity.

A developmental toxicity study was carried out with 20 females/dose level of the F2b generation of the multigeneration study described before at concentrations of 0, 200, 400 or 800 mg Sn/kg diet as stannous chloride (equivalent to 10, 20 or 40 mg Sn/kg bw per day (Sinkeldam et al., 1979, unpublished report). Females were mated with males of the same dose group. One-third of the fetuses were examined for skeletal examination and the other two-third of fetuses were used for visceral examination. No treatment-related differences in the number of corpora lutea, implantations, live and dead fetuses and fetal weight were observed. A visceral and skeletal examination showed no increase in the incidence of fetal abnormalities. The Panel identified the highest dose tested of 40 mg Sn/kg bw as the NOAEL.

Hamsters

Groups of 20–21 pregnant golden hamsters received via gavage daily doses of 0, 0.5 (0.3), 2.3 (1.4), 11 (6.9) or 50 (31.2) mg stannous chloride (Sn)/kg bw per day by gavage (vehicle was water; dose volume 1 mL/kg bw) from GD 6–10 (FDRL, 1972). Body weight was determined at GD 0, 8, 10, and 14. On GD 14, Caesarean section was carried out. There was no evidence for maternal or developmental toxicity. The Panel identified the highest dose tested of 31.2 mg Sn/kg bw as the NOAEL for maternal and developmental toxicity.

Other reproductive studies

Rabbits

Male mature New Zealand White rabbits (n = 6/group) were administered by gavage 20 mg $SnCl_2$/kg bw (purity 97%, $SnCl_2 \cdot 2H_2O$) (10.5 mg Sn/kg bw) every other day for 12 weeks. Body weight and food intake of the animals treated with stannous chloride were comparable to the controls (Yousef, 2005). At necropsy, relative weights of epididymis and testes were decreased. Treatment with stannous chloride caused a decrease in libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate, packed sperm volume, total functional sperm fraction, normal and live sperm and semen initial fructose. Dead sperm and initial hydrogen ion concentration (pH) were increased. Two extra groups (n = 6/group) were included; one group administered with ascorbic acid (AA; 40 mg/kg bw every other day) and another group administered with stannous chloride as described before and AA. Treatment with AA alone caused significant increase in body weight, food intake, relative weights of epididymis and testes, and semen characteristics compared to control group. The presence of AA in the animals administered stannous chloride decreased the effects reported in the stannous chloride-treated animals and the sperm parameters were comparable to controls. The Panel considered the results from this study to indicate a possible negative association between stannous chloride effects on reproductive performance and sperm parameters in rabbits. However, only one dose was tested and there were no data on reproductive outcome. This study cannot be used for risk assessment.

Overall, in a dietary multigeneration reproductive toxicity study in rats, no reproductive and developmental effects were observed up to the highest dose of stannous chloride tested (40 mg Sn/kg bw per day). Prenatal developmental studies (FDRL, 1972) in mice, rats and hamsters showed no maternal or developmental toxicity at the highest dose stannous chloride tested (31.2 mg Sn/kg bw per day).

3.4.7. Interaction with essential elements and ascorbic acid

Human studies

Eight adult males received mixed diets, either control diet with 0.11 mg Sn daily or a test diet with 49.7 mg Sn daily (added as stannous chloride to juices, consumed at lunch and dinner each day), equivalent to 0.7 mg Sn/kg bw in a 70-kg person, for 20 days in a cross-over design. This study is also described in Section 3.4.1. The faecal and urinary losses and serum levels of calcium were not affected by tin exposure (Johnson and Greger, 1982). After 40 days, effects on zinc, copper, iron, manganese and magnesium metabolism were studied (Johnson et al. 1982). The diet contained approximately 13.5 mg zinc/day. The retention of zinc was statistically significantly reduced in subjects fed the test diet, while retention of the other elements was not affected. The Panel noted that the current JECFA PTWI of 14 mg Sn/kg bw (corresponding to 2 mg Sn/kg bw per day) may not protect from the potential adverse effects of stannous chloride on absorption and status of zinc.

Solomons et al. (1983) studied the effect of a single tin dose on plasma zinc concentrations in humans administered 100 mL Coca-Cola with 12.5 mg Zn as zinc sulfate together with 25, 50 and 100 mg of tin as stannous chloride. Noxious gastrointestinal symptoms were induced by 100 mg tin. There were no effects on plasma zinc levels. In contrast, Valberg et al. (1984) reported reduced zinc absorption when stannous chloride was given at a dose of 36 mg Sn together with 4 mg of Zn with a turkey test meal.

Animal studies

Rats

In a feeding study in rats administered stannous chloride dihydrate in the diet at a concentration of 150 mg Sn/kg (equivalent to 13.5 mg Sn/kg bw per day) for 6 weeks, haemoglobin and serum iron levels were reduced. These effects disappeared when copper levels in the diet were increased from 3 to 6 or 50 mg/kg. Furthermore, growth depression and haematological changes induced by 500 mg Sn/kg diet (equivalent to 60 mg Sn/kg bw per day) were less marked when iron levels in the diet were increased from 35 to 250 mg/kg (De Groot, 1973).

