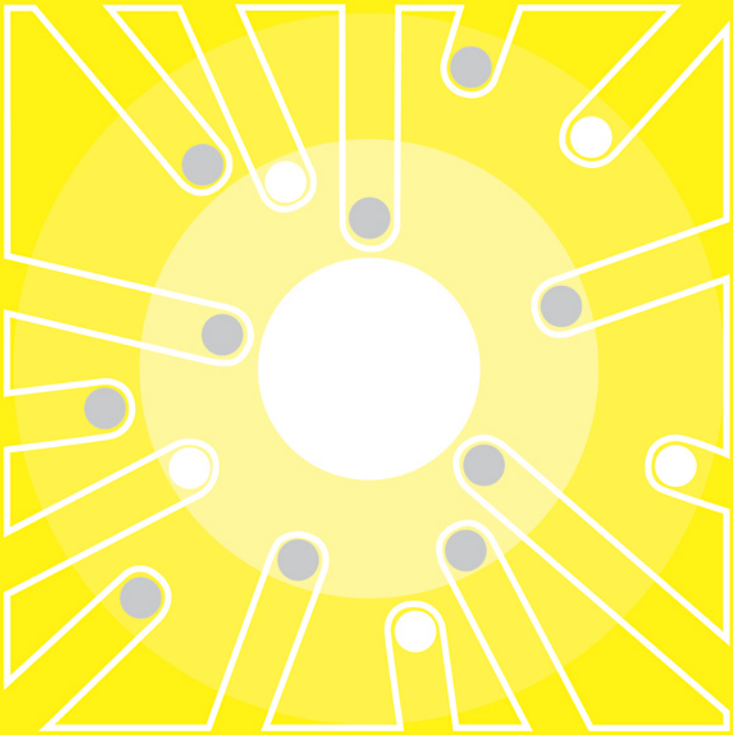
WHO FOOD ADDITIVES SERIES: 58

# Safety evaluation of certain food additives and contaminants

Prepared by the
Sixty-seventh meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)

Food and Agriculture Organization of the United Nations

World Health Organization



IPCS

International Programme on Chemical Safety

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WHO Library Cataloguing-in-Publication Data

Safety evaluation of certain food additives / prepared by the sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA).

(WHO food additives series ; 58)

1.Food additives - toxicity. 2.Food contamination. 3.Risk assessment. 4.Carotenoids. 5.Parabens. 6.Aluminum - toxicity. 7.alpha-Chlorohydrin - toxicity. 8.Methylmercury compounds - toxicity. I.Joint FAO/WHO Expert Committee on Food Additives. Meeting (67th : 2006 : Geneva, Switzerland) II.International Programme on Chemical Safety. III.Series.

ISBN 978 92 4 166058 7 (NLM classification: WA 712)
ISSN 0300-0923



Typeset in India
Printed in Spain

## CONTENTS

This publication is a contribution to the **International Programme on Chemical Safety (IPCS)**.

The IPCS, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessing the risk to human health and the environment to exposure from chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

## PREFACE

The monographs contained in this volume were prepared at the sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at FAO Headquarters in Rome, Italy, 20–29 June 2006. These monographs summarize the safety data on selected food additives and food contaminants reviewed by the Committee.

The sixty-seventh report of JECFA has been published by the World Health Organization as WHO Technical Report No. 940. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication; a summary of the conclusions of the Committee is given in Annex 4. Some of the substances listed in Annex 4 were evaluated at the meeting only for specifications.

Specifications that were developed at the sixty-seventh meeting of JECFA have been issued separately by FAO as *Compendium of food additive specifications*, FAO JECFA Monographs 3, 2006 (ISBN 92-5-105559-9). The monographs in the present publication should be read in conjunction with the specifications and the report.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States, and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food, and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The toxicological monographs contained in the volume are based on working papers that were prepared by Temporary Advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers.

Many proprietary unpublished reports are unreferenced. These were voluntarily submitted to the Committee by various producers of the food additives under review, and in many cases represent the only data available on those substances. The Temporary Advisers based the working papers they developed on all the data that were submitted, and all of these reports were available to the Committee when it made its evaluation. The monographs were edited by H. Mattock, Tignieu-Jameyzieu, France.

The preparation and editing of the monographs included in this volume were made possible through the technical and financial contributions of the Participating Organizations of the International Programme on Chemical Safety (IPCS), which supports the activities of JECFA.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in the IPCS concerning the legal status of any country, territory, city, or area or its authorities, or concerning the delimitation of its frontiers

or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

# FOOD ADDITIVES

## *ANNATTO EXTRACTS (addendum)*

***First draft prepared by***

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### *1. EXPLANATION*

Annatto extracts are obtained from the outer layer of the seeds of the tropical tree *Bixa orellana.* The principal pigment in annatto extract is *cis*-bixin, which is contained in the resinous coating of the seed itself. Processing primarily entails the removal of the pigment by abrasion of the seeds in an appropriate suspending agent. Traditionally, water or vegetable oil is used for this purpose, although solvent extraction is also employed to produce annatto extracts with a higher content of pigment. Microcrystalline bixin products of 80–97% purity have been developed in response to the need for more concentrated annatto extracts.

Annatto extracts were evaluated by the Committee at its thirteenth, eighteenth, twenty-sixth, forty-sixth, fifty-third and sixty-first meetings (Annex 1, references *19*, *35*, *59–61*, *122, 143* and *166*).

At its eighteenth meeting, the Committee considered the results of long-term and short-term tests in experimental animals fed an annatto extract containing 0.2–2.6% pigment expressed as bixin. A long-term study in rats provided the basis for evaluation; the no-observed-effect level (NOEL) in this study was 0.5% in the diet, the highest dose tested, equivalent to 250 mg/kg bw. A temporary acceptable daily intake (ADI) for this annatto extract was established at 0–1.25 mg/kg bw.

The Committee re-evaluated annatto extracts at its twenty-sixth meeting, when the results of the requested studies of metabolism became available. Studies of mutagenicity, additional long-term (1-year) studies in rats, and observations of the effects of annatto extract in humans were also considered. The metabolism studies were conducted on three different extracts—a vegetable oil solution, a vegetable oil suspension (containing mainly bixin pigment) and a water-soluble extract (mainly norbixin)—alone and in admixture. No evidence was found for the accumulation of annatto pigments in the tissues of rats fed with at low dietary concentrations (20–220 mg/kg bw per day) with annatto extracts containing up to 2.3% bixin/norbixin mixture for 1 year, and clearance from the plasma was rapid.

The NOEL in the original long-term study in rats was identified as 0.5% in the diet, equivalent to 250 mg/kg bw, and the ADI for these annatto extracts was set at 0–0.065 mg/kg bw expressed as bixin. At that time, the Committee considered the highest concentration of bixin in the material tested (i.e. 2.6%) and established an ADI on the basis of the content of bixin.

At its forty-sixth meeting, the Committee revised the specifications for annatto extracts and redesignated them according to their methods of manufacture into two general types: oil- or alkali-extracted products, and solvent-extracted products. The ADI was not changed at that meeting. At its fifty-third meeting, the Committee assessed intake of annatto extracts and concluded that the intake of annatto extracts would exceed the ADI for bixin if all foods contained annatto extracts at the maximum levels proposed in the Codex Alimentarius Commission draft General Standard for Food Additives (GSFA) (Codex Alimentarius Commission (2006). Intake assessments based on national permitted levels led to the conclusion that the ADI for bixin was unlikely to be exceeded as a result of the use of annatto extracts.

Table 1 describes the designation of the extracts.

At its sixty-first meeting, the Committee established temporary ADIs for annatto extracts B, C, E and F. As insufficient data on the potential toxicity of annatto D or annatto G were available, no ADIs could be established for those extracts.

At that meeting, additional information was requested to clarify the role that the non-pigment components of the extract play in the expression of the qualitative and quantitative differences in toxicity between the various extracts. In addition, the Committee requested data on the reproductive toxicity of an extract, such as annatto F, that contains norbixin.

At the present meeting, most of those data were available and were evaluated, and a re-evaluation of the overall database was performed.

## *2. BIOLOGICAL DATA*

Newly published and unpublished data regarding chemical composition, short term toxicity, genotoxicity and anti-genotoxicity, reproductive toxicity and cancer promotion became available.

***Table 1. Designation of annatto extracts***

| Annatto extract description[a] | Alternative designation[b] | Pigment content (%)[c] | | Specified pigment content[d] (%) |
|---|---|---|---|---|
| | | Bixin | Norbixin | |
| Solvent-extracted bixin | Annatto B | 89.2 | 1.6 | ≥ 85% pigment (as bixin) |
| | | (92) | (1.7) | ≤ 2% norbixin |
| Solvent-extracted norbixin | Annatto C | NR | (91.6) | ≥ 85% pigment (as norbixin) (includes $Na^+$ and $K^+$ salts) |
| Oil-processed bixin | Annatto D | 10.2 | 0.18 | ≥ 10% pigment (as bixin) |
| Aqueous processed bixin | Annatto E | 25.4 | 1.1 | ≥ 25% pigment (as bixin) |
| | | (26) | (4.2) | ≤ 7% norbixin |
| Alkali-processed norbixin (acid precipitated) | Annatto F | NA | 41.5 | ≥ 35% norbixin |
| | | NA | (38.4) | |
| Alkali-processed norbixin (not acid precipitated) | Annatto G | NA | 17.1 | ≥ 15% norbixin |

NA: not applicable; NR: not reported.
[a] Description used by the Committee at its present meeting.
[b] Designation used by the Committee at its sixty-first meeting.
[c] Analytical data on the bixin/norbixin content of various extracts. Values in parentheses are for extracts tested in 90-day studies.
[d] Specified by the Committee at its present meeting

### *2.1 Chemical composition*

Mass balance studies (Reading Scientific Service Ltd, 2005a, 2005b) have characterized the components of four annatto extracts listed in Table 1. These data indicate that the non-pigment material in these extracts consists of several well known plant constituents, none of which would be expected to raise any safety concerns under the intended conditions of use of the extracts.

### *2.2 Toxicological studies*

#### *2.2.1 Short-term studies of toxicity*

In its sixty-first report, the Committee described an unpublished 13-week study in Sprague-Dawley rats given annatto C, a purified annatto extract containing

***Table 2. Mass balances for various annatto preparations***

| Fraction / component | Annatto extract | | | | |
|---|---|---|---|---|---|
| | Solvent-extracted bixin (B) | Aqueous-processed bixin (E) | Alkali-processed norbixin (F) | Alkali-processed norbixin, not acid precipitated (G) | |
| | % w/w | % w/w | % w/w | % w/w | Calculated % w/dry w[a] |
| *Hexane solubles* | 2.4 | 26.8 | 12.6 | 0.75 | NC |
| Geranyl geraniol | ND | 8.4 | 10.0 | 0.75 | 7.7 |
| Aliphatic hydrocarbons (wax) | NQ | 1.6 | Present | NQ | — |
| Tocotrienols | < 0.01 | 3.4 | 0.02 | < 0.01 | < 0.1 |
| Other terpenoids by difference, including geranyl geranene | ND | 13.4 | ND | NQ | — |
| Aromatic component | NQ | Trace | Present | NQ | — |
| *Acetone solubles* | NC | NC | NC | NC | NC |
| Bixin | 87 | 29.2 | ND | ND | — |
| Bixin isomers[b] | 4.0 | — | ND | ND | — |
| Unknown bixins[c] | — | 1.1 | ND | ND | — |
| Norbixin | — | 0.9 | 39.0 (9-*cis*) | 1.8 (9-*cis*) | 18 |
| Norbixins[d] | 2.0 | —[e] | 8.0 (others) | 0.5 (others) | 5 |
| Fatty acid esters | < 0.01 | 4.1 | 1.9 | < 0.01 | < 0.1 |
| Polyphenols | ND | 4.0 | ND | ND | — |
| Moisture | 0.1 | 9.4 | 4.1 | 90.6 | 0 |
| *Acetone insolubles*[f] | < 1 | 20.4 | 34.5 | 7.4 | NC |
| Protein | 0.9 | 5.6 | 6.2 | 0.5 | 5 |
| Ash | 0.1 | 4.9 | 12.1 | 3.2 | 33 |
| Carbohydrate | 0.1 | 0.3 | 0.7 | 1.5 | 15 |
| Lignocellulose | < 0.1 | 9.6 | 15.5 | 1.5 | 15 |
| Total | 95 | 95.9 | > 95 | > 99 | > 99 |

NC: not calculated. ND: not detected; NQ: not quantifiable.

[a] Calculated from the preceding column, based on moisture content.

[b] 'Bixin isomers' refers to all isomers including bixin itself.

[c] 'Unknown bixins' refers to all isomers other than bixin itself, indicating that all other isomers were not separated and therefore not reported as 'Bixin isomers'.

[d] 'Norbixins' refers to all isomers including norbixin itself.

[e] This material contains only small amounts of norbixin and non-quantifiable amounts of other isomers of norbixin.

91.6% norbixin. This study has now been published (Hagiwara et al., 2003a), with no different information being available than was previously submitted to the Committee.

In a new short-term study of toxicity published by Bautista et al. (2004), groups of 15 male and 15 female Wistar rats were given annatto powder (bixin, 27%) suspended in corn oil and administered by gavage as a single dose at 2000 mg/kg bw per day for 4 weeks (no adverse effects having been noted in a preliminary experiment with annatto at a dose of 1000 mg/kg bw per day). Haematological and biochemical examination and necropsy were performed on 10 rats of each sex on day 29 and on the remaining 5 rats of each sex after recovery at day 43. Decreased erythrocyte counts and haemoglobin concentrations were observed in males and decreased leukocyte counts in males and females on day 29, but not in the recovery groups. At necropsy, no lesions were observed, but focal renal cell apoptosis occurred in 2 out of 10 treated female rats without proliferation or tubular damage.

### *2.2.2 Reproductive toxicity*

A study of prenatal developmental toxicity with annatto F was performed according to OECD guidelines and in compliance with good laboratory practice (GLP) (Huntingdon Life Sciences Laboratory, UK, 2005). Groups of 22 Sprague-Dawley rats were given alkali-processed norbixin (acid precipitated) (annatto F) with a norbixin content of 42.5% at a dose of 0, 20, 40, 80 or 160 mg/kg bw per day (equivalent to an intake of norbixin of 8.5, 17, 34 and 68 mg/kg bw per day) by oral gavage on days 6–19 of gestation. Controls were dosed with vehicle only (0.5% aqueous sodium carboxymethycellulose). Animals were killed on day 20, after mating for reproductive assessment and fetal examination. Adult females were examined macroscopically at necropsy on day 20 after mating, the uterus and contents were weighed, corpora lutea counted, and living and dead fetuses and resorptions were recorded. Fetuses were weighed and examined for externally visible abnormalities, and all fetuses were examined macroscopically at necropsy. Subsequently, detailed internal visceral examination followed by skeletal examination were performed for half the fetuses, and the other half were fixed and serially sectioned for examination for visceral abnormalities.

There were no deaths among the dams, and no clinical findings that were considered to be related to treatment, and there were no adverse effects of treatment on body-weight gain or food consumption. There was no increase in embryolethality and no reduction in fetal or placental weight. The annatto extract did not induce any increase in the incidence of externally visible, visceral, or skeletal abnormalities in the exposed offspring treated with does of up to 160 mg/kg bw per day. The amniotic sacs of the majority of litters in the groups at 80 and 160 mg/kg bw per day were stained yellow/orange, showing that the norbixin had been well absorbed. The no-observed-effect level (NOEL) for annatto F (alkali-processed norbixin, acid precipitated) in this study was 160 mg/kg bw per day, the highest dose tested.

### 2.2.3 Special studies

#### (a) Cancer promotion

In the report of its sixty-first meeting, the Committee described unpublished studies in which the effect of annatto extract (norbixin) containing 87% norbixin was examined in a medium-term assay for carcinogenesis. These studies have now been published (Hagiwara et al., 2003b).

A similar study has been published by a different group of workers studying the effects of an annatto extract containing bixin in the same medium-term assay for carcinogenesis. Agner et al. (2004) investigated the carcinogenic and anticarcinogenic effects of dietary annatto in male Wistar rat liver, using the preneoplastic glutathione *S*-transferase (GST-P) foci and DNA damage biomarkers. Groups of 14 male Wistar rats were given diets containing oil extract of annatto, containing 5% bixin, at a concentration of 20, 200, or 1000 ppm (annatto: 1.5, 16.45 and 84.8 mg/kg bw per day; bixin: 0.07; 0.80 and 4.23 mg/kg bw per day, respectively), continuously during 2 weeks before (pre-initiation protocol), or 8 weeks after treatment with diethylnitrosamine (DEN) at a dose of 200 mg/kg bw, given intraperitoneally (post-initiation protocol), with corresponding control groups. The comet assay was used to investigate the modifying potential of annatto on DEN-induced DNA damage. Groups of 10 male Wistar rats were dosed with annatto for 2 weeks before treatment with DEN injection (20 mg/kg bw, given by intraperitoneal infection) and sacrificed 4 h later with rats in the corresponding control groups. The results showed that annatto at up to the highest concentration tested (1000 ppm) neither potentiated nor ameliorated the effects of DEN in either GST-P foci development or in comet assays.

The same group of researchers also studied annatto in another anti-carcinogenicity model system (Agner et al., 2005). Based on evidence that has indicated carotenoids as possible agents that decrease the risk of colorectal cancer, the authors evaluated the activity of annatto on the formation of aberrant crypt foci induced by dimethylhydrazine (DMH) in the rat colon. They also investigated the effect of annatto on DMH-induced DNA damage, by the comet assay. Groups of 12 male Wistar rats were given subcutaneous injections of DMH at 40 mg/kg bw twice per week for 2 weeks to induce aberrant crypt foci. They also received experimental diets with annatto containing 5% bixin at 20, 200 or 1000 ppm (annatto: 1.22, 12.26 or 56.38 mg/kg bw per day; bixin: 0.06, 0.61 or 2.82 mg/kg bw per day) for 5 weeks before, or 10 weeks after treatment with DMH. In both protocols the rats were sacrificed in week 15. For the comet assay, the animals were fed with the same experimental diets for 2 weeks. Four hours before sacrifice, the animals received a subcutaneous injection of DMH at 40 mg/kg bw. Under such conditions, dietary administration of annatto at up to 1000 ppm did not induce DNA damage in blood and colon cells or in aberrant crypt foci in the rat distal colon. Conversely, annatto, mainly when administered after treatment with DMH, inhibited the number of crypts per colon (animal), but not the incidence of DMH-induced aberrant crypt foci. No antigenotoxic effect was observed in colon cells. These findings suggest possible chemopreventive effects of annatto through modulation of the cryptal

cell proliferation (anti-promotion effect) but not at the initiation stage of colon carcinogenesis.

*(b) Genotoxicity*

At its sixty-first meeting, the Committee reported on the large number of studies of genotoxicity with annatto extracts and with pure bixin or norbixin. Studies of genotoxicity in vitro revealed equivocal and inconsistent positive results only at concentrations that exceeded solubility or at concentrations that were cytotoxic. Since the results of tests on analytical-grade bixin and norbixin were negative, some weak positive results obtained with the concentrated annatto extracts in bacterial tests in the absence of an endogenous metabolic activation system were considered to be caused by other components in the annatto preparations. Results of tests for mutagenicity in mammalian cells and for chromosomal aberration were inconsistent. Weak positive results at toxic concentrations were noted for some preparations in tests for mutagenicity in mammalian cells in the absence of an endogenous metabolic activation system. Weak positive results were noticed only in the presence of an endogenous metabolic activation system in tests for chromosomal aberration. Annatto preparations B, E and F did not demonstrate any potential to cause genetic damage in the assay for micronucleus formation in mice in vivo.

