FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

Risk and Technical Assessment Report – Proposal P298

Benzoates & Sulphites Permissions in Food

Executive summary

In 2005 a potential public health and safety concern was identified in the 21st Australian Total Diet Study (ATDS). Estimated dietary exposures to the preservatives sulphites and benzoates for some groups in the Australian population potentially exceeded the relevant health based guidance values. This Risk and Technical Assessment was undertaken to:

- establish the current usage of sulphites and benzoates by industry
- update the dietary exposure estimates for sulphites and benzoates using new analytical and food consumption data available since the ATDS
- review the safety of these chemicals based on any new available toxicological evidence available since the ATDS.

'Sulphites' and 'benzoates' refer to classes of food preservatives that have a long history of use in food in Australia and New Zealand. There are a range of specific permissions for their addition to certain foods in the *Australia New Zealand Food Standards Code* (the Code). Generally, the addition level of sulphites and benzoates at the point of manufacture is at a lower level than the MPL in the Code and is consistent with good manufacturing practice.

The appropriate health based guidance value (HBGV)[1] for sulphites and benzoates, is an acceptable daily intake (ADI)[2]. The ADI values used by FSANZ for the dietary risk assessment for this proposal of 0-5 mg/kg bw/day for benzoates and 0-0.7 mg/kg bw/day for sulphites are concordant with those established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The subsequent consideration of the toxicology of sulphites concluded that this ADI may be inappropriate.

The dietary exposure assessment for benzoates and sulphites (DEA)[3] was conducted for Australia and New Zealand[4] using the most recent food consumption data and updated analytical concentration data for those foods previously identified as being important contributors to total estimated dietary exposure in the ATDS.

[1] A numerical value reflecting the level of a chemical that can be ingested over a defined time period (e.g. a day, weekly, monthly or lifetime) without appreciable health risk. Most health based guidance values are expressed on a per kilogram bodyweight basis.
[2] An acceptable daily intake (ADI) is an estimate of the amount of a substance in food or drinking-water, expressed on a body-weight basis that, on the basis of all the known facts at the time of the evaluation, can be ingested daily over a lifetime without appreciable health risk to the consumer.
[3] A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, may be exposed to from the diet.
[4] The dietary exposure assessment for sulphites and benzoates for New Zealand was commissioned by the then New Zealand Food Safety Authority, and published in 2009 by the New Zealand Institute of Environmental Science and Research Ltd (ESR), and is summarised in this report.

The DEA indicated that estimated dietary exposure to benzoates for Australian and New Zealand consumers was below the ADI for all populations assessed at both the mean and 90th percentile of benzoate exposures. Non-alcoholic beverages were the major contributor to benzoates exposure for all Australian population groups assessed, particularly juice and juice products, carbonated beverages and cordials. Carbonated soft drinks were the major contributor to benzoates exposure for all New Zealand population groups assessed. The conclusion of the risk and technical assessment for benzoates is that there is no public health and safety concern for the Australian and New Zealand populations arising from the consumption of foods containing benzoates.

For consumers of foods containing sulphites, estimated mean and 90th percentile exposures were below the ADI for all Australian and New Zealand population groups assessed except for Australian children aged 2–5 years and New Zealand boys aged 5–12 years, at the 90th percentile of estimated dietary exposure (130% and 110% of the ADI, respectively). Although nutrition survey data are not available, it is considered likely that younger New Zealand children, aged 2–4 years, would also exceed the ADI at the 90th percentile of exposure. The Australian data do show a reduction in exposure for the same age groups, compared with the previous Australian dietary exposure assessment reported in the 21st ATDS.

Major contributors to sulphites exposure for Australian children were beef sausages, dried apricots and cordials, while the major contributors for adults were white wine, beef sausages and dried apricots. Major contributors to dietary exposure to sulphites for New Zealand children were sausages and soft drinks. For New Zealand adults, dietary exposure was predominately through consumption of beer, sausages and white wine.

The evidence used by JECFA to establish the ADI for dietary sulphites was reviewed, together with a number of additional papers that have been published since JECFA last considered the toxicological database. There is no evidence that dietary sulphites are developmental or reproductive toxicants, or that they are carcinogenic. The current JECFA group ADI of 0–0.7 mg/kg bw for sulphites is based on gastric lesions observed in a combined three-generation reproduction and chronic feeding study where the LOAEL was ~150 mg/kg bw/day. This study had significant limitations in design and analysis, including considerable uncertainty regarding the actual dose of sulphites in the rat diets. In 1990 the same group of investigators who performed this pivotal study reported that the 'Minimum Observed Adverse Effect Level' (MOAEL) for gastric lesions in a 4-week oral toxicity study was 2000 mg/kg bw/day, highlighting uncertainty on the ADI.

FSANZ considered the outcomes of other studies performed on sulphites of varying durations but there was no consistency in findings of gastric lesions and no other study was considered suitable for determining an ADI. Therefore, FSANZ commissioned a seven-day dose-range finding study in rats which showed that the LOAEL was 1500 mg/kg bw/day in the absence of any gastrointestinal lesions. While this study was of shorter duration than the older studies, the proposed mode of action for sulphites is direct mucosal irritation. It is unlikely that the findings in chronic studies are due to chronic irritation when there was a lack of evidence of acute irritation in the dose-range finding study. A dose-range finding study is not designed to enable a different ADI to be established, because of the short duration, limited range of toxicological endpoints investigated and small group size. However, the evidence from both this study and the 4 week oral toxicity study conducted in 1990 suggest that the current group ADI is most likely to be higher if it was based on robust evidence from a definitive study conducted to modern experimental standards. Therefore FSANZ is of the opinion that the existing ADI is too low and that current levels of dietary exposure are unlikely to pose a risk for any consumer.

Contents

1 Introduction

'Sulphites' and 'benzoates' refer to classes of food preservatives that have a long history of use in food in Australia and New Zealand, with specific permissions for their addition to a range of foods, up to a maximum permitted level (MPL), contained in Schedule 15 – Substances that may be used as food additives (table to section S15—5) in the *Australia New Zealand Food Standards Code* (the Code).

These preservatives were included in the 21st Australian Total Diet Study (ATDS), conducted by Food Standards Australia New Zealand (FSANZ) in 2003 and published in 2005 (FSANZ, 2005), to examine usage patterns by the Australian food industry at the time and estimate the then current dietary exposure for Australian consumers. The outcomes of these assessments indicated that exceedances of the relevant health based guidance value for sulphites and benzoates could occur for some Australian population groups.

As a consequence of the outcomes of the 21st ATDS, FSANZ prepared Proposal P298 to consider amending MPLs in the Code relating to sulphites and benzoates.

Since the dietary exposure assessments of sulphites and benzoates were undertaken in the 21st ATDS, further data became available that allowed an updated risk assessment to be conducted for sulphites and benzoates for the Australian population. These included revised analytical data on the concentrations of sulphites in specific foods and the completion of the 2007 Australian Children's National Nutrition and Physical Activity Survey (2007 ANCNPAS) providing updated consumption data for Australian children.

Similar assessments of dietary exposure to sulphites and benzoates for the New Zealand population were undertaken in 2008 by the New Zealand Institute of Environmental Science and Research Ltd (ESR), commissioned by the then New Zealand Food Safety Authority (NZFSA), and published in 2009 (Cressey and Jones, 2009). The completion of the New Zealand 2002 National Children's Nutrition Survey provided updated consumption data for New Zealand children. Outcomes of these assessments are summarised in this report.

1.1 Objectives of the risk and technical assessment

Issues relating to sulphite sensitivity or allergies to sulphites have previously been considered by FSANZ and are not further addressed in this Risk and Technical Assessment report. Further information about sulphites in food is available on the FSANZ website[5]. Therefore, the objectives of this risk and technical assessment were, using the latest available data, to:

- assess current industry practices relating to use of the two preservatives in food in Australia and New Zealand
- assess the current dietary exposure to the two preservatives for the Australian and New Zealand populations
- assess the current risk to public health and safety from long term exposure to the two preservatives.

[5] http://www.foodstandards.gov.au/consumer/additives/sulphite/Pages/default.aspx

1.2 Key risk assessment questions

To meet the objectives of this risk assessment, the following key questions have been posed:

1. What is current industry practice with respect to manufacturers' levels of use of sulphites and benzoates?

2. Are the levels of sulphites and benzoates permitted to be added to foods and beverages consistent with good manufacturing practice?

3. Considering the latest consumption and concentration data available, what are the estimated total dietary exposures to sulphites and benzoates?

4. What are the major contributing food sources of sulphites and benzoate exposures?

5. Taking into consideration any new information relevant for assessing the risk of benzoates and sulphites in food that has become available since FSANZ completed its last review of their safety, is there a public health and safety concern for the Australian or New Zealand populations arising from the consumption of foods containing sulphites or benzoates?

2 Technological assessment of benzoates and sulphites

2.1 Technical description of benzoates and sulphites

'Benzoates' and 'Sulphites' are general terms for families of preservatives. Preservatives retard or prevent the deterioration of a food by microorganisms. The permitted addition rates of preservatives vary depending on the quantity required to achieve the specific function in a particular food type and take into account loss of the preservative over time due to chemical reactions and in some cases evaporation.

Benzoates - is the general term for benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate. Benzoates are added to foods as anti-microbial agents, and the benzoic acid form occurs naturally in a range of fruits. Benzoates are most effective in acidic solutions, although they may be used in other non-acidic foods, often in conjunction with other anti-microbial agents.

Sulphites - is the general term for sulphur dioxide, sodium sulphite, sodium bisulphite, sodium metabisulphite, potassium metabisulphite, potassium sulphite and potassium bisulphite. Sulphites are added primarily to inhibit and control microorganisms, to inhibit enzymic and non-enzymic browning, but also act as antioxidants.

2.2 Permitted levels of benzoates and sulphites

There is no general permission for the use of benzoates or sulphites in the Code - their specific permissions are found in the table to section S15—5. These permissions are summarised in Appendix 1. Overall, permissions in the Code are generally consistent with those published in the Codex General Standard for Food Additives[6] and are based on the maximum levels needed to achieve the technological purpose.

[6] http://www.fao.org/fao-who-codexalimentarius/standards/gsfa/en/

For benzoates, the Codex permissions for the addition to fruit juices, concentrates and nectars (1000 mg/kg) are higher than the current permissions in the Code (400 mg/kg) for fruit juices and fruit juice products, while all other benzoate permissions are similar.

For sulphites, a major difference between the Code and Codex levels is for dried fruit. Codex has a maximum level of 1000 mg/kg for most dried fruits with 2000 mg/kg for dried apricots and 1500 mg/kg for bleached raisins. The Code has a MPL of 3000 mg/kg for all dried fruits. The major reason for this difference has been to address the reported need of the New Zealand and Australian dried fruit industries to obtain a 12-month shelf-life once the product is packed for retail sale by maintaining the fruit's colour during shelf-life, as the level of sulphites drops significantly over time. However, dried apricots are a world-traded product and imported apricots now account for the majority of dried apricots sold in Australia and New Zealand. Both imported and domestic dried apricots have an average sulphite level as analysed at point of retail sale of around 1500 mg/kg (FSANZ, 2008).

With regard to sulphites in raw meat sausages the Code has a MPL of 500 mg/kg. In the Europe Union and Canada the sulphites permission levels for sausages are similar (Europe 450 mg/kg, Canada 500 mg/kg). These countries also have distribution chains and lead times which are similar to those in Australia and New Zealand.

In contrast to this, Codex and in some other countries, e.g. the United States of America (USA), there is no permission to add sulphites to sausages. For Codex, there is permission to add the antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) to sausages; additives which are currently not permitted in sausages by the Code.

A further difference between the Code and Codex permissions for sulphites is the higher permission in the Code (1000 mg/kg) for canned abalone (paua) compared with Codex (150 mg/kg). This reflects the use of sulphites for a different technological purpose (bleaching), as the abalone found in New Zealand and some parts of Australia have dark skin. Consumption of this product in Australia and New Zealand is negligible, with most being exported.

2.3 Current industry usage levels of benzoates and sulphites

2.3.1 Benzoates

A summary of benzoates levels for those foods previously identified as being major contributors to benzoates dietary exposure (key food groups) are shown below in Table 1, details for all foods are in Table A3.1 in Appendix 3. Major dietary contributors to benzoates exposure in Australia were identified in the 21st ATDS (FSANZ, 2005). For Australian children aged 2-5 years major contributors to estimated benzoate exposure were cordials, non-cola soft drinks and juice and juice products. Non-cola soft drinks were the greatest contributor to estimated benzoates exposure for Australian adults. In New Zealand, non-cola soft drinks were the biggest contributor to estimated benzoates exposures for 5–15 year olds and adults aged 15 years and above (Cressey and Jones, 2009).

Concentration levels of food 'as consumed' in Australia and New Zealand were taken from the 21st ATDS and Cressey and Jones reports respectively (FSANZ, 2005, Cressey and Jones, 2009); typical levels at manufacture were as notified by the industry and ingredient suppliers in 2008. While the product group 'juice and juice products' was identified as a major contributor to dietary exposure, benzoates are primarily only used in refrigerated juices because longer-life shelf-stable products use a heat or equivalent treatment to achieve their shelf life.

Key beverage industry members provided confidential information comparing their 2008 and 2003 (year of 21st ATDS survey) manufacturing levels of benzoates in the various drink types above. The information provided indicated two key changes:

- a higher proportion of juices in the market did not use benzoates in 2008 compared to 2003
- where benzoates are still added, a reduction since 2003 in the average benzoate use level was reported of approximately 10% in soft drinks, and approximately 25% in cordials.

Table 1: Levels of benzoates in key food groups

Product Group	Code Permission mg/kg	Typical Level at Manufacture[1] mg/kg	Average Level, as Consumed mg/kg	Data Source
Cordials (diluted ready to drink)	400	130	83	Australia[2]
			21	New Zealand[3]
Non-cola soft drinks	400	230	220	Australia[2]
			144	New Zealand[3]
Refrigerated juice	400	Not used; or 300	51	Australia[2]
			ND[4] orange 8 blackcurrant	New Zealand[3]

1 Data provided by Australian manufacturers and ingredient suppliers, 2008.
2 21st ATDS, 2005. Average of benzoate levels for a random sample of products on the Australian market in 2003, including products with no added benzoates.
3 Cressey and Jones (2009). Average of benzoate levels for a random sample of products on the New Zealand market in 2005, including products with no added benzoates.
4 Not detected.

2.3.2 Sulphites

A summary of sulphites levels for those foods previously identified as being major contributors to sulphites dietary exposure or for foods for which Australian permissions are different to Codex is included in Table 2. The level of sulphites added during manufacture is significantly higher than that still present at the point of consumption, as sulphites degrade during the products' shelf lives and during cooking.

The major dietary contributors to estimated dietary exposure to sulphites previously identified for Australian children were sausages, dried apricots and cordials (21st ATDS, 2005). Major contributors to estimated sulphites exposure for Australian adults were white wine, sausages and dried apricots. For the New Zealand population, major contributors to estimated sulphites dietary exposure for children were sausages and cola-based soft drinks. Beer, sausages and white wine were the major contributors to estimated sulphites dietary exposure for New Zealand adults (Cressey and Jones, 2009).

In order to update sulphites concentration data to reflect more recent manufacturers' usage in certain foods, a further analytical survey was conducted in April 2011 (FSANZ, 2012). This survey focussed on those foods that were previously identified as major contributors to sulphites exposure for children and that had not been re-surveyed since the 21st ATDS. Consequently, sausages, dried apples, sultanas and cordials were sampled. This survey indicated that, overall, mean sulphites levels in sausages had not changed greatly, levels of sulphites in sultanas had reduced substantially and levels in dried apples and cordials had increased slightly.

A summary of the analytical surveys, and how updated analytical data were used in the DEA, is provided at Appendix 3.

Table 2: Levels of sulphites in key food groups

Product Group	Code Permission mg/kg	Typical Level at Manufacture[1] mg/kg	Average Level, as Consumed mg/kg	Data Source[2]
Raw meat sausages, cooked as directed	500	500	275	21st ATDS, 2005
			267	updated analysis, Australia only (FSANZ, 2012)
			179[3]	New Zealand analyses[4]
Dried apricots	3000	3000	2097	21st ATDS, 2005
			1554	New Zealand analyses[4]
			1490	Updated analysis, Australia only (FSANZ, 2008)
Dried apples	3000	Not available	1334	updated analysis, Australia only (FSANZ, 2012)
Sultanas	3000	Not available	2	updated analysis, Australia only (FSANZ, 2012)
Cordials (diluted)	115	50	10	21st ATDS, 2005
			14	New Zealand analyses[4]
			19	Updated analysis, Australia only (FSANZ, 2012)
White wine	250-400 (depending on residual sugars)	20-200	123	21st ATDS, 2005 (Includes sulphites naturally present in wine)
			82	New Zealand analyses[4]

1 Data provided by manufacturers and ingredient suppliers, 2008
2 21st ATDS, 2005, average of sulphites levels for a random sample of products on the market in 2003.
3 Includes preservative free sausages
4 Cressey and Jones (2009) Average of sulphites levels for a random sample of products on the New Zealand market in 2005, including products with no added sulphites.

2.3.2.1 Sausages

For raw meat sausages (and other sulphite-containing smallgoods), the sulphite level is linked to the type of sausage, choice of raw materials, packaging system and finished product supply chains. There are now limited ranges of preservative-free speciality sausages in Australia, with sulphite-free sausages being more common in New Zealand. When sulphite-free sausages were included in the calculation of mean level of sulphites in sausages for New Zealand, the average sulphite level in sausages was 179 mg/kg, compared to 221 mg/kg when only sulphite containing sausages were included in the calculation (ESR, 2008). These levels in New Zealand (excluding preservative free sausages) were lower than that reported for sulphites in sausages in Australia of 267 mg/kg from the 2012 FSANZ survey and may be due to reported differences in raw material handling, supply chain length and ambient temperatures.

This survey indicated that the overall mean concentration of sulphites for all sausages (including all meat varieties), prepared to a ready to eat state, was basically unchanged since the 21st ATDS was conducted in 2003.

2.3.2.2 Dried apricots

Following publication of the 21st ATDS, FSANZ received advice from industry that the levels of sulphites in dried apricots for sale in Australia had changed since 2003 (when analytical

sampling had occurred), due to increased importation of dried apricots from Turkey. Therefore, in 2008 FSANZ conducted an analytical survey (FSANZ, 2008) to measure the current levels of sulphites in dried apricots and apricot-containing products in Australia. This further analysis, which included both Turkish and non-Turkish products, showed reduced sulphites levels in apricots (mean concentration of 1490 mg/kg) compared with the 21st ATDS (mean concentration of 2097 mg/kg). The new mean sulphites concentration for apricots was used in this DEA.

2.4 Capacity for manufacturers to reduce sulphites in certain foods

2.4.1 Sausages

Sausages are treated with sulphites to both retard microbial growth; primarily spoilage organisms, but also for pathogens and to act as an antioxidant, helping maintain the pink colour of the raw meat.

As the sulphite concentration drops to a level where it is ineffective to retard microorganisms it also becomes ineffective at maintaining the natural pink colour. The use of sulphites as a preservative is general purpose, it is not specifically effective against pathogens. However, it would be expected that as the preservative effect wanes and spoilage bacteria grow, there is a possibility of growth of pathogens. As raw meat sausages are cooked before consumption, this is not a risk.

In 2008, FSANZ commissioned a report: A *Review of Sulphites in Raw Meat Sausages*, from the South Australian Research and Development Institute (SARDI 2009) to better understand the current raw meat sausage industry (see

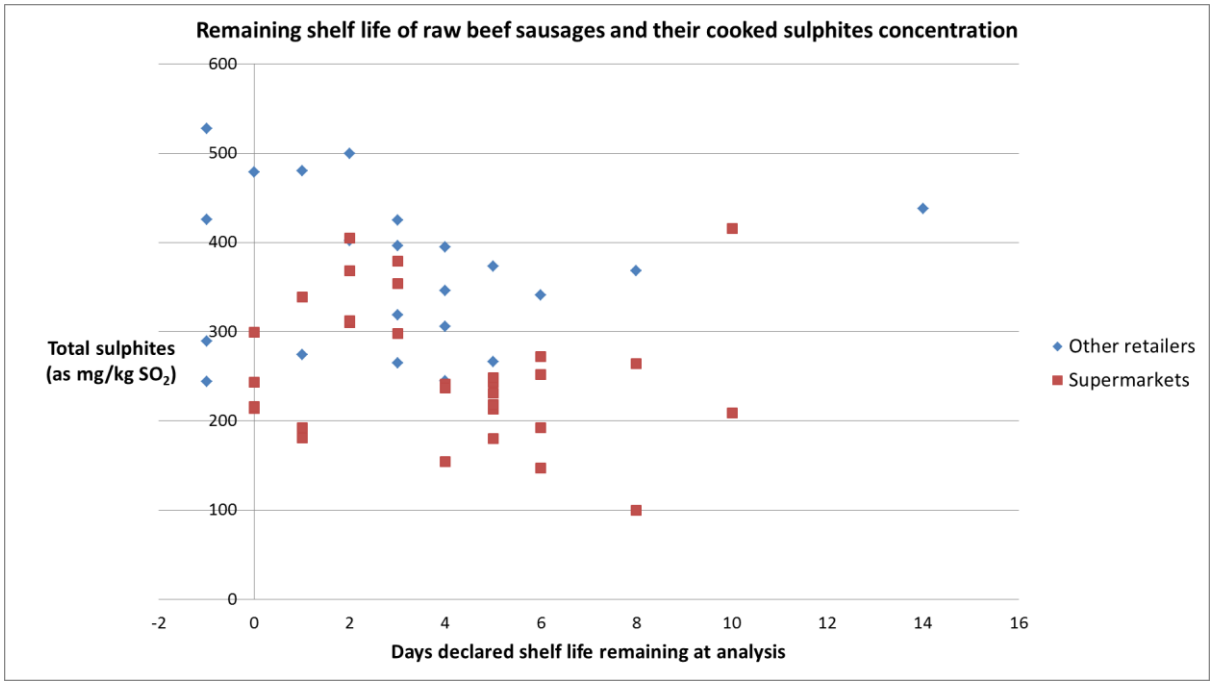
Appendix 2 for more information on the technological impact of reducing sulphites in sausages and Supporting Document 2 for the full SARDI report). SARDI was tasked to describe the uses of sulphites in sausages in Australia and overseas, and to consider the potential impacts of lowering levels of sulphites in raw sausages in Australia. In preparing the report, SARDI differentiated between sausages made by local butchers, these typically having a much shorter supply chain and shelf-life requirement and potentially needing less sulphites; and medium volume manufacturers and high volume suppliers of supermarket retail sausages which have a much longer supply chain, often up to 15 days.

The current level of sulphites in cooked sausages in Australia was assessed in an analytical survey, conducted by FSANZ in 2011, of a range of different meat based sausages (FSANZ, 2012), refer to Appendix 3 for mean concentration levels found.

Due to the significantly higher level of sampling in the 2011 analysis, it was possible to assess the relationship (if any) of the sulphites level of the cooked sausage with the remaining time left to the seller's 'best before' date. This indicated that a proportion of beef sausages, mostly purchased from butchers and similar small manufacturers, had a relatively high level of sulphites near the end of their shelf-lives (Figure 1, below). The average level of sulphites in cooked beef sausages purchased from butchers was 368 mg/kg compared with a level of 256 mg/kg in cooked sausages from supermarkets.

Most manufacturers use a sausage premix which contains, amongst other ingredients, the sulphites. The premixes may or may not be customised for culinary ingredients. FSANZ has been advised that the mixes for butchers generally contain sulphites at a concentration which is intended to provide 500 mg/kg in the sausage. Custom premixes for larger manufacturers generally provide sulphites in the sausage at 500–600 mg/kg, however, manufacturers target a level in the manufactured product of under 500 mg/kg, taking into account initial losses during manufacture. The general manufacturing procedure, based on information provided by the sausage industry, appears to be to manufacture fresh sausages with a sulphite level around 500 mg/kg, regardless of whether this level is required for a particular distribution chain.

It should be noted that there is no precise relationship between a sausage's raw and cooked sulphite level, as the final concentration in the cooked sausage is impacted by sausage formulation, preparation, degree of cooking, cooking type, shelf life remaining and other issues.



Note that the laboratory analysis commenced the day after sampling, and some samples were taken on the 'best before' date. Therefore, for those samples the cooked sausage sulphites concentration was one day after the best-before date (ie "-1" days remaining shelf life)

Figure 1: *Declared shelf life of raw beef sausages compared with cooked sausage sulphites concentration*

In their report, SARDI also described how long supply chain sausages are made in the USA, where sulphites are not permitted. Bulk packs and the use of modified atmosphere packaging, BHA, BHT or TBHQ and/or freezing are used to achieve a shelf-life of 12 days. The raw meat sausage industries in Australia, New Zealand, Europe and Canada have not adopted the USA approach for a number of reasons, including that the actual sausage products in the USA are generally a different style, increased costs of using different preservatives and lack of relevant additive permissions.