Johnson and Greger (1985) studied the effects of various dietary levels of stannous chloride on copper, iron and calcium metabolism in rats. Rats were fed diets containing < 1, or approximately 100, 200, 500 and 2,000 mg Sn/kg feed for 23–27 days. The doses are equivalent to < 0.12, 12, 24, 60 and 240 mg Sn/kg bw per day. Plasma copper levels were reduced to 13% of control levels at doses

at and above 500 mg Sn/kg feed. Minor effects were reported on iron and calcium metabolism. The same authors also studied the effects of dietary exposure to tin on zinc metabolism (Johnson and Greger, 1984). With the same design of the experiment, they found lower levels of zinc in the tibia, liver, kidney and plasma in rats treated at and above 500 mg Sn/kg feed.

The effect of tin intake on iron status was studied in rats, administered 1, 10, 50, 100 and 200 mg Sn/kg in the feed, equivalent to 0.12, 1.2, 6, 12 and 24 mg Sn/kg bw per day. After 28 days, iron concentrations in plasma and percentage transferrin saturation were decreased in the two highest dose groups (Beynen et al., 1992). Iron, copper and zinc levels in plasma were affected in a dose-dependent manner in rats fed stannous chloride dihydrate in the feed for 28 days at concentrations of 1, 10, 50, 100 and 200 mg Sn/kg feed, equivalent to 0.12, 1.2, 6, 12 and 24 mg Sn/kg bw per day (Pekelharing et al., 1994). Yu and Beynen (1995) reported reduced plasma copper levels in rats fed 100 mg Sn/kg feed for 4 weeks, equivalent to 12 mg Sn/kg bw per day. The estimated 'true copper absorption (intake – (faecal Cu – biliary Cu)' was statistically significantly reduced and the authors concluded that high tin intake reduces copper status in rats via inhibition of copper absorption.

The effects of stannous chloride on the mechanical strength of bone were tested in rats receiving the substance in drinking water at concentrations of (0, 50, 150, 300 and 600 mg Sn/L) for 4 weeks. The doses are equivalent to 0, 6, 18, 36 and 72 mg Sn/kg bw per day. The compressive strength of the distal epiphysis of the femur was significantly decreased in the two highest dose groups (Ogoshi et al., 1981). It can be noted that in this study the tin contamination of regular feed was analysed and a concentration of 52.4 mg Sn/kg was reported. This corresponds to a tin dose equivalent to 6.3 mg Sn/kg bw per day.

Rabbits

Chmielnicka et al. (1992) reported protective effects of zinc on tin effects on heme biosynthesis in rabbits, administered a single oral dose of stannous chloride dehydrate of 10, 100 or 200 mg Sn/kg bw and a subcutaneous injection of 50 mg Zn/kg bw as $ZnSO_4$. The tin-induced inhibition of δ-aminolevulinic dehydratase was prevented by zinc administration.

The effects of L-ascorbic acid on the toxicity of stannous chloride were studied in rabbits (El-Demerdash et al., 2005; Yousef, 2005 and Yousef et al., 2007). Male New Zealand White rabbits, were orally administered 20 mg $SnCl_2$/kg bw per day every other day for 12 weeks, either alone or together with 40 mg L-ascorbic acid/kg bw. Tin-induced markers of lipid peroxidation and antioxidant enzymes were alleviated by L-ascorbic acid. Histopathological examination of livers from tin-treated rabbits showed atypical hepatocytes, proliferation of duct epithelium, dilation and congestion of blood vessels and mononuclear inflammatory infiltrate. The histopathological effects were less marked in rats co-treated with L-ascorbic acid.

Overall, the limited data available in humans on tin effects on essential elements indicate that zinc status can be negatively affected by daily doses of 50 mg tin/day during 40 days, while no effects were observed on other essential elements. In rats, iron, copper, zinc and calcium status was negatively affected by dietary exposure to stannous chloride at 12 mg/kg bw per day for 4 weeks. Decreased bone strength was observed in rats at doses from 36 mg Sn/kg bw per day. The interaction is possibly due to inhibited absorption of the essential elements by stannous chloride.

3.4.8. Gastrointestinal effects in humans

Illness due to consumption of a vodka punch, containing 2,000 mg Sn/L, was reported in 31 of 38 women attending a banquet (Warburton et al., 1962). The punch had a pH of approximately 3 and had been stored in a re-tinned 5-gallon milk churn in which there were signs of corrosion. The symptoms included nausea, abdominal cramps, vomiting, headache, chills and diarrhoea. Onset of symptoms was observed within 2 h of consumption and the symptoms lasted for 2–48 h.