*(c) Anti-genotoxic activity*

At its sixty-first meeting, the Committee reported a number of studies regarding anti-mutagenic and anti-carcinogenic activities with annatto preparations.

Recently, the antigenotoxic and antimutagenic potential of annatto extract (norbixin) against oxidative stress in vitro was reported by Junior et al. (2005). The authors evaluated the effect of norbixin at 2 mmol/l on the response of *Escherichia coli* cells to DNA damage induced by UV radiation, hydrogen peroxide ($H_2O_2$) and superoxide anions ($O_2$*-) and found that norbixin protects the cells against these agents. Norbixin enhanced survival by at least 10 times. Norbixin at 2 mmol/l also inhibited the induction of genotoxicity by UVC in the SOS chromotest assay. Norbixin was also shown to have antimutagenic properties, with a maximum inhibition of $H_2O_2$-induced mutagenic activity of 87%, in a test for mutatgenicity in *Salmonella typhimurium* strain TA102.

Similar studies on the anticlastogenic effect of bixin on chromosomal damage induced by the clastogen cisplatin (cDDP) in human lymphocyte cultures have also been reported (Antunes et al. 2005). Blood samples were obtained from six healthy, non-smoking volunteers—two females and four males aged 18–35 years. The concentrations of bixin (1.0, 2.5, 5.0 or 10 g/ml) tested in combination with cDDP were established on the basis of mitotic index measurements. While bixin alone was not cytotoxic or clastogenic, it reduced the inhibitory effect of cDDP on the lymphocyte mitotic index in a concentration-dependent manner. Cultures simultaneously treated with bixin and cDDP showed a statistically significant reduction in total chromosomal aberrations and aberrant metaphases.

In its previous evaluations, the Committee had concluded that annatto extracts are not carcinogenic. This conclusion was based on the results of test with annatto preparations containing bixin at low concentrations. In a study of the initiation and promotion of liver carcinogenesis, solvent-extracted norbixin did not increase the incidence of preneoplastic lesions. A recent study showed that annatto extract (5% bixin) at dietary concentrations of up to 1000 mg/kg neither potentiated nor ameliorated the effects of DEN in the livers of male rats as measured by development of preneoplastic GST-P foci or DNA fragmentation using the comet assay. Together with the results of the tests for genotoxicity and the absence of proliferative lesions in the short-term tests for toxicity, these data support the earlier conclusions by the Committee that annatto extracts are not carcinogenic.

## *3. DIETARY INTAKE*

During its sixty-first meeting, the Committee performed an assessment of dietary exposure based on typical use levels of annatto extracts expressed as bixin and norbixin, provided by industry. Combining those levels with various average food consumption levels resulted in a dietary exposure ranging from 0.03 to 0.4 mg/day. Combining the use levels reported by industry with 97.5th percentiles of consumption by consumers in the United Kingdom of foods potentially containing annatto resulted in a dietary exposure to total bixin plus norbixin at 1.5 mg/day.

No additional data were provided for this meeting, therefore exposure scenarios were performed on the basis of the previous dietary exposure to pigments assuming a body weight of 60 kg.

## *4. COMMENTS*

Mass balance studies have characterized the components of the annatto extracts to the extent of greater than 95%, including non-pigment material, except for oil-processed bixin for which no new analytical data were provided.

A study of developmental toxicity in rats fed with an annatto extract with a norbixin content of 41.5% at doses of up to 160 mg/kg bw per day (equal to 68 mg/kg bw per day expressed as norbixin) confirmed the absence of developmental toxicity at this, the highest dose tested.

In its previous evaluations, the Committee had concluded that annatto extracts are not carcinogenic. This conclusion was based on the results of tests with annatto preparations containing low concentrations of bixin. In a study of the initiation and promotion of liver carcinogenesis, solvent-extracted norbixin did not increase the incidence of preneoplastic lesions. A recent study showed that annatto extract (5% bixin) at dietary concentrations of up to 1000 mg/kg had no influence on the development of preneoplastic GST-P-positive foci in livers of male rats treated with DEN, nor on DNA fragmentation in the livers using the comet assay. Together with the results of the tests for genotoxicity and the absence of proliferative

lesions in the short-term tests for toxicity, those data support the earlier conclusion made by the Committee, that annatto extracts are not carcinogenic.

During its sixty-first meeting, the Committee performed an assessment of dietary exposure based on typical use levels (provided by industry) of extracts expressed as bixin and norbixin. Combining those levels with various average levels of food consumption resulted in dietary exposures ranging from 0.03 to 0.4 mg/day. Combining the use levels reported by industry with 97.5th percentiles of consumption by United Kingdom (UK) consumers of foods potentially containing annatto resulted in a dietary exposure of 1.5 mg/day of total bixin plus norbixin.

No additional data were provided for this meeting, therefore exposure scenarios were performed on the basis of the previous dietary exposure to pigments, assuming a body weight of 60 kg.

## *5. EVALUATION*

At its present meeting, the Committee re-evaluated the 90-day studies of toxicity available for four of the extracts for which compositional data were provided. The results of those studies are summarized in Table 3.

In re-evaluating the studies of toxicity with solvent-extracted bixin (92% bixin) and solvent-extracted norbixin (91.6% norbixin) in the light of the additional compositional data, the Committee considered that ADIs could be allocated to those pigments on the basis of the studies conducted on the extracts summarized in Table 3.

The Committee established an ADI for bixin of 0–12 mg/kg bw on the basis of the NOEL of 1311 mg/kg bw per day from a 90-day study in male rats fed an extract containing 92% bixin, corrected for pigment content and applying a safety factor of 100.

The Committee established a group ADI for norbixin and its sodium and potassium salts of 0–0.6 mg/kg bw (expressed as norbixin) on the basis of the NOEL of 69 mg/kg bw per day from a 90-day study in male rats fed an extract containing 91.6% norbixin, corrected for pigment content and applying a safety factor of 100.

The Committee further evaluated compositional data on aqueous processed bixin and alkali-processed norbixin (acid-precipitated), together with toxicological data on annatto extracts for which NOELs had been identified in 90-day studies of toxicity. It concluded that the use of these annatto extracts as sources of bixin or norbixin would not raise safety concerns, provided that they complied with the relevant specifications. Accordingly, the ADIs given above could be applied to bixin and norbixin derived from those annatto extracts. The Committee noted that the pigment in alkali-processed norbixin (not acid-precipitated) consists of sodium or potassium salts of norbixin and that compositional data on this extract, complying with the specifications, did not raise safety concerns. Consequently, the Committee concluded that the group ADI for norbixin and its sodium and potassium salts could be applied to norbixin salts from this source.

***Table 3. Results of 90-day studies of toxicity with annatto extracts***

| Annatto extract | Pigment in extract tested (%) | | Extract NOEL[a] (mg/kg bw) | |
|---|---|---|---|---|
| | Bixin | Norbixin | Male | Female |
| Solvent-extracted bixin | 92 | 1.7 | 1311 | 1446 |
| Solvent-extracted norbixin | NR | 91.6 | 69 | 76 |
| Aqueous processed bixin | 26 | 4.2 | 734 | 801 |
| Alkali-processed norbixin (acid precipitated) | NA | 38.4 | 79 | 86 |

NA: not applicable; NR: not reported.
[a]As determined by the Committee at its sixty-first meeting.

As no NOEL could be identified for oil-processed bixin and no compositional data were available, the Committee decided that the above evaluation could not be applied to this extract.

If all the pigment ingested were bixin, the estimated dietary exposure of 1.5 mg/day would result in an intake of bixin of 26 µg/kg bw per day, corresponding to approximately 0.2% of the ADI (0–12 mg/kg bw). Similarly, if all the pigment were norbixin, the estimated dietary exposure of 1.5 mg/day would result in an intake of norbixin of 26 µg/kg bw per day, corresponding to approximately 4% of the ADI (0–0.6 mg/kg bw).

All previously established ADIs and temporary ADIs for bixin and annatto extracts were withdrawn.

The tentative specifications for all annatto extracts were revised and the tentative designations removed, with the exception of the specification for annatto extract (oil-processed bixin), which was maintained as tentative because the requested information on chemical characterization of the non-colouring-matter components of commercial products was not provided. The tentative specification for annatto extract (oil-processed bixin) would be withdrawn if the requested information is not received by the Committee before the end of 2008.

The Chemical and Technical Assessment prepared by the Committee at its sixty-first meeting was updated.

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## *LYCOPENE (SYNTHETIC)*

***First draft prepared by***

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### *1. EXPLANATION*

At the request of the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005), the JECFA Committee at its present meeting evaluated lycopene to be used as a food additive. Lycopene is a naturally-occurring pigment found in vegetables (especially tomatoes), fruits, algae and fungi. It can also be synthesized chemically.

The Committee had previously evaluated lycopene (both natural and synthetic) to be used as a food colour at its eighth, eighteenth, and twenty-first meetings (Annex 1, references *8*, *35* and *44*). The lack of adequate information available at those meetings precluded the Committee from developing specifications and establishing an acceptable daily intake (ADI) for lycopene to be used as a food colour. Under consideration at the present meeting were synthetic lycopene (the subject of this item) and lycopene from the fungus *Blakeslea trispora* (see monograph 'Lycopene from *Blakeslea trispora*', in this volume).

Synthetic lycopene is produced by the Wittig condensation of intermediates. The raw materials of this reaction are commonly used in the production of other carotenoids used in food.

## 2. BIOLOGICAL DATA

### 2.1 Biochemical aspects

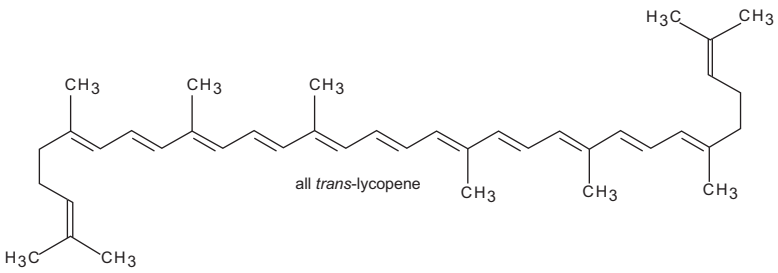
Lycopene belongs to the family of carotenoids and is a biochemical precursor of higher carotenoids, e.g. β-carotene. However, unlike β-carotene, lycopene lacks β-ionone ring structure and is therefore devoid of provitamin A activity.

In analogy with naturally occurring lycopene, the predominant isomer in synthetic lycopene is all-*trans*-lycopene (CAS No. 502-65-8). The chemical name for lycopene is 2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,-26,30-dotriacontatridecaene, and synonyms are *ψ*,*ψ*-carotene, all-*trans*-lycopene, and (all-*E*)-lycopene. Lycopene is an unsaturated acyclic hydrocarbon with the chemical structure shown in Figure 1.

#### *2.1.1 Stereochemical isomerism*

All carotenoids contain an extended conjugated polyene backbone. Although theoretically each of these carbon–carbon double bonds can exist in either *trans* or *cis* configurations (also referred to as *E*- or *Z*-configurations, respectively), the vast majority of carotenoids, including lycopene, exhibit predominantly the all-*trans* configuration. Lycopene, however, can undergo a conversion of one or more

***Figure 1. Chemical structure of lycopene***



of its *trans*-configured carbon–carbon double bonds to the corresponding *cis* forms, a conversion that occurs more readily than with most other carotenoids. The c*is* isomers of lycopene have physical and chemical characteristics that are different to those of all-*trans*-lycopene, e.g. lower melting points and decreased colour intensity (Nguyen & Schwartz, 1999).

In most natural matrices, all-*trans*-lycopene is thus quantitatively the most important isomer.[1] Among the naturally-occurring *cis* isomers, 5-*cis*-, 9-*cis*- and 13-*cis*-lycopene usually predominate. For illustrative purposes, the isomer composition of lycopene typically found in unprocessed, raw (red) tomatoes and in tomato-based foodstuffs is provided in Table 1 (Schierle et al., 1997). It is to be noted that in other fruits and vegetables, including tangerine-type tomatoes, other *cis* isomers and/or *cis*/*trans* isomer ratios may be prevalent. Processing and storage generally do not affect the lycopene content (Nguyen & Schwartz, 1999; Agarwal et al., 2001), but heat treatment has sometimes been reported to result in *trans* to *cis* isomerization, leading to a higher content of *cis* isomers in processed foods. Table 1 also contains data on lycopene in human plasma, in which it is one of the most abundant carotenoids. A significant proportion of lycopene in human plasma is present as *cis* isomers (Khachik et al., 1997a; Schierle et al., 1997).

### *2.1.2 Specifications*

The Committee received two applications for synthetic lycopene, submitted by DSM Nutritional Products Ltd (formerly Roche Vitamins Ltd) and BASF Aktiengesellschaft.

Synthetic lycopene is a high-purity crystalline product containing at least 96% total lycopene. Synthetic lycopene consists predominantly of all-*trans*-lycopene (not less than 70%) and 5-*cis*-lycopene, with minor quantities of other isomers. It may contain low concentrations of reaction by-products, such as TPPO, apo-12'-lycopenal (also referred to as lycopene $C_{25}$-aldehyde, lycopyl $C_{25}$-aldehyde or $C_{25}$-aldehyde of lycopene), and/or other lycopene-related substances, such as 1,2-dihydro-1-hydroxylycopene (rhodopin) or 1,2-dihydro-1-acetylycopene (acetylrhodopin). Apo-12'-lycopenal and TPPO may be present in synthetic lycopene at concentrations of up to 0.15% and 0.01%, respectively. Synthetic lycopene may also contain residual solvents at low concentrations.

The purity of synthetic lycopene is several times greater than that of lycopene extracted from tomatoes, which contains approximately 5% or more of total lycopene. Compared with natural lycopene in raw tomatoes, synthetic lycopene contains more 5-*cis*-lycopene. This difference is not considered to be toxicologically relevant, in view of the variation in *cis*/*trans* ratios in food sources and the fact that in processed foods the content of 5-*cis*-lycopene is also increased. Moreover, *cis* isomers of lycopene (in particular 5-*cis*-lycopene) are present in human blood plasma at relatively high concentrations.

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[1] Before 1992 it was not possible to separate 5-*cis*-lycopene analytically from all-*trans*-lycopene. Since the separation of the two stereoisomers became a standard only after 1998, earlier reported all-*trans*-lycopene contents may have been overestimated.

***Table 1. Isomer composition of lycopene in different samples***

| Sample | Isomer (as a percentage of total lycopene) | | | | |
|---|---|---|---|---|---|
| | All-*trans*-lycopene | 5-*cis*-lycopene | 9-*cis*-lycopene | 13-*cis*- + 15-*cis*-lycopene | Other *cis* isomers of lycopene |
| Raw tomatoes | 94–96 | 3–5 | 0–1 | 1 | < 1 |
| Cooked tomato-based foodstuffs | 35–96 | 4–27 | < 1–14 | < 1–7 | < 1–22 |
| Human blood plasma | 32–46 | 20–31 | 1–4 | 8–19 | 11–28 |

From Schierle et al. (1997)

Synthetic lycopene is a red crystalline powder. It is freely soluble in chloroform and tetrahydrofuran, sparingly soluble in vegetable oils, ether and hexane, and is insoluble in water. Lycopene is sensitive to light, heat and oxygen, but is stable when stored under inert gas in light-proof containers in a cool place. Since it is not possible to get crystalline lycopene into an aqueous solution, and because of its susceptibility to oxidative degradation in the presence of light and oxygen, lycopene crystals are not suitable for commercial use. Only formulated material is marketed for use in food. Lycopene crystals are formulated as suspensions in edible oils or as water dispersible powders, and are stabilized with antioxidants. The other substances present in the marketed formulations (such as sucrose, corn starch, gelatin, corn oil, ascorbyl palmitate and α-tocopherol) are common food ingredients and do not raise safety concerns.

The Committee noted that most of the available toxicological studies had been performed with synthetic-lycopene formulations complying with the specifications.

### *2.1.3 Absorption, distribution, and excretion*

#### *Rats*

Absorption, distribution and excretion of lycopene were determined in male and female Wistar rats given a 10% beadlet formulation of 6,7,6',7'-[$^{14}$C]-labelled lycopene (specific activity, 97.6 µCi/mg, equivalent to 3.61 MBq/mg; radiochemical purity, 98%) as a single dose by gavage. Except for the radiolabel, the beadlet formulation was comparable to the commercial formulation, Lycopene 10% CWS, used in the pre-treatment experiment. A series of experiments was performed:

- A balance and blood level study in groups of five male and five female rats that were given a lowest dose of 0.2 mg/kg bw or highest dose of 2.0 mg/kg bw, and were sacrificed at 96 h after dosing;

- A bioretention study in 16 male and 16 female rats given a dose of 2.0 mg/kg bw, and sacrificed at 2, 4, 8, or 12 h after dosing;
- A balance study in five male and five female rats that were given a dose of 2.0 mg/kg bw and that were pre-treated for 14 days by feed admix with non-radiolabelled test article at a concentration of 25 mg of lycopene/kg feed. Sacrifice was at 96 h after the last (radioactive) dosing;
- A balance study in four male and four female bile-duct cannulated rats given a dose of 2.0 mg/kg bw, and sacrificed at 48 h after dosing.

Depending on the study, radioactivity was analysed using liquid scintillation counting (LSC) in samples of expired air, urine, faeces, blood, plasma, and/or bile collected at several time-points during the study, and in organs/tissues sampled at sacrifice. The study was certified for compliance with good laboratory practice (GLP) and quality assurance (QA).

Overall recovery of radiolabel in the studies was more than 92.5%. Blood and plasma concentrations peaked at 2 h after administration of both the lowest and the highest dose, with maximum blood concentrations being approximately 60% of maximum plasma concentrations. Average half-lives in blood and plasma were 5.2 and 3.9 h, respectively. Plasma concentrations were not linear with dose, given that the area-under-the-curve (AUC) values only differed by a factor of approximately 5 instead of 10. During the 96 h after administration, radioactivity (as a percentage of administered dose) was mainly excreted via the faeces (88–97%). Only small amounts of radioactivity were recovered from the urine (3.1– 4%), expired air (1.1%) and organs/tissues (1.6–2.8%). In the study with bile-duct cannulated rats, of which only two males completed the experimental period of 48 h, radioactivity excreted in the bile in 48 h amounted to 1.3–2% of the administered dose. This low rate of biliary excretion indicates that the large amount of radioactivity found in the faeces represents mainly non-absorbed material. On average, only 8.7% of the administered dose was absorbed, of which 44% was excreted in urine, 20% in bile, 13% in expired air, and 24% remained in organs and tissues at 96 h after administration. Owing to poor absorption, low absolute amounts of radioactivity (<1 µg lycopene equivalents/g) were measured in organs/tissues, blood and plasma at any time. The highest levels of radioactivity in organs/tissues were found in the liver (with maximum reached at 8 h after administration, and somewhat higher levels in females than in males), followed by spleen, ovaries/uterus, fat, adrenals and intestinal tract, and levels were generally linear with dose. Pre-treatment for 14 days did not affect the excretion pattern, but reduced the amount of residual tissue radioactivity: only 0.9–1% of the administered dose was recovered from organs/tissues at 96 h after administration. No obvious sex differences were observed (Wendt & Bausch, 1995).

Analysis of plasma samples demonstrated systemic absorption of lycopene in toxicological studies in rats receiving Lycopene 10% CWS for 4 weeks at a lycopene dose of 1000 mg/kg bw per day or for 14 weeks at a dose of 50, 150, or 500 mg/kg bw per day (Niederhauser et al., 1996; Buser & Urwyler, 1996; see section 2.2.2).