2.4.2 Dried apricots

Dried apricots are treated with sulphites for the following reasons:

- to retard or prevent deterioration by microorganisms, specifically allowing an increased moisture content whilst controlling mould growth (i.e. producing a juicier product often preferred by consumers)

- as an antioxidant (to retard or prevent the oxidative deterioration of a food) and enzyme inhibitor which reduces the rate of darkening.

Apricots typically absorb around 3000 mg/kg sulphites with the initial treatment at harvest. This concentration then drops over time in the raw product warehouse. This annual harvest has to provide product for retail sale year-round. When processed for retail, the sulphite level is managed to be as close as possible to 2000 mg/kg at the time of packaging as the manufacturer can by continually testing and selecting appropriate raw material. The level of sulphites then drops further during retail shelf life.

The Australian dried apricot industry has indicated to FSANZ that there may be seasons when, due to fruit quality or quantity, it would not be possible to provide a supply of apricots at levels below 2000 mg/kg (the Codex maximum permitted concentration for sulphites in

dried apricots), and therefore they would not be able to supply retailers at that point in time, should FSANZ consider reducing the MPL to the Codex level.

Higher concentrations of sulphites in dried apricots could be required as a result of:

- environmental conditions impacting on fruit quality, sometimes resulting in apricots absorbing more than 3000 mg/kg sulphites so will contain more than 2000 mg/kg after final processing unless held back to allow time for the sulphites to degrade further.

- certain seasonal conditions resulting in poor sulphur retention in dried apricot with ingoing levels less than 3000 mg/kg resulting in quality downgrades and shorter shelf life for a high percentage of products. As the harvest and initial sulphiting is an annual, seasonal process, there is a desire to keep the initial dried apricot product in acceptable condition and ensure flexibility in the supply chain for up to 18 months before it is processed and packed for retail.

- apricots being packed relatively shortly after harvest (e.g. after a previous low harvest resulting in no initial dried fruit available for carry-over sales the following year), then the level of sulphites in the retail product may be over 2000 mg/kg.

Australian producers provided a view that the reported 21st ATDS sulphites levels for dried apricots were higher than the levels found in their own monitoring. As a consequence, FSANZ conducted an additional analytical survey in 2008 (FSANZ, 2008). A total of 120 dried apricot or apricot containing samples were analysed, including both Turkish and non-Turkish apricots. Sampling was done in two phases to maximise the variety of batch numbers and use by dates of the products purchased. Outcomes of the survey indicated that the average concentration for sulphites in dried apricots was lower (1490 mg/kg) in 2008 than that found in the 21st ATDS in 2005 (2097 mg/kg), and similar to levels found in New Zealand. This difference does not imply that less sulphites are being added to apricots – it is more likely to be due to seasonal variation and a larger sample size being taken in 2008. There was no material difference in average sulphites concentration between Australian and imported dried apricots. The survey data showed that even if there was a sulphite concentration over 2000 mg/kg at the time of initial drying the majority of dried apricots (Turkish or non-Turkish) were less than 2000 mg/kg at the point of retail sale.

2.4.3 Cordial and wine

Cordial manufacturers had previously indicated that there had been some reduction in levels of sulphites added to cordials since sampling for the 21st ATDS was carried out in 2003. However, this reduction was not reflected in the most recent sulphites analytical survey (FSANZ, 2012), where the mean concentration of sulphites in diluted cordial was found to be 19 mg/kg, higher than the level found in 2005 (10 mg/kg). Nevertheless, these levels are in the same range of values and it is expected that the concentration of sulphites in cordials would be highly variable, depending on the type of cordial, the level of sugar, the age of the cordial, how long it has been opened, and the length of time it has been diluted, as the sulphites rapidly evaporate.

With respect to white wine, the level of use of sulphites is highly dependent on the type of wine (sweeter wines requiring higher sulphite levels) and quality of ingoing raw material. The wine industry has advised that in some situations the maximum level permitted in the Code is required to be used, and that there is unlikely to have been a systemic change in the levels of sulphites used in wine since 2003.

Response to Risk Assessment Question 1: *What is current industry practice with respect to manufacturers' levels of use of sulphites and benzoates?*

For food groups that are major contributors to sulphite and benzoate exposure, the addition level at

the point of manufacture is generally at a lower level than the maximum level permitted in the Code, however there may be occasions where industry consider that the maximum permitted level is required (e.g. sulphites addition to white wine). The beverages industry has provided evidence of some reduction in levels of preservatives added to cordials but analysis indicates that this has not resulted in a lower concentration of sulphites in cordials sampled and tested as consumed.

The addition levels of sulphites to dried apricots and sausages, where used, at the time of manufacture are at or about the maximum permitted level in the Code. Generally the level of sulphites in the product at the point of sale or as consumed is significantly less than that, but for some sausages, generally those manufactured with a relatively short best-before date, the level of sulphites is relatively high by that date.

In all cases the average level of sulphites and benzoates in the product as consumed is less than that added at the point of manufacture due to natural degradation during storage, after opening or during preparation and cooking.

Response to Risk Assessment Question 2: *Are the levels of sulphites and benzoates permitted to be added to foods and beverages consistent with good manufacturing practice?*

Generally, the levels of benzoates and sulphites permitted to be added to foods and beverages are consistent with good manufacturing practice (that is, they are added at a level necessary to accomplish their desired effects) for the current production and distribution systems.

Regardless of any MPL in the Code, manufacturers must use additives, including sulphites, in accordance with good manufacturing practice. This means that the quantity of an additive added to food should be limited to the lowest possible level necessary to accomplish its desired effect. There is evidence that some sausage manufacturers are adding the maximum level of sulphites permitted in the Code regardless of their individual production and distribution systems.

As the harvest of apricots and initial sulphiting is an annual, seasonal process, there is a desire by the Australian industry to keep the initial dried apricot product in acceptable condition and ensure flexibility in the supply chain for up to 18 months before it is processed and packed for retail. While the level of sulphites initially added to apricots is, at 3000 mg/kg, consistent with the Code MPL for dried fruit, this level reduces over time and during processing so that the level in the product prepared for sale is usually close to 2000 mg/kg (the Codex MPL), and continues to reduce over storage.

3 Chemical hazard assessment

3.1 Benzoates

In 1998 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a group ADI of 0-5 mg/kg bw/day for benzoates based on a long-term exposure study in rats. Although no adverse effects were observed at the highest tested dose of 500 mg/kg bodyweight per day an ADI was established. This ADI incorporated a 100-fold uncertainty factor to take into account the intra- and interspecies variability in sensitivity to benzoates between laboratory animals and humans. EFSA recently reviewed the toxicological database for benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate and confirmed that there were no new studies available to change the basis or numerical value of the ADI which had been established by JECFA (EFSA 2016a)

3.2 Sulphites

3.2.1 Summary of sulphites toxicity

The current JECFA group ADI for sulphur dioxide and sulphites expressed as sulphur dioxide equivalents, set in 1973, is 0-0.7 mg/kg bw/day (WHO, 1974). The published studies used by JECFA to derive the group ADI, together with a number of additional papers which have been published since the ADI was established, have been reviewed by FSANZ. The NOAEL

established in rats by Til *et al*. (1972a) and used by JECFA at subsequent re-evaluations in 1986 and 1998 (WHO, 1987, 1999) to derive the group ADI was 0.215% sulphite (equivalent to 0.25% when unadjusted for loss and/or added thiamine). This concentration of dietary sulphite in the rat feed is approximately equivalent to 72 mg sulphite/kg bw/day.

The most sensitive target organ for effects of dietary sulphites was identified by Til *et al*. (1972a) as the mucosal lining of the stomach. A number of other studies from the same laboratory as the Til *et al*. study have identified the same target organ, although the dose-response relationship is not clear. Few studies of dietary sulphite have been completed by other laboratories since 1972, but the changes in stomach mucosa do not appear to be reproducible. The studies conducted by other laboratories are insufficient to enable a different ADI to be established, because of limitations such as short duration, limited range of toxicological endpoints investigated, and/or small group size. However, the evidence from those studies suggests it is likely that the current group ADI would be higher if it was based on robust evidence from a definitive study conducted to modern experimental standards. In 2015 FSANZ commissioned a toxicological study to confirm the toxicological endpoint observed in the Til *et al*. study (i.e. stomach lesions) and amount of sulphites in the feed at which it occurred. This study and other published information collectively supported a conclusion that adverse effects could occur only at doses that were at least 16 times higher than the level reported in the Till *et al.* study (Jonker *et al*. 1990; Cayzer 2015). The reason for this difference in outcome is not apparent. Although a new contemporary toxicological study will be required to establish a new robust ADI, FSANZ is of the opinion that the existing ADI is too low for the purposes of a risk characterisation.

The results of rodent carcinogenicity studies of sulphites are negative, and results of rodent developmental and reproductive toxicity studies are also negative.

In human beings hypersensitivity reactions, primarily among asthmatics, through exposure to sulphur dioxide from food containing sulphites are well documented. Consumers are advised of the presence of sulphites in foods with appropriate mandatory food labelling.

In 2016 the European Food Safety Authority (EFSA) published a Scientific Opinion on the re-evaluation of sulphites as food additives (EFSA, 2016b). The report highlighted several uncertainties and limitations in the toxicological database, in particular the absence of information around the chemical reactivity of sulphites in different foods and the resulting reaction products. However, it concluded that the current group ADI of 0.7 mg/kg bw/day should be considered temporary while the database was improved. The Panel noted that improving the toxicological database might result in either an increase or a decrease in the group ADI, depending on, for example, the effects detected, the identified point of departure and the use of chemical specific rather than default uncertainty factors. The Panel recommended that the database and the temporary group ADI should be re-evaluated and noted that recommended toxicological studies could require five years for completion.

A detailed hazard assessment of sulphites is available in Appendix 7.

4 Dietary exposure assessment

Dietary exposure assessments (DEAs) require data on chemical concentrations and on food consumption. The approach for this exposure assessment was to use the best available analytical data on benzoates and sulphites levels in the food supply along with the most recent consumption data available at the time the assessment was undertaken.

The results from the previous 21st ATDS for benzoates and sulphites dietary exposure estimates are summarised in Section 0.

DEAs for benzoates and sulphites have been conducted only for the Australian population by FSANZ (Sections 0 to 0) as the then New Zealand Food Safety Authority undertook an assessment of these preservatives for New Zealand populations (Cressey and Jones, 2009), based on the 1997 and 2002 New Zealand national nutrition surveys. A summary of the New Zealand DEA for sulphites and benzoates is provided in Sections 0 to 0.

4.1 Previous dietary exposure estimates for Australia

FSANZ previously assessed the dietary exposure of Australians to sulphites and benzoates in the 21st ATDS (FSANZ, 2005). The 21st ATDS dietary exposure estimates used the best available concentration data (21st ATDS analytical results) and consumption data (1995 NNS) of the time. The key findings of these assessments were:

4.1.1 Benzoates

For consumers of foods containing benzoates:

- Mean estimated dietary exposure to benzoates was less than 50% of the Acceptable Daily Intake (ADI) for all population groups assessed.
- 95th percentile estimated dietary exposures to benzoates exceeded the ADI for boys (approximately 140%) and girls (approximately 120%) aged 2–5 years, and was equivalent to the ADI for boys aged 6–12 years. All other population groups were below the ADI for 95th percentile estimated dietary exposures.
- 95th percentile estimated dietary exposure to benzoates for the population aged two years and over, representing lifetime exposure for a high consumer of benzoates, was approximately 60% of the ADI for males and approximately 50% of the ADI for females.
- Major foods contributing to dietary exposure to benzoates for young children aged 2–5 years were cordial, non-cola soft drinks and juice and juice products. For all other age groups assessed, non-cola soft drinks were the greatest contributor to dietary exposure to benzoates.

4.1.2 *Sulphites*

For consumers of foods containing sulphites:

- Mean estimated dietary exposure to sulphites was less than or equal to 80% of the ADI for all population groups assessed.
- 95th percentile estimated dietary exposures to sulphites exceeded the ADI for all population groups assessed, except females aged 13–18 years (85% of the ADI). Exceedances ranged up to 280% of the ADI for boys aged 2–5 years.
- 95th percentile estimated dietary exposure to sulphites for the population aged two years and above, representing lifetime exposure for a high consumer of sulphites, was approximately 130% of the ADI for males and females.
- Major foods contributing to dietary exposure to sulphites for children were sausages, dried apricots and cordial, and for adults were white wine, sausages and dried apricots.

More information on the 21st ATDS background, selection of foods, sampling and exposure estimates are provided in the 21st ATDS report (FSANZ, 2005).

4.2 Approach to updating estimated dietary exposures for Australian populations

The updated dietary exposure assessment for Australian populations prepared in this Risk and Technical Assessment report differs from the 21st ATDS as:

- new sulphites analytical concentration data (for dried apricots, dried apples, sultanas, cordials and sausages) have become available
- new food consumption data, the 2007 Australian National Children's Nutrition and Physical Activity Survey (2007 ANCNPAS), had become available
- the 90th percentile is used to best represent long term high exposure, instead of the previously used 95th percentile (FSANZ, 2009b).

An overview of the dietary modelling approach is shown in

Table 3.

A brief description of how FSANZ undertakes its DEAs is provided in Appendix 4. A detailed description of the DEA methodology is provided in the FSANZ document *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009a).

Table 3: Overview of dietary modelling approach for the estimation of dietary exposures to sulphites and benzoates in Australia

Key risk assessment question:	Dietary modelling approach
1. Considering the latest available data on levels of benzoates and sulphites as preservatives in the food supply, what is the estimated dietary exposure to these food additives, and how do they compare to the relevant health based guidance value?	• Estimate the dietary exposures to sulphites and benzoates using the - latest available analytical data (21st ATDS analysis and the updated sulphites analytical data for dried apricot, dried apple, sultanas, cordial and sausages) and - latest consumption data at the time the assessment was done: 2007 ANCNPAS for children aged 2–16 years, 1995 National Nutrition Survey (NNS) for people aged 17 years and over. • Estimate exposures to sulphites and benzoates compared to their relevant ADI.
2. What are the major contributing food sources to sulphites and benzoate exposures?	• Identify the major food groups contributing to the exposures for these preservatives

4.2.1 Concentration data used

The mean concentration levels used for the DEAs for sulphites and benzoates were obtained through analytical studies. The main source of analytical data used in this assessment was from the 21st ATDS. Where specific foods were analysed in more recent surveys (FSANZ analytical survey of sulphites concentrations in dried apricots (FSANZ, 2008)) and FSANZ analytical survey of sulphites in dried apples, sultanas, cordial and sausages (FSANZ, 2012)) the more recent mean analytical values were used.

Fifty nine types of foods were analysed in the 21st ATDS, representing mainly the processed foods for which there were permissions to add sulphites, benzoates and/or sorbates in the Code. Not all food types were analysed for all preservatives, depending on relevant Code permissions. Given that analytical surveys cannot survey all food in the food supply, foods reported as consumed in both the 1995 and 2007 Australian nutrition surveys were matched to the most appropriate analysed foods where possible, and allocated that analysed concentration of sulphites or benzoates. A summary of the concentration data used in the DEA is provided at Appendix 3, with Table A3.1 providing a summary of mean analytical values for each analysed food used in the assessment. Details of the analytical results for each survey are provided in the published reports.

4.2.1.1 Samples exceeding current maximum permitted levels

Updated sulphites analytical data for sausages (FSANZ 2012), indicated that three sausage samples contained sulphites at the point of analysis (cooked to a ready-to-eat state) at concentrations greater than the current MPL for raw sausages of 500 mg/kg. In addition, sampling from the 21st ATDS indicated that there were also a number of minced meat samples containing sulphites (three of the 15 composite samples analysed were found to have measurable concentrations of sulphites) even though there is no relevant permission in the Code. Given that these samples appeared to be inconsistent with Code requirements they were not included in the calculation of mean analytical concentrations for sulphites in sausages and minced meat for use in the DEA, assuming the Code is enforced.

Further sensitivity analysis showed that inclusion of these samples in the DEA resulted in very little impact on estimated dietary exposures, even though sausages were identified as a major contributor to sulphites exposures.

4.2.2 Consumption data used

Food consumption data used for the updated assessments for Australia included:

- 1995 Australian National Nutrition Survey (1995 NNS), a 24-hour dietary recall survey of 13,858 Australians aged 2 years and above for one day only
- 2007 Australian National Children's Nutrition and Physical Activity Survey (also known as 'Kids Eat Kids Play') (2007 ANCNPAS) a 24-hour dietary recall survey of 4,487 Australian children aged 2–16 years for two non-consecutive days.

The design of these two surveys varied somewhat and key attributes of each are set out in Appendix 4.

As the relevant health based guidance values for the preservatives do not differ by gender, separate assessments were not conducted for Australian males and females and the age groups selected for this assessment were 2–5 years, 6–12 years, 13–16 years and 17 years and above. These age groups were chosen to match with the food consumption data available and also to keep alignment with the 21st ATDS DEA for sulphites and benzoates where possible. Children are generally considered separately as their higher food consumption amounts per kilogram of body weight means their estimated exposure to food chemicals on a per-bodyweight basis is usually higher than that for adults.

Food consumption data from the Australian 2011–12 National Nutrition and Physical Activity Survey (NNPAS) (a component of the 2011–13 Australian Health Survey) was not incorporated into FSANZ's modelling program at the time of this assessment. However, an analysis of the proportion of children consuming sausages and dried apricots on day 1 of the 2011–12 NNPAS was undertaken, and compared with similar data derived from the 2007 ANCNPAS. This comparison may assist in providing insight into whether the newer data has the potential to change the conclusion of the dietary exposure assessment. It should be noted that for these calculations, for both surveys, only sausages and dried apricots reported as consumed in the respective surveys were included in the calculation of proportion of children consuming. Where these foods were included in recipes was not included in the calculations. This is likely to have an impact on the reported number of consumers of dried apricots in particular as this food is used in a range of recipes (e.g. breakfast cereals, confectionery, nut bars etc.).

The proportion of children aged 2–5 years consuming sausages on day 1 of the 2011–12 NNPAS was slightly lower (8.9%) compared with the 2007 ANCNPAS (10.9%). For children aged 6–12 years and 13–16 years, the proportion consuming sausages was similar between the 2011–12 NNPAS (9.8% and 6.7% respectively) and 2007 ANCNPAS (8.2% and 6.8%, respectively).

The proportion of children aged 2–5 years consuming dried apricots on day 1 of the 2011–12 NNPAS was slightly lower (1.2%) compared with the 2007 ANCNPAS (2.6%). The proportion consuming dried apricots was <1% for children aged 6–12 years and 13–16 years for both the 2011–12 NNPAS and the 2007 ANCNPAS.

4.2.3 Limitations and assumptions in the dietary exposure assessment

FSANZ aims to make as realistic an estimate of dietary exposure as possible. However, where significant uncertainties in the data exist, conservative (or 'worst-case') assumptions are generally used to ensure that the DEA does not underestimate exposure. Specific assumptions made in relation to this assessment are set out in Appendix 4.

DEAs based on the 1995 and 2007 nutrition survey food consumption data provide the best estimate of actual consumption of foods and resulting estimated dietary exposure to a food

chemical for the Australian population. However, it should be noted that nutrition survey data have limitations. In particular, the use of one or two days of 24-hour dietary recall data to estimate usual eating patterns tends to over-estimate habitual food consumption amounts for high consumers. Therefore, high percentile exposures estimated in the DEA are likely to be higher than actual high percentile exposures over a lifetime. In addition, for the 1995 NNS, there are limitations relating to the age of the data and the changes in eating patterns that may have occurred since these data were collected. These limitations are somewhat ameliorated by the availability of updated consumption data for children.

For more information on FSANZ dietary exposure assessment principles, methodology, assumptions and limitations and uncertainties of the concentration and food consumption data, see the FSANZ document, Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes (FSANZ, 2009a).

4.2.4 Dietary exposure assessment approach for 'high consumers'

As an outcome of an international peer review of FSANZ's dietary exposure assessment practices, FSANZ adopted a policy in 2009 that a high consumer's chronic dietary exposure was best represented by the 90th percentile of exposure. This replaced the previous standard use of the 95th percentile and is in line with international best practice. For further information on the use of the 90th percentile for dietary exposure assessments, refer to the FSANZ information paper: Protecting 'high consumers' (FSANZ, 2009b).

It is noted that the dietary exposure assessment conducted for the 21st ATDS reported high dietary exposure for consumers of foods containing sulphites and benzoates at the 95th percentile.

4.3 Results from updated dietary exposure estimates for Australia

4.3.1 Benzoates

Estimated dietary exposures to benzoates were below the ADI of 0.5 mg/kg bw for all Australian population groups, at both the mean and 90th percentile. Mean estimated dietary exposures to benzoates were at or below 20% of the ADI and 90th percentile estimated exposures were below 45% of the ADI for all the age groups assessed. These results are shown in Figure 2.

While comparisons are limited due to differences in population groups assessed, estimates of mean and high consumer benzoates dietary exposure have reduced for Australian children's population groups compared with estimated exposures reported in the 21st ATDS. As there were no updated analytical data collected relating to benzoates concentrations in foods, the apparent reductions in estimated exposures to benzoates for Australian children were a result of use of the more recent 2007 ANCNPAS food consumption data (average of 2 days), and, for high consumers, the use of the 90th percentile rather than the 95% percentile of exposure to represent the high consumer.

Full results for benzoates estimated dietary exposures for Australian population groups are presented in Appendix 5, Table A5.3.

4.3.2 Sulphites

Mean estimated dietary exposures for consumers of sulphites were at or below 50% of the ADI of 0.7 mg/kg bw for all population groups assessed. At the 90th percentile, all population groups were at or below 85% of the ADI except for children aged 2–5 years. The 90th percentile estimated exposure for the 2–5 year olds was 130% of the ADI. At both the

mean and 90th percentile, dietary exposures for 2–5 year-olds were approximately 40% higher than for 6–12 year olds on a body weight basis. Estimated dietary exposures to sulphites are shown in Figure 3.

While direct comparisons are limited due to assessment of different population groups, generally, mean estimated sulphites exposures for Australian population groups 13–16 years and 17 years and above have remained similar to mean exposures estimated in the 21st ATDS. For children aged 2–5 years and 6–12 years, mean estimated dietary exposures have reduced compared to the estimated exposures reported in the 21st ATDS. Previous mean estimated exposures to sulphites for boys and girls aged 2–5 years were 80% and 55% of the ADI, respectively, compared with 50% of the ADI for children aged 2–5 years (boys and girls combined) in this assessment. This reduction is likely to be due to a combination of:

- reductions in mean sulphites concentrations for some foods, particularly dried apricots, which is a major contributor to estimated sulphites dietary exposure for most Australian population groups
- additional concentration data for different types of sausages, allowing more refined 'mapping' of sausages made from different types of meat
- use of the more recent 2007 ANCNPAS, where the average of two days of food consumption data is used for chronic dietary exposure estimates.

Estimates of exposure for high consumers have reduced considerably since the 21st ATDS was conducted for the reasons listed above, as well as due to use of the 90th percentile rather than the 95% percentile of exposure to represent the high consumer.

Full results for sulphites estimated dietary exposures for Australian population groups are presented in Appendix 5, Table A5.4.