Case reports have described severe abdominal bloating, vomiting, diarrhoea and headache in persons after consumption of canned tomato juice with tin levels ranging from 131 to 405 mg tin/kg. The cans were de-tinned, probably due to unusually high nitrate levels in the tomatoes used to prepare the juice (Barker and Runte, 1972).

With the aim to determine the dose response of tin-induced acute gastrointestinal effects in man, two separate randomised, single-centre, double-blind, cross-over studies were performed (Boogaard et al., 2003). In the first study, 20 volunteers (12 males, 8 females, fasted for at least 7 h) received 250 mL tomato juice (concentration of tin < 0.5 mg/kg) spiked with stannous chloride dihydrate at concentrations of 0, 161, 264 and 529 mg Sn/kg. The tomato juice (250 mL) was consumed as a

bolus over a 3-min period. Washout periods between treatments were at least 48 h, which was considered sufficient to achieve washout of tin from the gastrointestinal tract. The observed adverse effects related to stannous chloride were gastrointestinal system disorders and a clear dose–response relationship was found. The percentage of persons with adverse events was 5.6, 17 and 80 in the tin-treated groups, compared to 0 in the controls. The proportion of related adverse effects was statistically significantly different between the groups as tested by chi-square test ($p < 0.001$). Treatment at the highest dose was discontinued due to the high incidence of reported adverse effects and only five persons were treated with this dose. Blood samples, taken before dosing and at 0.5–4 h after dosing, did not show increased levels of tin in serum, indicating a local irritating effect (Boogaard et al., 2003).

In the second study, 24 volunteers (14 female and 10 male, fasted for at least 6 h) received 250 mL tomato soup containing tin migrated from packaging in concentrations of < 0.5, 201 and 267 mg Sn/kg. The percentage of persons with tin-related adverse events was 13, 0 and 17 in the < 0.5, 201 and 267 mg/kg dose groups and no statistical significant difference in the incidence between the groups was reported. The distribution of tin in different fractions of the tomato juice and tomato soup differed and a higher percentage of tin was present in the solid fraction in the canned tomato soup (52%) than in the spiked tomato juice (14.5%). In contrast, a higher proportion of tin was present in the low molecular weight (< 1,000 Da) fraction of spiked tomato juice (58.5%) than in the canned tomato soup (31.5%). The authors suggested that the lower incidence of tin-related adverse events from exposure to canned tomato soup was likely due to the lower concentration of tin in the low molecular weight fraction (Boogaard et al., 2003).

The Panel noted a dose-related increase in incidence of acute gastrointestinal irritation which was already statistically significant at the lowest concentration of 161 mg Sn/kg tomato juice. This is in line with data from case reports, where gastrointestinal effects were described after consumption of food with concentration of tin from 131 mg/kg. The Panel also noted the discrepancy in results between the two studies, including the number of adverse events in the control group and the absence of a dose–response relationship in the second study. The Panel also noted that the totally consumed amount of tin in the first study, which showed a dose response, was 40, 66 and 132 mg tin. The development of acute effects is probably not due to the concentration in food, and would not occur at a low intake of the specific food with a certain concentration of tin, but is rather dependent on the total bolus dose of tin. Thus, it would be appropriate to express the dose as an acute bolus dose.

Overall, humans receiving oral doses of stannous chloride dihydrate showed dose-related gastrointestinal irritation with increased incidence from a single dose 161 mg Sn/kg food. From case reports, gastrointestinal adverse effects have been reported after doses from 131 mg tin/kg food. The symptoms included nausea, abdominal cramps, vomiting, headache, chills and diarrhoea. Onset of symptoms was within 2 h of consumption and the symptoms lasted for 2–48 h.

3.4.9. Hypersensitivity, allergenicity and food intolerance

In two older studies, effects of stannous chloride were investigated, by measuring antibody responses, delayed-type hypersensitivity to sheep erythrocytes and leukocyte adherence inhibition in mice after subcutaneous (Dimitrov et al., 1981) or intraperitoneal (Hayashi et al., 1984) exposure, as a measure of functionality of the immune system. The Panel considers that in neither study clear effects were noted. No studies have been reported that investigated potential induction of hypersensitivity or allergenicity by stannous chloride. The Panel noted that the available data do not raise a concern for immunotoxicity by stannous chloride.

3.5. Discussion

Stannous chloride was evaluated by JECFA in 1970, 1972, 1982, 1988, 2001 and 2005 (JECFA, 1970, 1972, 1982, 1989, 2001, 2006a,b). In 1982, a PMTDI of 2 mg/kg bw was established based on gastric irritancy with a threshold concentration of about 200 mg/kg in the food. In 1988, in its evaluation on tin levels as a contaminant from the use of canned foods (JECFA, 1989), the Committee allocated a PTWI of 14 mg/kg bw (converted from the PMTDI of 2 mg/kg bw) which was confirmed in the latest evaluation.

After oral administration, the absorption of stannous chloride from the gastrointestinal tract of experimental animals is low (< 5%). Unabsorbed ingested stannous chloride is mainly (90–99%) excreted with faeces within 48 h, while absorbed tin distributes mainly to the bone, but also to the

liver, kidneys and brain. In humans, the apparent percentage absorption is around 3% at moderate levels of tin in the diet and higher at low, background levels in the diet.