Systemic absorption was also demonstrated in toxicological studies in which lycopene was administered at lower doses than described above but over a longer duration. When rats were given lycopene (as Lycopene 10% WS beadlets) at a dose of 10, 50, or 250 mg/kg bw per day for 52 weeks, followed by a 13-week treatment-free period, lycopene was found in plasma and liver samples of all groups. Mean plasma concentrations of lycopene at weeks 13, 26, and 52 showed an approximate dose-related (but not dose-proportional) increase with the highest values occurring at 250 mg/kg bw per day. They tended to be higher for females than for the corresponding males at 50 and 250 mg/kg bw per day, and did not seem to increase after week 26. At week 6 of the recovery period, lycopene was only detectable (at a very low level) in plasma of 1 out of 10 animals at the highest dose. Liver samples of male rats showed a dose-related (but not dose-proportional) increase in lycopene concentration at week 52, while in liver samples of females the lycopene concentration reached a maximum at 50 mg/kg bw per day. The concentrations in liver were 3–4 times higher in females than in males (47, 176, and 200 mg/kg and 172, 709, and 657 mg/kg in males and females dosed with 10, 50, and 250 mg/kg bw per day, respectively). At the end of the recovery period, lycopene concentrations in the liver of animals at the highest dose had fallen to approximately 10% of the week 52 values (Smith et al., 2005).

When rats were given lycopene (as Lycopene 10% WS beadlets) at a dose of 2, 10, or 50 mg/kg bw per day for 104 weeks, mean lycopene plasma concentrations at weeks 52 and 104 showed a dose-related (but not dose-proportional) increase with higher values occurring at week 104 than week 52, and tended to be a little higher for males than for the corresponding females. Liver samples showed a dose-related (but not dose-proportional) increase in lycopene concentration at week 104, with concentrations in females being approximately two times higher than in males (33, 80, and 193 mg/kg and 54, 154, and 442 mg/kg in males and females given lycopene at a dose of 2, 10, and 50 mg/kg bw per day, respectively) (Edwards et al., 2006).

Zaripheh et al. (2003) examined the distribution of 6,7,6',7'-[$^{14}$C]-labelled lycopene (specific radioactivity, 1.83 µCi/mg, equivalent to 0.07 MBq/mg) in male F344 rats pre-fed a lycopene-enriched diet for 30 days and subsequently given 22 µCi (equivalent to 0.81 MBq) of [$^{14}$C]lycopene (in 0.5 ml cottonseed oil) via gavage. Lycopene was added to the diet in the form of water-dispersible beadlets containing 10% lycopene, at a target concentration of 0.25 g/kg. The dose of lycopene administered via gavage (0.246 mg) was approximately 5% of the daily dose of lycopene received via the diet, and contained 98% all-*trans*-lycopene. After gavage, each rat was placed in a metabolic cage and faeces and urine were collected every 24 h until sacrifice. At 0, 3, 6, 24, 72, and 168 h, eight rats per time-point were sacrificed, and blood, tissues and organs were collected. Radioactivity in excreta, serum and tissues was analysed using LSC. Over 168 h, 68% of the administered radioactivity was excreted via the faeces, the majority of this within the first 48 h. The amount of radioactivity retained in the body (tissues and serum) at 168 h was 3%. Total recovery at 168 h (including also urine and gastrointestinal contents) was 74%. Serum concentrations of radioactivity were highest between 3 and 24 h, and declined thereafter. In liver, peak concentrations were reached at

24 h. At all time-points, approximately 72% of tissue radioactivity was found in the liver. Hepatic radioactivity was made up of approximately 80% all-*trans*-lycopene and major *cis* isomers of lycopene (including 5-*cis*-lycopene) and approximately 20% polar metabolites. In extra-hepatic tissue, radioactivity was greatest in adipose tissue, spleen and adrenals.

In a follow-up study, 48 male F344 rats were divided into four groups pre-fed either a control or a lycopene-enriched diet for 30 days and killed at 5 or 24 h after receiving a single dose of 421.8 kBq of 6,7,6',7'-[$^{14}$C]-labelled lycopene (specific radioactivity 6771 kBq/mg) by gavage in 0.5 ml cottonseed oil. Lycopene was added to the diet in the form of 5% water-dispersible lycopene beadlets, at a target concentration of 0.25 g of lycopene/kg. The lycopene dose via gavage (0.152 mg) was approximately 2% of the daily lycopene dose via the diet. Radioactivity in excreta collected at 3, 6, and 24 h and in serum and tissues collected at sacrifice was analysed using LSC. Non-radioactive lycopene was detectable in serum and liver at both time-points. Irrespective of time, non-radioactive lycopene concentrations in serum and liver were higher in rats pre-fed with lycopene than in rats fed the control diet. Absorption of radioactivity at 24 h was less for rats pre-fed rats with lycopene (5.5%) than for control rats (6.9%). Irrespective of pre-treatment diet, elimination of radioactivity was primarily via faeces (10–12% in 24 h), with only minor elimination via urine (0.088–0.091% in 24 h). Total recovery at 24 h from all tissues, gastrointestinal contents, and excreta was 75 and 57% for rats pre-fed with lycopene and control rats, respectively. In tissues/organs, most radioactivity was found in the contents and tissues of the gastrointestinal tract, followed by liver and spleen. Other extrahepatic tissues contained only small amounts of radioactivity. In liver and spleen, radioactivity increased with time and was affected by the diet, such that rats pre-fed with the control diet had higher concentrations of radioactivity than the rats pre-fed with lycopene, particularly at 24 h. Hepatic radioactivity was made up of 71–76% all-*trans*-lycopene and major *cis* isomers of lycopene (including 5-*cis*-lycopene) and 24–29% polar metabolites, irrespective of pre-treatment diet and time (Zaripheh & Erdman, 2005).

Crystalline 6,7,6',7'-[$^{14}$C]-lycopene (specific activity, 101 µCi/mg, equivalent to 3.74 MBq/mg) was administered by gavage to three male and three female Sprague Dawley rats at a single dose of 20 µCi, equivalent to 0.74 MBq (in 1 ml olive oil containing α-tocopherol at 1 mg/ml). Blood was drawn before and at 4, 8, 24, 48, and 72 h after dosing, after which the rats were killed and organs were removed for radioactivity analysis. Maximal absorption into the blood of radioactive lycopene occurred between 4 and 8 h after administration. All organs examined contained radioactivity, but only trace or small amounts when compared with liver, which contained by far the highest amount (Mathews-Roth et al., 1990).

Special studies on the accumulation of lycopene in rat liver are described in section 2.1.5.

*Rabbits*

In order to establish absorption and tolerance for rabbits, four non-pregnant female New Zealand White rabbits were given Lycopene 10% WS beadlets (batch

No. UT00120002; 10.5% synthetic lycopene, of which 74% was all-*trans*- lycopene) at a maximum practical daily dose of lycopene of 131 mg/kg bw by gavage for 21 days. After a washout period of 20 days, this was followed by a single gavage dose of a 10% beadlet formulation of 6,7,6',7'-[$^{14}$C]-lycopene (specific radioactivity, 2.88 µCi/mg, equivalent to 0.11 MBq/mg; radiochemical purity, 98%) at a dose of 6 mg/kg bw. Blood samples were taken up to 24 h after dosing on days 1, 6, and 21 and up to 72 h after dosing the radiolabel.

One rabbit died after the first dose owing to a dosing error. The remaining three animals tolerated administration well, with no signs of ill health or effects on body weight. After the first dose, lycopene appeared in plasma after 1 h and a maximum concentration was reached after 2 h (56 µg/l). Plasma lycopene concentrations remained at this level thereafter, with the proportion of the all-*trans* isomer increasing from 19% at 1 h to 43% at 24 h. Plasma lycopene concentrations after 6 and 21 doses showed mean plasma lycopene concentrations of between 96 and 163 µg/l at all time-points (27–29% all-*trans*-lycopene) with a slight rise occurring at the 2 h time-point. Steady state was reached by day 6. After the radiolabelled dose, lycopene appeared in plasma after 1–2 h and a maximum concentration was reached after 10 h (18 µg/l), with the proportion of the all-*trans* isomer increasing from 36% after 2 h to 93% after 48 h. Radioactivity, however, appeared in plasma after 0.5 h, with maximum concentrations (approximately 50 µg equivalents/l) reached between 2 and 6 h. Lycopene as a percentage of radio-activity increased from 0% at 0.5 h to 36% at 10 h, and then declined to 33 and 5% at 24 and 72 h, respectively (Edwards et al., 2002).

In a further study, three female New Zealand White rabbits received lycopene at a higher dose of 400 mg/kg bw per day, achieved by dosing twice rather than once daily. Lycopene was administered as Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene, with 74% being all-trans-lycopene) for 21 consecutive days. One animal died after the first dose owing to a dosing error. The remaining two animals showed good tolerance for treatment with lycopene, but displayed dark red discoloured faeces, stomach and intestinal contents. Lycopene was observed in the plasma 2 to 4 h after the first dose, with a maximum of 69 µg/l reached at 8 h. On days 6 and 21 of dosing, maximum plasma lycopene concentrations were 171 (at 4 and 8 h) and 339 µg/l (at 4 h), respectively. Samples of liver taken at termination showed lycopene concentrations of 1 and 2.3 mg/kg (Edwards et al., 2004a).

*Dogs*

The pharmacokinetics and tissue distribution of lycopene was studied in male beagle dogs. The dogs were fed a lycopene-free dog food with a high fat content to aid lycopene absorption. The test material (a 5% lycopene-containing granular reddish powder) was administered in gelatin capsules 2 h after initiation of feeding, as single doses containing lycopene at 10, 30, or 50 mg/kg bw to two dogs per dose or at a dose of 30 mg/kg bw per day for 28 days to six dogs. In the single- and repeated-dose studies blood was collected for plasma lycopene analysis at several time-points. In the repeated-dose study, three dogs per time-point were killed at 1 and 5 days after the last dose, and tissues/organs were collected

for lycopene analysis. Peak plasma concentrations (56–129, 112–507, and 364–452 nmol/l from lowest to highest dose, respectively) were observed between 11 and 21 h in the single-dose studies, where half-lives ranged from 23 to 59 h. In the repeated-dose study, the plasma half-life of lycopene was approximately 30 h and a steady-state plasma concentration of approximately 800 nmol/l was reached approximately 4 days after the first dose. In tissues/organs, lycopene concentrations were highest in the liver, followed by adrenals, spleen, lymph nodes, and intestinal tissues. Tissue concentrations were generally higher at 5 days than at 1 day after the last dose. Although the test material contained 70% all-*trans*-lycopene, most of the lycopene identified in plasma and tissues was *cis*-lycopene, with only 23–41% all-*trans*-lycopene. Moreover, the percentage of all-*trans*-lycopene in many tissues, including liver, was significantly lower than that in plasma (Korytko et al., 2003).

*Monkeys*

Five female rhesus monkeys were given crystalline 6,7,6',7'-[$^{14}$C]-lycopene (specific activity, 101 µCi/mg, equivalent to 3.74 MBq/mg) by gavage as a single dose at 50 µCi, equivalent to 1.85 MBq (in 2.5 ml olive oil containing α-tocopherol at 1 mg/ml). Blood was drawn before and at 2, 4, 8, 24, and 48 h after dosing, after which the monkeys were killed and organs were removed for radioactivity analysis. The monkeys absorbed lycopene with considerable individual variation, both in blood, where peak concentrations were reached between 8 h and 48 h, and in organs. Liver contained the largest amount of radioactivity, but considerable amounts were also found in the spleen and organs of the digestive tract (Mathews-Roth et al., 1990).

*Humans*

In a randomized, parallel-group study design involving six male volunteers per group, the relative plasma response to lycopene intake from different sources was investigated, with restriction of intake of lycopene-rich food products during the study. After a 2-week lycopene-depletion phase, the subjects were given 20 mg of lycopene daily for 8 days as tomato juice, soup prepared from tomato paste, or lycopene tablets (lycopene 5% TG; batch No. UT990059720, containing a nominal content of 5 mg of synthetic lycopene per tablet) with dinner. For reasons of comparability, subjects received dinners with similar fat contents. Plasma concentrations of lycopene were monitored throughout the depletion and dosing phases and for 22 days after dosing, and kinetics were evaluated.

The three preparations of lycopene differed with respect to their isomer composition, although for all preparations the all-*trans* isomer was the predominant form (92%, 86%, and 73% for tomato juice, tomato soup, and tablets, respectively), and the 5-*cis* isomer was the predominant *cis* isomer (4%, 6%, and 19%, respectively). The plasma response was qualitatively the same for the three preparations, with a decline in lycopene concentrations during the depletion phase, a rise during treatment and peak concentrations reached after 8–9 days, and a decline in the after-dosing phase. Quantitatively, the plasma response of total and all-*trans*-lycopene was comparable for tablets and tomato soup, but was much lower for tomato juice. The plasma response of 5-*cis*-lycopene was relatively high for

tablets when compared with tomato soup and tomato juice, but this difference disappeared upon normalization to cholesterol concentrations. The latter was done to reduce the considerable interindividual variation observed in plasma concentrations of lycopene. The estimated half-lives were approximately 5 days and 9 days for all-*trans*- and 5-*cis*-lycopene, respectively, and did not differ between groups. The systemic availability of synthetic lycopene from a tablet formulation was comparable to that observed from processed tomatoes (soup from tomato paste) and superior to that from tomato juice (Cohn et al., 2000, 2004).

No other specific studies with synthetic lycopene in humans were available, but a number of publications were provided on the kinetics of lycopene in general. These have been summarized below.

Lycopene, like all carotenoids, is fat-soluble and follows the same digestion and intestinal absorption pathways as dietary fat (Rao & Agarwal, 1999). Absorption of dietary carotenoids begins with their release from the food matrix and dissolution in the lipid phase, followed by incorporation into lipid micelles in the small intestine, which is required for mucosal uptake, and finally, transport to the lymphatic and/or portal circulation (Erdman et al., 1993; Parker, 1996; Holloway et al., 2000). Movement of carotenoids from the mixed lipid micelle into the mucosal cells of the duodenum appears to occur via passive diffusion, and subsequent transport from the enterocytes to the blood stream involves incorporation into chylomicrons (with lycopene in the hydrophobic core), and secretion into the lymphatics. There is no evidence for significant portal absorption of carotenoids in humans (Parker, 1996). Carotenoids are transported in plasma exclusively by lipoprotein, with lycopene being transported primarily by low density lipoprotein (LDL) (Erdman et al., 1993; Parker, 1996; Holloway et al., 2000). Lycopene accumulates in tissues rich in LDL receptors, such as liver, adrenals and testes (Holloway et al., 2000). Other human tissues containing detectable concentrations of lycopene include blood plasma, adipose tissue, prostate, and lung (Schmitz et al., 1991; Redlich et al., 1996; Rao & Agarwal, 1999). Reported tissue concentrations of lycopene vary between individuals by about 100-fold (Rao & Agarwal, 1999).

Lycopene is the most predominant carotenoid in human plasma. Lycopene reaches its maximum concentration in the plasma 24–48 h after dosing (Stahl & Sies, 1992) but with repeated dosing the blood concentration continues to rise until a steady state is reached. Rao & Agarwal (1999) reported a half-life of lycopene in plasma in the order of 2–3 days, while Cohn et al. (2004) reported estimated half-lives of 5 days and 9 days for all-*trans*- and 5-*cis*-lycopene, respectively. When subjects were given a diet low in or without lycopene, their plasma lycopene concentrations were significantly lower after 1 to 2 weeks (Böhm & Bitsch, 1999; Porrini & Riso, 2000; Cohn et al., 2004); half-lives were then estimated to be between 12 and 33 days (Rock et al., 1992; Burri et al., 2001). Besides the effect of dietary fat (and dietary fibre content) on lycopene bioavailability, a number of conditions have been suggested to affect the absorption, metabolism or clearance of carotenoids, e.g. smoking and alcohol consumption (Bowen et al., 1993; Gärtner et al., 1997). Smokers have been reported to have lower plasma concentrations of most carotenoids than have non-smokers. This was also demonstrated for lycopene by Pamuk et al. (1994). However, other studies in smokers (Peng et al., 1995; Brady

et al., 1996; Driskell et al., 1996; Mayne et al., 1999) showed no effect of smoking on plasma lycopene concentrations. Chronic alcohol consumption has been reported to decrease dietary absorption of carotenoids (Leo & Lieber, 1999). However, Brady et al., (1996) found no interaction between alcohol and plasma lycopene.

Plasma lycopene concentrations reported for controls in several studies ranged from 0.2 to 1.9 µmol/l (Schierle et al., 1997; Paetau et al., 1998; Mayne et al., 1999; Porrini & Riso, 2000; Hoppe et al. 2003; Cohn et al., 2004). The most abundant geometrical isomers in human plasma are all-*trans*-lycopene and 5-*cis*-lycopene, with all the *cis* isomers contributing to more than 50% of total lycopene (see also Table 1; Schierle et al., 1997; Rao & Agarwal, 1999). The most prominent geometric isomer that occurs in plant sources is all-*trans*-lycopene. While heat treatment and processing may result in *trans*- to *cis*-isomerization, leading to increases (< 10%) in the *cis*-lycopene content of foods (Schierle et al., 1997; Boileau et al., 2002), this cannot fully explain the higher concentrations of *cis* isomers found in human blood (and tissues) when compared with those in the foods consumed. Hence, biological conversions may take place in humans after consumption (Holloway et al., 2000). Indeed, exposure to low pH in the stomach has been shown to result in a small increase in *cis* isomers of lycopene (Boileau et al., 2002). It is not known whether the proportion of *cis* isomers is increased in plasma because of their greater intrinsic bioavailability or because of a faster catabolism of the all-*trans* isomer in the body or both. It was speculated that probably owing to the shorter length of the molecule, their greater solubility in mixed micelles and lower tendency to aggregate, *cis* isomers are more readily bioavailable (Boileau et al., 1999, 2002).

The bioavailability of carotenoids is affected by food preparation in the presence of lipids, and the functional status of the intestine. In humans, depending on the presence of fat in the meal, appreciable quantities of carotenoids are absorbed and can be found in circulating plasma and later in adipose tissue (Su et al., 1998). Studies have demonstrated that the absorption of lycopene is increased when it is ingested with a high-fat diet. The addition of oil to tomato juice before heating also improves the bioavailability of lycopene (Stahl & Sies, 1992; Fielding et al., 2005). Heat treatment in the processing of raw tomatoes results in the release of lycopene from the cellular matrix, making it more bioavailable (Gärtner et al., 1997).

Studies on the bioavailability of synthetic lycopene and lycopene from natural sources have reported conflicting results. Some studies did not find a difference in bioavailability between supplements containing natural lycopene extracts and lycopene from tomato products (Paetau et al., 1998; Rao & Agarwal, 1998), while others reported that lycopene from tomato juice or lycopene in the form of a tomato oleoresin was clearly better absorbed than lycopene from fresh tomatoes (Böhm & Bitsch, 1999). Tang et al. (2005) reported that lycopene from cooked tomatoes was about three times less bioavailable than synthetic lycopene dissolved in corn oil. However, no oil was added to the lycopene extracted from tomatoes, which might have had a certain effect on the bioavailability, as was reported by Stahl & Sies (1992) and Fielding et al. (2005). Other studies reported that the bioavailability of synthetic lycopene was comparable to that of lycopene extracted from tomatoes in

the form of an oleoresin (Hoppe et al., 2003) or from tomato juice (Paetau et al., 1998). While the absorption of lycopene can be affected by other carotenoids (Wahlqvist et al., 1994; Gaziano et al., 1995), lycopene did not affect the absorption of other carotenoids like α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, and lutein (Hoppe et al., 2003).