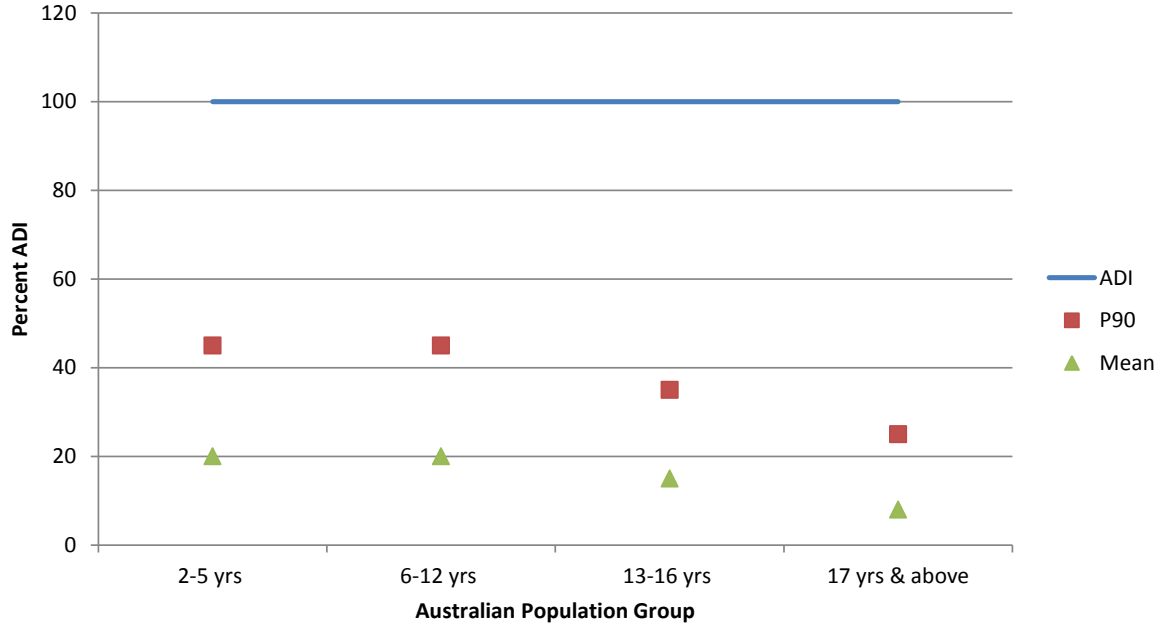


Figure 2: Mean and 90th percentile exposures for benzoates as a percentage of the ADI for Australian population groups (consumers only)

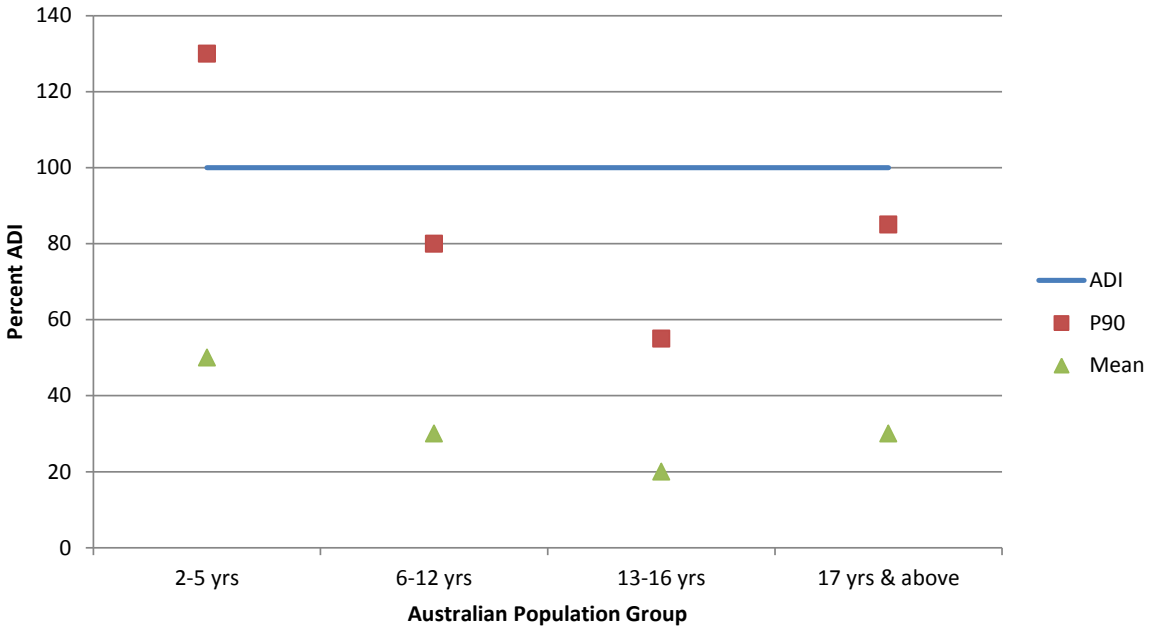


Figure 3: Mean and 90th percentile exposures for sulphites as a percentage of the ADI for Australian population groups (consumers only)

4.3.3 Major contributors to benzoates dietary exposures for Australian populations

Major contributors to benzoates estimated dietary exposures for Australian population groups were non-alcoholic beverages for all the age groups assessed. Within the non-alcoholic beverages group, juice and juice products, non-cola soft drinks, and cordials were the highest contributors, while cola soft drinks contributed more than 5% for those aged 13 years and above. The results are summarised in Figure 4. These major contributors were the same as those found in the 21st ATDS although the order of priority of the contributors has changed for some population groups. This reflects the use of the more recent consumption data for children only as updated concentration data for benzoates were not obtained for this assessment.

4.3.4 Major contributors to sulphites dietary exposures for Australian populations

Major contributors to estimated sulphites exposures for 2–16 year olds were beef sausages, dried apricots, cordials and dried apples. Beef sausages were the highest contributor for each age group, with the percentage contribution to sulphites exposure from beef sausages remaining about the same as age increased, from 34% for 2–5 year olds, to 38% for 6–12 and 13–16 year olds. Dried apricots were a higher contributor (23%) for children aged 2–5, than for older children (14% and 15% for 6–12 and 13–16 year olds, respectively), while cordial contributed approximately the same amount to sulphites exposure across all children's age groups (14%, 15% and 16% for children aged 2–5 years, 6–12 years and 13–16 years, respectively).

For adults aged 17 years and above, the major contributors to sulphites dietary exposure were white wine (30%), followed by dried apricots (17%) and beef sausages (14%). Red wine, cordial and dried apples all contributed 5% or more to sulphites dietary exposure.

Major contributors to sulphites dietary exposure are very similar to those found in the 21st ATDS, with changes to the order of priority for these contributors for some population groups. While still a major contributor, dried apricots are now contributing less than in the 21st ATDS for most age groups assessed, reflecting the use of revised (lower) concentration data and updated food consumption data for children. In the 21st ATDS, sausages were found to be the highest contributor for all except one children's population group assessed and this is still

the case for this assessment. White wine remains the highest contributor to estimated sulphites dietary exposure for Australian adults.

Major contributors to sulphites dietary exposure are summarised in Figure 5.

Detailed results of major contributors to dietary exposures for benzoates and sulphites are provided at Appendix 6, Table A6.3 and Table A6.4, respectively.

For this DEA additional analytical data for sulphites in a range of sausages were used. These data allowed some refinement of the mapping of analysed foods to foods consumed in the nutrition surveys. Sausages primarily made up of a particular type of meat were able to be mapped to those sausages reported as consumed (e.g. the mean analytical concentration for pork sausages was mapped to pork sausages identified as consumed in the nutrition surveys) (see Appendix 3). The majority of the contribution to sulphites dietary exposure from sausages came from beef sausages, however there was some contribution from all other types of sausages combined, ranging from about 6–10% across all population groups assessed. The total contribution to estimated sulphites exposure from all sausages was 43%, 45% and 44% for Australian children's population groups aged 2–5 years, 6–12 years and 13–16 years, respectively. All sausages contributed 21% to adults estimated sulphites dietary exposure. Details of the contribution of sausages to sulphites dietary exposure is provided in Table A6.5 of Appendix 6.

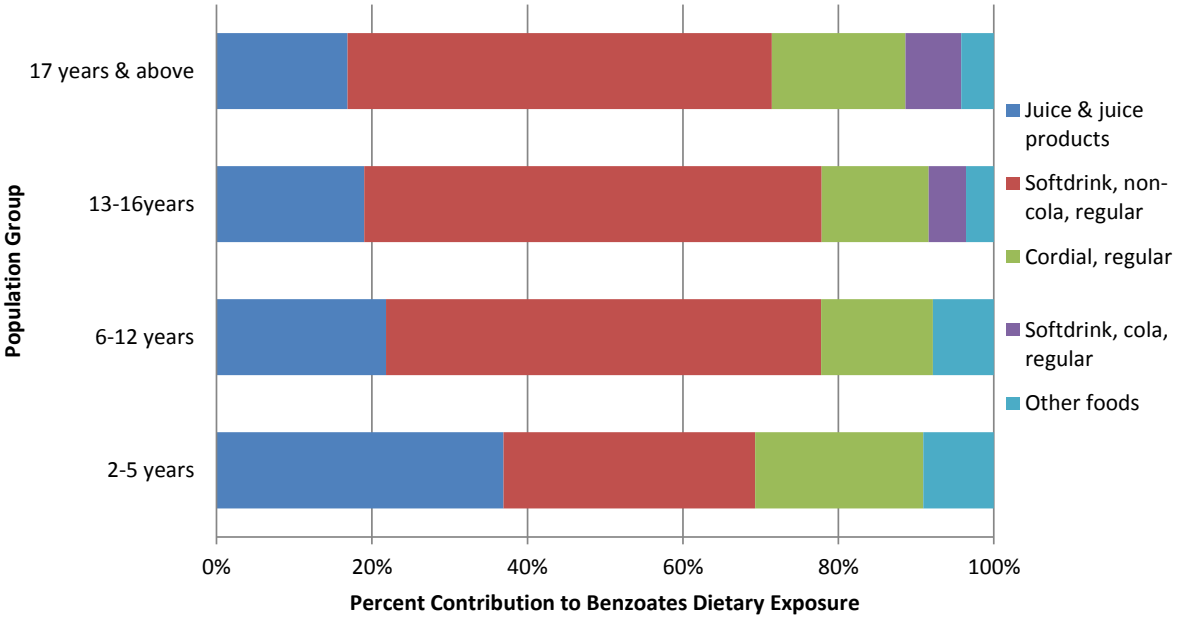


Figure 4: Major food contributors (>5%) to estimated benzoates exposures for Australian population groups

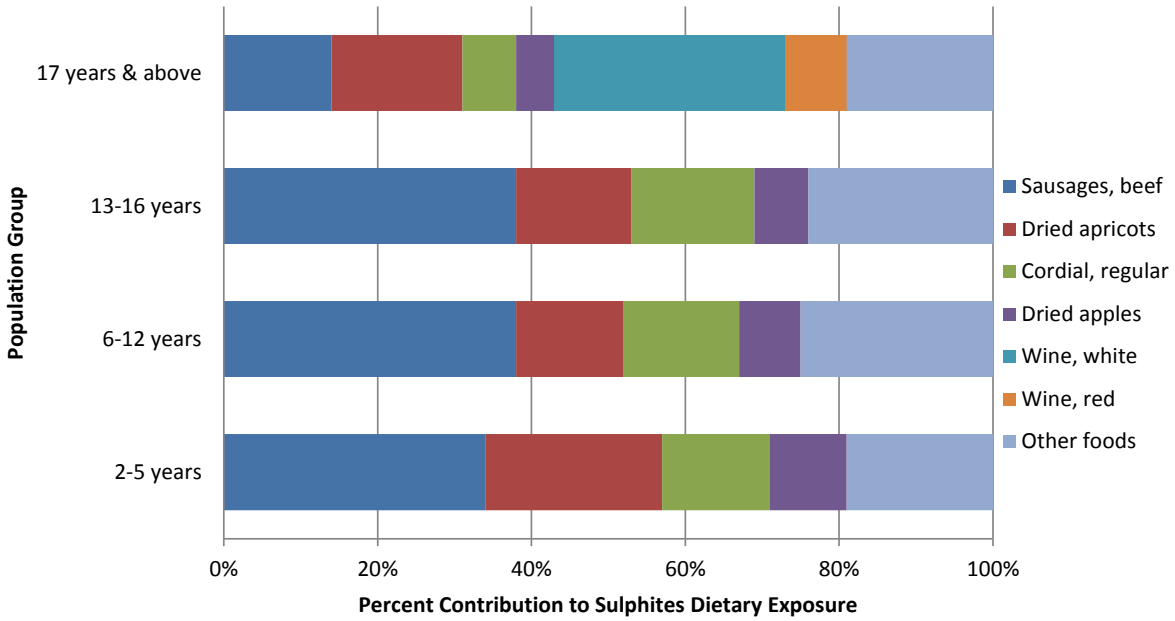


Figure 5: Major food contributors (>5%) to estimated sulphites exposures for Australian population groups

4.4 Approach to updating estimated dietary exposures for New Zealand populations

The lack of data relating to dietary exposure to sulphites and benzoates in New Zealand was expressed in submissions received following notification of the Initial Assessment Report for P298 in 2005. FSANZ subsequently sought to obtain data on the estimated dietary exposure to sulphites and benzoates of the New Zealand population. New Zealand dietary exposures assessments for sulphites and benzoates were performed by ESR on behalf of the then NZFSA, now the Ministry for Primary Industries (MPI) (Cressey and Jones, 2009). The New Zealand analytical study evaluated 30 selected foods available in New Zealand which were considered to be the predominant food sources of these preservatives.

Food consumption data used for these assessments included:

- New Zealand 2002 National Children's Nutrition Survey (2002 NCNS) covering 3,275 children aged 5–14[7] years for one day only
- New Zealand 1997 National Nutrition Survey (1997 NNS) covering 4,636 individuals aged 15 years and above for one day only.

The age groups selected for the New Zealand assessment aligned with age groups used in the 2002 children's and 1997 adult New Zealand nutrition surveys. The age groups were 5–12 years and 13–15 years from the 2002 NCNS and 16–18 years, 19–24 years, 25–44 years, 45–64 years and 65 years and above using the 1997 NNS. Males and females were reported separately.

The methodology for the New Zealand dietary exposure assessments is described in greater detail by Cressey and Jones (2009).

[7] The New Zealand 2002 National Children's Nutrition Survey was targeted at the age group 5-14 years and is represented as a Children's survey for 5-14 year olds. However, some 14 year olds turned 15 during the survey period.

4.5 Results from updated dietary exposure estimates for New Zealand

The New Zealand mean and 95th percentile exposure estimates for sulphites and benzoates are described in greater detail by Cressey and Jones (2009). In keeping with FSANZ's updated approach for reporting on the high consumer, further estimates of exposure for sulphites at the 90th percentile[8] were provided to FSANZ by ESR (ESR, 2008). The New Zealand dietary exposure estimates for consumers of benzoates (at the mean and 95th percentile) and sulphites (at the mean and 90th percentile) are presented below, expressed as a percentage of the relevant ADI, with results provided in Appendix 5, Table A5.1and Table A5.2.

4.5.1 Benzoates

The mean and 95th percentile estimated dietary exposures for consumers of foods containing benzoates were below the ADI of 0.5 mg/kg bw for all age groups assessed (Figure 6 and **Error! Reference source not found.**). Mean exposures were between 2–20% of the ADI and the 95th percentile exposures were 15–60% of the ADI.

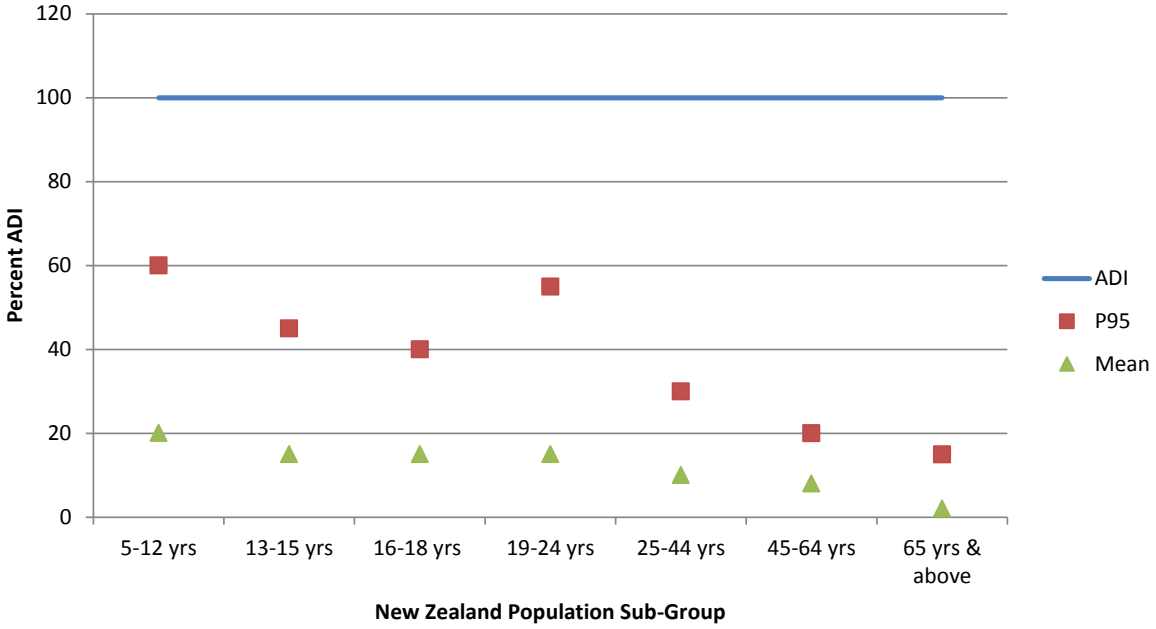


Figure 6: Mean and 95th percentile dietary exposures to benzoates as a percentage of the ADI for New Zealand males (consumers only)

[8] As 95th percentile estimated exposures to benzoates for all New Zealand populations were well below the relevant health based guidance value no calculation of the 90th percentile exposure was required.

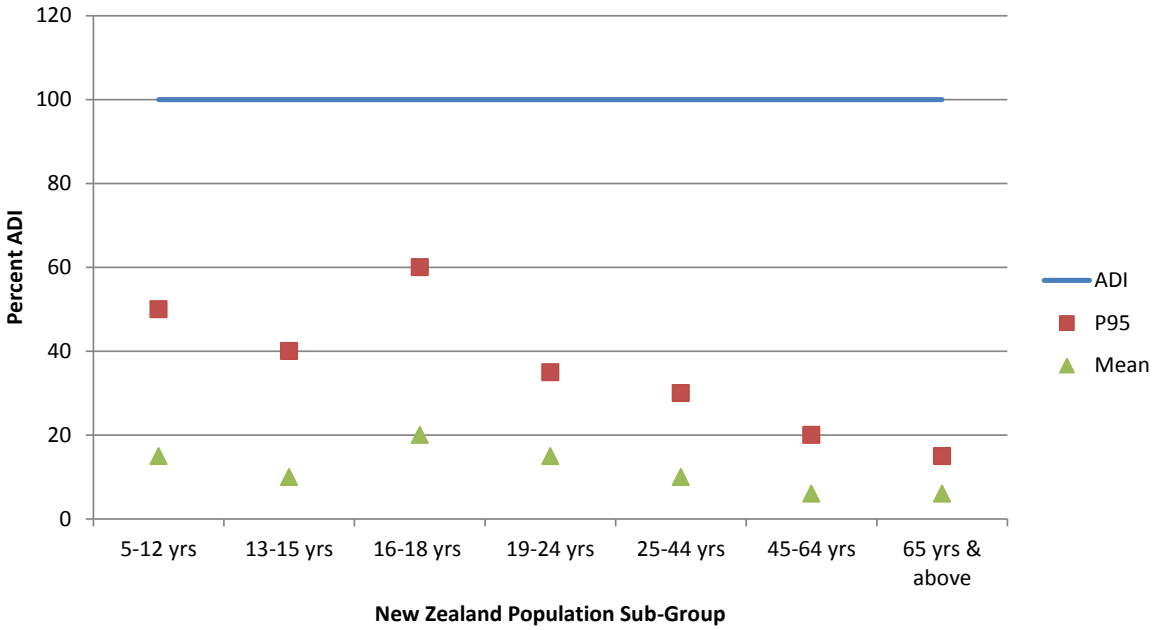


Figure 7: Mean and 95th percentile dietary exposures to benzoates as a percentage of the ADI for New Zealand females (consumers only)

4.5.2 Sulphites

Mean estimated dietary exposures for consumers of foods containing sulphites were below the ADI of 0.7 mg/kg bw (15–40%) for all age groups assessed; 90th percentile estimated dietary exposures for consumers of foods containing sulphites were below the ADI for all age groups except for 5–12 year olds boys where estimated dietary exposure to sulphites was 110% of the ADI (Figure 8 and Figure 9).

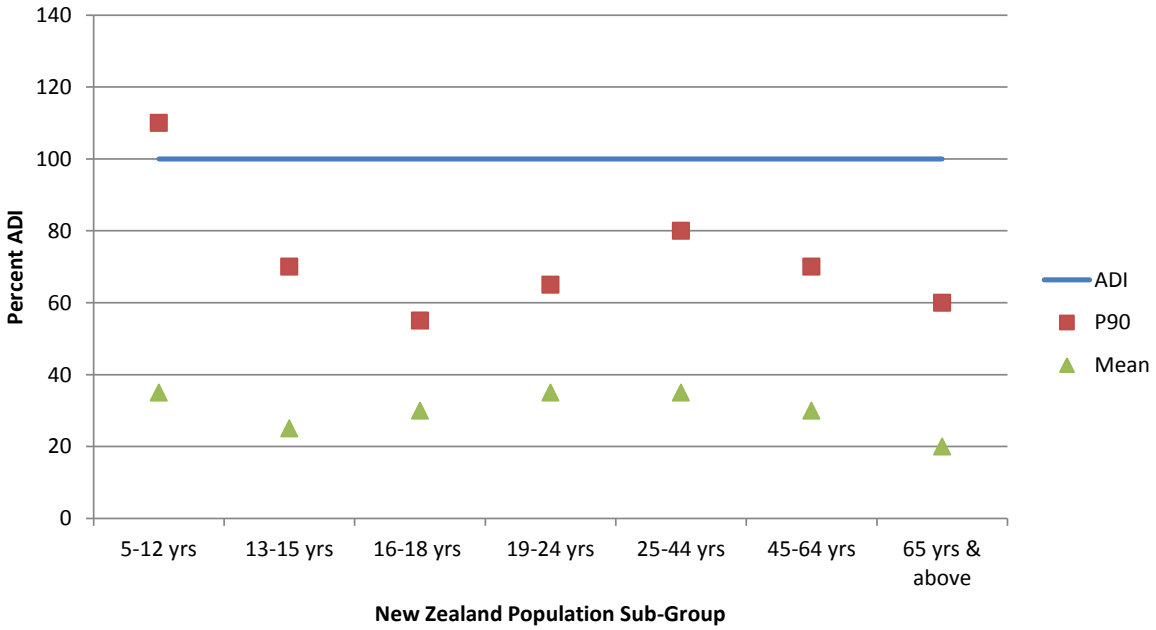


Figure 8: Mean and 90th percentile dietary exposures to sulphites as a percentage of the ADI for New Zealand males (consumers only)

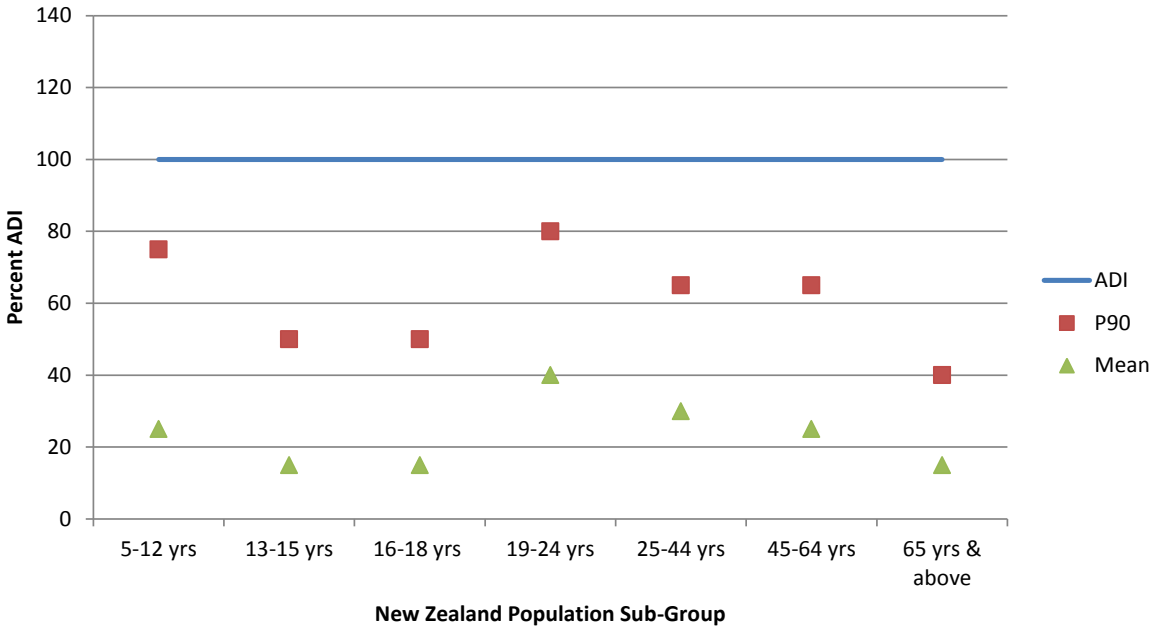


Figure 9: Mean and 90th percentile dietary exposures to sulphites as a percentage of the ADI for New Zealand females (consumers only)

4.5.3 Major contributors to benzoates dietary exposures for New Zealand populations

Major foods contributing to estimated dietary exposure to benzoates for both the 5–14 years and 15 years and above age groups were non-cola (72% and 62% respectively) and cola (23% and 34%, respectively) carbonated soft drinks. No other food group contributed more than 5% to estimated mean benzoates dietary exposure for any population group assessed.

Major contributors to benzoates estimated dietary exposure for New Zealand population groups are summarised in Figure 10, below. Detailed results of major contributors to dietary exposures for benzoates are provided at Appendix 6, Table A6.1.