In subchronic toxicity studies of stannous chloride, reduced body weight gain and reduced haemoglobin levels were reported. Gross distension of the caecum was observed in mice and rats. Rats were more sensitive than mice to the effects of stannous chloride on body weight gain and haemoglobin and the lowest NOAEL in rats in a 90 day study was 63 mg Sn/kg bw per day.

Effects on iron, copper, zinc and calcium status were observed after dietary exposure to stannous chloride in rats at 12 mg Sn/kg bw per day for 4 weeks. The adverse effects induced by stannous chloride on essential elements may explain the reduced body weight gain and haemoglobin levels, reported in subchronic toxicity studies, as well as reduced bone strength, reported in a short term study in rats. The interaction is possibly due to inhibited gastrointestinal absorption of the essential elements by stannous chloride.

The *in vitro* genotoxicity data on stannous chloride show a pattern of activity which suggest the involvement of ROS, even though the contribution by other mechanisms (inhibition of DNA repair, direct DNA binding) cannot be ruled out. *In vivo*, no indication of permanent DNA damage was obtained in rats and mice in experimental condition associated with significant internal exposure (i.p. and i.v. administration). The Panel considered that stannous chloride used as a food additive does not represent a concern with regard to genotoxicity.

Studies on chronic toxicity and carcinogenicity performed with rats and mice did not indicate concern for carcinogenicity of stannous chloride.

No reproductive and developmental effects were observed up to the highest dose of stannous chloride tested (40 mg Sn/kg bw per day) in a dietary multigenerational reproductive toxicity study in rats. Prenatal developmental studies in mice, rats and hamsters showed no maternal or developmental toxicity up to the highest dose tested (31.2 mg Sn/kg bw per day).

The limited data available in humans on effects of stannous chloride on essential elements indicate that zinc status can be negatively affected by daily doses of 50 mg tin/day during 40 days, while no effects were observed on other essential elements.

The Panel noted a dose-related increase in incidence of acute gastrointestinal irritation which was already statistically significant at the lowest concentration of 161 mg Sn/kg tomato juice (corresponding to a bolus dose of 40 mg Sn). The Panel considered that the development of acute effects was probably due to the total bolus dose of tin rather than the concentration in food per se. From case reports, gastrointestinal adverse effects were reported after exposure to concentrations from 131 mg Sn/kg food. The symptoms included nausea, abdominal cramps, vomiting, headache, chills and diarrhoea. Onset of symptoms was observed within 2 h of consumption and the symptoms lasted for 2–48 h.

The Panel also considered the effects of stannous chloride on absorption and status of essential elements. The limited human data available indicated that zinc status may be affected by continuous exposure to 50 mg Sn per day, corresponding to 0.7 mg Sn/kg bw per day.

Stannous chloride (E 512) is authorised as a food additive only in one food category. No reply on the actual use level of stannous chloride (E 512) as a food additive and on its concentration in food was provided by any interested party. According to the Mintel's GNPD, stannous chloride (E 512) was not labelled on any products in the EU or Norway. The regulatory maximum level exposure assessment scenario is based on the MPLs for stannous chloride (E 512), which is 25 mg Sn/kg. The Panel considered the exposure estimates derived following this scenario as the most conservative since it is assumed that the population will be exposed to the food additive present in food at the MPL over a longer period of time.

From the regulatory maximum level exposure assessment scenario, mean exposure to stannous chloride (E 512) from its use as a food additive was below 1.3 μg Sn/kg bw per day for all age groups. The 95th percentile of exposure to stannous chloride (E 512) ranged from 0.0 μg Sn/kg bw per day in all groups to 11.2 μg Sn/kg bw per day in adults. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to stannous chloride (E 512) as a food additive in European countries.

4. Conclusions

Considering that:

- absorption of stannous chloride from the gastrointestinal tract was low;
- adequate toxicity data were available. A reduction in body weight gain and haemoglobin was observed with a NOAEL of 63 mg/kg bw per day in rats in a 90-day study;

- there was no concern for genotoxicity and carcinogenicity;
- gastrointestinal irritation was reported in human after ingestion of a bolus dose of 40 mg Sn;
- stannous chloride is only permitted as food additives in one food category and no actual use was reported;
- the conservative estimated mean exposure based on MPL scenario was below 1.3 μg Sn/kg bw per day for all age groups,

the Panel concluded that stannous chloride (E 512) is of no safety concern in this current authorised use and use level.

References

AGS (Ausschuss für Gefahrstoffe), 2008. Zinn(II)-Verbindungen, anorganisch. Begründung zu Zinn(II)-Verbindungen, anorganisch in TRGS 900.

ATSDR (Agency for Toxic Substances and Disease Registry U.S. Public Health Service), 2005. Toxicological Profile for tin and tin compounds (update).