Diwadkar-Navsariwala et al. (2003) presented a physiological pharmacokinetic model, validated by a phase I study in groups of five healthy male subjects, describing the disposition of lycopene delivered as a tomato beverage formulation (i.e. tomato paste mixed with olive oil and distilled water) as single lycopene doses of 10, 30, 60, 90, or 120 mg. While the percentage absorption decreased with increasing dose, the absolute amount of lycopene absorbed was not statistically different between the doses and amounted to a mean value of 4.7 mg. Independent of the dose, 80% of the subjects absorbed less than 6 mg of lycopene, suggesting that lycopene bioavailability is saturated at doses of above 10 mg per person. The model comprised seven compartments: gastrointestinal tract, enterocytes, chylomicrons, plasma lipoproteins, fast-turnover liver, slow-turnover tissues, and a delay compartment before the enterocytes, with the slow-turnover tissues serving as a slow-depleting reservoir for lycopene.

### *2.1.4 Metabolism*

A post-mitochondrial preparation of rat intestinal mucosa with or without added soya bean lipoxygenase was used to investigate the metabolism of deuterated all-*trans*-lycopene (12,12',14,14',19,19,19,19',19',19'-$^{2}H_{10}$-lycopene or $^{2}H_{10}$-lycopene). Metabolites were identified by atmospheric pressure chemical ionization mass spectrometry (MS). The addition of lipoxygenase significantly increased the production of lycopene metabolites. All metabolites were formed after 15 min of incubation, and the amount was not significantly changed after 60–90 min of incubation. Both central and excentric cleavage of lycopene occurred, and cleavage products as well as oxidation products of the original all-*trans*-lycopene were observed, including *cis* isomers (Ferreira et al., 2004).

#### *Rats*

Three male bile-duct cannulated Wistar rats received 6,7,6',7'-[$^{14}$C]-lycopene (specific activity, 115 µCi/mg, equivalent to 4.26 MBq/mg; radiochemical purity, 98%) as a single dose at 2 mg/kg bw administered by gavage. The [$^{14}$C]lycopene was formulated as simulated beadlets with fish gelatin, similar to commercial lycopene formulations (radiochemical purity in formulation, 75%). Urine and bile were collected at 0–6, 6–12, and 12–24 h after dosing. The rats were sacrificed 24 h after dosing and radioactivity in blood plasma, urine, bile and liver was determined by LSC. The metabolite profile was determined in urine and bile extracts by reversed-phase radio high-performance liquid chromatography (HPLC). Twenty-four h after dosing, on average 1.4% of the administered dose was excreted via urine, and 0.84% and 0.78% was found in bile and liver, respectively. The chromatograms of urine and bile were comparable, and both showed three broad peak regions, all showing more polarity than lycopene. The isolation and

characterization of individual metabolites was not achieved. Lycopene itself was not detected in urine and bile samples (Mair et al., 2005).

*Humans*

The metabolic pathway of lycopene has not been fully described. A number of oxidative metabolites of lycopene have been identified in tomato paste, tomato juice and in human serum (Khachik et al., 1998a). The major metabolites were identified as lycopene 1,2-epoxide and lycopene 5,6-epoxide. Other minor metabolites were also identified, including 2,6-cyclolycopene-1,5-diol I and 2,6-cyclolycopene-1,5-diol II. A few of the metabolites are naturally found in tomato products at low concentrations. In human serum and human milk only 2,6-cyclolycopene-1,5-diol I and 2,6-cyclolycopene-1,5-diol II were found (Khachik et al., 1997a, 1998a). These two epimeric isomers are dihydroxylycopenes with a five-member ring end-group. Apparently, lycopene undergoes oxidation to yield lycopene 5,6-oxide followed by cyclization and enzymatic reduction to form the two epimeric isomers of 2,6-cyclolycopene-1,5-diol (Khachik et al., 1997b, 1998b). None of the products formed by cleavage in the respective 11 conjugated double bonds of lycopene (as described by Kim et al., 2001) were detected in the blood of humans.

### *2.1.5 Special studies on the accumulation of lycopene in rat liver*

Since the liver was shown to be the main target organ after lycopene administration, a series of studies was performed to investigate possible differences between lycopene administered as beadlets or as tomato concentrate, and reversibility of changes observed. Most studies were conducted with female rats, since they showed higher accumulation of lycopene in liver than did male rats.

In a pair-feeding experiment, groups of eight male Wistar rats were given daily 20 g of feed enriched with lycopene, either using beadlets or tomato concentrate, at a target concentration of lycopene of 25 mg/kg diet for 5 weeks or for 5 weeks followed by control diet for 3 weeks. This dose was equivalent to approximately 2 mg/kg bw per day. The beadlet formulation (Lycopene 10% CWS; batch No. 11/12 7/95-1) contained lycopene as 75% all-*trans* and 23% 5-*cis* configuration at 104 mg/g, the tomato concentrate contained lycopene as a 91% all-*trans* and 5% 5-*cis* configuration at 435 µg/g. Control groups of four male rats received control diet for 5 or 8 weeks. Accumulation of lycopene was monitored in rat tissues and plasma, and liver was examined histopathologically.

After 5 weeks, the plasma concentration of lycopene was lower in rats fed beadlets (2.2 ng/ml, 36% all-*trans*-lycopene) than in rats fed tomato concentrate (3.8 ng/ml, 57% all-*trans*-lycopene). In contrast, lycopene concentrations in tissues were higher after feeding with beadlets than with tomato concentrate. In tissues, the highest concentration of lycopene was found in the liver, followed by spleen and small intestine. Total mean concentrations of lycopene in liver were 2.5 times higher in beadlet-fed rats (18 µg/g, 62% all-*trans*-lycopene) than in rats fed with tomato concentrate (7.1 µg/g, 82% all-*trans*-lycopene). In the spleen, total mean concentrations of lycopene were five times higher in the group receiving beadlets

(6.3 µg/g, 78% all-*trans*-lycopene) than in the group receiving tomato concentrate (1.2 µg/g, 80% all-*trans*-lycopene). In the small intestine, total mean concentrations of lycopene were almost three times higher in the group receiving beadlets (2.8 µg/g, 61% all-*trans*-lycopene) than in the group receiving tomato concentrate (1.0 µg/g, 68% all-*trans*-lycopene). For all other tissues investigated (adrenal, heart, lung, kidney, brain, fat, eye, muscle, skin, testes, thyroid gland), the concentrations of lycopene found were less than 1 µg/g or not detectable. Compared with the feed given, supplementation resulted in lower relative amounts of the all-*trans* isomer in all tissues with detectable lycopene, except for spleen where feeding with beadlets resulted in a somewhat higher proportion of the all-*trans* isomer. Depletion for 3 weeks led to significantly lower concentrations of lycopene in tissues in both groups receiving beadlets or tomato concentrate, with a significant increase in the relative amount of the all-*trans* isomer in liver, spleen, and small intestine. Histological examination of the liver did not reveal carotenoid inclusions in the treated or control animals (Glatzle et al., 1997).

The time-course of lycopene levels in liver, spleen, and plasma was investigated in a pair-feeding experiment in which female Wistar rats were given 20 g/day of feed enriched with lycopene beadlets (lycopin 10% CWS; batch No. 511821, containing 113 mg/g lycopene of a 72% all-*trans* and 22% 5-*cis* configuration) at a target concentration of lycopene of 110–120 mg/kg diet for up to 64 weeks (equivalent to approximately 10 mg/kg bw per day). The concentration of lycopene was monitored in liver, spleen, and plasma of rats sacrificed after 2, 4, 6, 8, 10, 12, 14, 18, 22, 26, and 64 weeks of treatment (3–10 rats per time-point). Control animals (three to –six per time-point) were sacrificed at the start of the study, and after 20, 26, and 64 weeks. Histopathological and morphological examination of liver and spleen was also performed.

Lycopene was found in the liver and spleen of all animals receiving supplementation and in the plasma in most animals. Liver showed the highest concentration of lycopene, mean concentrations increasing from 80 µg/g after 2 weeks of treatment to 248 µg/g after 64 weeks; no steady state was reached. The spleen showed mean concentrations of lycopene of 33 µg/g after 2 weeks, 87 µg/g after 14 weeks, 104 µg/g after 22 weeks, and 78 µg/g after 64 weeks; a steady state was found after approximately 14 weeks of treatment. There was a large variability in plasma concentrations, with mean concentrations of lycopene of 29–54 ng/ml in weeks 2 to 10 and 57–120 ng/ml in weeks 12 to 64. Compared with the feed given, 5-*cis*-lycopene was significantly increased in tissues and plasma. The proportion of all-*trans*-lycopene was 52–62% in the liver, 61–71% in spleen, and 40–47% in plasma. At necropsy, brown-orange staining of the abdominal adipose tissue and red-orange to dark red discoloration of the liver were increasingly noted after 26 and 6 weeks of treatment, respectively. Upon histopathology, deposits were found in the liver and, to a lesser extent, spleen of all treated animals. To further investigate these deposits, morphological examinations were performed by semi-quantitative light microscopy and electron microscopy. Light microscopy revealed a time-related increase in pigment deposition in hepatocytes between week 2 (average grading 1.2) and week 18 (average grading 3.8), but no apparent further increase thereafter (average grading 3.8–4 between weeks 18 and 64). Pigments appeared as

brown-orange gritty deposits in the cytoplasm in normal light and as birefringent material in polarized light. There was a significant correlation between total liver lycopene concentration and birefringent deposits. Despite significant accumulation of pigment in liver cells, there was no evidence of lycopene-related liver damage after 64 weeks of continuous exposure. The deposits in the spleen were also birefringent pigments (average grading, 1–1.3). Upon ultrastructural localization of lycopene, the intracellular inclusions, varying in size from submicron sizes up to several micrometres, were found to be present in a compartment with a clearly discernable membrane bilayer, indicating a pathway that involves the endocytotic system of the hepatocytes (Glatzle et al., 1998a; Urwyler & Bohrmann, 1998).

In another pair-feeding experiment, female Wistar rats were given 20 g/day of feed enriched with lycopene beadlets (lycopin 10% CWS; batch No. 511821, containing 11% lycopene of a 72% all-*trans* and 22% 5-*cis* configuration) at target concentrations of lycopene of 50 or 100 mg/kg diet for 4 weeks (equivalent to approximately 5 or 10 mg/kg bw per day), followed by a depletion period of up to 21 weeks. Accumulation and depletion of lycopene was monitored in liver, spleen, and plasma of six rats of each group per time-point after 2 weeks and 4 weeks of supplementation and after 1, 2, 3, 5, 7, 10, 13, 17, and 21 weeks of depletion. Control animals received control diet and were examined after 4, 6, 11, 17, and 25 weeks (three rats per time-point). The liver was also examined histopathologically.

After 4 weeks of treatment, rats in both treated groups had accumulated lycopene in liver (72 µg/g, 61% all-*trans*-lycopene and 105 µg/g, 53% all-*trans*-lycopene at 5 and 10 mg/kg bw per day, respectively) and spleen (38 µg/g, 72% all-*trans*-lycopene and 35 µg/g, 64% all-*trans*-lycopene at 5 and 10 mg/kg bw per day, respectively). Plasma concentrations were approximately 30 ng/ml at both doses, with approximately 50% and 41% all-*trans*-lycopene at 5 and 10 mg/kg bw per day, respectively. After 1 week of depletion, lycopene was no longer detectable in the plasma. During the depletion period, a steady decrease of total lycopene in liver and spleen was observed, while the proportion of the all-*trans* isomer steadily increased in both tissues. Histological examination showed intracellular brown-orange gritty pigments in livers of treated animals (average grading 1), up to 7 weeks (5 mg/kg bw per day) or 10 weeks (10 mg/kg bw per day) of depletion. Thereafter, deposits were no longer observed. In polarized light, the pigments occurred as birefringent deposits. There was no indication of treatment-related morphological changes in the liver (Glatzle et al., 1998b).

The occurrence of liver deposits after administration of tomato concentrate or beadlets was investigated in a pair-feeding experiment in which female Wistar rats were fed lycopene at doses of approximately 20 mg/kg bw per day as tomato concentrate mixed with feed for 4, 8, or 13 weeks, followed by a depletion period of 4, 8, or 12 weeks for those rats that were treated for 13 weeks. Other rats were fed lycopene at a dose of approximately 4 mg/kg bw per day (intended dose 5 mg/kg bw per day) as beadlets (lycopin 10% CWS; batch No. 511821, containing lycopene at 113 mg/g of a 72% all-*trans* and 22% 5-*cis* configuration) mixed with feed for 4 weeks or 8 weeks. Accumulation and depletion of lycopene were monitored in rat liver (six rats per time-point), and liver was examined histopathologically.

Liver concentrations of lycopene were comparable in the groups fed with tomato concentrate (39, 45, and 43 µg/g after 4, 8, and 13 weeks) and in the groups fed with beadlets (42 and 49 µg/g after 4 and 8 weeks), just like the presence of birefringent deposits (in all but one animals of the groups receiving tomato concentrate and in all animals of the groups receiving beadlets, with average gradings of 1.3 and 1.6, respectively). However, the proportion of all-*trans*-lycopene in the groups receiving tomato concentrate was higher (68–72%, with 25% 5-*cis*-lycopene) than in the groups receiving beadlets (55–59%, with 38% 5-*cis*-lycopene). During the depletion period for the group receiving tomato concentrate, the concentration of lycopene in the liver steadily decreased, with an increase in the proportion of all-*trans*-lycopene (up to 82%). The birefringent deposits, however, did not reduce appreciably (Glatzle et al., 1998c).

Ten female rats from the 64-week experiment by Glatzle et al. (1998a) were transferred into a new study protocol to investigate the long-term effects of continuous feeding with lycopene at 10 mg/kg bw per day over a total of 2 years (Urwyler & Riss, 1999; see also section 2.2.3). Compared with the liver lycopene content of 248 µg/g (55% all-*trans*-lycopene) observed after 64 weeks, a decrease in liver lycopene content was observed from week 64 to week 104 of treatment (78–215 µg/g), while no change in the proportion of all-*trans*-lycopene was noted (50–57%). The authors attributed this decrease to the relationship between age, general health status and feed consumption. Comparing this decrease with the observations on liver lycopene content in the long-term studies of toxicity by Smith et al. (2005) and Edwards et al. (2006) (see section 2.1.3), a similar reduction between 1 year and 2 years of treatment was found in female rats; however, only at a dose of 50 mg/kg bw per day (from 709 µg/g after 1 year to 442 µg/g after 2 years), not at 10 mg/kg bw per day (from 172 to 154 µg/g). In male rats no reduction was observed, neither at 10 mg/kg bw per day (from 47 to 80 µg/g) nor at 50 mg/kg bw per day (from 176 to 193 µg/g). Pigment deposition was observed in the hepatocytes of all rats, with an average grading of 5 in five prematurely sacrificed rats and 3 in the remaining five rats. Pigments appeared as brown-orange gritty deposits in the cytoplasm with normal light, and as birefringent crystals with polarized light. Despite the high pigment load and the long duration of exposure, there was no indication of associated microscopic changes in the liver.

### *2.1.6 Effects on enzymes and other biochemical parameters*

Three publications were provided in which the effect of lycopene (mostly from natural sources) on the activities of drug-metabolizing and/or antioxidant enzymes was investigated (Gradelet et al., 1996, Jewell & O'Brien, 1999; Breinholt et al., 2000). These studies have been summarized in section 2.1.5 of the monograph 'Lycopene from *Blakeslea trispora*', in this volume. The studies showed that lycopene has only minor modifying effects, if any, on phase I and phase II xenobiotic-metabolizing enzymes and on antioxidant enzymes.

## 2.2 Toxicological studies

### 2.2.1 Acute toxicity

One study of acute toxicity has been performed with synthetic lycopene. In this study, three male and three female Wistar rats received Lycopene 10 CWD (batch No. OD-12-04 6.Spr.A; 9.81% synthetic lycopene) as a single oral dose at 5000 mg/kg bw by gavage. This study followed OECD test guideline 423 (acute toxic class method; 1996), and was certified for compliance with GLP and QA. No animals died and the median lethal dose ($LD_{50}$) was > 5000 mg/kg bw (BASF, 2001a).

Studies of acute toxicity have also been provided for lycopene extracted from tomatoes, administered via oral, subcutaneous or intraperitoneal route to mice (Milani et al., 1970). These studies have been summarized in Table 2 of the monograph 'Lycopene from *Blakeslea trispora*', in this volume. Lycopene extracted from tomatoes was not acutely toxic ($LD_{50}$ > 3000 mg/kg bw).

### 2.2.2 Short-term studies of toxicity

#### Rats

Groups of six male and six female Wistar rats were given diets mixed with Lycopene 10% CWS (batch No. 20/93-2; 9.9% synthetic lycopene, < 0.001% apo-12'-lycopenal) at concentrations intended to provide lycopene at 1000 mg/kg bw per day for 4 weeks. A control group of six males and six females received a diet mixed with the beadlet formulation without lycopene (batch No. 19/93-3; placebo group) and another control group of six males and six females received the pure diet for rodents (standard control group). To compare the toxicity of this Lycopene 10% CWS formulation containing < 0.001% of the impurity apo-12'-lycopenal (i.e. < 0.01% relative to lycopene) with that of a Lycopene 10% CWS beadlet formulation containing 0.2% of this impurity (i.e. 2% relative to lycopene), an additional group of six males and six females received a diet providing an intended dose of 1000 mg of lycopene + 20 mg of apo-12'-lycopenal/kg bw per day for 4 weeks. The latter formulation was obtained by mixing a Lycopene 10% CWS formulation (batch No. 7/95-1; 11.5% synthetic lycopene, 0.025% apo-12'- lycopenal) with a lycopene-$C_{25}$-aldehyde 2% CWS formulation (batch No. RS2/95-69; 0.305% synthetic lycopene, 2.16% apo-12'-lycopenal). Owing to a mistake in feed preparation, this group received only 0.3% instead of the intended 2% apo-12'-lycopenal, so two additional groups of of six males and six females were introduced, receiving either a diet providing 1000 mg of lycopene + 20 mg of apo-12'-lycopenal/kg bw per day or a placebo diet. Observations included clinical signs, body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry, urine analysis, organ weights, macro- and microscopy, and plasma concentrations of lycopene, apo-12'-lycopenal, vitamin A and E. The study was certified to comply with GLP and QA.

The only treatment-related changes observed were a red discoloration of the faeces and a brown-orange discoloration of the liver in most animals receiving either

lycopene only or lycopene supplemented with apo-12'-lycopenal. Histologically, the discoloration of the liver correlated with deposits of brown-yellow fine granulated pigment in hepatocytes with no other associated histopathological alterations. The quantity of pigment was significantly higher in females than in males; the average grading being moderate in females and slight in males. No consistent and toxicologically relevant changes were observed in haematology, clinical chemistry, and urine analysis parameters. Except for a statistically significantly increased relative liver (15%) and kidney (12%) weight in males of the group receiving 1000 mg of lycopene + 20 mg of apo-12'- lycopenal/kg bw, organ weights were not affected. In the absence of any histopathological changes, these weight changes were not considered to be toxicologically relevant. The analysis of plasma samples collected on the day of necropsy from non-fasted rats showed group mean concentrations of lycopene of 120–250 µg/l in males and 140–190 µg/l in females in the groups treated with lycopene, with concentrations being significantly higher with increasing supplementation with apo-12'-lycopenal. This effect was more pronounced in males than in females. Apo-12'-lycopenal was only detectable in plasma of rats receiving 1000 mg of lycopene + 20 mg of apo-12'-lycopenal/kg bw, with group mean concentrations of 10 and 8 µg/l in males and females, respectively (or approximately 4% of the lycopene content). Vitamin A concentrations in plasma were not consistently affected, while vitamin E concentrations were consistently higher in all groups receiving feed together with formulation (approximately 2.3-fold in males and 3-fold in females). This effect was most probably due to the high concentration of vitamin E in the basic formulation. It was concluded that the lycopene beadlet formulation was well tolerated and that in the absence of histopathological liver damage, the treatment-related pigment deposition was not considered to be toxicologically relevant. The no-observed-effect level (NOEL) was 1000 mg of lycopene/kg bw per day, the only dose tested. The presence of apo-12'-lycopenal impurity at a concentration of 2% in the formulation (relative to lycopene) did not result in additional findings (Niederhauser et al., 1996; McClain & Bausch, 2003) .