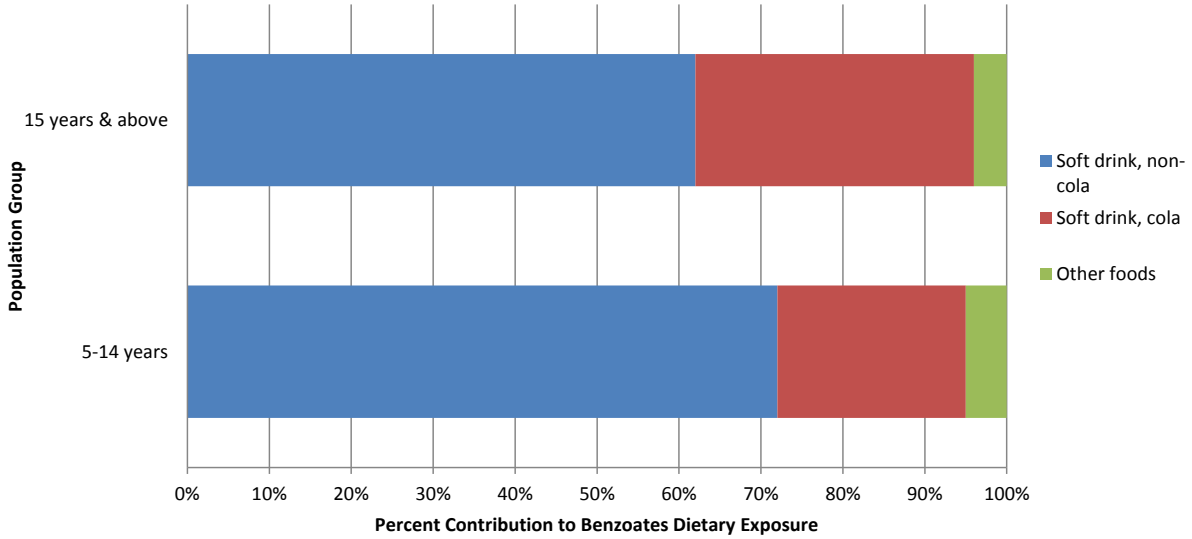


Figure 10: Major food contributors (>5%) to estimated benzoates exposures for New Zealand population groups

4.5.4 Major contributors to sulphites dietary exposures for New Zealand populations

Major foods contributing to estimated dietary exposure to sulphites for children 5–15 years were sausages (65%) and soft drinks (20% for cola and non-cola soft drinks combined). For adults 15 years and above dietary exposure was predominately through consumption of beer (24%), sausages (22%) and white wine (22%), while soft drinks (14% for cola and non-cola soft drinks combined), dried apricots (7%) and red wine (5%) were also major contributors (>5%).

Major contributors to sulphites estimated dietary exposure for New Zealand population groups are summarised in Figure 11, below. Detailed results of major contributors to dietary exposures for sulphites are provided at Appendix 6, Table A6.2.

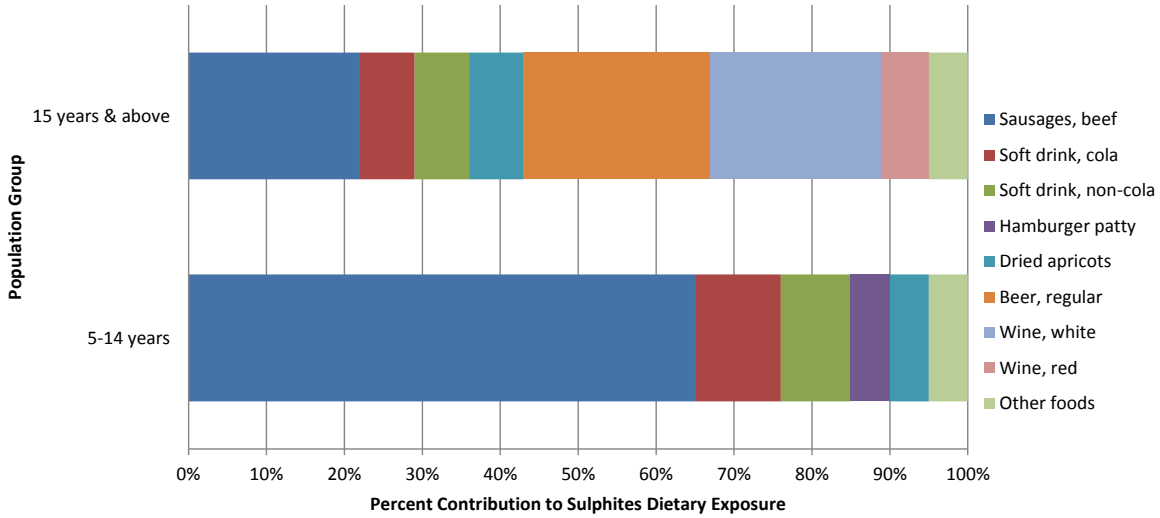


Figure 11: Major food contributors (>5%) to estimated sulphites exposures for New Zealand population groups

4.6 Differences in data inputs to dietary exposure assessments between Australia and New Zealand

There were some differences in the approach to the DEAs for sulphites and benzoates between Australia and New Zealand. These differences included:

- Both the 1997 NNS and the 2002 NCNS use a single 24-hour recall, as is the case for the 1995 NNS. In contrast, the 2007 ANCNPAS provides data on two 24-hour recalls on non-consecutive days, which provides a better estimate of long term exposure to food chemicals by taking the average food consumption per day over the two days.

- The number of foods analysed was lower in New Zealand (30 types of food) compared to Australia (59 food types). For the New Zealand study a preliminary exercise was carried out to determine foods that were likely to be the major contributors to dietary exposure. The subsequent survey was believed to capture 98% of estimated exposure to sulphites and benzoates.

- Concentrations of both preservatives in food varied between the New Zealand and Australian studies. For example, the benzoate levels in cordials found in the Australian and New Zealand analyses were 83 mg/kg and 21 mg/kg respectively; average levels of sulphites in cooked sausages were lower in New Zealand (179 mg/kg) than in

Australia (267 mg/kg although it is noted this is partly due to the greater presence of sulphite free sausages in the New Zealand market).

- More Australians (97–100%) were estimated to be consumers of foods containing sulphites and benzoates than New Zealanders (44–76% for sulphites, 25–64% for benzoates). This may be due to: the number of foods sampled and analysed in each survey; the number of New Zealand nutrition survey foods represented by the sampled foods compared with Australian nutrition survey foods represented by the sampled foods; and/or differences in the proportion of the population that consume foods containing the preservatives.

- The age and gender groups assessed for New Zealand in this evaluation were different to the groups used for Australia and thus direct comparisons between the assessments were limited.

- Major contributors (>5%) to estimated dietary exposure to sulphites and benzoates for the New Zealand assessments were only available for combined New Zealand survey populations for each of the 1997 NNS and 2002 NCNS (i.e. 5–14 years and 15 years and above).

4.7 Comparison of Australian and New Zealand estimates of dietary exposure

DEA results for sulphites and benzoates for both Australian and New Zealand were calculated for 'consumers' only, that is, those people who reported consuming food containing the preservative. For Australia, almost all survey respondents (97–100%) were identified as consumers of sulphites and benzoates. There were lower numbers of consumers of sulphites (44–76%) and benzoates (25–64%) reported for New Zealand populations (Cressey and Jones, 2009).

For all assessments, estimated mean and high percentile exposures for sulphites and benzoates expressed per kilogram body weight were higher for young children compared to adults. This is expected due to children's higher food intake on a body weight basis.

Direct comparisons of sulphites dietary exposure estimates between the Australia and New Zealand are limited by differences in population groups assessed and assessment methodologies (discussed above). However, there are some general similarities between the estimates of exposure. Mean and 90th percentile estimated exposures for children decrease as age increases, with young children having the highest exposures per kilogram bodyweight. However, exposures for young adults increase compared to older children, due to the introduction of beer and wine to the diet, and then these exposures again drop off with age.

At the 90th percentile, New Zealand boys aged 5–12 were estimated to exceed the ADI for sulphites by approximately 10% and New Zealand girls were estimated to have a 90th percentile exposure of 75% of the ADI. While it is not possible to average the exposure estimate across these two population groups, it is likely that New Zealand children aged 5–12 years (boys and girls combined) would have a 90th percentile estimated exposure below 100% of the ADI. This difference in estimated sulphites exposures between 5–12 year old New Zealand boys and girls does not appear to be reflected in the equivalent Australian populations, with estimated 90th percentile exposures for Australian boys and girls aged 6–12 years at 85% and 75% of the ADI, respectively. It should also be noted that New Zealand estimated exposures for 5–12 year olds were based on consumption data derived from one day of dietary recall data, which is likely to over-estimate exposure (FSANZ, 2009a). The

potential to exceed the ADI for high consuming 5–12 year old New Zealand boys would be reduced if estimates were based on more than one day of dietary data.

The New Zealand dietary exposure assessment did not consider younger children aged <5 years, as consumption data are not collected for this population group in the New Zealand nutrition surveys. However, smaller children usually have a higher exposure to food chemicals when expressed per kilogram bodyweight, as their food consumption per kilogram of body weight is higher. In addition, the major contributors to sulphites dietary exposure for New Zealand children aged 5–14 years (sausages and soft drinks) are also foods commonly consumed by younger children. Therefore, it is likely that New Zealand children aged 2–4 years would have a higher exposure to sulphites than New Zealand children aged 5–12 years, and therefore are also likely to exceed the ADI.

4.8 Conclusion - updated dietary exposure assessment

The updated DEAs for benzoates and sulphites for Australian populations indicate a reduction in the overall estimated mean dietary exposure to both preservatives, in comparison to the previous 21st ATDS assessment. Mean and 90th or 95th percentile exposures for consumers of foods containing benzoates were well below the relevant health based guidance value (ADI) for all Australian and New Zealand population groups. Mean exposures for consumers of foods containing sulphites were well below the relevant health based guidance value (ADI) for all population groups, and 90th percentile exposures below the ADI for most population groups.

For Australia, children aged 2–5 years were the only population group that had an estimated dietary exposure to sulphites above the health based guidance value. For this population group, although mean exposure was well below the ADI, 90th percentile exposure to sulphites was estimated at 130% of the ADI. Similarly, for New Zealand, boys aged 5–12 years were the only population group for which estimated dietary exposure to sulphites exceeded the relevant health based guidance value. For this group dietary exposure to sulphites was approximately 110% of the ADI at the 90th percentile of exposure, while mean estimated exposure was well below the ADI.

There are a number of limitations and uncertainties inherent in the DEAs, including assumptions made when mapping the NNS foods to the analysed foods, certain limitations of the analytical data and sampling, and the design of the food consumption surveys. In particular, there are limitations in using one or two day dietary recall surveys to represent usual food intake over the longer term. Where there are uncertainties in the data, FSANZ generally makes conservative 'worst-case' assumptions to ensure that dietary exposure is not underestimated.

Despite these uncertainties, the DEA represent the best estimate of dietary exposure for benzoates and sulphites using the available data. Overall, the DEAs for sulphites and benzoates are conservative and likely to lead to an overestimate of actual dietary exposure.

Response to Risk Assessment Question 3: *Considering the latest consumption and concentration data available, what are the estimated total dietary exposures to sulphites and benzoates?*

For consumers of foods containing benzoates, estimated benzoates dietary exposures were below the health based guidance value for all Australian and New Zealand population groups assessed at both mean and 90th percentile levels of exposure (for Australia) or mean and 95th percentile (for New Zealand).

For consumers of foods containing sulphites, estimated dietary exposures to sulphites were below the health based guidance value for all the Australian and New Zealand population groups assessed at

the mean and 90th percentile level of exposure, except for Australian children aged 2–5 years and New Zealand boys aged 5–12 years. For both these population groups, mean estimated exposures to sulphites were well below the ADI, while 90th percentile exposure was 130% and 110% of the ADI, respectively.

Response to Risk Assessment Question 4: *What are the major food sources of sulphites and benzoate exposures?*

Major contributors to benzoates exposures for the Australian population were the non-alcoholic beverages for all the age groups analysed. Within this larger food group, juice and juice products, non-cola soft drinks, and cordials were the highest contributors while cola soft drink also contributed more than 5% for those aged 13 years and above. Carbonated soft drinks were the major contributor to benzoates exposure for all New Zealand population groups assessed.

For Australian populations, major contributors to sulphites exposure for 2–16 year olds were beef sausages, dried apricots and cordials while the major contributors for adults aged 17 years and above were white wine, beef sausages and dried apricots. Major contributors to dietary exposure to sulphites for New Zealand children aged 5–15 years were sausages and soft drinks. For New Zealand adults aged 15 years and above, dietary exposure was predominately through consumption of beer, sausages and white wine.

5 Chemical risk characterisation

5.1 Benzoates

Mean estimated dietary exposures for consumers of foods containing benzoates were between 2–20% of the ADI and 90th and 95th percentile exposures ranged from 15–60% of the ADI for Australian population groups using 2007 ANCNPAS data, and for New Zealand population groups (ESR, 2008; Cressey & Jones, 2009).

The conclusion of the risk and technical assessment for benzoates was that there is no public health and safety concern for the Australian and New Zealand populations arising from the consumption of foods containing benzoates.

5.2 Sulphites

A full hazard assessment has been prepared for sulphites, and is attached (Appendix 7). The hazard characterisation is confounded by the considerable uncertainties concerning the toxic mechanism, target organ or system, and NOAEL of sulphites in animal models. The current JECFA group ADI for sulphites (expressed as sulphur dioxide) is 0–0.7 mg/kg bw (LOAEL~150 mg/kg bw/day). FSANZ has concluded that robust evidence from a definitive study conducted to modern experimental standards would be necessary to derive a revised group ADI. It is likely that a revised group ADI would be higher than the current group ADI.

Dietary exposure assessments were undertaken using the more recent Australian and New Zealand data and dietary exposure assessment methodology. From this analysis, the estimated mean and 90th percentile exposures for sulphites were found to be higher for children compared to adults when expressed on a body weight basis, which is primarily related to their higher food consumption to body weight ratio. Nonetheless, the estimated *mean* dietary exposure for sulphites was below the ADI for all Australian and New Zealand population groups. However, sulphite exposure at the *90th percentile* exceeded the ADI (130% and 110%) for children aged 2–5 years in Australia and boys aged 5–12 years in New Zealand, respectively. While consumption data for New Zealand children aged <5 years is not collected and a DEA for this population group was not conducted, it is likely that younger New Zealand children aged 2–4 years would also exceed the ADI at the 90th percentile.

Direct irritation of the gastric mucosa, with consequent mucosal injury, has been considered to be the most sensitive endpoint associated with acute dietary exposure to sulphites in experimental animals, but this effect has not been shown to be reproducible in laboratories other than the laboratory in which it was characterised. In the absence of a clearly identified mechanism of toxicity, it is not possible to determine if findings in experimental animals are relevant to human beings.

Response to Risk Assessment Question 5: *Taking into consideration any new information relevant for assessing the risk of benzoates and sulphites in food, that has become available since FSANZ completed its last review of their safety, is there a public health and safety concern for the Australian or New Zealand populations arising from the consumption of foods containing sulphites or benzoates?*

The existing ADI values for benzoates and sulphites for Australia and New Zealand (FSANZ) are concordant with international standards (JECFA), and no new, robust information relevant to the potential toxicity of benzoates and sulphites has been identified from the scientific literature. However, it is noted that the ADI for sulphites established by JECFA in 1974 is based on animal studies that suffer from significant design flaws, which place uncertainty on the toxic mechanism, the organ or system most sensitive to toxic effects, and the lowest dose at which such toxic effects are observed. Subsequent toxicological studies, although limited, cast further doubt on the reproducibility of the established toxicological endpoint at levels of dietary exposure that are likely to occur.

Updated dietary exposure estimates indicated that exposure to benzoates for those consuming foods containing these additives in Australia and New Zealand was below the ADI for all age groups at both the mean and 90th percentile. The conclusion of the risk and technical assessment for benzoates is that there is no public health and safety concern for the Australian and New Zealand populations arising from the consumption of foods containing benzoates.

Estimated dietary exposures to sulphites were below the ADI for most age groups assessed, with the exceptions of children aged 2–5 years in Australia and boys aged 5–12 years in New Zealand at or above the 90th percentile exposure level for sulphites. Based on the Australian 2007 ANCNPAS data and the New Zealand 2002 NCNS, sulphite exposures, for the high consumer at the 90th percentile, were estimated at 130% and 110% of the ADI for both of these population groups, respectively. It is also considered likely that younger New Zealand children, aged 2–4 years, would also exceed the ADI at the 90th percentile of exposure, similar to Australian children aged 2–5 years. Whilst this level of exposure is above the ADI, it does show a distinct reduction for the same age groups, compared with the previous Australian dietary exposure assessment, reported in the 21st ATDS.

In 2016 the European Food Safety Authority (EFSA) published a Scientific Opinion on the re-evaluation of sulphites as food additives (EFSA, 2016b). The Panel concluded that exposure estimates to sulphur dioxide-sulphites were higher than the group ADI of 0.7 mg SO_2 equivalent/kg bw per day for all population groups.

The period of time in which exceedances may occur is limited when compared to whole of life exposure on which the ADI is based. Because the target organ or system, and toxic mechanism of action of sulphites are both uncertain, it is difficult to estimate the severity of any adverse effects that may develop over a limited time, or whether they would have long-term consequences. This consideration may now be moot in view of new information suggesting that the toxicological endpoint of the current ADI (i.e. stomach lesions) is not reproducible at likely levels of dietary sulphite exposure (Jonker et al. 1990; Cayzer 2015). Although a new toxicological study will be required to establish a new robust ADI, FSANZ is of the opinion that the existing ADI is too low and that current levels of dietary exposure are unlikely to pose a risk for all consumers.

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Appendix 1: Current benzoates and sulphites permissions in Schedule 15 (table to section S15—5)

Table A1.1: Summary of benzoates (INS 210, 211, 212 and 213) permissions for Australia and New Zealand

Food	Maximum permitted levels (mg/kg)
Preparations of food additives	1000
Rennetting enzymes	9000
Oil emulsions (<80% oil)	1000
Ice confection sold in liquid form	400
Mushrooms in brine or water and not commercially sterile	500
Preserved cherries (maraschino cherries, cocktail cherries or glace cherries)	1000
Fruits and vegetables in vinegar, oil, brine or alcohol	1000
Low joule chutneys, low joule jams and low joule spreads	1000
Fruit and vegetable preparations including pulp	1000
Chilli paste	3000
Other fruit and vegetable based products – imitation fruit	400
Icings and frostings	1000
Semi preserved fish and fish products	2500
Table top sweeteners – liquid preparation	GMP (Good Manufacturing Practice)
Solid formulated supplementary sports foods	400
Liquid formulated supplementary sports foods	400
Fruit and vegetable juices and fruit and vegetable juice products	400; GMP principle precludes the use in juices represented as not preserved by chemical or heat treatment.
Coconut milk, coconut cream and coconut syrup	1000
Water based flavoured drinks	400
Formulated Beverages	400
Fruit wine, vegetable wine and mead (including cider and perry)	400
Mixed alcoholic beverages.	400
Dairy and fat based desserts, dips and snacks	700
Sauces and toppings (including mayonnaises and salad dressings)	1000

Table A1.2: Summary of Sulphites (INS 220, 221, 222, 223, 224, 225, 228) permissions for Australia and New Zealand

Food	Maximum permitted levels (mg/kg)
Preparations of food additives	350
Cheese and cheese products	300
Ice confection sold in liquid form	25
Peeled and/or cut fruits and vegetables – products for manufacturing purposes; apples and potatoes only.	200
Peeled and/or cut fruits and vegetables – root and tuber vegetables	50
Frozen unprocessed fruits and vegetables – frozen avocado	300
Processed fruits and vegetables (ginger only)	20
Dried fruits and vegetables	3000
Desiccated coconut	50
Products made from bleached vegetables	750
Low joule chutneys, low joule jams and low joule spreads	285
Candied fruits and vegetables	2000
Fruit and vegetable preparations including pulp	350
Fruit and vegetable preparations for manufacturing purposes	1000
Other fruit and vegetable based products – imitation fruit	3000
Flour products (including noodles and pasta)	300
Biscuits, cakes and pastries	300
Processed comminuted meat, poultry and game products	500
Sausage and sausage meat containing raw, unprocessed meat	500
Edible casings	500
Unprocessed fish and fish fillets – uncooked crustacea	100
Processed fish and fish products – cooked crustacea	30
Fully preserved fish including canned fish products	30
Canned abalone (paua)	1000
Sugars and syrups	450
Vinegars and related products	100
Solid formulated supplementary sports foods	115; sulphur dioxide only
Liquid formulated supplementary sports foods	115; sulphur dioxide only
Fruit and vegetable juices and fruit and vegetable juice products	115; GMP principle precludes the use in juices represented as not preserved by chemical or heat treatment.
Water based flavoured drinks	115
Formulated Beverages	115
Beer and related products	25
Wine, sparkling wine and fortified wine containing >35 g/L residual sugar	400
Wine, sparkling wine and fortified wine containing <35 g/L residual sugar	250
Fruit wine, vegetable wine and mead containing > 5 g/L residual sugar	300
Fruit wine, vegetable wine and mead containing < 5 g/L residual sugar	200
Mixed alcoholic beverages	250
Sauces and toppings (including mayonnaises and salad dressings)	350
Note: some unprocessed fruits and vegetables may have sulphites present due to agricultural chemical residues	Typically 10

Appendix 2: Technological impact of a reduction of sulphites in sausages

In order to better understand the current raw meat sausage industry and investigate options for reducing levels of or replacing sulphites in sausages, in 2008, FSANZ commissioned a report; A *Review of Sulphites in Raw Meat Sausages*, from the South Australian Research and Development Institute (SARDI 2009) (see Supporting Document 2). SARDI was tasked to describe the uses of sulphites in sausages in Australia and overseas and to consider the potential impacts of lowering levels of sulphites in raw sausages in Australia, either through reduction of in-going sulphites from 500 mg/kg to 200 mg/kg or its complete elimination from sausage formulations. In preparing the report, SARDI differentiated between sausages made by local butchers, these typically having a much shorter supply chain and shelf-life requirement and potentially needing less sulphites; and medium volume manufacturers and high volume suppliers of supermarket retail sausages which have a much longer supply chain.

Most manufacturers use a sausage premix which contains, amongst other ingredients, the sulphites. The premixes may or may not be customised for culinary ingredients. FSANZ has been advised that the mixes for butchers generally contain sulphites at a concentration which is intended to provide 500 mg/kg in the sausage. Custom premixes for larger manufacturers generally provide sulphites in the sausage at 500–600 mg/kg, however, manufacturers target a level in the manufactured product of under 500 mg/kg, taking into account initial losses during manufacture. The general manufacturing procedure, based on information provided by the sausage industry, appears to be to manufacture fresh sausages with a sulphite level around 500 mg/kg, regardless of whether this level is required for a particular distribution chain. This allows simpler management of production, distribution and sales.

The SARDI report included a summary of trials conducted by the Australian sausage manufacturing industry. While based on limited data, this report and its associated graphs, for example

Figure 12 below, showed a direct relationship between the level of sulphites added at manufacture and residual sulphites remaining after 15 days storage. It showed that for sausages produced by high-volume, long supply chain manufacturers, a significant reduction of the ingoing sulphite concentration at the time of manufacture to 200 mg/kg[9] would effectively halve shelf-life to around nine days.

[9] A reduction to 200 mg/kg was arbitrarily chosen by SARDI because it (i) represented a significant (60%) reduction in ingoing sulphite level; (ii) allowed some shelf-life extension, to around 9 days; and (iii) had a low residual sulphite level at the end of shelf-life (around 40 mg/kg after cooking).