Barker Jr WH and Runte V, 1972. Tomato juice-associated gastroenteritis, Washington and Oregon 1969. American Journal of Epidemiology, 96, 219–226.

Bernardo-Filho M, Cunha MC, Valsa JO, Araujo AC, Da Silva FCP and Fonseca AS, 1994. Evaluation of potential genotoxicity of stannous chloride: inactivation, filamentation and lysogenic induction of *Escherichia coli*. Food and Chemical Toxicology, 32, 477–479.

Beynen AC, Pekelharing HLM and Lemmens AG, 1992. High intakes of tin lower iron status in rats. Biological Trace Element Research, 35, 85–88.

Biégo GH, Joyeux M, Hartemann P and Debry G, 1999. Determination of dietary tin intake in an adult French citizen. Archives of Environmental Contamination and Toxicology 36, 227–232.

Blunden S and Wallace T, 2003. Tin in canned food: a review and understanding of occurrence and effect. Food Chemical Toxicology, 41, 1651–1662.

Boogaard PJ, Boisset M, Blunden S, Davies S, Ong TJ and Taverne J-P, 2003. Comparative assessment of gastrointestinal irritant potency in man of tin(II) chloride and tin migrated from packaging. Food and Chemical Toxicology, 41, 1663–1670.

Cabral RE, Leitao AC, Lage C, Caldeira-de-Araujo A, Bernardo-Filho M, Dantas FJ and Cabral-Neto JB, 1998. Mutational potentiality of stannous chloride: an important reducing agent in the Tc-99m-radiopharmaceuticals. Mutation Research, 408, 129–135.

Calvery HO, 1942. Trace elements in foods. Food Research, 7, 313–331.

Chmielnicka J, Zareba G and Grabowska U, 1992. Protective effect of zinc on heme biosynthesis disturbances in rabbits after administration per os of tin. Ecotoxicology and Environmental Safety, 24, 266–274.

Codex Alimentarious, 2017. Available online: http://www.fao.org/fao-who-codexalimentarius/en/

Conine DL, Yum M, Martz RC, Stookey GK, Muhler JC and Forney RB, 1975. Toxicity of sodium pentaflourostannite, a new anticarcinogenic agent. I. Comparison of the Acute Toxicity of Sodium Pentafluorostannite, Sodium Fluoride, and Stannous Chloride in Mice and/or Rats. Toxicology and Applied Pharmacology, 33, 21–26.

Cotton FA, Wilkinson G, Murillo CA and Bochmann M, 1999. *Advanced Inorganic Chemistry*, 6th Edition. A Wiley-Interscience Publication, John Wiley & Sons, Inc., New York.

Damati A, Vlastos D, Philippopoulos AI and Matthopoulos DP, 2014. Inorganic tin compounds do not induce micronuclei in human lymphocytes in the absence of metabolic activation. Drug and Chemical Toxicology, 37, 213–220.

Dantas FJS, de Mattos JCP, Moraes MO, Viana ME, Lage CAS, Cabral-Neto JB, Leitao AC, Bernardo M, Bezerra RJAC, Carvalho JJ and Caldeira-de-Araujo A, 2002. Genotoxic effects of stannous chloride (SnCl2) in K562 cell line. Food and Chemical Toxicology, 40, 1493–1498.

De Groot AP, 1973. Subacute toxicity of inorganic tin as influenced by dietary levels of iron and copper. Food and Cosmetics Toxicology, 11, 955–962.

DeGroot AP, Til HP and Willems MI, 1973. Short-term (90 day) toxicity of tin in rats on semi-purified diets with excessive or marginal iron content. Submitted to the World Health Organization by Centraal Instituut voor Voedingsonderzoek, R4264. Cited in JECFA, 1982.

Dimitrov NV, Meyer C, Nahhas F, Miller C and Averill BA, 1981. Effect of tin on immune responses of mice. Clinical Immunology and Immunpathology, 20, 39–48.

van Dokkum W, de Vos RH, Muys T and Wesstra JA, 1989. Minerals and trace elements in total diets in The Netherlands. British Journal of Nutrition 61, 7–15.

ECHA, online. Available online: https://echa.europa.eu/substance-information/-/substanceinfo/100.133.348?_disssubsinfo_WAR_disssubsinfoportlet_backURL=https%3A%2F%2Fecha.europa.eu%2Fhome%3Fp_p_id%3Ddisssimplesearchhomepage_WAR_disssearchportlet%26p_p_lifecycle%3D0%26p_p_state%3Dnormal%26p_p_mode%3Dview%26p_p_col_id%3Dcolumn-1%26p_p_col_count%3D2%26_disssimplesearchhomepage_WAR_disssearchportlet_sessionCriteriaId%3D