In a 14-week study of toxicity, Wistar rats were treated with Lycopene 10% CWS (batch No. 20/93-2; 9.9% synthetic lycopene) as a dietary admixture at concentrations intended to provide lycopene at 50, 150, and 500 mg/kg bw per day. A control group received a diet mixed with the beadlet formulation without lycopene (batch No. 19/93-3; placebo group) and another control group received the pure diet for rodents (standard control group). There were 26 males and 26 females per group, with 6 males and 6 females assigned to recovery groups for a 5-week treatment-free period. The study was certified for compliance with GLP and QA. Observations included clinical symptoms and mortality (daily), body-weight development and food consumption (weekly), ophthalmoscopy (before dosing on all animals, and in week 9 on animals in the control groups and the group receiving the highest dose), haematology and clinical chemistry (in blood taken from 10 animals per group before dosing and in weeks 7 and 14), and urine analysis (in urine sampled from 10 animals per group in weeks 6 and 13). In addition, blood was taken from all animals in the recovery groups just before the start and at the end of the recovery period for clinical chemistry determinations, and from six males and six females of each group in weeks 2, 8, 12, and 17 for plasma concentration

determinations. At necropsy, a macroscopic examination was performed on all animals, and absolute and relative weights of adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thyroid were determined. Microscopy was carried out on a number of organs and tissues from 20 males and 20 females in the control groups and in the group receiving the highest dose, on the liver from 20 males and 20 females in the groups receiving the lowest and intermediate dose, and on all gross lesions.

There were no treatment-related deaths or adverse effects on general health and behaviour, eyes, or urine analysis parameters. The only sign associated with the intake of lycopene was a reddish discoloration of the faeces of all animals from the group receiving the highest dose and to a lesser extent from the groups at the intermediate and lowest dose. Food consumption was comparable in all groups. Body-weight gain was slightly increased (7–10%) in females treated with lycopene when compared with standard controls, but not when compared with females treated with placebo. In the recovery period, females at the intermediate and highest dose also had an increased body-weight gain, compared with the standard controls (6–18% difference) and the females treated with placebo (12–25% difference). Statistically significant increases in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were observed in male, but not female rats in the groups at 50, 150, and 500 mg/kg bw per day after 93 days of treatment (ASAT, twofold at the lowest dose and threefold at the intermediate and highest dose; ALAT, no change at the lowest dose and twofold at the intermediate and highest dose). A trend for elevated ASAT and ALAT activities was still seen in male rats after 5 weeks of recovery, although this was not statistically significant. Also, statistically significant changes were observed in some other biochemical parameters as well as in some haematological parameters after 93 days of treatment. However, as these changes were either small, not dose-related and/or found in one sex only, they were considered not to be related to treatment. In all groups the plasma concentration of lycopene was continuously high, with higher mean concentrations in females (133–344 µg/l) than in males (102–197 µg/l). In male rats, plasma concentrations were comparable between the three dose groups, while in female rats plasma concentrations increased non-proportionally with dose. After 2 weeks of recovery, complete elimination of lycopene in plasma was shown in all animals. Statistically significant changes in organ weights were only observed in animals receiving the highest dose, where females showed a decrease of 15% in relative thyroid weight, and males a 2% increase in relative brain weight. These changes were not associated with morphological changes and did not persist beyond the recovery period. Necropsy revealed an orange-red discoloration of the liver (in males at 500 mg/kg bw per day and in females at 50, 150, and 500 mg/kg bw per day) and the adipose tissue (in males and females at 150 and 500 mg/kg bw per day). There were no microscopic findings that were considered to be related to treatment, except for the deposition of orange-brown, birefringent pigment in the hepatocytes of animals of all lycopene-treated groups, with females more severely affected than males (average grading, severe and slight to moderate, respectively). There was, however, no histopathological evidence of any hepatocellular damage. The pigment deposits were still present after 5 weeks of recovery. In the absence of histopathological liver damage, the relatively small increase in ASAT and ALAT

activities and the pigment deposition were not considered to be toxicologically relevant. The NOEL was 500 mg of lycopene/kg bw per day, the highest dose tested (Buser & Urwyler, 1996; McClain & Bausch, 2003).

In a 3-month study of toxicity, groups of 10 male and 10 female Wistar rats were dosed daily by gavage with one of the following: water, Lycopene 10 CWD (500, 1500, or 3000 mg/kg bw per day), Lycopene 10 CWD formulation matrix (3000 mg/kg bw per day), LycoVit 10% (3000 mg/kg bw per day), or LycoVit 10% formulation matrix (3000 mg/kg bw per day). These doses correspond to approximate doses of lycopene of 50, 150, and 300 mg/kg bw per day from Lycopene 10 CWD (batch No. OD-12-04 6.Spr.A, containing 9.81% synthetic lycopene) and 300 mg/kg bw per day from LycoVit 10% (batch No. 20014372, containing 10.8% synthetic lycopene). Additional satellite groups of five males and five females received water or 3000 mg/kg bw per day of either Lycopene 10 CWD or LycoVit 10% for an interim evaluation at 4 weeks. The study followed OECD guideline 408 (1998), and was certified to comply with GLP and QA. Plasma concentrations of lycopene were not determined in this study.

There were no significant differences in body weight, food consumption, organ weights, or haematological evaluations between treatment groups, and there were no treatment-related deaths or effects seen in ophthalmoscopic evaluations. Similarly, a battery of behavioural and reflex tests, motor activity assessments, clinical chemistry evaluations, and urine analysis did not reveal any biologically relevant findings. Treatment-related clinical findings were limited to a red discoloration of the faeces of animals in the Lycopene 10 CWD and LycoVit 10% treatment groups. Consistent with this finding was a red discoloration in the gastrointestinal tract (jejunum, caecum, and at highest dose stomach) of these animals, which was not associated with changes in gross pathology or histopathology. No other remarkable or substance-related abnormalities were observed in any of the other tissues examined, nor were there any significant histopathological findings. Given the absence of relevant toxicological findings with both lycopene formulations, the NOEL was approximately 300 mg of lycopene/kg bw per day, the highest dose tested (BASF, 2001b; Mellert et al., 2002).

In a study evaluating the biodistribution of a single dose of [$^{14}$C]lycopene, groups of 24 male F344 rats (mean body weight, 113 g) were pre-fed either a lycopene-enriched or a control diet for 30 days. The test diet contained synthetic lycopene (as 5% water-dispersible lycopene beadlets) at a concentration of 0.25 g/kg, and it was calculated that rats consumed approximately 8 mg of lycopene per day from the diet. Food intake and weight gain were not affected by treatment, and no effects were apparent other than a red discoloration of the liver and faeces (Zaripheh & Erdman, 2005).

A 100-day feeding study was conducted in which 10 male and 10 female Wistar rats were given synthetic lycopene (purity, 97–98.5%) at a dose of 1000 mg/kg bw per day, mixed with a small amount of food. A control group of 20 animals was also included. No adverse clinical signs were observed, and treatment did not affect weight gain or haematology parameters (haemoglobin, erythrocyte and

leukocyte counts, and differential blood counts). Liver, kidneys, adrenals, spleen, pancreas, testes, ovaries, skin, bone, and bone marrow were histologically normal. Signs of tissue storage of lycopene were not detected by microscopic examination (Zbinden & Studer, 1958).

A 90-day feeding study in rats given lycopene derived from *B. trispora* (Jonker et al., 2003) was also available. Studies of 14–16 days or 10 weeks duration in rats given lycopene oleoresin from tomato were also available, but were not designed to examine adverse health effects (Zhao et al., 1998; Gradelet et al., 1996; Jewell & O'Brien, 1999; Breinholt et al., 2000). All these studies have been summarized in section 2.2.2 of the monograph 'Lycopene from *Blakeslea trispora*', in this volume. Lycopene from natural sources was well tolerated, without relevant adverse effects. The only treatment-related finding in the full study of toxicity with lycopene from *B. trispora* was a red discoloration of the contents of the gastrointestinal tract, caused by ingestion of the red-staining test substance. The NOEL was approximately 600 mg of lycopene/kg bw per day, corresponding to the highest dose tested in that study.

*Dogs*

In a study evaluating the pharmacokinetics and tissue distribution of lycopene, six male beagle dogs were given a 5% lycopene-containing granular reddish powder in gelatin capsules providing lycopene at a dose of 30 mg/kg bw per day for 28 days. Food consumption and body weight were not affected by treatment, and, other than red material in faeces, no clinical signs were observed (Koryotko et al., 2003).

One female mongrel dog was treated orally with gelatin capsules containing synthetic lycopene (purity, 97–98.5%) at a dose of 100 mg/kg bw, once daily for 192 days. In total, the animal received 272 g of lycopene without displaying signs of intolerance. Body weight increased from 13 to 15 kg, and haematology, clinical chemistry and urine analysis parameters investigated were normal. Macroscopy did not reveal treatment-related changes. Chemical analysis showed the presence of lycopene in the liver and, to a much lesser extent, kidneys. The pigment was also detectable histologically in liver cells. Otherwise, the liver was histologically normal, as were other tissues and organs (Zbinden & Studer, 1958).

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

*Rats*

Zbinden & Studer (1958) investigated the toxicity of lycopene as part of a two-generation study of reproductive toxicity. Groups of 15 male and 15 female Wistar rats were found to develp normally when given a diet containing 0 or 0.1% synthetic lycopene (purity, 97–98.5%) for 200 days. There were no mortalities or great differences in food consumption and body-weight gain, and there were no remarkable haematological findings. Histopathological examinations in tissues and organs of two rats of each sex per group did not reveal abnormalities, with the exception of pigment deposition (in females marked and in males slight) in the liver

of animals treated with lycopene. Continuation of treatment of the remaining 13 rats of each sex per group for a further 200 days was reported not to result in any sign of toxicity or spontaneous tumours (Zbinden & Studer, 1958).

In a 52-week study of toxicity, groups of 20 male and 20 female Wistar rats were treated with Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene) mixed in the diet at target doses of lycopene of 10, 50, and 250 mg/kg bw per day. A control group received a diet mixed with placebo beadlets (batch No. UT02111005; placebo group) and another control group received the pure diet for rodents (standard control group). Five rats of each sex in the standard control group and the group at the highest dose were assigned to recovery groups for a 13-week treatment-free period. The study followed OECD guideline 452 (1981), and was certified to comply with GLP and QA. In addition to the standard observations, a functional observational battery and motor activity assessment were included, plasma and liver samples were analysed for total lycopene content (i.e. sum of all determined isomers), and liver from all animals in the study was examined microscopically.

Analysis of plasma and liver samples demonstrated systemic absorption of lycopene in all groups receiving Lycopene 10% WS beadlets (see section 2.1.3). Discoloured faeces or red staining was noted in both sexes in all groups given lycopene. There were no treatment-related deaths, ophthalmic changes, or neurological changes. Body-weight gain, food consumption, and food conversion efficiency were unaffected by treatment after 52 weeks. Haematology parameters were not consistently affected by treatment, nor were clinical chemistry parameters other than ASAT and ALAT activities. At week 13, slight increases in the group mean activities of ASAT and ALAT were recorded at the highest dose only. At weeks 26 and 52, group mean activities of these enzymes were increased in a dose-dependent manner in males (up to 1.7- and 2.6-fold, respectively) and females (up to 1.4- and 2-fold, respectively) at the lowest, intermediate and highest dose, although not always statistically significantly and generally without real progression between week 26 and week 52. At the end of the treatment-free period, ASAT and ALAT activities had declined, but still tended to be higher in the group at the highest dose than in the standard control group. At week 51 there was an increase in urinary volume and decrease in urinary specific gravity in both sexes at 250 mg/kg bw per day. These findings were not accompanied by related changes in the kidney or increased water consumption. The weights of the adrenals, kidneys, liver, brain, testes and epididymides, and ovaries were unaffected by treatment. Macroscopy revealed abnormal contents (often a thick, red substance) in the stomach and caecum, and yellow connective tissue in the abdominal cavity of animals receiving the intermediate or highest dose at terminal kill, but not at the end of the treatment-free period. These findings did not correlate with any microscopic observation and were probably associated with the colour of the test article. The macroscopic finding of mottled liver in both control and treated animals, especially males, correlated with agonal congestion/haemorrhage seen microscopically. Treatment-related findings upon microscopy were confined to the liver, particularly of females.

In females at terminal kill, there were increases in the incidences and severity of hepatocyte pigment (in treated and placebo-fed groups, without a

obvious dose–response relationship) and pigmented histiocytes (in a dose-related manner in all groups treated with lycopene) compared with those in of standard controls. In females at the intermediate and highest dose there was also a greater incidence and severity of basophilic foci. After the treatment-free period, these findings were still observed, although the incidences of hepatocyte pigment and basophilic foci were slightly lower and similar, respectively, in females at the highest dose than in standard controls. In males, hepatocyte pigment was rarely seen, and the incidences of pigmented histiocytes and basophilic foci were only marginally increased in groups treated with lycopene. The pigment in hepatocytes and histiocytes was seen as fine golden brown granular deposits, which stained variably positive for both haemosiderin and lipofuscins. It was concluded that treatment with lycopene for 52 weeks resulted in liver pigmentation with associated histopathological alterations of basophilic foci (in females at the intermediate and highest dose), while in the short-term studies of toxicity the latter were not observed.

The significance of these treatment-related alterations for humans is unclear, given that there was no apparent sign of liver dysfunction and that, in contrast to humans, basophilic foci are commonly found at high incidences in the ageing rat. The latter is, for example, reported for F344 rats by Eustis et al. (1990), and is also observed in the present study in female control animals (standard and placebo). Basophilic foci were also observed in control animals (especially females) of other (26-week and 104-week) studies by the same laboratory using the same strain of rats (see also 104-week study below). However, the Committee also noted that the alterations were observed in a single, non-standardized liver section investigated, which would not necessarily be representative of other sections of the liver (Smith et al., 2005).

In a study of carcinogenicity, groups of 50 male and 50 female Wistar rats were treated with Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene) mixed in the diet at target doses of lycopene of 2, 10, and 50 mg/kg bw per day for 104 weeks. Two control groups of 50 males and 50 females received either a diet mixed with placebo beadlets (batch No. UT02111005; placebo group) or the pure diet (standard control group). The study followed OECD guideline 451 (1981), and was certified for compliance with GLP and QA. In addition to the standard observations, plasma and liver samples were analysed for total lycopene content (i.e. sum of all determined isomers), and liver, mesenteric and mandibular lymph nodes from all animals in the study were examined microscopically, as well as kidney from all female rats.

Analysis of plasma and liver samples demonstrated systemic absorption of lycopene in all groups receiving Lycopene 10% WS beadlets (see section 2.1.3). Survival was acceptable, being 86, 80, 74, 66, and 82% and 78, 80, 78, 84, and 74% for males and females, respectively, from the standard control group to the group at the highest dose, respectively. There was no influence of treatment on incidences and causes of morbidity and mortality, or on incidences of clinical signs commonly seen in laboratory rats. Red discoloured faeces were noted in both sexes at 50 and 10 mg/kg bw per day, from the onset and week 67 of treatment, respectively. Food consumption, overall body-weight gain and food conversion efficiency of males and females given lycopene in the diet were similar to that of the

standard control and placebo groups. Erythrocyte and leukocyte counts were not consistently affected by treatment.

Macroscopically, several findings in the gastrointestinal tract (e.g. abnormal or dark contents in the stomach and caecum, red discoloration of jejunum) and yellow connective tissue, especially in males and females at the highest dose, were probably associated with the colour of the test article. Likewise, treatment with lycopene resulted in dark/red discoloration in the kidney (especially in females at the highest dose) and lymph nodes, and variable discolorations in the liver, corresponding with non-neoplastic findings observed microscopically. In the kidney of females at the highest dose, the incidence and severity of tubular pigment were increased compared with values for controls and other treatment groups. In males, renal tubular pigmentation was significantly less than in females and there was no difference between control and treated groups. In the mesenteric lymph node, all animals, including controls, displayed pigment or pigmented histiocytes, but the severity of pigmentation was increased in the placebo group (females only) and in lycopene-treated animals. In the mandibular lymph node, the incidence of pigment/pigmented histiocytes was increased in the placebo and treated groups, with the severity also being increased in females. The increases in lymph node pigmentation were not consistently dose-dependent. Pigmentation, seen as fine granular or globular golden-brown deposits, was also observed in the liver, especially in females. In females treated with placebo or lycopene, there were increases in the incidences and severity of hepatocyte pigment (without an obvious dose–response relationship) and pigmented histiocytes (severity only increased in the group at the highest dose) above that in standard controls. In males, hepatocyte pigment was rarely seen, but the incidence of pigmented histiocytes was also increased in the groups receiving placebo or lycopene above that in standard controls. Other non-neoplastic findings in the liver included a greater incidence and severity of multinucleate cells (dose- related, in all treated males, albeit marginally at the lowest dose), and of histologically altered cell foci. Altered cell foci of the eosinophilic type were of greater severity but their incidence was without an obvious dose–response relationship in groups of males given placebo or lycopene. The incidence and severity of basophilic, normochromic and clear cell foci was greater in females at the intermediate and/or highest dose (albeit that for basophilic foci the incidences were only slightly above the high background incidence of spontaneous). In placebo controls and in treated animals at all doses, the incidence of vacuolated foci was greater than that in controls receiving pure diet only, but was without an obvious dose–response relationship. For eosinophilic and basophilic foci it was stated by the authors that there was no evidence of an increase in cellular pleomorphism, distorted architecture or increased mitotic activity, but no data were provided to support this statement.

No liver carcinomas were observed in this study, and there was no treatment-related increase in liver adenomas. In fact, the types and incidences of tumours observed were generally similar in control and treated groups. The highest incidences were found for pituitary adenomas, mesenteric lymph node haemangiomas, uterus adenocarcinomas and stromal polyps, and mammary gland fibroadenomas (of which the incidence seemed to be slightly increased in females treated with

lycopene). It was concluded that there were no indications to suggest an association betwwen carcinogenicity and administration of lycopene.

As to the non-neoplastic findings, it was concluded that treatment with lycopene for 104 weeks, as with treatment for 52 weeks, resulted in liver pigmentation with associated histopathological alterations of hepatocellular foci, in particular eosinophilic, normochromic and basophilic foci, and mainly at the intermediate and highest dose. Again, the significance of these treatment-related alterations for humans is unclear, given that there was no apparent sign of liver dysfunction, they were without a consistent dose–response relationship, and placebo controls were in some cases also affected. Moreover, in contrast to humans, hepatocellular foci are commonly found in the ageing rat at high incidences, and while experimental models suggest that some foci may be precursors of hepatocellular neoplasia, it is also known that only a very small proportion of foci progress to neoplasia even after continued administration. Indeed, treatment with lycopene did not result in an increase in liver tumours. However, the Committee also noted that the alterations were observed in a single, non-standardized liver section investigated, which would not necessarily be representative for other sections of the liver (Edwards et al., 2006).

Ten female Wistar rats from a 64-week exploratory study investigating the time-course of lycopene concentrations in liver, spleen, and plasma under steady-state conditions (see study by Glatze et al., 1998a, section 2.1.5) were transferred to a study investigating the long-term effects of continuous feeding with lycopene over a total of 2 years. The rats were pair-fed a diet enriched with lycopene (as lycopin 10% CWS beadlets (batch No. 511821; 113 mg of synthetic lycopene/g) at a concentration of 113 mg/kg. The amount of feed served was adapted weekly to the body weights measured, so as to provide lycopene at a dose of approximately 10 mg/kg bw per day throughout the study. Animals were observed daily for clinical signs and weekly for body-weight development. At 18 and 21 months, blood was collected from non-fasted rats and analysed for ASAT, ALAT, alkaline phosphatase, triglycerides, phospholipids, and total cholesterol. At necropsy, the liver and all tissues with macroscopic changes were removed and examined microscopically.