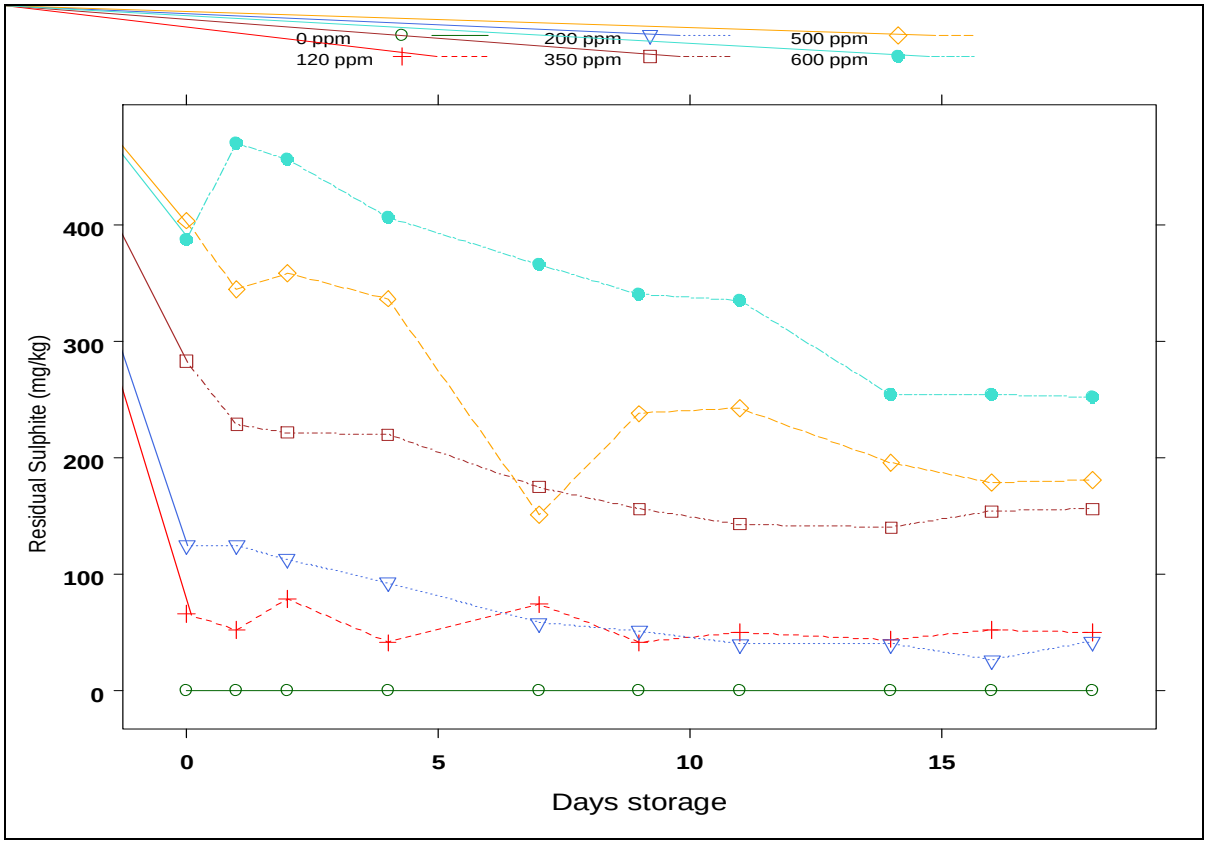

Figure 12: Effect of in-going sulphite level on residual sulphite in sausages stored at 4°C

Impact of a reduction of the MPL for sausages to 350 mg/kg

If the MPL for sausages were to be reduced to 350 mg/kg, on the basis of when sold by the manufacturer, the level of sulphites added at manufacture would need to more precisely relate to the expected shelf life and distribution chain, that is, sausages with a shorter expected shelf life would need to add less sulphites at the point of manufacture. This is in accordance with the requirement in the Code that additive use be consistent with GMP.

According to the SARDI report (p 34) sausages made with an ingoing level of 350 mg/kg sulphite had a shelf life of 11 days, which is indicative of a conservative shelf life for sausages containing 350 mg/kg sulphites. This was based on their defined end-point of a Total Viable Count of 10^7, although industry members have advised that in their experience it may be shorter than this when colour and natural variability are taken into account.

To achieve the MPL of 350 mg/kg in sausages that are made for in-store sales or for local distribution a reduced input level from 500 mg/kg to, presumably, 350 mg/kg would be needed as the sausages are often ready for immediate sale. This would reduce the sausages shelf life by approximately one third (from 18 to 11 days, according to the SARDI report, but noting the SARDI shelf-life end-point may not be the same as industry's). For many smaller manufacturers who make sausages twice or more a week this should not be a technical issue, but for those who only make sausages once per week it might increase their manufacturing costs.

For sausages that are made for regional or national distribution, because of the natural decay in sulphite levels in the sausages, product made with an ingoing level of 500 mg/kg could still be made provided they were not sold (i.e. still in manufacturer's storage or distribution) until after around three days. These manufacturers would need to manage their formulations and supply chains – including for sausages that may also be sold locally – to ensure that the sausages they sell have a sulphites level under 350 mg/kg.

Alternatives to the use of sulphites

SARDI identified current and potential methods to reduce or eliminate sulphites in long supply chain sausages, for example semi-freezing the final product, addition of other antimicrobial compounds such as chitin, or the use of modified atmosphere packaging. However, it was recognised that these alternative methods of preservation would lead to increases in costs. These costs were not quantified over the short-term in transitioning to alternatives or following implementation because companies have different business models. To properly evaluate any change to sulphites use could involve individual enterprises embarking on in-depth analysis of their businesses practices and those of their suppliers.

The report also described how long supply chain sausages are made in the USA, where sulphites are not permitted. Bulk packs and the use of modified atmosphere packaging, BHA, BHT or TBHQ and/or freezing are used to achieve a shelf-life of 12 days.

The raw meat sausage industries in Australia, New Zealand, Europe and Canada have not adopted the USA approach. In Australia and New Zealand the reasons given included that the actual sausage products in the USA are generally a different style; that freezing would cost considerably more, may change the sausage's attributes, and would impact on labelling; modified atmosphere packaging would cost more than current packaging systems, and BHA and BHT are not currently permitted in sausages.

Maintaining supply of low or no-sulphites sausages to major supermarkets would involve significant research and development by the small number of manufacturers which currently service this market, with a proportion of the cost passed on to consumers. Most likely solutions involve use of antimicrobial ingredients such as lactate and diacetate, coupled with modified atmosphere packaging. Improvements to temperature control during production, storage by the manufacturer, transport to distribution centre, storage at distribution centre, transport to retail store, storage back-of-house and display may also be effective in extending shelf-life.

FSANZ has held a number of discussions with Australian and New Zealand sausage manufacturers, marketers and ingredient suppliers in order to establish the levels of sulphites used, whether a reduction is possible, and what the cost and safety implications would be of reducing or eliminating sulphites. The outcomes of these discussions indicate that the Australian industry, along with ingredient suppliers, has been conducting research into alternatives to sulphites, and while there has been some progress to date the ingredient suppliers have not been able to meet the existing product characteristics through the current supply chain when trialling the alternatives. These ingredient suppliers are active internationally, so can incorporate the latest technological options.

The SARDI report noted that completely eliminating sulphites from raw meat sausages would give no more than four days shelf life before the bacterial loading becomes excessive and that sulphites-free sausages may quickly (two days) take on a grey appearance, further limiting their acceptability.

Appendix 3: Concentration data used in the dietary exposure assessment

The mean concentration levels used for sulphites and benzoates assessments were obtained through analytical studies. The main sources of analytical data used in this report were:

- 21st Australian total diet survey results (FSANZ, 2005)
- dried apricot concentration data for sulphites (FSANZ, 2008)
- dried apple, sultana, cordial and sausage concentration data from a 2011 analytical survey of these foods (FSANZ, 2012).

Levels of chemicals in foods can be highly variable, even within the same type of food, as there are many factors that can affect this level. Data derived from analytical surveys were assessed to determine the quality and appropriateness for use in dietary exposure estimates. It is common that food chemical concentration data available for dietary exposure assessments are imperfect and not fully representative of the chemical and food being studied (FSANZ, 2009a).

Concentration data used in the dietary exposure assessment are shown in Table A3.1.

Summary of dried apple, sultana, cordial and sausage survey

This survey was undertaken to supplement the analyses undertaken as part of the 21st Australian Total Diet Study (ATDS), with the view to update and extend the analytical evidence base, given the limited number of samples analysed for the 21st ATDS and the reported contribution of these foods to sulphites exposures, particularly for children (>5%).

The analytical survey of sulphites levels in sausages, cordial and dried fruit was conducted between April and June 2011. As additional data had already been collected on sulphite levels in dried apricots in 2008, this survey analysed dried apple and sultana samples. A total of 207 samples were purchased, including 156 sausage samples, 30 cordial samples, 9 dried apple samples and 12 sultana samples. Market research was conducted to determine the amounts, brands and types of samples that were collected.

To take into account the variation of sulphites usage in the sausage industry, sausage samples were purchased from supermarkets, butchers shops and poultry shops in each State and Territory in Australia. Thick and thin beef varieties were included, as well as beef, lamb, chicken, pork, and other mixed meat and flavoured varieties. In order to give a representative sample, different brands and use by/best before dates were selected. Sausages labelled or sold as sulphite/preservative free, organic, vegetarian, salami, chorizo, chipolatas, frankfurts, smoked, cured, liver, parboiled and blood sausages were excluded from the survey.

As dried fruit and cordial samples are generally manufactured locally and distributed nationally, samples were collected from supermarkets in the Australian Capital Territory (ACT). Different brands and use by/best before dates were selected to give a representative sample. For the cordial samples, an assortment of flavours were purchased, as well as regular, concentrated and no added sugar or light (diet) varieties. Alcoholic cordials such as liqueurs were excluded.

As with foods analysed in the 21st ATDS, all foods purchased in this survey were prepared to a ready-to-eat state prior to analysis.

The updated mean concentrations for cordials, dried apple and sultanas were incorporated into the dietary exposure assessment by replacing the existing mean concentration data derived from the 21st ATDS.

Mean concentration values for use in the modelling were derived for sausages grouped according to the type of meat (or type of meat that made up the highest percentage for mixed-meat sausages e.g. pork and veal). Where the type of meat was unable to be identified, these sausages were grouped with beef sausages. A mean concentration value derived from the entire sausage sample (including all meat varieties) was allocated to those sausages consumed in the nutrition surveys that were not identified by meat variety (ie sausage, not further specified).

Results less than the limit of reporting

For analytical results from the 21st ATDS, the Limit of Detection (LOD) [10] for each of the food chemicals evaluated was 2 mg/kg. Given that the food chemicals assessed are specifically added to certain foods, it was assumed that the chemicals were not added and were not present when the analytical result was less than the LOD. Therefore the chemical concentration of the food was assumed to equal zero. Assumptions have also been made about analytical results reported as being between the LOD and the limit of reporting (LOR)[11]. It may not be reasonable to assume that the food chemical is not present in the food when the analytical results are between the LOD and LOR as they are specifically added in for technological purposes. Therefore when the food chemical concentrations were between the LOD and LOR, it can be assumed that concentrations may equal to the LOR. The LOR was 5 mg/kg for sulphites and 10 mg/kg for benzoates. Given that there were few foods for which the analytical results were between the LOD and LOR for benzoates and sulphites, the dietary exposure assessment was conducted and reported using mean concentration data calculated assuming those foods with a chemical concentration between the LOD and LOR were at the LOR. This may result in a slight over-estimation of sulphites and benzoates concentrations used in the dietary exposure assessment.

Mapping analysed foods to consumed foods

A major step in the calculation of estimated dietary exposures is matching (or mapping) the approximately 4,367 foods reported as consumed in the 2007 ANCNPAS and 4,551 foods reported as consumed in the 1995 NNS to the foods analysed for sulphites and benzoates. For example, white bread may be assumed to represent a whole group of foods such as wholemeal bread, multigrain bread, rolls and fancy breads. Recipes are used for mixed foods to assign their ingredients to the appropriate analysed foods, for example, the proportion of dried apricots and apples in breakfast cereal. Table A3.1 also outlines the 63 foods surveyed and which foods these represent in the Australian nutrition surveys.

As only beef sausages were analysed for the 21st ATDS, the mapping of sausages for this dietary exposure assessment was amended slightly to take into account the range of analytical data available for sausages made from different meats (beef, chicken, pork and lamb).

[10] The LOD is the lowest concentration of a chemical that can be detected and quantified, with an acceptable degree of certainty, using a specified laboratory method and/or item of laboratory equipment.
[11] The LOR is the lowest concentration of a chemical that can be reported with an acceptable degree of certainty, using a specified laboratory method and/or item of laboratory equipment.

Table A3.1: Mean concentration levels of benzoates and sulphites in foods analysed[#] and foods represented in the Australian NNS

Food analysed	Food represented in NNS	Benzoates	Sulphites
		(mg/kg)	
Beverages, alcoholic			
Alcoholic cider	Ciders	0	78
Beer, regular alcohol	All beers, reduced alcohol, low alcohol, stout	NA	3
Wine, white	All white wines and de-alcoholised and non-alcoholic wines	1	123
Wine, red	All red wines and fortified wines	0	55
Beverages, non-alcoholic			
Blackcurrant juice syrup	Blackcurrant juice drinks	94	2
Cordial, regular*	All cordials, those made from dry base	83	19
Orange juice, refrigerated	All fruit and vegetable juices and fruit and vegetable juice products, except home squeezed juices and cordials	51	1
Soft drink, cola regular	All cola soft drinks and non-fruit flavoured drink bases	17	5
Soft drink, non-cola regular	All non-cola soft drinks, flavoured mineral waters & sports drinks/electrolyte drinks	220	1
Cereal and cereal products			
Bread, white	All breads, and rolls, English-style muffins, crumpets, flat breads, buns and yeast-based products including doughnuts (uniced), fancy breads, bread-based stuffings, tortilla, taco shells, and corn bread.	0	0
Chocolate cake, iced (cake only)	All uniced cakes and muffins, excluding dry mixes, muffins/cakes made from dry mixes, and homemade muffins/cakes	3	NA
Fruit cake, un-iced	All commercial uniced fruit cakes, sultana cakes, fruit muffins and dried fruit containing cake-style desserts, fruit breads, fruit buns	23	6
Pie/danish, fruit	Fruit containing pastries (pies, danish, strudels)	NA	2
Lamingtons	Coconut biscuits/slices/bars, lamingtons & macaroons, coconut creams & milks, coconut	41	3
Muesli bars, containing fruit	All breakfast bars and all muesli bars, muesli slices, commercial fruit biscuits except-chocolate chip	3	35
Noodles, egg fresh	All wheat, rice & egg noodles, except dry and instant noodles	1	0

Food analysed	Food represented in NNS	Benzoates (mg/kg)	Sulphites (mg/kg)
Pasta, fresh	All pasta, except dry pasta, filled pasta and corn pasta	0	1
Pasties, meat and vegetable	Savoury pastry products – single/double crust, including pies, pasties, quiche, vol au vent, pastry roll, spring roll, dim sim, wontons	4	9
Pikelets	Unfilled pancakes, crepes and pikelets, except home-made pancakes/ pikelets/ crepes and pancakes/ pikelets/ crepes made from dry mix	0	NA
Pizza, meat and vegetable topped	All pizza, all toppings, thick/thin base, including commercial, homemade & frozen	3	1
Condiments			
Barbeque sauce	Barbeque sauce, tomato sauce, commercial	3	7
Chutney, fruit	All chutneys	1	7
Dressing, oil and vinegar based	Italian and French dressings and vinegar	1	7
Soy sauce	All soy sauces, including Asian style fish/oyster sauces	7	11
Dairy products			
Cheese, cheddar, full fat	Ripened cheeses	9	1
Cheese, cottage	Unripened cheeses	11	0
Cheese, processed cheddar type	Processed cheeses	12	2
Dip, cream cheese based	All cheese-, cream- and yoghurt based dips (excluding home-made)	9	3
Fats and oils			
Margarine spread, <80% fat, polyunsaturated	All "margarines" with <80% fat content	1	NA
Fish, seafood and fish products			
Prawns, cooked fresh	All cooked and raw crustacea	NA	1
Smoked cod	All smoked fish	0	NA

Food analysed	Food represented in NNS	Benzoates (mg/kg)	Sulphites (mg/kg)
Fruit			
Apples, dried*	Dried apples, bananas, pears, pineapple, fruit medley, and glace pineapple and ginger	NA	1334
Apricots, dried^	All dried and glace "orange" coloured tree fruits, mixed peel	NA	1490
Fruit filled bars, cereal coated	Fruit filled biscuits and breakfast cereals, tartlet biscuits, fruit mince pies	0	16
Fruit fingers	Fruit based bars except cereal/grain coated, including fruit leathers	0	243
Fruit salad, canned	All canned fruits, excluding infant fruit	NA	2
Grapes, green seedless	All fresh grapes	NA	1
Jam, low joule	All low joule jams and conserves	9	1
Prunes	All dried "darker" tree fruits, glace cherries	9	NA
Sultanas*	All dried vine fruits, chocolate covered dried fruits, mixed dried fruit	NA	5
Meat and meat products			
Frankfurts	All plain frankfurts, saveloys, and hot dog sausages	NA	55
Hamburger, patties/rissoles	All hamburger patties	NA	129
Luncheon sausage, uniform mixture	All uniform mixture meat pastes, except ham paste	NA	28
Mince, red meat	All minced meats, including chicken	NA	12
Salami	All salamis, cabanossi	1	3
Sausages, beef*	All beef sausages, sausages where beef was identified as being the major meat source, and sausage patties	NA	282
Sausages, chicken*	All chicken sausages and sausages where chicken was identified as being the major meat source	NA	211
Sausages, lamb*	All lamb sausages and sausages where lamb was identified as being the major meat source	NA	264
Sausages, pork*	All pork sausages and sausages where pork was identified as being the major meat source	NA	249
Sausages, not specified as to type*	All sausages where the meat source was not identified	NA	267
Strasburg	Brawn, mortadella, strasburg	NA	3

Food analysed	Food represented in NNS	Benzoates (mg/kg)	Sulphites (mg/kg)
Snack foods			
Potato crisps	All potato crisps and extruded snacks	NA	3
Sugar/confectionery			
Chocolate cake, iced (icing only)	All glace icings, "frostings and icings with added fat", except homemade and those containing cream cheese or chocolate	28	NA
Ice confection, sold in liquid form	All water ice confections in stick/bar form, excluding those containing beverage whitener	163	2
Ice cream topping	All "toppings"	134	4
Lollies, soft jelly type	All lollies and other confectionery except compound yoghurts and carbohydrate modified confectionery	NA	5
Vegetables			
Coleslaw, with dressing	All coleslaw & coleslaw style dressing, except homemade	2	7
Instant vegetable soup, dry	All soups made from dry mix	NA	1
Olives	All olives	9	1
Onion, pickled or cocktail	Pickled onion, other pickled vegetables, except olives	0	50
Potato chips, hot, takeaway	All commercially cooked fries, chips, wedges and hash browns	NA	NA
Potato chips, frozen	All fries, chips, wedges and hash browns, cooked from frozen	NA	3
Potato salad	All potato salads, except homemade	1	6

[#] Concentration data derived from the 21st ATDS analytical survey, conducted in 2003, unless otherwise indicated.
^ Concentration data from 2009 analytical survey of dried apricots
* Concentration data from 2011 analytical survey of dried apples, sultanas, cordial and sausages.
NA – not analysed

Appendix 4: Dietary exposure assessments at FSANZ

Dietary modelling was conducted using the FSANZ custom-built computer program which multiplied the chemical concentration for each food consumed in national nutrition surveys with the amount of that food that each survey respondent consumed to estimate each individual's exposure to that chemical from each food. Once this had been completed for all foods determined as containing a particular chemical, the total amount of the chemical consumed from all foods was summed for each individual. Population statistics (e.g. mean and 90th percentile exposures) for each age group were derived from the individual ranked exposures. Where the results are expressed on a body weight basis, each individual's exposure from all foods was divided by their own body weight before population summary statistics were derived.

A4.1 Food consumption data used

The most recent food consumption data available at the time the assessment was undertaken were used to estimate exposures to sulphites and benzoates for the Australian population. The national nutrition survey (NNS) data used for these assessments were:

- the 2007 Australian National Children's Nutrition and Physical Activity Survey (also known as '*Kids Eat Kids Play*') (2007 ANCNPAS)
- 1995 Australian National Nutrition Survey (1995 NNS).

The results for children aged 2–16 years were reported using the 2007 ANCNPAS and for the population 17 years and above used the 1995 NNS. The design of each of these surveys varies somewhat and key attributes of each are set out below.

A4.1.1 2007 ANCNPAS

The 2007 ANCNPAS collected data on nutrition and physical activity for 4,487 children aged 2–16 years across Australia. The survey was conducted over a seven-month period, from February to August 2007.

In contrast to other national nutrition surveys used to date by FSANZ (the 1995 Australian and 1997 and 2002 New Zealand surveys), for the 2007 ANCNPAS each respondent completed two 24-hour recalls on non-consecutive days. The availability of two days of food consumption data provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes account of those who may eat a food on one day of the survey but not on the other. Using one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat.

In this assessment, exposure of sulphites and benzoates were estimated from each consumer's average exposures per day from foods containing preservatives across Day 1 and Day 2. The results of the 2007 ANCNPAS were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used.

A4.1.2 1995 NNS

The 1995 NNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged from 2 years and above (McLennan and Podger, 1998). At the time the DEA was conducted it was the most recent NNS for Australians aged 17 years and above. The survey used a 24-hour recall method for all respondents, with 10% of

respondents also completing a second 24-hour recall on a second, non-consecutive day. Food frequency data are available for a subset of the national sample (respondents aged 12 years and above) as are responses to a series of short dietary questions about food habits. These data are used unweighted.

The 1995 NNS Day 1 records were used to estimate exposure of sulphites and benzoates for adults and children in previous assessments. These findings are summarised in the 21st ATDS report (FSANZ, 2005). The Day 1 records were also used for this assessment.

A4.1.3 Updated national nutrition survey data

Since this DEA was completed, data from the 2011–12 National Nutrition and Physical Activity Survey (NNPAS) component of the 2011–13 Australian Health Survey and the 2008–09 New Zealand Adult Nutrition Survey (NZANS) have become available. The DEA has not been updated using these more recent nutrition surveys as the area of greatest uncertainty in the risk assessment is in relation to the current sulphites HBGV. It is likely that a revised group ADI for sulphites would be higher than the current group ADI (refer to Section 5.2).

In addition, the 2007 ANCNPAS still provides a good estimate of chronic dietary exposure for Australian children as there are two days of dietary data for each respondent and the average exposure from day 1 and day 2 was used making it more applicable for estimating chronic dietary exposure than using one day of data only. Comparisons of consumption data for major contributing foods for children can be done between 2007 and 2011 nutrition surveys to assist in determining if it is likely that the new data would change the DEA conclusion (see section 4.2.2 above). Further, an updated sulphites dietary exposure assessment using the most recent data for Australian and New Zealand adults is considered unlikely to change risk assessment outcomes as estimated mean and 90th percentile dietary exposures based on the 1995 NNS and 1997 NNS were well below the HBGV.

A4.2 Assumptions in the dietary exposure assessments

The aim of the DEA was to make as realistic an estimate of dietary exposure as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the DEA did not underestimate exposure.

Assumptions made in the dietary modelling include:

- Where permissions to use sulphites and/or benzoates are given to a food classification code, all foods in that group contain sulphites and/or benzoates.
- All the foods within the group contain sulphites/benzoates, at the levels specified in Table A3.1 in Appendix 3. Unless otherwise specified, the mean analysed concentration of sulphites and benzoates in each food category has been used.
- Consumption of foods as recorded in the NNSs represents current food consumption patterns.
- Consumers always select products containing sulphites and benzoates, where permitted to be used in those foods as recorded in the NNS.
- Consumers do not increase their consumption of foods when foods containing sulphites and benzoates become available.
- All sulphites and benzoates present in food are absorbed by the body.
- Endogenous production of sulphites and benzoates has not been included in the dietary exposure assessment.
- Where a food or food group has a zero concentration of sulphites or benzoates, it was not included in the exposure assessment.

- Where a food or food group was not mapped to an analysed food (eg fresh fruit), it was assumed to contain a zero concentration of sulphites and benzoates.
- Where a food has a specified sulphite and benzoate concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient e.g. dried apricot in muesli bars.
- For the purpose of this assessment it is assumed that 1 millilitre is equal to 1 gram for all liquid and semi-liquid foods (e.g. milk, yoghurt).
- There is no contribution to sulphite and benzoate exposure through the use of complementary medicines.
- All refrigerated juice contains benzoates as a preservative.

These assumptions are likely to lead to a conservative (i.e. protective) estimate for sulphites and benzoates dietary exposure.

A4.3 Limitations of dietary exposure assessments

DEAs based on 2007 ANCNPAS, 1995 NNS food consumption data and 1997 NZNNS provided the best estimate of actual consumption of all foods and the resulting estimated dietary exposure assessments, at the time the assessment was conducted, for Australian children aged 2–16 years, Australian adults aged 17 years and above and New Zealanders aged 15 years and above, respectively. However, it should be noted that NNS data do have limitations. Further details of the limitations relating to dietary exposure assessments undertaken by FSANZ are set out in the FSANZ document, Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes (FSANZ, 2009a).

Appendix 5: Estimated dietary exposures for consumers of foods containing benzoates and sulphites

Table A5.1: Mean and 95th percentile estimated exposures for consumers of benzoates for New Zealand population groups

Age group	mg/kg bw/day (%ADI) ADI=5 mg/kg bw/day			
	Males		Females	
	Mean	P95	Mean	P95
5–12*	0.9 (20)	2.9 (60)	0.7 (15)	2.4 (50)
13–15*	0.8 (15)	2.2 (45)	0.6 (10)	1.9 (40)
16–18[#]	0.7 (15)	1.9 (40)	0.9 (20)	3.0 (60)
19–24[#]	0.7 (15)	2.8 (55)	0.8 (15)	1.8 (35)
25–44[#]	0.5 (10)	1.5 (30)	0.5 (10)	1.5 (30)
45–64[#]	0.4 (8)	1.1 (20)	0.3 (6)	1.1 (20)
65+[#]	0.1 (2)	0.7 (15)	0.3 (6)	0.8 (15)

Cressey and Jones (2009), All estimated exposures for consumers only.
***** Based on 2002 NCNS 24-hour dietary recall records and sulphite concentration data from the 2003-04 New Zealand analytical survey .
[#]Based on 1997 NNS 24-hour dietary recall records and sulphite concentration data from the 2003-04 New Zealand analytical survey.