ECHA, 2014. Available online: https://www.echa.europa.eu/documents/10162/13564/ir_csa_r7a_muta_caracal_conclusion_fina_draft_201408_en.pdf
EDQM (European Directorate for the Quality of Medicines & HealthCare), 2016. Annual report 2016. Available online: https://www.edqm.eu/sites/default/files/annual_report_edqm_2016.pdf
EFSA (European Food Safety Authority), 2007. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438
EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspectzrs of Risk Assessments carried out by EFSA. Part 2: General Principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051
EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097
EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970
EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. https://doi.org/10.2903/j.efsa.2012.2760
EFSA NDA Panel, 2006. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Tin. (Request N° EFSA-Q-2003-018), (adopted on 6 July 2005). EFSA Journal 2006;254, 1–25.
EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579
EGVM (Expert Group on Vitamins and Minerals), 2002. Draft report on "Safe upper levels for vitamins and minerals", pp. 205–214. August 2002. London.
El-Demerdash FM, Yousef MI and Zoheir MA, 2005. Stannous chloride induces alterations in enzyme activities, lipid peroxidation and histopathology in male rabbit: antioxidant role of vitamin C. Food and Chemical Toxicology, 43, 1743–1752.
El-Makawy AI, Girgis SM and Khalil Wagdy KB, 2008. Developmental and genetic toxicity of stannous chloride in mouse dams and foetuses. Mutation Research, 657, 105–110.
FDRL, 1972. Teratologic evaluation of FDA 71-33 (stannous chloride) in mice, rats and hamsters. Food and Drug Research Laboratories Rept., Maspeth, NY, Unpublished report submitted to the World Health Organization, FDA Contract 71-260. Cited in JECFA, 1982.
Fritsch P, De Saint Blanquat G and Derache R, 1977. Etude Nutritionnelle et Toxicologique, chez le rat, d'un Contaminant Alimentaire: l'Etain. Toxicology, 8, 165–175.
Furchner, 1976. Comparative metabolism of radionuclides in mammals - XI. Retention of Sn in the mouse, rat, monkey and dog. Health Physics, 31, 219–224.
Ganguly BB, 1993. Cell-Division, chromosomal aberration, and micronuclei formation in human peripheral-blood lymphocytes - effect of stannic chloride on donors age. Biological Trace Element Research, 38, 55–62.
Gitlitz MN and Moran MK, Updated by Staff 2006, 2007. Tin compounds. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, 5th Edition. pp. 800837. Wiley-Interscience, a John Wiley & Sons, Inc., Publication (Hoboken).
Gulati DK, Witt K, Anderson B, Zeiger E and Shelby MD, 1989. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro 111: results with 27 chemicals. Environmental and Molecular Mutagenesis, 13, 133–193.
Halacka B, 1970. Beitrag zur alimentären Toxizität der einfachen Zinnverbindungen. Vitalst. Zivilisationskr., 15, 188–192.
Hayashi O, Chiba M and Kikuchi M, 1984. The Effects of stannous chloride on the humoral immune response of mice. Toxicology Letters, 21, 279–285.
IPCS INCHEM, 2004. IPCS Risk Assessment Terminology (Part 1: IPCS/OECD Key Generic terms used in Chemical Hazard/ Risk Assessment. Available online: http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf
Janssen PJ, Bosland MC, van Hees JP, Spit BJ, Willems MI and Kuper CF, 1985. Effects of feeding stannous chloride on different parts of the gastrointestinal tract of the rat. Toxicology and Applied Pharmacology, 78, 19–28.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1970. Toxicological evaluation of some extraction solvents and certain other substances. 210. Tin and stannous chloride. FAO Nutrition Meetings Report Series 48a, WHO/FOOD ADD/70.39.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972. WHO Technical Report Series, Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives. No. 505. World Health Organization, Geneva, Switzerland.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1982. 552. Tin and stannous chloride. WHO Food Additives Series, No. 17.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1989. 663. Tin. WHO Food Additives Series, No. 24.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Tin (addendum). WHO Food Additives Series, No. 46.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Stannous chloride. FAO JECFA Monographs 4. Joint FAO/WHO Expert Committee on Food Additives.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006b. Monograph 1. Combined compendium of food additive specifications. Available online: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
Johnson MA and Greger JL, 1982. Effects of dietary tin on tin and calcium metabolism of adult males. American Journal of Clinical Nutrition, 35, 655–660.
Johnson MA and Greger JL, 1984. Absorption, distribution and endogenous excretion of zinc by rats fed various dietary levels of inorganic tin and zinc. Journal of Nutrition 114, 1843–1852.
Johnson MA, Baier MJ and Greger JL, 1982. Effects of dietary tin on tin and calcium metabolism of adult males. American Journal of Clinical Nutrition, 35, 655–660.
Johnson MA and Greger JL, 1985. Tin, copper, iron and calcium metabolism of rats fed various dietary levels of inorganic tin and zinc. Journal of Nutrition 115, 615–624.
Kada T, Hirano K and Shirasu Y, 1980. Screening of environmental chemical mutagens by the rec-assay system with bacillus subtilis. In: De Serres FJ and Hollaender A (eds.). Chemical mutagens: principles and methods for their detection. Vol 6. Plenum Press, New York, NY. pp. 149–173.
Martinez A, Urios A and Blanco M, 2000. Mutagenicity of 80 chemicals in Escherichia coli tester strains IC203, deficient in OxyR, and its oxyR(+) parent WP2 uvrA/pKM101: detection of 31 oxidative mutagens. Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 467, 41–53.
de Mattos JC, Dantas FJ, Bezerra RJ, Bernardo-Filho M, Cabral-Neto JB, Lage C, Leitão AC and de Caldeira- Araújo A, 2000. Damage induced by stannous chloride in plasmid DNA. Toxicology Letters 116, 159–163.
de Mattos JC, Matos VC, Rodrigues MP, Oliveira MB, Dantas FJ, Santos-Filho SD, Bernardo-Filho M and Caldeira-de-Araujo A, 2012. Evaluation of deoxyribonucleic acid toxicity induced by the radiopharmaceutical ^{99m}technetium-methylenediphosphonic acid and by stannous chloride in Wistar rats. Molecules, 17, 12974–12983.
McLean JRN, Birnboim HC, Pontefact R and Kaplan JG, 1983a. The effect of tin chloride on the structure and function of DNA in human white blood cells. Chemico-Biological Interactions, 46, 189–200.
Mclean JRN, Blakey DH, Douglas GR and Kaplan JG, 1983b. The effect of stannous and stannic (Tin) chloride on DNA in Chinese-Hamster Ovary Cells. Mutation Research, 119, 195–201.
Merck, 1996. Stannous Chloride. The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals.
Mino Y, 2006. Determination of tin in canned foods by X-Ray fluorescence spectrometry. Journal of Health Science, 52, 67–72.
Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis, 8 (Suppl 7), 1–119.
Myhr BC and Caspary WJ, 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: results for 31 coded compounds in the National Toxicology Program. Environmental and Molecular Mutagenesis, 18, 51–83.
Nishioka H, 1975. Mutagenic activities of metal compounds in bacteria. Mutation Research, 31, 185–189.
NTP (National Toxicology Program), 1982. Carcinogenesis Bioassay of Stannous Chloride (CAS No. 7772-99-8) in F344/N rats and B6C3F1/N Mice (Feed Study). National Toxicology Program technical report series, 231, 0888-8051 (Print) 0888-8051 (Linking), 1–149.
Ogoshi K, Kurumatani N, Aoki Y, Moriyama T and Nanzai Y, 1981. Decrease in compressive strength of the femoral bone in rats administered stannous chloride for a short-period. Toxicology and Applied Pharmacology, 58, 331–332.
Olivier P and Marzin D, 1987. Study of the genotoxic potential of 48 inorganic derivatives with the SOS chromotest. Mutation Research, 189, 263–269.
Ostrakhovitch EA, 2015. Chapter 56 on Tin. In: Nordberg G, Fowler BA and Nordberg M (eds.). Handbook on the Toxicology of Metals, 4th edition. Elsevier, Amsterdam. pp. 1242–1276.
Pekelharing HL, Lemmens AG and Beynen AC, 1994. Iron, copper and zinc status in rats fed on diets containing various concentrations of tin. British Journal of Nutrition, 71, 103–109.
Pelikan Z, Halacka K and Cerny E, 1968. Acute toxic effects of stannous chloride on white mice. Science and Medicine. 41, 351–356. Cited in JECFA, 1982.
Prival MJ, Simmon VF and Mortelmans KE, 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutation Research, 260, 321–329.
Pungartnik C, Viau C, Picada J, Caldeira-de-Araujo A, Henriques JA and Brendel M, 2005. Genotoxicity of stannous chloride in yeast and bacteria. Mutation Research, 583, 146–157.
Savolainen H and Valkonen S, 1986. Dose-dependent brain tin concentration in rats given stannous chloride in drinking water. Toxicology Letters, 30, 35–39.
Sax NI and Lewis RJ, 1987. Stannous Chloride. In: Hawley's Condensed Chemical Dictionary, 11th Edition.
SCF (Scientific Committee for Food), 1991. *Reports of the Scientific Committee for Food*. Twenty-fifth series. First series of food additives of various technological functions.