Except for the pathology data, the results for the observations performed were described with little detail or not at all, as was the case for body-weight development. It was stated that no clinical signs were recorded throughout the study, and that five rats were sacrificed prematurely owing to deterioration of their general condition. In absence of a concurrent control group, clinical chemistry values for the non-fasted rats were compared with historical control values for fasted female Wistar rats. Clinical chemistry parameters were elevated, which was most likely to be caused by the non-fasting state of the animals. Upon macroscopy, staining of the mammary gland and abdominal fat tissue (yellow) and liver (orange) was noted in some animals, as well as enlarged pituitary glands (in four out of five prematurely sacrificed rats and in two out of five rats surviving to the end). Histopathology showed brown-orange gritty deposits in hepatocytes of all rats, with the amount of pigment deposited higher in prematurely sacrificed animals than in animals at terminal kill. No microscopic changes associated with the liver pigmentation were observed, nor with the discoloration of other tissues. Pituitary

adenomas were found in all animals with enlarged pituitary glands, and were the cause of the moribund condition of prematurely sacrificed animals (Urwyler & Riss, 1999).

### *2.2.4 Genotoxicity*

The results of studies of genotoxicity with synthetic lycopene are summarized in Table 2. The studies followed OECD test guidelines 471 (1983 + update/1997), 473 (1983 + update/1997), 474 (1987 + update/97), 476 (1984 + update), or (proposal for) 486 (1997), and were certified to comply with GLP and QA.

The results of some studies of genotoxicity in vitro and in vivo with lycopene from natural sources have also been provided (He & Campbell, 1990; Pool-Zobel et al., 1997; Riso et al., 1999; Aizawa et al., 2000; Guttenplan et al., 2001; McClain & Bausch, 2003). The studies in vitro concerned tests for gene mutation in bacteria (*Salmonella typhimurium*, *Escherichia coli*), the studies in vivo included a test for micronucleus formation in mouse peripheral blood, tests for DNA damage in human lymphocytes and a test for spontaneous mutation in LacZ mouse DNA. These studies have been summarized in Table 3 of the monograph 'Lycopene from *Blakeslea trispora*', in this volume. Lycopene from natural sources gave negative results in all studies.

### *2.2.5 Reproductive toxicity*

#### *(a) Multigeneration study*

##### *Rats*

In a two-generation study of reproductive toxicity, groups of 15 male and 15 female Wistar rats received a diet containing 0 or 0.1% synthetic lycopene (purity, 97–98.5%) for 14–16 weeks before mating and subsequently throughout pregnancy and beyond, for a total of 238 treatment days. After weaning, pups of the first generation were fed either the same diet containing lycopene (13 male and 18 female rats) or the control diet (38 animals) for 50–80 days. One of the dams treated with lycopene aborted; the number of offspring was slightly but not significantly lower than in the control group, in which two animals aborted. The duration of gestation was normal, and no malformations were seen. Pup weight at birth was slightly but not significantly higher in the treated group than in the control group. Continuation of treatment of the first generation for a further 200 days, while producing a second generation, was reported to have resulted in a normal reproduction (Zbinden & Studer, 1958).

Sprague-Dawley rats were treated with diets mixed with Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene) to provide lycopene at target doses of 50, 150, or 500 mg/kg bw per day for two consecutive generations ($F_0$ and $F_1$). $F_0$ animals (30 of each sex per dose) received the test diet 10 weeks before pairing, throughout mating and gestation, and throughout to necropsy after weaning. $F_1$ animals (25 of each sex per dose) were mated after 10 weeks of

exposure and females were allowed to rear their young to weaning. Two control groups were included: one (standard) control group received the basic powdered diet, the other (placebo) control group received a diet mixed with placebo beadlets (batch No. UT02111005). The study followed OECD guideline 416 (2001). Additionally, blood and liver samples from selected $F_0$ and $F_1$ adults (five of each sex per group) and their weanlings (two of each sex per litter) were taken shortly before sacrifice for determination of lycopene content. The study was certified to comply with GLP and QA.

The achieved intakes during pre-mating, gestation and lactation were consistent with the target doses. Data on exposure showed detectable concentrations of lycopene in the plasma of rats in all groups treated with Lycopene 10% WS beadlets, with higher values in adult females than in adult males in each generation. There was no dose-dependency for adult males in either generation, while for adult females concentrations increased with dose but proportionally to a lesser degree than the dose. Plasma concentrations in the weaning pups were greater than those in the adults for both generations, were consistent between the sexes and increased with dose but proportionally to a lesser degree than the dose. All treated groups of adult males and females from each generation had detectable concentrations of lycopene in the liver. As noted for plasma, levels in females were much higher than in males, with no dose- dependency for males and an increase with dose for females but proportionally to a lesser degree than the dose. Liver concentrations for the weaning pups were again consistent between the sexes for both generations. Values for the group at the lowest dose were comparable with those noted for the adult males and then increased slightly with dose but proportionally to a lesser degree.

Observations not related to reproduction in adults included red-coloured faeces in all groups receiving lycopene throughout the study, and orange-stained fur/skin for $F_0$ and $F_1$ animals at 500 mg/kg bw per day and for $F_0$ males at 150 mg/kg bw per day. In the $F_0$ generation, treatment with lycopene generally had no effect on mean body-weight change and food consumption. In the $F_1$ generation, males and females at 500 mg/kg bw per day occasionally had a marginally lower food consumption during pre-mating, resulting in a marginally lower body weight during the first 7 or 2 weeks, respectively, but overall there was little or no effect on body weight and body-weight gain. $F_1$ females at 500 and 150 mg/kg bw per day also had slightly reduced food consumption during days 7– 20 of gestation and days 1–7 of lactation. Compared with standard controls, mean body-weight gain was reduced in all groups given beadlets, including placebo, during the last week of gestation and the first week of lactation but recovered thereafter, resulting in only slightly lower body weights (< 5%) at day 21 of lactation. In the groups receiving lycopene, macroscopic examination showed dark adipose tissue in some animals of the $F_0$ generation, and yellow discoloration of fat and organs of the abdominal cavity and liver streaks in some animals of the $F_1$ generation. Treatment-related histological changes were, however, not observed.

Some occasional small changes (generally less than 10% change) in absolute and/or relative organ weights were observed (e.g. for coagulating glands/seminal vesicles and uterus), without histopathological correlate.

With respect to reproductive findings, no treatment-related effects on mating performance and fertility were observed. Gonadal function, as assessed by determination of estrus cycle, detailed sperm analysis (motility, counts, morphology), and microscopic examination of ovaries, testes and epididymes, was also unaffected.

The survival and growth of $F_0$ offspring was not affected by treatment, while the number of $F_1$ offspring dying during days 1–4 was increased (13, 21, and 14 in groups treated with lycopene versus 5 in control groups). Live litter sizes, however, were unaffected and comparable with those for controls throughout lactation. $F_2$ pup body weights were not affected by treatment, except for a slightly decreased weight for female pups at the highest dose at day 1. Although some changes in physical development of $F_1$ and $F_2$ pups (measured as mean day of attainment of pinna unfolding, incisor eruption and eye opening) reached statistical significance, they were only of a minor nature. During lactation, an increased number of $F_2$ pups at the highest dose showed clinical signs (thin, lacrimation, hair-loss, pale), but only one or two litters were affected.

Aside from red-coloured faeces and yellow-orange stained fur/skin/fat/abdominal organs owing to the colour of lycopene, it can be concluded that in the parental generation treatment with lycopene was only associated with marginal effects on body weight and food consumption ($F_1$ only), with no influence on mating performance and fertility. Hence, the NOEL for parental and reproductive toxicity was 500 mg of lycopene/kg bw per day, the highest dose tested. The NOEL for effects on growth and development of the offspring was also 500 mg of lycopene/kg bw per day, as the clinical findings in $F_2$ pups at the highest dose can be considered to be incidental (Edwards et al., 2005).

*Developmental toxicity*

*Rats*

In order to establish the influence of the impurity apo-12'-lycopenal on the potential developmental toxicity of lycopene, groups of up to 14 pregnant female Wistar rats were treated with Lycopene 10% CWS containing a low concentration of apo-12'-lycopenal (batch No. 20/93-2) or Lycopene 10% CWS with an approximate 10-fold excess of apo-12'-lycopenal (mixture of batch Nos 7/95-1 and RS2/95-70). The formulations were given as a dietary admixture at concentrations calculated to provide lycopene at a dose of 1000 mg/kg bw per day or 1000 mg of lycopene + 20 mg of apo-12'-lycopenal/kg bw per day, respectively, from days 6 to 18 of gestation, followed by caesarian section on day 21 of gestation. A control group receiving a placebo beadlet formulation (batch No. 19/93-3) was also included, following the same protocol. Observations included clinical signs and measurement of body weight and food consumption. At necropsy, uterus and ovaries were examined for reproductive parameters and internal organs were examined macroscopically. Fetuses were examined for external, visceral and skeletal deviations. The study followed OECD guideline 414 (1981), however with less than the recommended number of pregnant animals, and was certified to comply with GLP and QA.

***Table 2. Studies of genotoxicity with lycopene (synthetic)***

| End-point | Test system | Test substance | Concentration | Result | Reference |
|---|---|---|---|---|---|
| *In vitro* | | | | | |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA* | Lycopene 10 CWD[a] (in water) | 20–5 000 μg/plate, ± S9 | Negative[b] | BASF (2000) |
| Reverse mutation | *S. typhimurium* TA97, TA98, TA100, TA102, TA1535, TA1537 *E. coli* WP2 *uvrA* | Lycopene[c] (in DMSO) | 1.6–1 000 μg/plate, ± S9 | Negative[d] | Gocke (1996); McClain & Bausch (2003) |
| Reverse mutation | *S. typhimurium* TA97, TA100 | Lycopene, degraded[e] (in DMSO) | 250–1 000 μg/plate, ± S9 | Positive[f] | Gocke (1996); McClain & Bausch (2003) |
| Reverse mutation | *S. typhimurium* TA97, TA98, TA100, TA102, TA1535, TA1537 *E. coli* WP2 *uvrA* | Lycopene 10% CWS[g] (in water) | 100–10 000 μg/plate, ± S9 | Negative[h] | Gocke (1996); McClain & Bausch (2003) |
| Reverse mutation | *S. typhimurium* TA97, TA100 | Lycopene 10% CWS, aged[i] (in water) | 100–10 000 μg/plate, ± S9 | Negative[h] | Gocke (1996); McClain & Bausch (2003) |
| Reverse mutation | *S. typhimurium* TA97, TA98, TA100, TA102, TA1535 | Lycopene 10% WS[j] (in water) | 100–10 000 μg/plate, ± S9 | Negative[k] | Gocke (1999); McClain & Bausch (2003) |
| Reverse mutation | *S. typhimurium* TA97, TA98, TA100, TA102, TA1535 | Lycopene 10% FS[l] (in acetone) | 100–10 000 μg/plate, ± S9 | Negative[m] | Gocke (2000); McClain & Bausch (2003) |
| Gene mutation | Mouse lymphoma L1578Y $Tk^{+/-}$ cells | Lycopene 10% CWS (in treatment medium) | 6.25–400 μg/ml, – S9; 12.5–800 μg/ml, + S9 | Negative[n] | Muster (1996a); McClain & Bausch (2003) |

***Table 2*** (contd)

| End-point | Test system | Test substance | Concentration | Result | Reference |
|---|---|---|---|---|---|
| Chromosomal aberration | Chinese hamster V79 cells | Lycopene 10 CWD (in water) | First experiment: 156.25, 312.5, or 625 µg/ml, –S9; 625, 1250, or 2500 µg/ml, +S9 | Negative[o] | BASF (2001c) |
| | | | Second experiment: 300, 600, or 1200 µg/ml, –S9;[p] 1200 µg/ml, –S9;[q] 600, 1200, or 2400 µg/ml, +S9[q] | Weak positive[r] | |
| | | | Third experiment: 1000, 1200, or 1400 µg/ml, –S9 | Positive[s] | |
| Chromosomal aberration | Human lymphocytes | Lycopene 10% CWS (in culture medium) | 250–1000 µg/ml, –S9; 250–2500 µg/ml, +S9 | Negative[t] | Miller (1996a); McClain & Bausch (2003) |
| *In vivo* | | | | | |
| Micronucleus formation | Mouse bone marrow | Lycopene 10 CWD (in water) | 500, 1000, or 2000 mg/kg bw, 2 x intraperitoneal dose with an interval of 24 h | Negative[u] | BASF (2001d) |
| Micronucleus formation | Mouse bone marrow | Lycopene 10% CWS (in water) | 1070, 2140, or 4280 mg/kg bw, 2 x oral dose with an interval of 24 h | Negative[v] | Miller (1996b); McClain & Bausch (2003) |
| Micronucleus formation | Mouse bone marrow | Lycopene 10% CWS, aged[w] (in water) | 2140 or 4280 mg/kg bw, 2 x oral dose with an interval of 24 h | Negative[v] | Miller (1996b); McClain & Bausch (2003) |

***Table 2*** (contd)

| End-point | Test system | Test substance | Concentration | Result | Reference |
|---|---|---|---|---|---|
| Micronucleus formation | Mouse peripheral blood | Lycopene 10% CWS in soft drink | 50 mg of lycopene/l, ad libitum for 14 days[x] | Negative | Muster (1999); McClain & Bausch (2003) |
| Micronucleus formation | Mouse peripheral blood | Lycopene 10% CWS in soft drink, aged[y] | 25 or 50 mg of lycopene/l, ad libitum for 14 days[z] | Negative | Muster (1999); McClain & Bausch (2003) |
| Unscheduled DNA synthesis | Rat hepatocytes | Lycopene 10 CWD (in water) | 1000 or 2000 mg/kg bw, single oral dose | Negative[aa] | BASF (2001e) |
| Unscheduled DNA synthesis | Rat hepatocytes | Lycopene 10% CWS (in water) | 6000 mg/kg bw, single oral dose | Negative[ab] | Muster (1996b); McClain & Bausch (2003) |
| Unscheduled DNA synthesis | Rat hepatocytes | Lycopene 10% CWS, aged[w] (in water) | 6000 mg/kg bw, single oral dose | Negative[ab] | Muster (1996b); McClain & Bausch (2003) |

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rat liver

[a] Batch No. OD-12-04 6.Spr.A, containing 9.81% lycopene, except for the assay for unscheduled DNA synthesis in vivo, where the same batch was reported to contain 9.3% lycopene.

[b] With and without metabolic activation from S9, using both the plate incorporation method and the preincubation method. No cytotoxicity or precipitation was observed.

[c] Batch No. DV-111255; purity, 99.74%.

[d] With and without metabolic activation from S9, using both the plate incorporation method and the preincubation method. No cytotoxicity was observed. Precipitation was observed at 200 and 1000 µg/plate. Previous experiments with a less pure batch of crystalline lycopene (purity, 96%) showed positive effects, especially with strains TA100 and TA97. This was owing to a mutagenic impurity, as demonstrated by the negative results for pure crystalline lycopene.

***Table 2*** (contd)

[e] Batch No. DV-111255; purity, 99.74%, degraded by exposure to air or a combination of air, light and room temperature.
[f] Only without metabolic activation from S9, and more pronounced in TA100 than in TA97. Preincubation method was used, with precipitation observed at all concentrations, but no cytotoxicity. Additional experiments showed that the formation of mutagenic degradation products by oxidative processes can be reduced by adding antioxidants like DL-α-tocopherol.
[g] Batch No. 7/95-1, containing 11.5%/10.9% lycopene (by UV/HPLC, respectively).
[h] With and without metabolic activation from S9, using the preincubation method. No cytotoxicity was observed. Red particles observed at 10 000 µg/plate did not allow evaluation of background growth.
[i] Batch No. 7/95-1, containing 11.5%/10.9% lycopene (by UV/HPLC, respectively), after stress storage (under air in dark bottle at 45 C for 1, 2, 4, or 8 weeks).
[j] Batch No. RS2/99-105, containing 10.4% lycopene and 0.19% $C_{25}$-lycopyl-aldehyde (by HPLC).
[k] With and without metabolic activation from S9, using the preincubation method. Reddish precipitation was observed at 10 000 µg/plate, not allowing evaluation of background growth and necessitating manual counting. Cytotoxicity was only observed at 3160 µg/plate for TA102 without S9.
[l] Batch No. UE00002004, containing 10.5% lycopene (by UV).
[m] With and without metabolic activation from S9, using both the plate incorporation method and the preincubation method. Reddish precipitation was observed at 316 µg/plate and greater. Cytotoxicity was not observed.
[n] With and without metabolic activation from S9. Cytotoxicity was observed at 400 (without S9) and 800 µg/ml (with S9).
[o] With and without metabolic activation from S9. The cell cultures were treated for 4 h without and with S9 and were harvested 14 h later. Effects on mitotic index were only observed without S9, at 625 µg/ml.
[p] The cells were exposed continuously for 18 h and were then harvested. Effects on mitotic index were observed at 600 and 1200 µg/ml.
[q] The cells were exposed continuously for 18 h (without S9) or 4 h (with S9), and were harvested at 28 h. No effects on mitotic index were observed.
[r] Only without metabolic activation from S9, at a harvest time of 28 h. A slight, not statistically significant increase in structural aberrations was observed.
[s] The cell cultures were treated for 18 h and were harvested 10 h later. A dose-dependent, statistically significant increase in structural (but not numerical) aberrations was observed at which the test substance was insoluble and distinct precipitation occurred. Effects on mitotic index were observed at 1400 µg/ml.
[t] With and without metabolic activation from S9. In the first experiment, cells were treated continuously for 22 h without S9 and then harvested or were treated for 3 h with S9 and harvested 19 h later. In the second experiment, the cell cultures were treated for 3 h without and with S9 and were harvested 19 h later. Cytotoxicity was obvious at the highest concentrations tested, as indicated by a reduction of the mitotic index and the number of cells that could be evaluated.
[u] No toxic signs or symptoms were observed, and no effects on the ratio of polychromatic versus normochromatic erythrocytes.

***Table 2*** (contd)

[v] No toxic signs or symptoms were observed, but the ratio of polychromatic vs normochromatic erythrocytes was slightly reduced.
[w] Batch No. 7/95-1, containing 11.5%/10.9% lycopene (by UV/HPLC, respectively), after stress storage (under air in dark bottle at 45 ˚C for 8 weeks).
[x] Equivalent to a dose of lycopene of 3.5-11.5 mg/kg bw per day, estimated based on an average (water) intake of 3-10 ml/day for growing mice and an average body weight of 43.5 g. No toxic effects were observed and the ratio of polychromatic vs normochromatic erythrocytes was not affected.
[y] After storage at 45 ˚C for 4 weeks in the dark.
[z] Equivalent to a dose of lycopene of 1.7-5.8 or 3.5-11.5 mg/kg bw per day, estimated based on an average (water) intake of 3-10 ml/day for growing mice and an average body weight of 43.5 g. No toxic effects were observed and the ratio of polychromatic vs normochromatic erythrocytes was not affected.
[aa] No toxic signs or symptoms were observed.
[ab] No signs of toxicity or cytotoxicity were observed.

No effects were observed on body weight and food consumption. In 13 out of 14 females of the group receiving lycopene + apo-12'-lycopenal, yellowish fat in the abdominal cavity was noted. Reproductive parameters evaluated (numbers of corpora lutea, implantations, resorptions and live/dead fetuses, fetal body weight and sex ratio) were not affected. Fetal examination showed an increased incidence of extra-thoracic (14th) ribs in the lycopene group compared with the group receiving lycopene + apo-12'-lycopenal and the control group (26.1, 16.8, and 14.7%, respectively). Co-administration of apo-12'-lycopenal at a concentration of 2% in the formulation (relative to lycopene) did thus not result in substance-related effects (Eckhardt, 1996; McClain & Bausch, 2003).