Table A5.2: Mean and 90th percentile estimated exposures for consumers of sulphites for New Zealand population groups

Age group	mg/kg bw/day (%ADI) ADI=0.7 mg/kg bw/day			
	Males		Females	
	Mean	P90	Mean	P90
5–12*	0.24 (35)	0.74 (110)	0.18 (25)	0.54 (75)
13–15*	0.17 (25)	0.50 (70)	0.12 (15)	0.35 (50)
16–18[#]	0.22 (30)	0.39 (55)	0.12 (15)	0.34 (50)
19–24[#]	0.23 (35)	0.47 (65)	0.27 (40)	0.57 (80)
25–44[#]	0.24 (35)	0.55 (80)	0.22 (30)	0.44 (65)
45–64[#]	0.20 (30)	0.50 (70)	0.19 (25)	0.45 (65)
65+[#]	0.13 (20)	0.41 (60)	0.11 (15)	0.28 (40)

Extracted from Cressey and Jones (2009), and further analysis provided to FSANZ by ESR, 2008. All exposures for consumers only.
***** Based on 2002 NCNS 24-hour dietary recall records and sulphite concentration data from the 2003-04 New Zealand analytical survey.
[#]Based on 1997 NNS 24-hour dietary recall records and sulphite concentration data from the 2003-04 New Zealand analytical survey.

Table A5.3: Mean and the 90th percentile estimated exposures for consumers of benzoates for Australian population groups

Australia	Age Group	mg/kg bw/day (%ADI) ADI=5 mg/kg bw/day	
		Mean	P90
2007 ANCNPAS	2–5 yrs	0.9 (20)	2.3 (45)
	6–12 yrs	0.9 (20)	2.2 (45)
	13–16 yrs	0.7 (15)	1.8 (35)
1995 NNS	17+ yrs	0.4 (10)	1.2 (25)

Note: 100% of survey respondents were consumers of benzoates

Table A5.4: Mean and the 90th percentile estimated exposures for consumers of sulphites for Australian population groups

Australia	Age Group	mg/kg bw/day (%ADI) ADI=0.7 mg/kg bw/day	
		Mean	P90
2007 ANCNPAS	2–5 yrs	0.4 (50)	0.9 (130)
	6–12 yrs	0.2 (30)	0.6 (80)
	13–16 yrs	0.2 (20)	0.4 (55)
	2–16 yrs	0.2 (35)	0.6 (85)
1995 NNS	17+ yrs	0.4 (30)	1.0 (85)

Note: 100% of survey respondents were consumers of sulphites for all 2007 ANCNPAS population groups. For Australians aged 17 years and above, 97% of respondents were consumers of sulphites.

Appendix 6: Major food contributors to estimated dietary exposures for benzoates and sulphites

Table A6.1: Major food contributors (>5%) to estimated benzoates exposures for New Zealand population groups

Food group name	% Contribution	
	5-14 yrs*	15 years & above#
Soft drink, non-cola	72	62
Soft drink, cola	23	34
Other foods	5	4

***** 2002 NCNS
# 1997 NNS

Table A6.2: Major food contributors (>5%) to estimated sulphites exposures for New Zealand population groups

Food group name	% Contribution	
	5-14 yrs*	15 years & above#
Sausages, beef	65	22
Soft drink, cola	11	7
Soft drink, non-cola	9	7
Hamburger patty	5	0
Dried apricots	5	7
Beer, regular	0	24
Wine, white	<1	22
Wine, red	<1	6
Other foods	5	5

***** 2002 NCNS
# 1997 NNS

Table A6.3: Major food contributors (>5%) to estimated benzoates exposures for Australian population groups

Food group name	% Contribution			
	2-5 yrs*	6-12 yrs*	13-16yrs*	17 yrs & above#
Juice and juice products	37	22	19	17
Soft drink, non-cola, regular	32	56	59	55
Cordial, regular	22	14	14	17
Soft drink, cola, regular	<5	<5	5	7
Other foods	8	6	<5	<5

***** 2007 ANCNPAS
# 1995 NNS

Table A6.4: Major food contributors (>5%) to estimated sulphites exposures for Australian population groups

Food group name	% Contribution			
	2–5 yrs*	6–12 yrs*	13–16yrs*	17 yrs & above[#]
Sausages, beef	34	38	38	14
Dried apricots	23	14	15	17
Cordial, regular	14	15	16	7
Dried apples	10	8	7	5
Wine, white	<1	<1	<1	30
Wine, red	<1	<1	<1	8
Other foods	19	25	24	19

***** 2007 ANCNPAS
[#] 1995 NNS

Table A6.5: Contribution of all types of sausages to total estimated sulphites exposures for Australian population groups

Food group name	% Contribution			
	2–5 yrs*	6–12 yrs*	13–16yrs*	17 yrs & above[#]
Sausages, beef	34	38	38	14
Sausages, pork	4	2	2	3
Sausages, chicken	3	<1	<1	<1
Sausages, lamb	<1	<1	NC	<1
Sausages, not further specified	3	4	4	4
Total contribution from all sausages	43	45	44	21

***** 2007 ANCNPAS
[#] 1995 NNS
NC – not consumed

Appendix 7: Hazard assessment: Sulphites

A7.1 Summary

The evidence used by JECFA to establish the ADI for dietary sulphites is reviewed, together with a number of additional papers that have been published since JECFA last considered the toxicological database. There is no evidence that dietary sulphites are developmental or reproductive toxicants, or that they are carcinogenic. Known human toxicity is limited to hypersensitivity reactions, primarily in subpopulations that are also asthmatic. The current JECFA group ADI of 0–0.7 mg/kg bw (LOAEL~150 mg/kg bw/day) for sulphites (expressed as sulphur dioxide) is based on gastric lesions observed in a combined three-generation reproduction and chronic feeding study (Til *et al*. 1972a). The Til *et al.* (1972a) study has significant limitations in design and analysis, including considerable uncertainty regarding the actual dose of sulphites in the rat diets. In 1990 the same group of investigators reported that the 'Minimum Observed Adverse Effect Level' (MOAEL) for gastric lesions in a 4-week oral toxicity study was 2000 mg/kg bw/day. A recent seven-day dose-range finding study showed that the LOAEL was 1500 mg/kg bw/day in the absence of any gastrointestinal lesions (Cayzer 2015). While the Cayzer study was of shorter duration than the older studies, the proposed mode of action for sulphites is direct mucosal irritation, and it is unlikely that the findings in chronic studies are due to chronic irritation when there was a lack of evidence of acute irritation in a seven-day dose-range finding study. A dose-range finding study is not designed to enable a different ADI to be established, because of the short duration, limited range of toxicological endpoints investigated and small group size. However, the evidence from both studies suggest that the current group ADI is most likely to be higher if it was based on robust evidence from a definitive study conducted to modern experimental standards.

A7.2 Introduction

"Sulphites" (or "sulphiting agents") when used as a food additive refers to sulphur dioxide or the various inorganic forms that liberate sulphur dioxide such as sodium, potassium and calcium bisulphites or metabisulphites. The concentration of sulphites in food is generally expressed as 'total' sulphur dioxide equivalents. Sulphur dioxide and sulphites have been reviewed by JECFA several times, the initial evaluation being in 1961 and the most recent in 1997. At its 17th meeting in1973, JECFA established a group ADI for sulphites of 0–0.7 mg/kg bw, expressed as sulphur dioxide and the numerical value of the ADI has remained unchanged since then (Leclerq *et al*. 2009). This review describes the pivotal study used by JECFA to derive the ADI, and also summarizes other important studies, particularly the studies which have become available since JECFA last reviewed sulphites, to determine whether there is additional information that could justify revising the JECFA ADI.

A7.2.1 Chemistry (Taylor *et al*.1986; Leclerq *et al*. 2009)

For unknown reasons sulphite residues in foods are commonly referred to as 'free' or 'total' sulphur dioxide although it does not actually exist in food in this form. Sulphites may be present in food as sulphurous acid, inorganic sulphites and a variety of reversibly and irreversibly combined forms. Sulphites react rapidly with a variety of food constituents, including reducing sugars, aldehydes, ketones and proteins, to form various combined sulphites, such as the highly stable hydroxysulphonate adducts. The amount of compound in each of these states is dependent on a number of factors, including the food matrix and the pH. Sulphites reversibly bound may dissociate into free sulphite when the food pH is raised above 10 or when acidified (pH <4). Adducts irreversibly bound are usually formed by the

reaction of sulphites with alkanes or aromatic compounds, giving rise to sulphonic acids, which are not recovered when the sulphited food is submitted to alkaline conditions or upon distillation in acid medium. The fraction of sulphites that does not bind to food constituents is called “free sulphite”, constituting a mixture of sulphur dioxide, bisulphite and sulphite ions in a dynamic equilibrium. This fraction is rapidly converted to molecular sulphur dioxide when the sulphited food is acidified (pH <4).

A7.3 Non-human Studies

Owing to the reactivity of sulphites there are a number of practical study complications which hamper the interpretation of older studies, some which date back to around 1900. The destruction of thiamine by sulphites in the diet and their reactivity and conversion into other chemical forms (eg sulphur dioxide off-gassing) when added to drinking water or animal feed was not understood. Toxic effects noted at low doses in several feeding studies conducted early in the 20th century are now considered likely to have been confounded by thiamine deficiency (Hui *et al*. 1989, Til and Feron 1992). One approach to avoid this problem is to add the sulphite either to the drinking water or the feed, to avoid direct contact of sulphite and thiamine. However, sulphites impart a dose related unpleasant taste that results in decreased water intake (Hui *et al*. 1989). In addition, sulphites are unstable in water resulting in the release of sulphur dioxide. This loss of sulphite levels in drinking water needs to be quantified but can be minimised by regular preparation. Another approach has been to fortify the feed with thiamine prior to addition of sulphites to ensure the rats are not deficient (Til *et al.* 1972a; Til and Feron 1992). Since sulphites readily react with many dietary components and are converted to other forms (bound and unbound) there may be an apparent loss of added sulphites usually in the form of sulphur dioxide that makes it difficult to ensure correct dosages in the test animals (Hui *et al.* 1989).

A7.3.1 Short-term and subchronic toxicity studies

Seven-day range-finding study in rats (Cayzer, 2015)

Pilot, preliminary, sighting or dose-range finding studies are usually undertaken to identify dosing regimens for subsequent regulatory studies. A 7-day repeat-dose range finding study with sodium metabisulphite was performed in 10-week old male Sprague Dawley rats (4/group). Sodium metabisulphite was prepared fresh daily at concentrations in the diet of 0, 0.25, 0.5, 1.0 or 4% (w/w; equal to 0, 90, 198, 390 and 1478 mg/kg bw/day expressed as SO_2 equivalents). Dose analysis and appropriate sampling confirmed that daily feed preparation resulted in adequate stability and homogeneity of sodium metabisulphite in the feed over 24 hours. Thiamine was administered in the drinking water (50 ppm).

Measured endpoints were survival, feed intake, water intake, clinical observations, bodyweight changes, haematology, serum total protein, serum albumin and albumin:globulin ratio, gross necropsy findings, and histopathology of the gastrointestinal tract. Individual and group mean sulphite consumptions (expressed as SO_2 equivalents) were calculated using the estimated midweek bodyweight of each rat. The total and unbound sulphite concentrations were measured by the modified Monier-Williams method, in which SO_2 is released from sulphites when the sample is mixed with an acid (usually HCl) and heated. The SO_2 is distilled using a stream of nitrogen gas which carries it into an absorbing solution of H_2O_2 which oxidises it to sulphuric acid. The sulphuric acid is quantified by titration with sodium hydroxide (Egan *et al.* 1981)**.** Free sulphite represented 79.3 to 86.6% of total sulphite shortly after mixing, but was more variable after 24 hours, ranging from 64.3– 94.7% of total sulphite.

All rats survived to scheduled termination on day 8 and there were no treatment-related clinical signs, gross necropsy findings or microscopic findings. There were no treatment-

related effects on food intake, water intake or measured serum protein parameters. The bodyweight gain of the rats in the 1478 mg/kg bw/day group was only 23.6% that of the control group over the week of treatment. The group mean values for erythrocyte count, haemoglobin and haematocrit were also marginally lower in this group compared to the control group (91%, 90% and 89.4% those of the controls, respectively) but there was no evidence of a compensatory response. No treatment-related effects were found in the rats treated with 1% dietary sodium metabisulphite (390 mg/kg bw/day) or lower doses. It was recommended that the dietary concentrations for a longer term repeat-dose study are 2% for the low dose group, and 4%, 6%, and 8% for the mid-low, mid-high and high dose groups respectively.

Eight-week study in rats (Hui et al. *1989)*

The effects of sodium metabisulphite and acetaldehyde hydroxysulphonate in normal or sulphite oxidase (SOX)-deficient female Sprague Dawley rats (8/group) was measured after 8 weeks of exposure to 0, 7, or 70 mg/kg bw/day for the control, low and mid-dose groups respectively, and 3 weeks of 350 mg/kg/bw/day followed by 5 weeks of 175 mg/kg bw/day for the high dose group. Sulphites (purities not specified) were administered via drinking water and all doses are expressed as sulphur dioxide equivalents. The reason for reducing the highest dose after 3 weeks was not reported. The rats were fed a diet fortified with 50 ppm thiamine. The dose rates of sulphites were based on the chronic dietary study of sodium metabisulphite in rats by Til *et al*. (1972a), which found a NOAEL of 72 mg sulphur dioxide/kg bw/day. SOX deficiency was induced by adding sodium tungstate (200 ppm) to the drinking water for 3 weeks prior to treatment and continuing throughout the sulphite treatment period. Thiamine sufficiency was confirmed at necropsy. The sodium tungstate treatment depressed hepatic SOX activity to below the level of detection, in comparison to an average SOX activity of 348 units/g liver in rats not treated with sodium tungstate. SOX is an essential enzyme in the pathway of the oxidative degradation of sulfite to sulfate thereby protecting cells from sulphite toxicity.

All rats survived to scheduled termination after 8 weeks of sulphite treatment. Body weights at scheduled termination were comparable between all groups except for the SOX-deficient rats that received the highest dose of sodium metabisulphite. In this group, body weight was significantly lower (group mean 86% of SOX-deficient control group mean) than that of controls. This group also had significantly decreased water consumption (group mean 38% of control group mean). Food consumption was generally comparable between groups although the rats treated with sodium metabisulphite tended to have higher food consumption than controls (112 to 132%).

Gross lesions were confined to “white patches” noted in the lungs of SOX-deficient rats treated with sulphites, but these appeared to have no histopathological correlate. In sodium metabisulphite-treated normal and SOX-deficient rats, microscopic lesions were noted in the stomach only at the highest dose. Moderate hyperkeratosis was observed in the forestomach while lesions in the glandular stomach included dilation of deep fundic glands, and eosinophilic staining of chief cells in normal rats. In SOX-deficient rats, some nuclear and cytoplasmic hypertrophy of chief cells was also noted. In high-dose SOX-deficient rats, 5/8 also had oedematous submucosa, thinning of the fundic mucosa, and subjective appearance of reduced parietal cell numbers. Two of these five affected rats also had foci of chief cell hyperplasia in fundic glands. Based on histological findings in the stomach the no observed adverse effect level (NOAEL) for sodium metabisulphite in this study was 70 mg /kg bw/d.

Treatment with acetaldehyde hydroxysulphonate was associated with gastric and hepatic lesions which were more severe in SOX-deficient rats, and found only at the highest treatment regimen in SOX-normal rats. Gastric lesions were those of forestomach

hyperkeratosis and a number of changes in the fundic portion of the stomach, including thinner mucous membrane, distension of the basal part of the fundic glands, fewer parietal and mucous cells, and chief cell hypertrophy. Some of the SOX-deficient rats in the mid and high-dose groups had gastric pits with oedema and some bleeding. Hepatic lesions included hepatocellular vacuolation, pyknosis and karyorrhexis.

A significant limitation of this study is that the sulphites were administered in the drinking water and therefore the study does not model intake in the food, in which some sulphites will be bound to other dietary constituents. Another important limitation is that it cannot be determined, in retrospect, whether the gastric changes developed during the first three weeks of the study when the rats were fed 350 mg/kg bw/d, or during the subsequent five weeks when that dose was halved.

Twelve-week study in rats (Mahmoud et al. *2015)*

Sodium metabisulphite (96%) at 0, 200, 500 or 1000 ppm was administered to young female Wistar rats (5/group) in their drinking water (Mahmoud *et al*. 2015). Blood was collected immediately prior to scheduled termination for haematology and serum chemistry. Body weights were recorded at the beginning and end of the 12-week in-life phase of the study. Livers and kidneys were collected and weighed from three rats in each group at scheduled termination, and then processed for histopathological examination. The other two rats in each group were treated with 0.025% colchicine (1 mL/100 g bw) by intraperitoneal injection prior to termination, and bone marrow was collected at termination for a chromosomal aberration assay. Measured parameters were survival, bodyweight gain, Hb, Hct, RBC, MCV, MCH, MCHC, WBC, platelet count, total protein, albumin, AST, ALT, ALP, creatinine, urea, cholesterol, glucose, a range of chromosomal aberrations, relative weights of liver and kidney, and histopathology. It appears that water consumption and food consumption were not measured. Because water consumption was not measured, the actual amount of sodium metabisulphite consumed cannot be calculated.

There was a dose-related decrease in bodyweight gain, in all three treated groups relative to the control group. The group mean weight gain of the 200 ppm group was only 8.8% that of controls, while the 500 ppm group lost 17.3% of their bodyweight and the 1000 ppm group lost 31.3% of their bodyweight. Two rats in the 1000 ppm group died during the last week of the in-life phase, but the cause of death was not specified. Mean Liver and kidney weights, relative to bodyweight, did not differ to a statistically significant extent from those of controls, but were consistently higher than those of controls. The relative kidney weight increased with increasing dose level. These findings are consistent with loss of adipose tissue and probably also muscle mass, with relative preservation of mass of parenchymal organs.

Group mean HCT, RBC and Hb were mildly to moderately increased (17%, 49% and 13% increases respectively) in the 200 ppm group relative to the control group, but decreased in the 500 (16%, 5.8% and 11% decrease respectively) and 1000 ppm groups (23%, 17% and 24% decrease respectively). MCHC was also significantly decreased in the 500 and 1000 ppm groups (19% decrease and 32% decrease respectively). The authors attributed the haematological changes in the 200 ppm group to increased release of erythrocytes into the circulation, i.e. a true polycythaemia, but did not appear to consider the likelihood that the findings could be due to dehydration. The 200 ppm group mean WBC and platelet counts were also significantly decreased (63% and 50% decrease respectively) but the authors did not comment on those changes. The decreases in HCT, RBC and Hb in rats administered ≥500 ppm Na_2SO_3 were accompanied by statistically significant decreases in WBC and platelet counts (79% and 56% decrease respectively at 500 ppm, and 79% and 65% decrease respectively at 1000 ppm), and were attributed by the authors to oxidative stress induced by Na_2SO_3.

Serum albumin was significantly lower at ≥ 500 ppm, being 36% lower than that of controls at 500 ppm, and 53% lower than that of controls at 1000 ppm. Group mean blood glucose was significantly lower than that of controls in all treated groups, being 31% lower than that of controls at 200 ppm, 45% at 500 ppm and 61% at 1000 ppm. AST was significantly increased in all treatment groups, with a 83% increase at 200 ppm, 159% increase at 500 ppm and a 227% increase at 1000 ppm. ALT was also significantly increased above the control group mean, with a 24% increase at 200 ppm, a 43% increase at 500 ppm and a 76% increase at 1000 ppm. Creatinine showed a significant dose-related increase; 52% higher than that of controls at 200 ppm, 78% higher at 500 ppm and 146% higher at 1000 ppm. Urea was significantly increased at ≥ 500 ppm; 81% higher than that of controls at 500 ppm and 134% higher at 1000 ppm. Groups administered ≥ 500 ppm Na_2SO_3 showed significant dose-related decreases in total protein (12% decrease at 500 ppm and 38% decrease at 1000 ppm) and increases in ALP (34% increase at 500 ppm and 64% increase at 1000 ppm). Cholesterol was significantly higher (32% increase) at 1000 ppm. The authors interpreted these findings as evidence of liver and kidney dysfunction but did not discuss the effects of the profound decline in body weight at ≥ 500 ppm Na_2SO_3, on the values. Nor did the authors discuss the possibility that Na_2SO_3 may have decreased the palatability of the water and resulted in a dose-related decrease in water intake, which in rodents is also recognized to decrease food intake.

Increases in a number of chromosomal aberrations were reported. Overall, 4 examples of aberrant metaphase were found in bone marrow of control rats, compared with 25 at 200 ppm, 34 at 500 ppm and 57 at 1000 ppm. However these totals included a number of types of aberration that did not show a dose-response relationship when considered in isolation. Furthermore, starvation has been shown to increase the incidence of chromosomal aberrations in rats (Alu and Murthy 1993), so this finding may not be a direct effect of Na_2SO_3 but rather a consequence of the profound decrease in bodyweight gain, and absolute bodyweight loss at higher doses.

A number of microscopic findings were claimed to be present in liver and kidney but the published photomicrographs purported to show these findings are not convincing.

A critical defect of this study is the absence of any data on water consumption. The mild increases in HCT, RBC and Hb in the 200 ppm group are consistent with mild dehydration and decreased water consumption was noted in the Hui *et al*. (1989) study which also administered sulphites via drinking water. Rodents that do not consume adequate water also do not eat normally, and the group mean bodyweights of the rats in this study suggest starvation. The clinical pathology and chromosomal aberration findings are likely to be attributable to starvation and dehydration rather than a direct effect of Na_2SO_3. This study is not considered to be of value in the assessment of the toxicity of dietary sulphites.

Short-term and subchronic studies of dietary sodium metabisulphite in rats (Ribera et al. *2001)*

In this Good Laboratory Practice (GLP)-compliant study, sodium metabisulphite was added to dough for the manufacture of biscuits so that the administered dose in rats was equal to 1.2, 3.3, 12.6 or 27.5 mg/kg bw/day expressed as sulphur dioxide over 28 days and 1.3, 3.2, 13.3 or 26.4 mg/kg bw/day over 85 days (Ribera *et al*. 2001). The authors contend that toxicity studies should focus on the bound sulphites as found in manufactured rat biscuits, rather than using a source of free sulphites as the test article. After baking, the biscuits were ground and mixed with supplements of protein, sugar, vitamins and minerals to prepare nutritionally adequate rat diets. Sulphite levels, and stability under conditions of storage, were confirmed by GLP-compliant dose analysis.

The prepared sulphite-containing diet was fed to Sprague-Dawley rats (10/sex/group) for either 28 or 85 days. Rats were housed at 5/sex/cage and food consumption was measured by daily weighing. Endpoints measured in the 28-day study were survival, clinical signs, body weights, food consumption, food conversion efficiency, renal concentrating ability, haematology, clinical chemistry, urinalysis, gross necropsy findings, weights of selected organs, histopathology of selected organs, and liver levels of vitamins A, C and B_1 and E.

Endpoints in the 85-day study were survival, clinical signs, body weights, food consumption, water consumption, ophthalmology, haematology, clinical chemistry, urinalysis, gross necropsy findings, weights of selected organs, and histopathology of an extensive organ list.

Diet analysis associated with the 28-day study showed that the nutrient composition of the four diets was similar and that dietary peroxide levels remained low, consistent with negligible sulphite-induced lipid peroxidation. Stability of sulphite levels for at least 7 weeks at room temperature was established.

All rats on the 28-day study survived to scheduled termination and no treatment-related clinical observations were noted. There were no treatment-related effects on group mean body weights, food consumption, food conversion efficiency, haematological parameters, clinical chemistry parameters, renal concentrating ability, urinalysis, organ weights, gross necropsy findings or histopathological findings. Group mean hepatic concentrations of vitamins A, C and B_1 (=thiamine) and E were comparable between groups, with the exception of an increased concentration of group mean α-tocopherol in high-dose male rats, relative to male controls.

Similarly, in the 85-day study there were no treatment-related effects on mortality, clinical observations, body weights, food consumption, water consumption, ophthalmoscopic findings, haematology values, clinical chemistry values, urinalysis values, organ weights, gross necropsy findings or histopathology findings. Although diets were not thiamine-supplemented, there was no sign of thiamine deficiency at any dose level as shown by the normal growth and the absence of changes in hepatic levels of several vitamins.

The authors noted the absence of adverse clinical signs in their studies compared to previous subchronic and chronic rat studies in which free sulphites were administered, and concluded that the irritant and toxic properties of sulphites are modified during food processing. However, while the investigators acknowledged that the administered doses were all substantially less than the NOAEL (70 mg/kg bw/d) reported in other studies (eg Til *et al.* 1972a, 1972b) they did not appear to recognize that it was unlikely that the study protocol would have enabled them to conclude much else.