SCF (Scientific Committee for Food), 2001. Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out82_en.pdf
SCF (Scientific Committee for Food), 2002. Opinion of the Scientific Committee for Food on ACute Risks Posed by Tin in Canned Foods. SCF/CS/CNTM/OTH/18 Final.
Schroeder HA and Balassa JJ, 1967. Arsenic, germanium, tin and vanadium in mice: effects on growth, survival and tissue levels. Journal of Nutrition, 92, 245–252.
Schroeder HA, Kanisawa M, Frost DV and Mitchener M, 1968. Germanium tin and arsenic in rats: effects on growth, survival, pathological lesions and life span. Journal of Nutrition, 96, 37–45.
Shelby MD and Witt KL, 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environmental and Molecular Mutagenesis 25302-313.
Shelby MD, Erexson GL, Hook GJ and Tice RR, 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environmental and Molecular Mutagenesis, 21, 160–179.
Singh I, 1983. Induction of reverse mutation and mitotic gene conversion by some metal-compounds in saccharomyces-cerevisiae. Mutation Research, 117, 149–152.
Sinkeldam EF, Koeter HBWM and Willems MI, 1979. Multigeneration study with stannous chloride in rats. Central Institute for Nutrition and Food Research, Netherlands, Unpublished report submitted by Thomassen Z Drijver-Verblifa, N.V. Deventer, Netherlands to WHO/FAO, R6281. Cited in JECFA, 1982.
Sinkeldam EF, Dreef-van der Meulen EJ and Willems ML, 1981. Chronic (115-week) oral toxicity study with stannous chloride in rats. Central Institute for Nutrition and Food Research, Netherlands, Unpublished report submitted by. Thomassen Z Drijver-Verblifa, N.V. Deventer, Netherlands to WHO/FAO, R6372. Cited in JECFA, 1982.
Sisman T, 2011. Early life stage and genetic toxicity of stannous chloride on zebrafish embryos and adults: toxic effects of tin on zebrafish. Environmental Toxicology 26, 240–249.
Solomons NW, Marchini JS, RS Duarte Favaro, Vannuchi H and de Dutra Oliveira JE, 1983. Studies on the bioavailability of zinc in humans: intestinal interaction of tin and zinc. The American Journal of Clinical Nutrition, 37, 566–571.
Sumitani H, Suekane S, Nakatani A and Tatsuka K, 1993. Inductively coupled plasma atomic emission spectrometric determination of tin in canned food. Journal of AOAC International, 76, 1374–1377.
TemaNord (Nordic Council of Ministers), 2002. Stannous chloride. Food Additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU.
Valberg LS, Flanagan PR and Chamberlain MJ, 1984. Effects of iron, tin, and copper on zinc absorption in humans. American Journal of Clinical Nutrition, 40, 536–541.
Viau CM, Guecheva TN, Sousa FG, Pungartnik C, Brendel M, Saffi J and Henriques JA, 2009. SnCl(2)-induced DNA damage and repair inhibition of MMS-caused lesions in V79 Chinese hamster fibroblasts. Archives of Toxicology, 83, 769–775.
Warburton S, Udler W, Ewert RM and Haynes WS, 1962. Outbreak of foodborne illness attributed to tin. Public Health Reports 798-800.
WHO (World Health Organisation), 1980. Tin and organotic compounds: a preliminary review. IPCS - Environmental Health Criteria, 15, 15.
WHO (World Health Organisation), 2004. Inorganic tin in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/115.
Yamaguchi M, Saito R and Okada S, 1980. Dose-effect of inorganic tin on biochemical indices in rats. Toxicology, 16, 267–273.
Yamamoto A, Kohyama Y and Hanawa T, 2002. Mutagenicity evaluation of forty-one metal salts by the umu test. Journal of Biomedical Materials Research, 59, 176–183.
Yousef MI, 2005. Protective role of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. Toxicology, 207, 81–89.
Yousef MI, Awad TI, Elhag FA and Khaled FA, 2007. Study of the protective effect of ascorbic acid against the toxicity of stannous chloride on oxidative damage, antioxidant enzymes and biochemical parameters in rabbits. Toxicology, 235, 194–202.
Yu S and Beynen AC, 1995. High tin intake reduces copper status in rats through inhibition of copper absorption. British Journal of Nutrition 73, 863–869.