In a study to examine the effects of lycopene on embryonic and fetal development, groups of mated female Wistar rats were given diets admixed with Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene) from day 5 (implantation) through to day 21 post coitum (the day of scheduled caesarean section), at target doses of lycopene of 50, 150, and 500 mg/kg bw per day. There were two control groups, one receiving standard diet, and the other receiving placebo-control beadlets (batch No. UT02111005). Each group consisted of 22 mated female rats, with an additional three mated female rats per group in the three groups receiving lycopene for exposure monitoring. From the latter animals, blood was taken on days 6 and 21 post coitum. The study followed OECD guideline 414 (2001), and was certified to comply with GLP and QA.

Analysis of plasma samples showed systemic absorption of Lycopene 10% WS beadlets in all three treated groups, without clear dose-dependency. For all groups, higher mean values were observed in the plasma on day 20 than on day 6. No mortalities occurred and no clinical signs were observed, except for discoloured faeces in all animals treated with lycopene. No treatment-related effects on body weight and food consumption were observed. In beadlet controls and females at the highest dose, postimplantation loss (which was caused by embryonic rather than fetal resorptions) was slightly increased above control and historical control values. Although examination of fetuses showed some differences in individual visceral and skeletal findings between treated groups and (historical) controls (among which left-sided umbilical artery, abnormally shaped sternebrae and vertebral bodies, and non-ossified cervical vertebral bodies), treatment with lycopene did not increase the overall number of external, visceral and skeletal abnormalities and variations. Most findings were common variations or abnormalities for the rat strain used. The NOEL for maternal and developmental toxicity was 500 mg/kg bw per day, the highest dose tested (Edwards et al., 2004b).

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley rats were given water, or Lycopene 10 CWD (500, 1500, or 3000 mg/kg bw per day), Lycopene 10 CWD formulation matrix (3000 mg/kg bw per day), LycoVit 10% (3000 mg/kg bw per day), or LycoVit 10% formulation matrix (3000 mg/kg bw per day) by gavage on day 6 to day 19 post-coitum. These doses correspond to approximate lycopene dosages of 50, 150, and 300 mg/kg bw per day for Lycopene 10 CWD (batch No. OD-12-04 6.Spr.A, containing 9.81% synthetic lycopene) and 300 mg/kg bw per day for LycoVit 10% (batch No. 20014372, containing 10.8% synthetic

lycopene). The animals were sacrificed on day 20 post coitum. The study followed OECD guideline 414 (draft of 1999), and was certified for compliance with GLP and QA.

Red-coloured faeces were observed from day 7 or 8 post-coitum at all females receiving Lycopene 10 CWD or LycoVit. Some other clinical observations (e.g. regurgitation) reflected systemic responses to the high viscosity and large volumes of the test and control substances administered. At necropsy, the animals treated with lycopene animals showed thick reddish contents in the intestines (with a dose-dependent increase) or stomach. Maternal food consumption, body-weight gain and gravid uterus weight were not affected by treatment. At caesarian section, some small, not statistically significant changes were observed in the numbers of implantation sites and fetuses per dam (slight decrease) and in early resorptions and postimplantation loss (slight increase) in females receiving Lycopene 10 CWD (no dose–response relationship) and LycoVit when compared with placebo and vehicle controls. Fetal body weights were not affected by treatment. No treatment-related effects on external and soft tissue malformations and variations were observed. Treatment with lycopene also did not affect skeletal malformations. There was a large number of skeletal variations and cartilage in fetuses from all groups, mostly related to incomplete or absent ossification. Generally, fetal and litter incidences were similar in treated and control groups, and were consistent with historical control data. Despite some differences in individual findings (including incomplete ossification and absence of cartilage of the supraoccipital, parietal and hyoid bones), the overall rate of skeletal variations and cartilage did not differ between groups. Given the absence of relevant toxicological findings with both lycopene formulations, the NOEL for both maternal and developmental toxicity was approximately 300 mg of lycopene/kg bw per day, the highest dose tested (BASF, 2001f; Christian et al., 2003).

*Rabbits*

In order to find appropriate doses for the main study, a preliminary study of prenatal developmental toxicity was conducted in groups of five mated Himalayan rabbits receiving either placebo beadlets (batch No. UT02111005) or Lycopene 10% WS beadlets (batch No. UT02070002; 13.2% synthetic lycopene) at a daily dose of lycopene of 0 or 400 mg/kg bw (divided between two doses), respectively, by gavage from day 6 until day 27 of gestation. Since analysis of plasma samples and liver samples at termination demonstrated the presence of systemic absorption, it was concluded that the maximum practical dose caused no apparent maternal and embryo-fetal toxicity and was an appropriate highest dose for the main study (Edwards et al., 2004c).

In the main study, groups of 20 mated female Himalayan rabbits were given Lycopene 10% WS beadlets (batch No. UT02070001; 13.2% synthetic lycopene) at a dose of lycopene of 0 (beadlet control), 50, 150, or 400 mg/kg bw per day by gavage (twice daily) from day 6 until to day 27 post coitum, with caesarean section carried out on day 28 post coitum. Exposure was monitored by blood sampling from four animals per group on days 6 and 27 post coitum. The study followed OECD guideline 414 (2001), and was certified to comply with GLP and QA

Systemic absorption of lycopene was demonstrated in all groups receiving Lycopene 10% WS beadlets, both on day 6 and day 27 post coitum, without clear dose-dependency. For all groups, the mean plasma concentrations of lycopene were slightly higher on day 6 than on day 27 post coitum. At 50, 150, and 400 mg/kg bw per day, red faeces were noted from day 6 post coitum until termination. During necropsy, reddish stomach content was noted for one female at 50 mg/kg bw per day and for most females at 150 and 400 mg/kg bw per day. At 50 and at 400 mg/kg bw per day, a single female was found dead in each group; these deaths were assumed to be caused by oesophageal reflux and aspiration of the very viscous suspension of the test item. Treatment with lycopene had no apparent effect on food consumption and body-weight gain. One female at the highest dose delivered pre-term on day 28 post coitum. One female in the beadlet control group and one female at the highest dose had total postimplantation loss. Excluding these two females, postimplantation loss (which concerned embryonic rather than fetal resorptions) was slightly increased in females at the lowest and intermediate dose when compared with the control group and with historical controls, but not in females at the highest dose. The number of fetuses per dam and fetal weights were not affected by treatment. Identical to the preliminary study, examination of the fetuses did not reveal any differences in total numbers of litters and fetuses with external and fresh visceral findings, although some individual findings differed between groups (including sutural bone between frontals, absence of accessory lung lobe, and additional ossification of forelimb and hindlimb). Given the absence of relevant toxicological findings, the NOEL for maternal and developmental toxicity was 400 mg of lycopene/kg bw per day, the highest dose tested (Edwards et al., 2004d).

In a study of the potential developmental toxicity of two synthetic lycopene formulations (Lycopene 10 CWD and LycoVit 10%), groups of 25–34 pregnant New Zealand White rabbits were given water, Lycopene 10 CWD (500, 1500, or 2000 mg/kg bw per day), Lycopene 10 CWD formulation matrix (2000 mg/kg bw per day), LycoVit 10% (2000 mg/kg bw per day), or LycoVit 10% formulation matrix (2000 mg/kg bw per day) by gavage from day 6 until day 28 post-coitum. These correspond to approximate lycopene dosages of 50, 150, and 200 mg/kg bw per day from Lycopene 10 CWD (batch No. OD-13-10 Fass 1, containing 10% synthetic lycopene) and 200 mg/kg bw per day from LycoVit 10% (batch No. 80000007, containing 10% synthetic lycopene). The animals were sacrificed on day 29 post coitum. The study followed OECD guideline 414 (draft of 2000), and was certified to comply with GLP and QA.

Red-coloured faeces were observed for all females receiving Lycopene 10 CWD or LycoVit. There was a high rate of mortality in the groups given Lycopene 10 CWD, which was, in part, related to regurgitation after gavage and accidental aspiration of stomach contents into the lungs, owing to the high viscosity, large volume and possibly bad taste/odour of the test substance. Other observations in the animals treated with lycopene included breathing problems and altered gastointestinal motility and, at necropsy, red material in stomach, intestines and, in some decedent animals, lungs. Compared with vehicle controls, food consumption was reduced in the group receiving Lycopene 10 CWD at the highest dose during days 6 to 25 of gestation, accompanied by a slightly reduced body-weight gain

(−9%) over the whole treatment period in this group. The reduced food consumption was most probably associated with the high viscosity of the preparation at the highest dose. Food consumption was also slightly reduced in both matrix control groups during days 6 to 29, resulting in a slight decrease in body-weight gain (−10%) only for the LycoVit matrix group. No treatment-related effects on external and soft tissue malformations and variations were observed. There were a large number of skeletal variations and cartilage in fetuses from all groups, including controls, mostly related to incomplete or absent ossification. For most findings there was no relationship with lycopene treatment. For some individual findings (e.g. unossified or incomplete ossification of the 5th and 6th sternebrae) the incidences were slightly increased in the group receiving LycoVit, while for the overall rate of skeletal variations and cartilage this was not the case. Given the inherent variability in ossification in term fetuses, the skeletal findings are not considered to be related to treatment. It can be concluded that in rabbits, more than in rats, gavage administration of the viscous, gelatinous suspensions of Lycopene 10 CWD or LycoVit 10% at maximum dosage volumes affected the animals' ability to retain the intubated test material and altered the animals' gastrointestinal motility, contributing to maternal stress and in some cases maternal deaths. Despite these difficulties, no direct toxic effect of lycopene on maternal animals or fetuses was observed. Noting also that the amount and mode of administration is not representative for human exposure to lycopene from food or dietary supplements, for both formulations the NOEL for both maternal and developmental toxicity was approximately 200 mg of lycopene/kg bw per day, the highest dose tested (BASF, 2001g; Christian et al., 2003).

### *2.2.6 Additional toxicological data on impurities/reaction by-products*

Since most of the available toxicological studies were performed with formulations of synthetic lycopene complying with the specifications, the safety of any impurities/reaction by-products present (if any) has been implicitly tested at their maximum permissible levels. Additional data were, however, available for apo-12'-lycopenal and TPPO.

Three studies were available that were conducted with 10% synthetic lycopene formulations containing an enhanced content of apo-12'-lycopenal (up to 2% relative to lycopene). These studies, i.e. a 4-week study of toxicity in rats (Niederhauser et al., 1996), a study of developmental toxicity in rats (Eckhardt, 1996), and a test for gene mutations in vitro in *Salmonella typhimurium* (Gocke, 1999), have been summarized in sections 2.2.2, 2.2.4 and 2.2.5(b) of this monograph, respectively. In these studies, apo-12'-lycopenal did not induce substance-related effects.

TPPO gave negative results in a test for gene mutations in vitro in *S. typhimurium* strains TA97, TA98, TA100, TA102, and TA1535, at concentrations of 31.6–3160 µg/plate with and without metabolic activation, and using both the plate incorporation method and the preincubation method (Gocke, 1998). Additionally, limited information on the toxicity of TPPO was available from a public database (EC-ECB, 2000). The original studies were, however, not available to the Committee. In a 3-month feeding study in Sprague-Dawley rats given TPPO at a

dose of 20, 100, 500 or 2500 mg/kg feed, followed by a 7-week recovery period, TPPO was reported to have resulted in clinical signs, reduced feed consumption and body-weight gain, haematological and biochemical alterations, increased organ weights, hepatic degeneration, and death. The NOEL was 20 mg/kg feed, equivalent to 1 mg/kg bw per day. When administered to beagle dogs at doses of 20, 200, 2000, or 10 000 mg/kg feed for 3 months, no effects were observed at 20 and 200 mg/kg feed, while clinical signs, haematological and biochemical alterations, emaciation, liver damage, atrophy of the skeletal musculature, and death were observed at 2000 and 10 000 mg/kg feed. The NOEL was 200 mg/kg feed, equivalent to 5 mg/kg bw per day. TPPO gave negative results in a test for gene mutations in vitro in *S. typhimurium* strains TA98, TA100 and TA1537, at concentrations of 4–2500 µg/plate with and without metabolic activation.

### *2.3 Observations in humans*

A number of epidemiological studies were provided on the purported health benefits of lycopene. A review of these studies is however beyond the scope of this monograph dealing with the safety aspects of lycopene.

Most studies in humans reported in literature (see summaries in section 2.3 of the monograph 'Lycopene from *Blakeslea trispora*', in this volume), although not specifically designed to assess the safety of lycopene, revealed no adverse effects after administration of dietary lycopene. There are, however, case reports of yellow-orange skin discoloration and/or gastrointestinal discomfort after prolonged high intakes of lycopene-rich food and supplements. This so-called lycopenodermia is reversible upon cessation of lycopene ingestion.

## *3. DIETARY INTAKE*

### *3.1 Introduction*

Lycopene is a naturally-occurring pigment found in a number of fruits and vegetables, notably tomato, watermelon, guava, pink grapefruit, and apricot. It is also produced by a number of algae and fungi. These foods are common to most cultures; therefore, lycopene is already part of the diet. Because of evidence that the consumption of lycopene is associated with some potential health benefits, food processors are seeking to add lycopene to processed foods. Such foods include: flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings, sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. Additionally, lycopene is available directly as a dietary supplement. The Committee considered the additional exposure to lycopene from its proposed food uses in context with the naturally-occurring background intake from food.

The Committee received dossiers concerning lycopene exposure from three food-ingredient processors and obtained additional information from the published literature and national government sources (BASF, 2006; DSM, 2006; Vitatene, 2006). Lycopene can be obtained from a number of sources: extracted from fruits, such as tomatoes or watermelons, extracted from fungi, such as *B. trispora*, or

chemically synthesized from smaller organic starting materials. Although the Committee was asked to evaluate both synthetic lycopene and lycopene derived from *B. trispora*, the proposed food uses/use levels for lycopene from *B. trispora* encompass those of synthetic lycopene (with the single exception of cereal bars), therefore the estimated exposure was considered together. Although purified lycopene is unstable in the presence of light and temperatures above room temperature, it is known to be stable in a food matrix (processed tomato products) (Agarwal et al., 2001).

### *3.2 Background exposure to lycopene from food*

Concentrations of lycopene in fruit and vegetables typically vary from approximately 1 mg/100 g of fresh fruit to 20 mg/100 g, with some specially-bred tomatoes having concentrations reaching 40 mg/100 g. In processed tomato products, such as ketchup, juice, and pastes, the lycopene can be concentrated to up to 100 mg/100 g of food (Lugasi et al., 2003). National dietary exposures to lycopene have been estimated and reported in the literature. Table 3 summarizes some of these estimates. The estimates consistently range from approximately 1 to 10 mg/person per day, with the sole exception of a Canadian study that showed background intakes of approximately 25 mg/day. The Committee noted that the concentrations of lycopene measured in foods and used in this exposure study were consistently three to five times higher than those used in the other studies. No explanation of these high levels was reported. An international estimate of lycopene intake from food was prepared using the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets. Based primarily on intake of tomatoes, the estimates ranged from 1 to 7 mg/person per day in the five regional diets (DSM, 2006).

***Table 3. National estimates of lycopene intake from natural dietary sources***

| Country | Intake(s) (mg/person per day) | Reference |
|---|---|---|
| Canada | 6.3, 25 | Johnson-Down et al. (2002); Agarwal et al. (2001) |
| Finland | 0.8 | Järvinen (1995) |
| France | 2.0 | DSM (2006) |
| Germany | 1.3 | Pelz et al. (1998) |
| Hungary | 4.4 | Lugasi et al. (2003) |
| Netherlands | 1.3 | Goldbohm et al. (1998) |
| United Kingdom | 1.0, 5.0 | Scott et al. (1996); DSM (2006) |
| United States of America | 0.6, 1.6 | Vanden Langenberg et al. (1996) |
| | 3.7, 3.1, 11 | Forman et al. (1993); Yong et al. (1994); McClain & Bausch (2003) |

In a submission to the US Food and Drug Administration's Generally Recognized As Safe (GRAS) notification programme, a food-ingredient processor estimated that mean background intake of lycopene from food in the USA was 8.2 mg/day (GRN 156; US Food and Drug Administration, 2002b). According to a intake (GRN 119; US Food and Drug Administration, 2002a, 2002b). These estimates are consistent with the reported national estimates and the GEMS/Food international estimates.

### *3.3 Exposure to added lycopene from proposed food uses*

The Committee considered the proposed uses for lycopene in numerous food categories, including flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings, sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. Table 4 contains proposed food categories and associated use levels for the lycopene from *B. trispora*. The food category system (Annex B) of the General Standard for Food Additives (GSFA) of the Codex Alimentarius Commission [CODEX STAN 192-1995 (Rev. 6-2005)] was used to classify the uses. The highest use level proposed (either for the USA or the EU) appears in the table. These food category/use level combinations were used with food intake information from the US Continuing Survey of Food Intake by Individuals (1994–6, 1998 supplement) and the UK National Diet and Nutrition Survey (NDNS; 1992–1993, 1997) to derive estimates of added lycopene intake for the USA and European populations (Table 5 and Table 6, respectively). Food intakes from a Dutch Survey (DNFCS-3, 1997–8) were combined with food category/use level combinations (Table 7) from the synthetic-lycopene dossier to estimate intake from that source (Table 8). Additional analyses using food intake data from France and the UK were provided; estimated intakes were less than those presented in Table 8 and are not presented in this monograph (DSM, 2006).

***Table 4. Proposed food uses for lycopene from* Blakeslea trispora**

| Food category | Use level (mg/kg) |
|---|---|
| *01.0 Dairy products and analogues* | |
| 01.1.2 Cultured dairy drinks | 20 |
| 01.1.2 Dairy-based fruit drinks | 20 |
| 01.7 Gelatin desserts, puddings, and custards | 25 |
| *02.0 Fats, oils, and fat emulsions* | |
| 0.2.2.2 Table fat spreads | 5 |
| *05.0 Confectionery* | |
| 05.2.1 Hard candy | 25 |
| 05.2.2 Jelly products | 25 |
| 05.4 Decorations, fillings, and icings | 25 |
| *06.0 Cereals and cereal products* | |
| 06.3 Ready-to-eat cereals | 50 |

| Food category | Use level (mg/kg) |
|---|---|
| *07.0 Bakery wares* | |
| 07.1.2 Crackers | 30 |
| *12.0 Salts, spices, soups, salads, protein products, and fermented soybean products* | |
| 12.5.1 Prepared and condensed soups | 7 |
| 12.5.2 Dry soup mixes | 575 |
| 12.6.1 Low-fat salad dressings | 20 |
| 12.6.2 Tomato-based gravies and specialty sauces | 50 |
| 12.6.2 Non-emulsified sauces, seasonings, relishes, and pickles | 50 |
| 12.9 Meat substitutes | 50 |
| *13.0 Foodstuffs intended for particular nutritional uses* | |
| 13.4 Dairy- and non-dairy-based meal replacement beverages | 25 |
| *14.0 Non-dairy beverages* | |
| 14.1.2.1 Fruit juices | 25 |
| 14.1.3.1 Fruit nectars | 25 |
| 14.1.4 Energy, sport, and isotonic drinks | 25 |
| 14.1.4.2 Fruit-flavoured drinks | 25 |
| *15.0 Ready-to-eat savouries* | |
| 15.1 Nutrient bars | 50 |
| 15.1 Salty snacks | 30 |

From General Standard for Food Additives of the Codex Alimentarius Commission (CODEX STAN 192-1995, Rev. 6, 2005).