A7.3.2 Chronic Toxicity Studies

Rats

Sodium metabisulphite added to the stock diet at 0, 0.125, 0.25, 0.5, 1.0 or 2.0% was fed to groups of Wistar rats (20/sex) (Til *et al*. 1972a). The stock diet was fortified with 50 ppm thiamine to compensate for thiamine destruction by sulphite. To avoid losses of thiamine and sulphite with storage, diets were prepared freshly every two weeks, and stored frozen. Diets were analysed for sulphite and for thiamine. Rats were group-housed at 5/cage, with food and water provided *ad libitum*. All rats in the F_0 generation were mated within their dose group at Week 21, and half of the rats in each group in the F_0 generation were mated within their dose group at Week 34. Thereafter, all F_0 generation rats were maintained on their diets to a total of 104 weeks. Ten males and 10 females from the first mating of each diet group were maintained as the F_{1a} generation to 104 weeks on study, while all other pups from matings of the F_0 generation were discarded at weaning. The rats of the F_{1a} generation

were mated at their Weeks 12 and 30 to generate the F_{2a} and F_{2b} generations. Ten males and 15 females from each dose group of the F_{2a} generation were mated at their Weeks 14 and 22. All pups from those matings were discarded at weaning, whereas the F_{2a} parents were maintained on their diets to a total exposure of 30 weeks. An interim necropsy was performed on 5 rats/sex from each dose group in the F_0 generation at 52 weeks. Remaining rats of the F_0 generation, and all survivors of the F_{1a} generation, were terminated and necropsied at their respective Week 104, and all survivors of the F_2 generation were terminated and necropsied at their Week 30.

Endpoints measured in this study included body weights, food consumption, litter sizes at birth (prior to culling to equalize litter sizes), pup weight gains, haematology, faecal occult blood, serum SGOT and SGPT in F_0 rats, renal function, urinalysis, urinary thiamine, selected organ weights, gross necropsy findings and histopathology, including special stains, of a comprehensive tissue list.

No test article-related findings were noted in most endpoints examined, with the following exceptions. There was a marginal reduction in bodyweight gain in both sexes of the F_1 and F_2 generation rats in the 2% group, both before and after weaning, and this was attributed to treatment. Sulphite treatment was also associated with occult blood in the faeces, indicative of intestinal blood loss, at ≥ 1% $Na_2S_2O_5$. Hyperplastic changes in the forestomach and glandular stomach were found at ≥ 1% $Na_2S_2O_5$ in the F_0 and F_1 generations, and similar but slight alterations were also found in the 0.5% $Na_2S_2O_5$ group in the F_2 generation rats. On the basis of these findings, the no observed adverse effect level (NOAEL) was identified at the intended dose of 0.25% $Na_2S_2O_5$, although, on the basis of measured losses of $Na_2S_2O_5$ from the diet, the dose was estimated to be, on average, 0.215%. This concentration in the diet would be equal to 72 mg/kg bw/d.

Pigs

This study was a 48 week feeding study in Dutch Landrace pigs (20/sex/group), with an interim necropsy of cohorts from each group commencing at 15 weeks (Til et al. 1972b). Subjects were female and castrated male pigs that were placed on study at weaning, although age was not specified. Pigs were group-housed by gender and fed a commercial diet with thiamine supplementation. Sodium metabisulphite dissolved in water was added to the feed at intended levels of 0.0, 0.125, 0.25, 0.5, 1.0 and 2.0% $Na_2S_2O_5$, although due to loss of SO_2 during the 8-16 hours between food preparation and consumption, the actual mean levels consumed were calculated to be 0.0, 0.06, 0.16, 0.35, 0.83 and 1.72% $Na_2S_2O_5$.

Interim necropsies of 14 pigs/sex/group were performed over the course of weeks 15–19. Endpoints measured included bodyweights, presence of occult blood in faeces in Week 6, terminal haematology, organ weights of heart, liver, kidneys and spleen, and histology of heart, liver, kidneys, spleen, pancreas, stomach, duodenum, ileum, and mesenteric lymph nodes.

Food consumption, body weight gain and terminal body weight were significantly reduced at the high dose of 1.72% $Na_2S_2O_5$. A slight, nonsignificant reduction in these parameters was also noted in the 0.83% group. Liver thiamine levels were adversely affected at the high dose of 1.72% $Na_2S_2O_5$. There were no treatment-related differences in haematology parameters or in occult blood determinations in faeces. Significant increases in the relative weights of heart, liver, kidneys and spleen were found in the 1.72% $Na_2S_2O_5$ group. Absolute weights were not reported, and the increases in relative weights of these organs may be artefacts of the significantly lower terminal body weights. Treatment-related gross findings were confined to the stomach and caecum. Pigs fed ≥0.83% $Na_2S_2O_5$ were found to have prominent mucosal folds in the cardiac and pyloric parts of the stomach, and black

discoloration of the caecal mucosa. Histologically, the stomachs of pigs in the 1.72% $Na_2S_2O_5$ group exhibited hyperplasia of the cardiac and pyloric epithelium. Stomachs of pigs in the 0.83% $Na_2S_2O_5$ group were not subject to microscopic examination following the interim necropsies, and nor were caeca from either group.

The 6 pigs/sex/group which remained after the interim necropsy were maintained on study until scheduled necropsy during Week 48 to 51. Endpoints for this cohort included bodyweights, presence of occult blood in faeces in Weeks 26 and 47, terminal haematology, organ weights of heart, liver, kidneys and spleen, and histology of heart, liver, kidneys, spleen, pancreas, stomach, duodenum, ileum, mesenteric lymph nodes, brain, urinary bladder, adrenals, diaphragm, pituitary, thyroids, oesophagus, caecum and colon from all pigs.

There were no treatment-related effects on survival. Group mean terminal body weights were significantly decreased, relative to those of controls, at ≥ 0.35% $Na_2S_2O_5$. Food consumption and thiamine levels in urine and liver were significantly reduced at the high dose of 1.72% $Na_2S_2O_5$. There were no treatment-related differences in haematology parameters or in occult blood determinations in faeces. Significant increases in the relative weights of heart, kidneys and spleen were found in groups fed ≥ 0.83% $Na_2S_2O_5$, and the relative weight of liver was increased in the 1.72% $Na_2S_2O_5$ group. Absolute weights were not reported, and the increases in relative weights of these organs may be artefacts of the significantly lower terminal body weights. The same hyperplastic changes of the cardiac and pyloric gastric epithelium that were observed at 1.72% $Na_2S_2O_5$ at the interim necropsy, as well as inflammatory and hyperplastic changes in the oesophageal region, were found at ≥ 0.83% $Na_2S_2O_5$ at the final necropsy. Additional lesions found at the final necropsy and attributed to sulphite treatment were increased numbers of fat-containing Kupffer cells in the liver of 4 of the 12 pigs fed 1.72% $Na_2S_2O_5$, and a dose-related increase in the number of pigs with heavy infiltrations of pigment-laden macrophages in the lamina propria of the caecum in pigs fed ≥ 0.35% sulphite.

The authors concluded the no observed effect level (NOEL) of sulphite established in this study was 0.35% sulphite. It is not clear why the infiltration of pigment-laden macrophages in the caecal lamina propria found in one pig at this exposure level was not considered to be a treatment-related effect, particularly since this effect increased in incidence with increasing dose, being found in 6/12 pigs fed 0.83% $Na_2S_2O_5$ and 8/12 pigs fed $Na_2S_2O_5$. The authors remarked that the caecal infiltration appeared to be equivalent to pseudomelanosis coli in man, which is known to be an innocuous condition, but this would justify identifying 0.35% sulphite as the no observed adverse effect level (NOAEL) rather than the no observed effect level (NOEL). A major drawback of this study is the lack of correction of test article administration for bodyweight, so that pigs ingested a progressively smaller dosage in mg/kg bw terms as they grew larger. Another weakness of this study is that pigs were group-housed, and homogeneity of the $Na_2S_2O_5$ in the diet was not demonstrated, raising the possibility that individual exposures may have been influenced by position within the social hierarchy.

A7.3.3 Reproductive Toxicity

A small number of developmental and reproductive studies of sulphites were reviewed by JECFA (WHO 1986). Intraperitoneal injections of sodium bisulphite (sodium metabisulphite) had no specific adverse effects on spermatogenesis in mice at doses up to or in excess of the Maximum Tolerated Dose. Consumption of diets containing up to 2% $Na_2S_2O_5$ had no adverse effects on fertility, birth weight or postnatal survival in rats in a multigenerational study. Gavage studies of teratogenicity of various sulphites in a number of laboratory species were also reviewed. No significant effects on implantation, maternal or fetal survival, or incidence of developmental abnormalities were discovered (WHO, 1983).

Reproductive toxicity study in rats (Dulak et al*. 1984)*

Generally consistent with those results, a reproductive toxicology study in rats with induced SOX deficiency did not identify any developmental or reproductive toxicity of sulphites. SOX deficiency was induced by means of a low-molybdenum (Mo), high-tungsten (W) regime commencing 3 weeks before mating. Group sizes were variable, ranging from 14 to 29 individuals. With the exception of the first control group, that was fed Purina Lab Chow, all control groups and treatment groups were fed a low-Mo diet. The second control group comprised SOX-normal rats fed the low-Mo diet, but with no other interventions. The third control group comprised rats given W supplement in their water but also supplemented via the diet with Mo, so that they were SOX-normal, and their drinking water also contained sodium metabisulphite to 25 mM sulphite. The fourth control group was maintained in a SOX-normal state in the same way, and differed from the third control group only in that the sulphite concentration in their water was 50 mM sulphite.

The three treatment groups were all rendered SOX-deficient by simultaneous high W and low Mo, and the water of the second and third treatment groups contained 25 and 50 mM sulphite, respectively. Water containing sulphite was prepared daily to minimise losses by auto-oxidation, and such losses were determined to be less than 5%/24 h. 42 days after the start of induction of SOX deficiency, and 21 days after sulphite supplementation was commenced, rats were pair-mated to untreated males for four consecutive nights or until copulatory plugs were observed. Dams were terminated on Day 21 of gestation.

Endpoints for determination of toxicity included maternal food consumption, maternal weight gain, examination for resorptions in the uteri of non-pregnant females, numbers of corpora lutea, numbers and positions of live and dead fetuses, and examinations of the foetuses including gross appearance, weight, crown-to-rump length, gender, visceral examinations of 33% of the foetuses and skeletal examination of the remaining foetuses. Exposure to sulphite did not result in any dose-related trends in any of the reproductive parameters assessed. The mean number of corpora lutea was moderately higher in SOX-deficient females in the high sulphite group, which translated to a statistically greater pre-implantation loss ($P < 0.05$) but the absolute difference was minimal. There were no significant dose- or treatment-related trends with respect to any fetal malformation. In discussing their results, the authors remarked that their findings were consistent with previous studies, conducted by other researchers, in rabbits and in mice (Dulak et al 1984).

A7.3.4 Genotoxicity

JECFA has recognized that sulphites can interact with DNA and may induce mutations in bacteria, and chromosomal aberrations in mammalian cells, in genotoxicity experiments conducted *in vitro*. JECFA also cited an *in vivo* mutagenicity study conducted in mice given intraperitoneal (IP) injections of sodium metabisulphite. No dominant lethal mutations were observed as a result of female mice receiving 550 mg/kg bw in a single IP injection prior to mating with untreated males. Neither heritable translocations nor dominant lethal mutations occurred when male mice were mated to untreated females after IP regimes of either 400 mg/kg bw 20 times over a 26-day period or 300 mg/kg bw 38 times over a 54-day period (WHO 1987).

The Cosmetic Ingredient Review Expert Panel published a review of sodium sulphite, potassium sulphite, ammonium sulphite, sodium bisulphite, ammonium bisulphite, sodium metabisulphite and potassium metabisulphite in 2003. This review covered genotoxicity as well as *in vivo* toxicity including oral toxicity, inhalation toxicity, developmental and reproductive toxicity, and carcinogenicity (Nair *et al.* 2003). The results are reported under separate headings below.

A7.3.4.1 In vitro

Review (Nair et al*. 2003)*

Review of *in vitro* genotoxicity studies found that both positive and negative results were reported for sodium bisulphite, but three bacterial assays of sodium sulphite, an Ames test of sodium metabisulphite and a sister chromatid exchange assay in Chinese hamster cells of potassium metabisulphite were all negative (Nair *et al*. 2003)

Genotoxicity of $K_2S_2O_5$ in human lymphocytes (Yavus-Kocoman et al*. 2008)*

Potassium metabisulphite induced structural and numerical chromosome aberrations, sister chromatid exchanges and micronucleus formations in a dose-related manner in human lymphocytes *in vitro* as a result of treatments for 24 or 48 hours. Concentrations of potassium metabisulphite used were 25, 50, 100 or 200 µg/mL (Yavus-Kocoman *et al.* 2008).

A7.3.4.2 In vivo

Review (Nair et al*. 2003)*

Sodium sulphite did not induce abnormalities in mouse oocytes *in vivo*. Sodium bisulphite was negative for *in vivo* genotoxicity in assays including a host-mediated assay in mice, a cytogenetic assay in rats, two dominant lethal assays in rats and one in mice, and a translocation assay in mice*. In vivo* assays of sodium metabisulphite included in the review were a host-mediated assay in mice, a cytogenetics assay in rats, assays in SOX-deficient hamsters and NMRI mice, a bone marrow chromosomal aberration assay in mice, a micronucleus assay in mice, and two dominant lethal assays in rats. In all of these studies, the result was negative. No *in vivo* studies of the genotoxicity of potassium metabisulphite were reported (Nair *et al.* 2003)

Genotoxic effects of sodium sulphite/sodium bisulphite in mice (Meng et al*. 2004)*

A mixture of sodium sulphite and sodium bisulphite (3:1 M:M) in normal saline was administered by IP injection to male mice (6/group) at a dose of 0, 125, 250 or 500 mg/kg bw once daily for 7 days. The mice were terminated 24 hours after the last injection and brain, lung, heart, liver, stomach, spleen, thymus, kidney and bone marrow were each processed to single cell suspensions. Genotoxicity was assessed using the single cell gel electrophoresis technique (SCGE). The SCGE, or comet assay, is especially sensitive for the detection of single-strand breaks, alkaline-labile damage and excision repair sites in single cells. There was a dose-related increase in DNA damage at all doses of sulphite in all cell types.

Genotoxicity of sodium metabisulphite in rat bone marrow cells (Kayraldis and Topaktas 2007)

$Na_2S_2O_5$, dissolved in distilled water, was administered to rats (3/sex) by IP injection or by gavage, at doses of 0, 250, 500, 750 or 1000 mg/kg bw. A positive control group was dosed with ethyl carbamate. Rats were terminated at time-points of 6, 12 or 24 hours. Colchicine was administered by IP injection 2 hours prior to scheduled termination. Bone marrow cells in metaphase were examined. Parameters determined were percentage of abnormal cells, chromosomal aberrations/cell (CA/cell), and mitotic index (MI).

In rats dosed by IP injection, as compared to negative controls, frequency of abnormal cells and CA/cell were increased at all $Na_2S_2O_5$ dose levels at all termination time-points. A dose-

response relationship was apparent at 12 and 24 hours, but not at 6 hours. Treatment with IP $Na_2S_2O_5$ decreased in the MI at all dose levels at the 12 and 24 time-points, but decreased the MI only at doses of 750 and 1000 mg/kg bw at the 6-hour termination time-point. A dose-response relationship in MI reduction was apparent only at 24 hours.

In gavaged rats, no effect on frequency of abnormal cells or CA/cell was observed at 6 hours. Frequency of abnormal cells was significantly increased, relative to controls, at all dose levels at the 12 hour time point. At the 24-hour time-point, a significant dose-related increase in CA/cell was reported in the cells from rats dosed with 750 and 1000 mg/kg bw, but the negative control data are not included in the paper to allow calculation of the percent increase. MI was reported to be decreased in 1000 mg/kg bw rats at 6 hours, in ≥500 mg/kg bw rats at 12 hours, and at all dose levels of $Na_2S_2O_5$ at 24 hours.

Genotoxicity of $K_2S_2O_5$ in rat bone marrow (Yavus-Kocoman et al*. 2008)*

Rats (2/sex/group) were dosed IP with potassium metabisulphite in distilled water at a dose of 150, 300 or 600 mg/kg bw and sacrificed at 12 or 24 hours. Urethane (400 mg/kg bw) was used as the mutagen for a positive control group, and a negative control group received nothing IP. Mitosis was arrested using colchicine 2 hours prior to scheduled termination. Bone marrow cells were assessed for the occurrence of chromosome aberrations (CAs), and the mitotic index (MI) was assessed.

Treatment with $K_2S_2O_5$ caused a statistically significant increase in CA at all dose levels at both time points. At the 12 hour time point, the increase in CA at doses of $K_2S_2O_5$ ≥ 300 mg/kg bw was greater than the increase, relative to negative controls, of 400 mg/kg bw urethane. The percentage of abnormal cells was greater, relative to that for the negative controls, at all dose levels at both time points, and a dose-response relationship was evident. The MI was significantly decreased relative to both negative and positive controls at all doses of $K_2S_2O_5$ at the 12 hour time point, and was lower than that of the negative control at 600 mg/kg bw at the 24 hour time point.

Genotoxicity of $Na_2S_2O_5$ in mouse tissues by comet assay and micronucleus test (Carvalho et al*. 2011)*

Sodium metabisulphite was shown to be genotoxic in the blood, liver and bone marrow cells of mice by use of the comet assay and the micronucleus test. $Na_2S_2O_5$ was administered by gavage to 5 mice/sex/group at a dose of 0, 500, 1000 or 2000 mg/kg bw. A negative control group was gavaged with water while a positive control group was treated similarly with cyclophosphamide. Mice were sacrificed 24 hours after dosing. Statistically significant increases in both group mean damage index (DI) and damage frequency (DF) in blood, liver and bone marrow cells were seen in the comet assay at doses of 1 and 2 g/kg $Na_2S_2O_5$. In addition, there was an increase in micronucleus frequencies in both blood and bone marrow cells from mice treated with $Na_2S_2O_5$ at 2 g/kg bw.

A7.3.5 Carcinogenicity

In this two-year study ICR/JCL mice (50/sex/group) were treated with potassium metabisulphite in drinking water at concentrations of 0, 1% (equivalent to 1500 mg/kg bw/day) or 2% (equivalent to 3000 mg/kg bw/day)(Tanaka *et al*. 1979). The high dose of 2% had previously been established as a maximum tolerated dose (MTD) in a dose-range finding study. The report of this study is very brief, but it appears that survival and histopathology were the only endpoints measured. There were no treatment-related effects on survival, and there were no significant differences in tumour incidence between the treated and control groups.

A7.4 Human studies

There is relatively little information of oral toxicity of sulphites in humans, excluding sensitivity reactions (Yang and Purchase 1985; Simon 1998). The findings of a number of reports in the old medical literature indicate high oral doses of sulphites caused signs of nausea and vomiting, and possibly gastric haemorrhage, at doses of 4 to 6 g/day (reviewed by Schwartz and Klein 1984), which would be 57 to 86 mg/kg bw in a 70 kg person. A mechanism of gastric irritation was assumed.

No additional or subsequent reports of toxic effects of oral sulphites to non-sensitised human beings were located for the purpose of this review.

Ban *et al.* (2014) described of two major phenotypes of sulphite hypersensitivity in human beings, asthma and urticaria. Asthma is the more common phenotype. In their small (n=26) retrospective study of patients with sulphite-sensitive asthma and sulphite-sensitive urticarial, they found that 100% (n=18) of those with sulphite-sensitive asthma had a comorbidity with chronic asthma, whereas only 60% of those with sulphite-sensitive urticaria (n=8) had chronic asthma. Thus, sulphite hypersensitivity is not confined to people with a history of asthma. Only 50% of those with sulphite-sensitive urticaria had a history of chronic urticaria, although 100% of them had a history of allergic rhinitis. Patients with sulphite-sensitive asthma given sodium metabisulphite orally in gelatin capsules developed wheezing, cough, and dyspnoea, consistent with bronchoconstriction. Patients with sulphite-sensitive urticaria, given the same oral challenge, developed hives (Ban *et al*. 2014).

A7.5 Other studies

Four-week study in rats (Jonker et al. 1990)
In a study intended to investigate possible additive or synergistic effects of chemical mixtures when administered at their respective NOAELs, a combination of eight unrelated compounds comprising sodium metabisulphite, Mirex (a chlorinated hydrocarbon insecticide), loperamide, metaldehyde, di-*n*-octyltin dichloride, stannous chloride, lysinoalanine and potassium nitrite was orally administered to Wistar rats (10/sex/group). Except for potassium nitrite, which was administered in the drinking water the mixture of compounds was added to the diet. The minimal observed adverse effect level (MOAEL) and NOAEL of each compound had been previously established for the strain of rat in the same laboratory as the study was conducted. There was one untreated control group. The doses administered to the treatment groups comprised NOAEL/10, NOAEL/3, NOAEL and MOAEL. Endpoints measured were clinical observations, bodyweight changes, food consumption, water intake, haematology, clinical chemistry, urinalysis, gross necropsy, selected histopathology, and selected organ weights.

There was little evidence for any additive or synergistic effects for the tested chemical mixture. However, this report does provide some additional data on the effects of sodium metabisulphite in the diet alone. The reported MOAEL for sodium metabisulphite alone was 20,000 ppm (equivalent to 2000 mg/kg bw/d), at which the only reported effect was a slight, unspecified effect on the morphology of the stomach. However, as there were no significant observed changes observed at three times the MOAEL (60,000 ppm; equivalent to 6000 mg/kg bw/d) the effect on stomach morphology was not more severe, but slight unspecified effects on the morphology of spleen and liver, a moderate change in spleen weight, and a ‘mild’ increase in WBC were also observed.

The following eight studies in this section were conducted to investigate possible neurotoxic effects of sulphites in rats. The rationale for investigating neurotoxicity of sulphites is that hereditary deficiency of SOX in humans, a rare autosomal recessive disorder, is a neurological disorder. Clinical signs of this hereditary deficiency include mental retardation,

attenuated growth of the brain, seizures, spastic quadriparesis, progressive destruction of brain tissue, and early death. Furthermore, the brain has low SOX activity relative to other organs.

Effects of sulphites on EEGs in rats (Ozkaya et al*. 2006)*

Adult male Wistar rats were divided into four groups of 10 rats/group. SOX deficiency was induced in the rats of two of these groups by the low Mo/high W regime, commencing three weeks before study start. Group assignments were a control group of SOX-normal rats not treated with sulphite, a SOX-normal group treated with sulphite, a SOX-deficient group not treated with sulphite, and a SOX-deficient group treated with sulphite. Sulphite treatment was by administration of $Na_2S_2O_5$ in drinking water, at a calculated dose of 25 mg/kg bw/day, for six weeks. EEG recordings of the parietal region of the brain were made under chloral hydrate anaesthesia.

All rats remained healthy for the duration of the study and exhibited similar weight gain. Plasma S-sulphonate levels confirmed exposure to sulphite and were higher in SOX-deficient groups than in SOX-normal groups. Hepatic SOX activity at the end of the study confirmed the effectiveness of the low Mo/high W regime. Sulphite administration to SOX-normal rats had no effect on mean EEG power, although delta power was increased relative to the control group. Mean EEG power was increased two-fold in the SOX-deficient group relative to the control group, with increases in all bands (delta, theta, alpha and beta). Sulphite administration to SOX-deficient rats reduced the mean EEG power relative to untreated SOX-deficient rats, although still higher than the mean control value, and activity of all bands was lower in sulphite-treated SOX-deficient rats than in SOX-deficient rats that had not received sulphite. There were no clinical signs of neurotoxicity such as behavioural changes or seizures.

Study of effects of sulphites on hippocampal enzymes in rats (Küçükatay et al. *2007)*

This study was also of adult male rats, of unstated strain, with the same group assignments, treatments and duration as in the study of Ozkaya *et al*. (2006). Although both studies were undertaken as part of the PhD thesis of V Küçükatay, they do not appear to be reports of separate results from the same study, because the mean group hepatic SOX activity values were different, although induction of SOX deficiency was effective in both studies. Parameters examined in this study were activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxide (GPx) in hippocampi of the rats after six weeks of treatment. Ingestion of sulphite was associated with a marked increase in the activities of all these enzymes in SOX-normal rats, relative to SOX-normal controls. Enzyme activities in SOX-deficient rats were similar to those of SOX-normal controls regardless of whether or not they had been ingesting sulphite. Whether the lack of response to sulphite in the SOX-deficient rats was a direct consequence of SOX deficiency or an unrelated consequence of the low Mo/high W regime used to induce the SOX-deficient state was unclear.

Effect of sulphite exposure on spinal reflexes in rats (Küçükatay et al*. 2008)*

This study was conducted using 28 male albino rats, 7rats/group. The group assignments were the same as those used in the studies of Ozkaya *et al*. (2006) and Küçükatay *et al.* (2007). As in those studies, SOX deficiency was induced in 14 of the rats by means of the low Mo/high W regime, commencing three weeks before the start of sulphite treatment, and the duration of sulphite treatment was 6 weeks. However, in contrast to those studies, the sulphite treatment in this study was 70 mg/kg bw/day $Na_2S_2O_5$ in drinking water. At the end of the study, spinal reflexes in response to sciatic nerve stimulation were measured in anesthetized rats, after which they were terminated and the livers removed to confirm their SOX activity status.