Abbreviations

AAS	atomic absorption spectrophotometry
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CA	chromosomal aberrations
CAS	Chemical Abstracts Service

CHO	Chinese hamster ovary
CONTAM	EFSA Panel on Contaminants in Food Chain
EINECS	European Inventory of Existing Chemical Substances
FAAS	furnace atomic absorption spectrometry
FAO	Food and Agriculture Organization of the United Nations
FCs	food categories
FCS	food categorisation system
GD	gestation day
GFAAS	graphite furnace atomic absorption spectrometry
GNPD	Global New Products Database
ICP-AES	inductively coupled plasma atomic emission spectrometry
i.p.	intraperitoneal
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose, median
MMS	methyl methane sulfonate
MN	micronuclei
MPL	maximum permitted level
MS	mass spectrometry
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse effect level
NOEL	no-observed-effect-level
OECD	Organisation for Economic Co-operation and Development
QS	*quantum satis*
PMTDI	Provisional Maximum Tolerable Daily Intake
PTWI	Provisional Maximum Tolerable Weekly Intake
ROS	reactive oxygen species
SCE	sister chromatid exchanges
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 total estimated exposure of stannous chloride (E 512) from its use as a food additive for the maximum permitted level exposure scenario per population group and survey: mean and 95th percentile (μg/kg bw per day)

Appendix A can be found in the online version of this output ('Supporting information' section)