Reflex response amplitudes were significantly increased in SOX-deficient rats that had not been treated with sulphite, relative to SOX-normal controls. Exposure to sulphite also caused a statistically significant increase in reflex response amplitude, with the result that the group mean amplitudes were as follows: [SOX-normal, no sulphite] <[SOX-normal, sulphite]<[SOX-deficient, no sulphite]<[SOX-deficient, sulphite].

Hippocampal neuron studies in rats exposed to sulphite (Akdogan et al. *2011; Kocamaz* et al. *2012)*

Male Wistar rats (5/group) were treated with either 0 or 70 mg/kg bw/day $Na_2S_2O_5$ in drinking water for 8 weeks (Akdogan *et al.* 2011). Rats were terminated at the end of the study, brains were frozen and sectioned, and microscopic images were obtained from pyramidal cell layers at matching levels of the hippocampus. Pyramidal neurons from three areas of the hippocampus were counted systematically, but it is not clear how many sections from each area from each rat were included. It was not reported whether there any clinical signs during the in-life phase of the study. It was found that that the sulphite-treated rats had approximately 39% fewer total pyramidal neurons than the controls. The authors described this as a “decrease” and a “loss”. However, no evidence of cellular degeneration or death, or of inflammatory changes associated with cellular necrosis, was described. The photomicrographs are not convincing, because those supposedly showing the reduced number of pyramidal neurons also show considerably more artefactual changes including disruption of neuropil and shrinkage or neurons in the surrounding tissue.

In a similar study (*Kocamaz* et al. *2012)*, SOX deficiency was induced in 12 male Wistar rats by means feeding a low molybdenum diet while supplying 200 ppm of tungsten as sodium in the drinking water. This low Mo/high W regime was commenced three weeks prior to administration of sulphite, and was maintained throughout the experiment. Twelve SOX-normal male Wistars were also used on the study. Four groups, with 6 rats/group, were used on the study. SOX-normal rats were used in the control group, Group C, and in the second group, group S, in which 70 mg/kg/day sulphite was administered in drinking water. The SOX-deficient rats made up the third group, Group D, with sulphite-free water, and the fourth group, Group DS, which was administered 70 mg/kg/day sulphite in their water. The sulphite administration was maintained for six weeks, after which all rats were terminated. Analysis of liver SOX activity confirmed that the rats in groups D and DS were SOX-deficient. Hippocampal neurons were counted in frozen sections. The number of hippocampal neurons in both SOX-normal and SOX-deficient rats was moderately lower, to a statistically significant effect, than that in controls. Again, no degenerating or apoptotic neurons were reported. There were no correlating clinical signs in the rats. Photomicrographs included in the paper show a variable degree of artefactual fracturing and vacuolation of the neuropil that could be a confounding factor.

Visual evoked potentials and lipid peroxidation in rats exposed to sulphite (Ozturk et al. *2011)*

Young male Wistar rats (13/group) were treated daily by oral gavage with 0, 10, 100 or 260 mg $Na_2S_2O_5$/kg bw in water for 35 days. Using a theoretical yield of 67% SO_2 from $Na_2S_2O_5$, this was equivalent to a mean intake of 0, 7, 67 and 175 mg SO_2/kg bw/d. Control rats were gavaged with distilled water.

Endpoints of the study were survival, clinical observations, food consumption, body weights, visual evoked potentials (VEPs) in anesthetised rats, plasma S-sulphonate analysis, levels of thiobarbituric acid reactive substances (TBARS) in retina and brain, levels of 4-hydroxy-2-noneal (HNE) in retina and brain, and levels of glutathione (GSH) and oxidised glutathione (GSSG) in retina and in brain.

There were no treatment-related effects on survival, clinical observations, food consumption or body weights. Group mean values for latency of VEPs, level of plasma S- sulphonate, and level of TBARS in both retina and brain, were significantly increased at ≥100 mg/kg bw/d, and showed a dose-response relationship. Significant correlations were obtained between VEP latencies and plasma S-sulphonate levels. There was no treatment-related effect on VEP amplitude. There appeared to be a dose-related increase in amino acid-HNE adducts, as recognized by immunostaining, in both brain and retina. Mean retina GSH levels were comparable between groups, but the mean retina GSSG level was significantly decreased in the 260 mg/kg bw/day group relative to that of the control group. Group mean brain levels of GSH and GSSG were significantly lower in the 260 mg/kg bw/day group as compared to the control group. The authors concluded that there was a treatment-related increase in lipid peroxidation in brain and retina at ≥100 mg/kg bw/d, although they acknowledged that many biological substances may give false positives in the TBA assay. They suggested that increased ingestion of sulphite may be detrimental to the visual system.

Sensory evoked potentials and lipid peroxidation in rats exposed to sulphite (Kencebay et al*. 2013)*

This study was a follow-up study to that of Ozturk *et al*. (2011). Male Wistar rats (10/group), were gavaged daily for 5 weeks with 0 or 100 mg/kg bw/day freshly prepared solution of $Na_2S_2O_5$. Another group was also treated with 10 mg/kg bw/day quinacrine by daily IP injection. Quinacrine is a non-specific inhibitor of phospholipase A2 (PLA2), a family of enzymes that catalyse the cleavage of fatty acids from membrane phospholipids to release free fatty acids. Parameters measured at the end of treatment were sensory evoked potentials (SEPs) in anesthetised rats, plasma S-sulfonate levels, and TBARS, secretory PLA2 (sPLA2), Caspase-3 and other apoptosis markers in brain tissue. Rats treated with sulphite alone had prolonged latencies of SEP components, although amplitudes were not affected, but SEPs of rats treated with sulphite and quinacrine were comparable to those of controls. Plasma S-sulfonate levels and brain sPLA2 were significantly increased, relative to those of controls, in sulphite-treated rats, and quinacrine had no ameliorating effect. On the other hand, TBARS were significantly increased (159%) in rats treated with sulphite alone, but not in rats treated with sulphite and quinacrine. Similarly, caspase-3 positive neurons and other markers of apoptosis were observed in the brains of rats treated with sulphite alone, but not in the brains of control rats or rats given sulphite and quinacrine. The authors concluded that this study supports their hypothesis that the detrimental effects of sulphite on rat brain are mediated by phospholipase A2 enzymes.

35-day oral gavage study of sodium metabisulphite in rats (Ercan et al*. 2010)*

Young male Wistar rats (10/group) were dosed by oral gavage for 35 days with sodium metabisulphite ($Na_2S_2O_5$) in distilled water at doses of 0, 10, 100 or 260 mg/kg bw/day $Na_2S_2O_5$. Stomachs were removed at termination and assessed for apoptosis using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) technique and for lipid peroxidation using the thiobarbituric acid assay. No significant differences were observed in the 10 mg/kg bw/day group. A dose-related increase in thiobarbituric acid reactive-substances (TBARS) was found in groups treated with ≥ 100 mg/kg bw/d, and the number of TUNEL-positive cells also showed a dose-related increase in groups treated with ≥ 100 mg/kg bw/d. In a subsequent study (Ercan *et al.* 2013) the same research team reported that ghrelin, a peptide produced by the stomach and a number of other organs, inhibits the induction of oxidative stress and apoptosis in rat stomach by sodium metabisulphite.

The significance of this study is difficult to assess because it is known that the homeostasis of gastric epithelial cells is maintained by a balance between cell proliferation and apoptosis. This homeostasis is a very effective protective measure against damage induced by a range

of chemical constituents in food. Since no other chemicals were tested for apoptosis the relative potency of sulphites relative to other food constituents is not possible to determine.

A7.6 Discussion and Conclusions

There have been relatively few publications which could better characterise the hazard of oral sulphites since JECFA established the current ADI in 1973.

A7.6.1 General toxicity in laboratory animals

In 1973 JECFA established an ADI (published in 1974) based on the study by Til *et al.* (1972a), who reported that the most sensitive toxicological endpoint observed following either 8 or 104 weeks of dietary exposure to sulphites was gastric mucosal lesions. In an addendum to the toxicological data in 1999 JECFA stated that the gastric mucosal lesions "reported in rats and pigs arise from local irritation" (WHO 1999). That is, the lesions are likely to be the result of direct contact rather than absorbed and systemically distributed sulphite. This mode of action was considered likely because dietary sulphites are very rapidly and completely biotransformed to sulphates with none being detected in blood or urine in all species tested including humans. Moreover, small amounts of sulphite are formed in the intermediary metabolism of the body in the catabolism of cystine by the non-enzymatic decomposition of 8-sulphinyl pyruvic acid to pyruvic acid and sulphur dioxide. The steady-state concentration of sulphite in the cells is usually too small to be measured. However, 0.10-0.12 molar equivalent/100 mL was found in bull seminal fluid (WHO 1987).

As shown in **Table A7.1**, the presence of gastric mucosal lesions in rat and pig studies of metabisulphites is not a consistent finding.

Table A7.1: Summary of animal studies of metabisulphite

Duration & route	***Number/ group***	***Doses (SO_2-equiv.)****	***Findings***			***Reference***
			Histological	***Bodyweight***	***Haematology***	
Rat studies						
7 d Diet	4 males	Equal. to 0, 90, 200, 400, 1500 mg/kg bw/day	None	Marked decrease in bw gain at 1500 mg/kg bw/day. NOAEL 390 mg/kg bw/day	Slight decrease in HCT. No compensatory response.	Cayzer 2015
3 w Diet	Not stated	Equal to 0, 300, 600 and 1700 mg/kg bw/day	Not examined	Slightly decreased food consumption and bw gain at ≤ 600 mg/kg bw/day. Markedly decreased food consumption and actual bw loss at 1700 mg/kg bw/day.	Significant dose-related decreases in Hb and Hct at ≥600 mg/kg bw/day; decreased Hb/Hct at 1700 mg/kg bw/day	Gunnison *et al.* 1981
4 w Diet	10/sex	Equal to 1, 3, 13 or 28 mg/kg bw/day	None	None	None	Ribera *et al.*1981

Duration & route	***Number/ group***	***Doses (SO_2-equiv.)****	***Findings***			***Reference***
			Histological	***Bodyweight***	***Haematology***	
4 w Diet	10/sex	Equal to 2000 or 6000 mg/kg bw/day	Epithelial hyperplasia and slight hyperkeratosis observed at 2000 mg/kg bw/day when administered in combination with other chemicals was not increased at 6000 mg/kg bw/day	None	None	Jonker *et al.* 1990
8 w Water	8 females	0, 7, 70, 350/175 mg/kg bw/day	Hyperkeratosis of forestomach, and histological lesions of glandular stomach, at 350/175 mg/kg bw/day. NOAEL 70 mg/kg bw/day.	Decreased bw gain and water consumption in SOX-deficient cohort at 350/175 mg/kg bw/day.	None	Hui *et al.* 1989
10 d to 8 w Diet	Not stated	Equal to 0, 150, 300, 600, 1200, 1700, 2300 mg/kg bw/day	Dose-related hyperkeratosis of limiting ridge at ≥ 300 mg/kg bw/day. Reddish material in mucus layer at > 600 mg/kg bw/day. Microscopic erosions, necrosis, glandular hyperplasia and eosinophil infiltration in fundic mucosa at ≥ 1200 mg/kg bw/day for ≥ 4 w.	Not addressed	Not addressed	Feron & Wensvoort 1972
9 w Water	11 to 20/group	0, 300, and 600 in SOX-normal rats. 600 and 1700 mg/kg bw/day in SOX-deficient rats.	Not addressed; not examined?	Significantly decreased bw gain at 300 and 600 mg/kg bw/day in SOX normal rats. No dose-related effect in SOX-deficient rats.	None	Gunnison *et al*. 1981
Up to 12 w Diet		Equal to 0, 1200 and 1700 mg/kg bw/day	Hyperplastic glands in fundic mucosa. Hypertrophy of	Not measured	Not measured	Beems *et al.* 1982

Duration & route	Number/ group	Doses (SO_2-equiv.)*	Findings			Reference
			Histological	Bodyweight	Haematology	
			chief cells. Decreased number of parietal cells. Changes commenced from 1 week.			
12 w Diet	10/sex	Equal to 1.3, 3.2, 13.3 or 26.4 mg/kg bw/day	None	None	None	Ribera *et al.*1981
8, 12 and 24 m Diet	Not stated	Equal to 0, 36, 72, 144, 288 and 576 mg/kg bw/day	Dose-related hyperkeratosis of limiting ridge at ≥ 144 mg/kg bw/d Mild chronic submucosal inflammation at ≥144 mg/kg bw/day in nonglandular stomach and at ≥ 288 mg/kg bw/day in glandular stomach. Hyperplastic fundic glands at ≥ 288 mg/kg bw/day. Mild atrophic changes in glandular stomach at 576 mg/kg bw/day after 2 y	Not addressed	Not addressed	Feron & Wensvoort 1972
20 months Water	40/sex/ group	0 and 53 mg/kg bw/day	None		Increase number of leucocytes in males, and spleen weight in females.	Cluzan *et al.* 1965
2 y (3 generat-ions) Diet	20/sex/ group	Equal to 0, 36, 72, 150, 300 or 600 mg/kg bw/day	Hyperplasia in forestomach and glandular stomach at ≥300 mg/kg bw/day in F0 and F1, and ≥150 mg/kg bw/day in F2. NOAEL= 72 mg/kg bw/day.	Decreased bw gain in both sexes in F1 and F2 rats.	Occult blood in faeces at ≥300 mg/kg bw/day.	Til *et al.* 1972a
2.5 y (3 generat-ions) Water	variable	30 and 60 mg/kg bw/day	None	None	Not measured	Lockett & Natoff 1960
Up to 1 y (4 generat-ions) gavage	10/sex/ Generat-ion	0 and 14 mg/kg bw/day	None	None	None	Lanteaume *et al*. 1965
Pig study						

Duration & route	Number/ group	Doses (SO_2-equiv.)*	Findings			Reference
			Histological	Bodyweight	Haematology	
Up to 48 weeks, diet	20/sex/ Group, decreased to 6/sex/ group after interim necropsies at weeks 15/19	Equiv. to 0, 16, 43, 95, 225 and 465 mg/kg bw/day	At 465 mg/kg bw/day at interim necropsy, and ≥ 225 mg/kg bw/day at final necropsy, hyperplasia of gastric epithelium.	Bw gain decreased at ≥95 mg/kg bw/day but food intake only decreased at 465 mg/kg bw/day	None	Til *et al*. 1972b

*For rat dietary studies that included food consumption, in order to facilitate comparison, used the conversion rate of Til *et al* 1972a; 0.25% added = 72 mg/kg bw/d, then rounded off. This conversion includes loss due to sulphite breakdown.
For Jonker *et al.* 1990 and Til *et al.* 1972b, used conversion factor from
http://apps.who.int/iris/bitstream/10665/44065/14/WHO_EHC_240_14_eng_Annex2.pdf?ua=1
For administration via water, assumed 450 g rat consuming 35 mL water/day therefore 77.8 mL/kg ~ 78 mL/kg bw

All the dietary studies in which gastric lesions have been reported, that is Feron and Wensvoort (1972), Til *et al.* (1972a,b), Beems *et al.* (1982) and Jonker *et al.* (1990) were performed at the same laboratory, the Central Institute for Nutrition and Food Research in the Netherlands. The paper by Feron and Wensvoort (1972) is based on essentially the same data as the rat study by Til *et al.* (1972a).

Jonker *et al.* (1990) reported no increase in the severity of gastric findings in rats between 2000 and 6000 mg sodium metabisulphite/kg bw/day after 4 weeks of dietary exposure. This absence of a dose-response effect casts considerable doubt on the conclusion that the unspecified gastric findings were caused by dietary sodium metabisulphite. Moreover, Jonker *et al.* (1990) identified 2000 mg/kg bw/day as the minimum observed adverse effect level, a much higher dose than the LOAELs reported for subchronic rat studies in other publications from the same laboratory. Thus, a threshold dose for the gastric findings does not appear to be reproducible even within the one laboratory.

The findings of the seven day study of Cayzer (2015) cast further doubt on the reproducibility of the findings in the Central Institute for Nutrition and Food Research studies. It is not plausible that an agent that causes chronic gastric irritation would not cause lesions of acute irritation in a shorter study when administered at much higher doses. While the reproducibility of the gastric findings has proven to be poor, the effects on bodyweight and on haematology in Gunnison *et al*. (1981) and Cayzer (2015) show reasonably good inter-laboratory reproducibility.

The lack of effects on the gastric mucosa in the Cayzer (2015) study at oral doses up to 1500 mg/kg bw/day does not support a direct irritant mode of action of sulphites. However, at very high sodium metabisulphite concentrations (ie. 38% w/v or 2 Molar; pH between 3.9 and 4.4) it can damage epithelial membranes as shown by corneal opacity, erythema and oedema if instilled into the eye (Kirsch and Kieczka 1984).

The authors of the Til *et al*. (1972a) three-generation rat study reported that microscopic haemorrhages from the gastrointestinal tract were occurring, although no sites of haemorrhage were discovered histologically, on the basis of occult blood in the faeces at sulphite doses of about 140-150 mg/kg bw/d. However, the test for occult blood in the faeces was the benzidine test, which is now regarded as obsolete because of a high rate of false positive results. No corresponding evidence of microscopic haemorrhages was found in the pig study performed by the same research group, and no histological evidence of gastrointestinal haemorrhage was observed in the study by Cayzer (2015). Although both the Cayzer (2015) study and the Gunnison *et al.* (1981) study found decreased haematocrit,

there was no evidence of decreased albumin in the Cayzer (2015) study, as might be expected if the decreased haematocrit was due to blood loss.

There was no evidence of a compensatory haematopoietic response to decreased haematocrit, such as reticulocytosis or increased average erythrocyte diameter, in the Cayzer (2015) study. The haematology data in the Gunnison *et a*l. (1981) study were limited to only reporting for haemoglobin, haematocrit and Hb/Hct. At 1700 mg/kg bw/day, the Hb/Hct was decreased, consistent with some increase in average erythrocyte diameter, which is in turn consistent with compensatory haematopoiesis. Gunnison et al. (1981) attributed the significant decreases in haematocrit in their study to degradation of cyanocobalamin by sulphites. Deficiency of cobalamins is usually associated with a macrocytic response. Til *et al*. (1972a) principally reported the three-generation rat study but also briefly described short-term studies of 10-56 days duration, at higher doses of sulphite. Decreases in Hb and Hct were observed at ≥ 600 mg/kg bw/day. Haematological changes consistent with compensatory haematopoiesis and/or cobalamin deficiency, including hypochromasia, polychromasia and anisocytosis, were observed at approximately 1800 mg/kg bw/day (6% dietary sulphite).

The reproducibility of effects in the Til *et al* (1972a) three-generation rat study is difficult to assess because the incidence of the gastric lesions in the F0 and F1 generations at 104 weeks are reported together, so that they cannot be distinguished from each other, and the frequency of measurement of food consumption is not clear. The greater sensitivity of the F2 generation to gastric lesions, relative to the F0 and F1 generations, is inexplicable, and so is the decrease in bodyweight gain in the F1 and F2, but not the F0, rats.

The study in pigs (Til *et al*. 1972b) is of limited value for the purpose of establishing effect levels because of the uncertainty around the actual doses of sodium metabisulphite which gave rise to the observed effects. However it is noteworthy that the microscopic lesions in the pigs were dissimilar to those found in rats by the same laboratory. Microscopic lesions in the pigs, after 48 weeks of sulphite exposure, were glandular and surface epithelial hyperplasia in the cardiac and pyloric regions, and epithelial hyperplasia, microabscesses and neutrophilic infiltrations in the pars oesophagea. In the glandular stomach regions of rats, on the other hand, lesions attributed to chronic dietary sulphite exposure were hyperplastic fundic glands, eosinophilic infiltration, and mild mucosal atrophy. The histology and function of the glandular stomach is very similar between mammals, so it is difficult to explain why different species would have such different responses to direct irritation by dietary sulphites.

On the basis of the variable findings summarized in **Table 1**, it is apparent that there is considerable uncertainty around the ADI, because the adverse effect on which it is based has not been established to be reproducible in laboratories other than the laboratory in which it was first reported. Mild gastric lesions were found in some high-dose (350/175 mg/kg bw/day) rats in the Hui *et al*. (1981) study, but the route of exposure was via drinking water rather than in diet. The apparent greater sensitivity of SOX-deficient rats to this effect would be inexplicable if the effect is due to direct irritation of the gastric mucosa.

In the seven-day study of Cayzer (2015), the most sensitive toxicological effect was a decrease in bodyweight gain. This occurred at 1500 mg/kg/day, a dose about 10 times greater than the LOAEL (~140 mg/kg bw/day) observed in the Til *et al.* (1972a) study. A decrease in growth rate was observed only in the F1 and F2 rats in the multi-generational study by Til *et al* (1972a), and only at the highest dose, 600 mg/kg bw/day. If decreased bodyweight gain is used as the most sensitive endpoint that is reproducible by other laboratories, this means that the NOAEL in the long term Til *et al*. (1972) study would be 300 mg/kg bw/day.

The 3-week dietary study by Gunnison *et al*.(1981) also found a decrease in bodyweight gain, in SOX-normal rats only, with a concomitant decrease in food intake, which did not occur in the Cayzer (2015) study. The decrease in bodyweight gain in the Gunnison *et al.* (1981) study did not show a clear dose-response relationship between the low- and high-dose groups and was not observed in the SOX-deficient groups, although the highest dose used in the SOX-deficient rats was double the highest dose used in the SOX-normal rats. A significant decrease in bodyweight was found in the SOX-deficient high-dose (350/175 mg/kg bw/d) group in the study by Hui *et al.* (1981) but not in the corresponding SOX-normal group. These observations make it highly unlikely that the sulphite was the cause of the decrease in bodyweight gain in the Gunnison *et al*. (1981) study, and much more likely that the effect in the SOX-normal rats reflected the presence of supplemental molybdenum in their water supply, supplementation given only to the SOX-normal rats treated with sulphite and not to SOX-normal controls.

Decrease in haematocrit is another possible candidate for the most sensitive endpoint, but this endpoint has been measured in relatively few studies and results are conflicting. In the Cayzer (2015) study, slightly decreased erythrocyte count, haematocrit and haemoglobin were observed only at the highest dose of 1500 mg/kg bw/d, the dose at which decrease in bodyweight gain was profound. In contrast, Gunnison *et al.* (1981) reported that decreased haemoglobin and haematocrit were observed at ≥ 600 mg/kg bw/day but decreased bodyweight gain was observed only at 1700 mg/kg bw/d. Decreased erythrocyte count, haematocrit and haemoglobin were observed only in the F0 females consuming 600 mg/kg bw/day in the Til *et al*. (1972a) study. It is possible that the short duration of the Cayzer (2015) study meant that the threshold for haematology changes was higher than in the longer studies. If decreased haematocrit is the most sensitive endpoint, then this means that the NOAELs from the Gunnison et al. (1981) study and the Til *et al*. (1972a) study are the same; 300 mg/kg bw/d.

A7.6.2 Developmental and Reproductive Toxicity

No new evidence was found that sulphites are developmental or reproductive toxicants by the oral route.

A7.6.3 Genotoxicity and Carcinogenicity

Further genotoxicity studies have been published which confirm the genotoxic nature of sulphites *in vitro*, but there remains a lack of evidence that sulphites act as tumour initiators *in vivo.* The study of Takahashi *et al* (1986) was interpreted to suggest that sulphites may promote development of tumours of the stomach or duodenum in tissue that has already undergone neoplastic transformation. Promotion of tumours by exerting an irritant effect on the mucosa of stomach or intestine is a mechanism recognized for a number of chemically unrelated substances, but the study did not report any evidence of a chronic irritant effect. The chronic multigenerational study of Til *et al.* (1972a) did not find any evidence of carcinogenicity in rats.

A7.6.4 Human toxicity

Recent evidence shows that sulphite sensitivity is not limited to asthmatics. People with no history of asthma may develop urticaria following oral challenge with sodium metabisulphite. No additional or subsequent reports of toxic effects of oral sulphites to non-sensitised human beings were located for the purpose of this review.

A7.6.5 Other studies

No studies were found that provide evidence that sulphites cause neurotoxicity in the absence of congenital SOX deficiency.

A7.6.6 Conclusions

The current JECFA ADI is based on a NOAEL for gastric lesions in rats, but these findings are not consistently reproducible, and this means that there is great uncertainty around the dose at which adverse findings are likely to occur. Limited evidence suggests that selection of a more reproducible adverse effect, such as impaired bodyweight gain or decreased haematocrit, would result in a higher NOAEL and therefore a higher ADI. There is no evidence that sulphites are developmental or reproductive toxicants, and although sulphites are genotoxic *in vitro*, there is no evidence that they are carcinogenic *in vivo*. Human toxicity is limited to hypersensitivity reactions in limited subpopulations. This uncertainty around the most relevant toxicological endpoint to establish a suitable health based guidance value can only be overcome if a robust long-term repeat dose study is completed.