***Table 5. Summary of the estimated daily intake of lycopene from* Blakeslea trispora *in the USA, 1994–1996, 1998 USDA CSFII data***

| Population group | Age group (years) | Percentage of users | Actual No. of total users | Intake (mg/person per day) | | | |
|---|---|---|---|---|---|---|---|
| | | | | All-person | | All-user | |
| | | | | Mean | 90th percentile | Mean | 90th percentile |
| Infants | 0–2 | 78.7 | 2591 | 6.7 | 15.4 | 8.5 | 17.0 |
| Children | 3–11 | 99.6 | 6132 | 10.7 | 19.5 | 10.8 | 19.5 |
| Female teenagers | 12–19 | 98.8 | 707 | 10.6 | 21.3 | 10.7 | 21.6 |
| Male teenagers | 12–19 | 98.1 | 714 | 13.1 | 27.1 | 13.3 | 27.1 |

| Population group | Age group (years) | Percentage of users | Actual No. of total users | Intake (mg/person per day) | | | |
|---|---|---|---|---|---|---|---|
| | | | | All-person | | All-user | |
| | | | | Mean | 90th percentile | Mean | 90th percentile |
| Female adults | 20 and older | 97.2 | 4534 | 7.1 | 15.2 | 7.3 | 15.3 |
| Male adults | 20 and older | 96.5 | 4834 | 9.6 | 20.4 | 10.0 | 20.9 |

From 1994–1996, 1998 USDA CSFII data

***Table 6. Summary of the estimated daily intake of lycopene from* Blakeslea trispora *in the UK, 1992–1993 and 1997***

| Population group | Age group (years) | Percentage of users | No. of total users | Intake (mg/person per day) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | All-person consumption | | | All-user consumption | | |
| | | | | Mean | 90th %ile | 95th %ile | Mean | 90th %ile | 95th %ile |
| Children | 1.5–4.5 | 95.5 | 1574 | 7.6 | 14.6 | 18.7 | 7.9 | 14.7 | 19.0 |
| Young people | 4–10 | 97.5 | 816 | 8.3 | 15.8 | 18.3 | 8.5 | 15.9 | 18.6 |
| Female teenagers | 11–18 | 93.5 | 417 | 6.4 | 13.6 | 15.9 | 6.7 | 14.1 | 16.5 |
| Male teenagers | 11–18 | 92.3 | 384 | 7.6 | 16.6 | 20.8 | 8.3 | 17.0 | 21.5 |
| Female adults | 16–64 | 72.4 | 694 | 3.1 | 7.7 | 10.6 | 4.0 | 8.6 | 12.0 |
| Male adults | 16–64 | 71.5 | 548 | 3.5 | 9.0 | 11.5 | 4.5 | 10.0 | 13.2 |

%tile: percentile
From UK National Diet and Nutrition Survey data, 1992–1993 and 1997

***Table 7. Proposed food uses for synthetic lycopene***

| Food category | Use level (mg/kg) |
|---|---|
| Fruit and vegetable juice | 25 |
| Fruit juice drinks | 25 |
| Soft drinks | 25 |
| Squash | 25 |

| Food category | Use level (mg/kg) |
|---|---|
| Sports and energy drinks | 25 |
| Dairy fruit drinks | 25 |
| Yogurt, yogurt drinks | 50 |
| Cereals | 80 |
| Cereal bars | 80 |
| Crackers | 100 |
| Margarine, low-fat spreads | 40 |
| Hard candy | 70 |

***Table 8. Estimated daily intake of synthetic lycopene, Netherlands 1997– 1998***

| | Intake (mg/person per day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All-person | | | | All-user | | | |
| Population group (years) | N | Mean | 50th %ile | 95th %ile | N | Mean | 50th %ile | 95th %ile |
| Child aged 1–3 | 254 | 13.2 | 11.8 | 29.2 | 253 | 13.2 | 11.8 | 29.2 |
| Boys aged 4–9 | 242 | 15.9 | 15.0 | 29.6 | 241 | 16.0 | 15.0 | 29.6 |
| Girls aged 4–9 | 272 | 15.0 | 14.1 | 28.4 | 272 | 15.0 | 14.1 | 28.4 |
| Males aged 10–18 | 391 | 18.9 | 17.7 | 36.8 | 390 | 18.9 | 17.7 | 36.8 |
| Females aged 10–18 | 380 | 16.5 | 14.9 | 32.7 | 380 | 16.5 | 14.9 | 32.7 |
| Males aged 19 and older | 2117 | 8.8 | 6.5 | 24.9 | 2042 | 9.1 | 6.7 | 25.2 |
| Females aged 19 and older | 2544 | 8.3 | 6.2 | 22.7 | 2431 | 8.7 | 6.6 | 22.9 |

%ile: percentile
From Dutch DNFCS-3, 1997–98 data

Each of the analyses suggests that an intake of approximately 30 mg/day could be expected for a consumer at or above the 95th percentile of the intake distribution. This high-percentile intake is approximately 10 times greater than mean background intakes of lycopene (it would be expected that high-percentile background intake might be two or more times greater than the mean). The conservatisms in each analysis have been noted: all foods that might contain added lycopene would do so, and at the proposed maximum use level. Because the concentrations for food additive use are approximately four times higher than the concentrations needed to colour food, the estimated intake of 30 mg/person per day covers uses of lycopene as a food colour.

## 4. COMMENTS

*Toxicological data*

The Committee considered the results of a large number of studies of pharmacokinetics and metabolism, acute toxicity, short- and long-term studies of toxicity, and studies of carcinogenicity, genotoxicity and reproductive toxicity with lycopene. Most of these studies had been performed with formulations of synthetic lycopene complying with the specifications as prepared at the present meeting, and met appropriate standards for study protocol and conduct.

In rats given a single oral dose of a formulation containing 10% radiolabelled synthetic lycopene, lycopene was rapidly but poorly absorbed. Owing to the poor absorption (less than 10% of the administered dose), concentrations of radioactivity in organs and tissues were low, with highest concentrations being found in the liver, and lower concentrations in the spleen, adipose tissue and adrenals. In rats, repeated oral doses of formulations containing 10% synthetic lycopene and of lycopene from tomato concentrate also resulted in the accumulation of lycopene in the liver (with higher concentrations in females than in males), and to a lesser extent in spleen and adipose tissue. This accumulation in the liver was associated with pigment deposits in hepatocytes, both with synthetic lycopene and with lycopene from tomato concentrate, although higher doses of the latter were necessary to induce the same level of effect. In the rat body, the isomeric ratio changed to favour *cis* isomers, the percentage of *cis* isomers of lycopene being higher in plasma and most tissues, including liver, than in the test material. *Trans* to *cis* isomerization was also observed in dogs. Studies in dogs and monkeys confirmed that the highest concentrations of lycopene accumulate in the liver.

In humans, absorption of formulated synthetic lycopene was comparable to absorption of lycopene contained in tomato-based products. As in laboratory species, the systemic availability of lycopene in humans is generally low, but can be increased in the presence of dietary fat. The most abundant isomers in human plasma are all-*trans*-lycopene and 5-*cis*-lycopene, with all the *cis* isomers contributing to more than 50% of total lycopene. This isomer ratio differs from that of synthetic lycopene and lycopene in food, indicating that conversions take place after ingestion, as was also shown in laboratory species.

Little is known about the metabolism or degradation of lycopene in mammals. It is not converted to vitamin A. In rats, non-characterized polar metabolites are present in tissues and excreta. In humans, the proposed metabolic pathway involves oxidation of lycopene to lycopene 5,6-oxide, which subsequently undergoes cyclization and enzymatic reduction to form an epimeric mixture of 2,6-cyclolycopene-1,2-diol.

When administered orally as a formulation containing 10% synthetic lycopene, the $LD_{50}$ for lycopene was more than 500 mg/kg bw in rats.

The toxicity of synthetic lycopene was evaluated using the results of short-term studies in which rats were given one of several 10% formulations, either in the diet for 4 or 14 weeks, or by gavage for 3 months. Synthetic lycopene was well

tolerated in those studies. A reddish discoloration of the faeces was observed in the feeding and the gavage studies, owing to excretion of the red-staining test substance. The gavage study also showed a red discoloration of contents of the gastrointestinal tract, and the feeding studies showed an orange-reddish discoloration of the liver and adipose tissue. The observed discoloration in the liver was associated with orange-brown pigment deposits in the hepatocytes, with female rats being more affected than males. There was, however, no histopathological evidence of liver damage. The Committee considered that the changes observed in the short-term studies of toxicity did not represent adverse effects. The NOELs for lycopene were 1000, 500 and 300 mg/kg bw per day for the 4-week, 14-week and 3-month study, respectively, corresponding to the highest doses tested in those studies.

Observations made in short-term studies of toxicity in dogs were consistent with the findings in rats. When administered in capsules at a dose of 30 mg/kg bw per day for 28 days or 100 mg/kg bw per day for 192 days, synthetic lycopene caused only a red discoloration of the faeces and liver, respectively, with pigment being detectable in the latter, without associated hepatocellular alterations.

In a long-term study of toxicity, rats received diet mixed with a beadlet formulation containing 10% synthetic lycopene at target doses of 0 (untreated control), 0 (beadlet control), 10, 50, or 250 mg/kg bw per day for 52 weeks, followed by a recovery period of 13 weeks for some of the animals. Treatment-related findings were confined to discoloured faeces/red staining at the lowest, intermediate and highest dose, red contents in the stomach and caecum and yellow connective tissue in the abdominal cavity at the intermediate and highest dose, and (particularly in female rats at all doses) golden brown pigment deposits in the liver. The pigment deposits were still observed after recovery, albeit to a lesser degree. There was no apparent sign of liver dysfunction but, in contrast to the findings in the short-term studies of toxicity, the liver pigmentation in hepatocytes and histiocytes was associated with a greater incidence and severity of basophilic foci in females at the intermediate and highest dose than in females at the lowest dose or in the control groups. The histopathological alterations were considered to be treatment-related.

In a study of carcinogenicity in which diets were mixed with the same beadlet formulation containing 10% synthetic lycopene, rats received synthetic lycopene at target doses of 0 (untreated control), 0 (beadlet control), 2, 10, or 50 mg/kg bw per day for 104 weeks. Again, treatment resulted in a red discoloration of the faeces, red contents in the gastrointestinal tract, and yellow connective tissue at the intermediate and/or highest dose, golden brown pigment deposits in the liver (at all doses), as well as pigmentation in kidneys (females at the highest dose) and mesenteric and mandibular lymph nodes (at all doses). Liver pigmentation was observed in females (in hepatocytes and histiocytes) and, to a lesser degree, in males (in histiocytes). Histopathologically, the liver pigmentation was associated with a greater incidence and severity of eosinophilic foci in males and of normochromic and basophilic foci in females, especially at the intermediate and highest dose, albeit without a consistent dose–response relationship. There was no apparent sign of liver dysfunction. Also, no increase in the incidence of liver tumours was observed, nor was treatment with lycopene associated with an increase in the

incidence of tumours in any other tissue or organ. The histopathological alterations of liver foci mainly observed at the intermediate and highest dose were considered to be treatment-related.

Synthetic lycopene has been tested in vitro for its capacity to induce reverse mutations in *S. typhimurium* and *E. coli*, gene mutations in mouse lymphoma L1578Y $Tk^{+/-}$ cells, and chromosomal aberrations in Chinese hamster V79 cells and human lymphocytes. It has also been tested in vivo for its ability to induce micronucleus formation in bone marrow and peripheral blood cells of mice and unscheduled DNA synthesis in rat hepatocytes. In those studies, several formulations containing 10% synthetic lycopene were tested, and the outcomes were predominantly negative. In contrast, when oxidatively degraded, unformulated synthetic lycopene was tested for capacity to induce gene mutations in *S. typhimurium*, the outcome was positive. On the basis of those data and the results of the study of carcinogenicity in rats, the Committee concluded that synthetic lycopene, when formulated and, as such protected against oxidative processes, has no genotoxic or carcinogenic potential.

In a two-generation study of reproductive toxicity, rats received a diet mixed with a formulation containing 10% synthetic lycopene at target doses of 0, 50, 150, or 500 mg/kg bw per day. In the parental generation, apart from red-coloured faeces and yellow-orange staining of fur/skin/fat/abdominal organs attributable to the colour of lycopene, treatment with lycopene was only associated with marginal effects on body weight and food consumption ($F_1$ generation only). Mating performance and fertility, and survival and growth of the pups were not affected by treatment with lycopene. The NOELs for parental, reproductive and offspring toxicity were all 500 mg/kg bw per day, the highest dose tested.

The developmental toxicity of synthetic lycopene was evaluated via studies in which one of several 10% formulations was administered orally to rats (via diet and via gavage) and rabbits (via gavage) at up to maximum practical doses. Administration via the diet was better tolerated than was administration of large volumes of the highly viscous test substance via gavage. In all studies, dams showed red discoloured faeces, and in the gavage studies the contents of the gastrointestinal tract were red. Synthetic lycopene did not affect reproductive or fetal parameters in the studies in rats and rabbits, nor did it increase the overall number of external, visceral and skeletal abnormalities and variations. Given the absence of significant toxicological findings, the NOELs for maternal and developmental toxicity were 500 and 300 mg/kg bw per day in the feeding and gavage studies in rats, respectively, and 400 and 200 mg/kg bw per day in the gavage studies in rabbits, corresponding to the highest doses tested in those studies.

In reports in the literature, most studies in humans, although not specifically designed to assess the safety of lycopene, revealed no adverse effects after administration of dietary lycopene. There are, however, case reports of yellow-orange skin discoloration and/or gastrointestinal discomfort after prolonged high intakes of lycopene-rich food and supplements, those effects being reversible upon cessation of lycopene ingestion.

Since most of the available toxicological studies were performed with formulations of synthetic lycopene complying with the specifications, the safety of any impurities/reaction by-products present (if any) has been implicitly tested at their maximum permissible levels. Additional toxicological data available on apo- 12'-lycopenal and TPPO did not raise safety concerns.

*Dietary exposure assessment*

Lycopene is a normal constituent of the human diet owing to its presence in a number of vegetables and fruits. Dietary intakes of lycopene range from 1 to 10 mg/person per day, based on published estimates from eight countries. Additional exposure to lycopene would result from its proposed uses in a variety of food types, including flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings, sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. An estimate of high exposure (greater than 95th percentile), which includes intake from fruits and vegetables, is 30 mg/person per day. This estimate is based on food intake data from a number of national surveys, combined with proposed maximum levels for use of lycopene in food. This estimate is conservatively high in that it is assumed that lycopene would be present in all foods within a food type, at the maximum use level.

## *5. EVALUATION*

After ingestion, synthetic lycopene is considered to be equivalent to naturally-occurring dietary lycopene. Being a normal constituent of the human diet, background intake ranging from 1 to 10 mg/person per day, lycopene has a long history of consumption. Available data indicate that dietary lycopene is generally well tolerated in humans. After prolonged high intakes of lycopene-rich food and supplements, effects limited to yellow-orange skin discoloration and/or gastrointestinal discomfort have been reported. In the available toxicological studies, histopathological alterations of liver foci were observed in rats with synthetic lycopene at doses of greater than or equal to 50 mg/kg bw per day for 1 year and 10 mg/kg bw per day for 2 years. The significance of these treatment-related alterations for humans is not clear, given that there was no apparent sign of liver dysfunction and that they were without a consistent dose–response relationship. Moreover, although hepatocellular foci are commonly found at a high incidence in the ageing rat, they are extremely rare in humans. Only in parts of the world where, for example, hepatitis is endemic, low incidences of hepatocellular foci are found. Although foci can be precursors of liver neoplasia in rats, the Committee noted that treatment with synthetic lycopene did not cause progression of the foci to neoplasia in the 2-year study of carcinogenicity. The Committee also noted that many substances that are known to induce liver foci in rodents do not have a similar effect in humans. Taking all this into account, the Committee concluded that the observed histopathological alterations of liver foci in rats do not raise a safety concern for humans.

The Committee established an ADI of 0–0.5 mg/kg bw for synthetic lycopene based on the highest dose of 50 mg/kg bw per day tested in the 104-week study in

rats (at which no adverse effects relevant to humans were induced), and a safety factor of 100. This ADI was made into a group ADI to include lycopene from *B. trispora*, which was also under consideration at the present meeting and which was considered to be toxicologically equivalent to chemically synthesized lycopene. The estimate of high exposure (greater than 95th percentile) of 30 mg/person per day, equivalent to 0.5 mg/kg bw per day, and which includes background exposure, is compatible with the ADI.

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## *LYCOPENE FROM* BLAKESLEA TRISPORA

***First draft prepared by***

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### *1. EXPLANATION*

At the request of the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005), the JECFA Committee at its present meeting evaluated lycopene to be used as a food additive.

Lycopene is a naturally-occurring pigment found in vegetables (especially tomatoes), fruits, algae and fungi. It can also be synthesized chemically. The Committee had previously evaluated lycopene (both natural and synthetic) to be

used as a food colour at its eighth, eighteenth, and twenty-first meetings (Annex 1, references *8*, *35* and *44*). The lack of adequate information at those meetings precluded the Committee from developing specifications and establishing an acceptable daily intake (ADI) for lycopene to be used as a food colour. Under consideration at the present meeting were lycopene from the fungus *Blakeslea trispora* (the subject of this item) and synthetic lycopene (see monograph 'Lycopene (synthetic)', in this volume).

Lycopene from *B. trispora* is obtained by cofermentation of the (+) and (-) sexual mating types of the fungus. It is an intermediate in the biosynthesis of β-carotene from *B. trispora*, the safety of which was evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*). The Committee concluded at that meeting that the source organism *B. trispora* is neither pathogenic nor toxigenic, and that the production process and composition of β-carotene from *B. trispora* do not raise safety concerns.

Lycopene is extracted from the biomass of *B. trispora* and purified by crystallization and filtration, using the solvents isobutyl acetate and isopropanol. The process by which lycopene is produced from *B. trispora* is nearly identical to that used to manufacture β-carotene from *B. trispora*, the only difference being the addition of imidazole to the fermentation broth to inhibit the formation of β- and γ-carotene from lycopene.

## *2. BIOLOGICAL DATA*

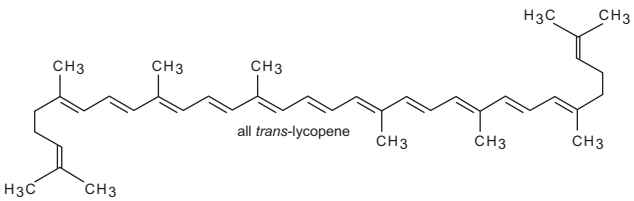
### *2.1 Biochemical aspects*

Lycopene belongs to the family of carotenoids and is a biochemical precursor of higher carotenoids, e.g. β-carotene. However, unlike β-carotene, lycopene lacks β-ionone ring structure and is therefore devoid of provitamin A activity.

Lycopene from *B. trispora* is biosynthesized via the same pathway as lycopene produced in the tomato, with the predominant lycopene isomer in the fermentation material being all-*trans*-lycopene (CAS No. 502-65-8).

The chemical name for lycopene is 2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene, and synonyms are *ψ,ψ*-carotene, all-*trans*-lycopene, and (all-*E*)-lycopene. Lycopene is an unsaturated acyclic hydrocarbon with the chemical structure shown in Figure 1.

***Figure 1. Chemical structure of lycopene***



### *2.1.1 Stereochemical isomerism*

All carotenoids contain an extended conjugated polyene backbone. Although theoretically each of these carbon–carbon double bonds can exist in either *trans* or *cis* configurations (also referred to as *E*- or *Z*-configurations, respectively), the vast majority of carotenoids, including lycopene, exhibit predominantly the all-*trans* configuration. Lycopene, however, can undergo conversion of one or more of its *trans*-configured carbon–carbon double bonds to the corresponding *cis* forms, a conversion that occurs more readily than with most other carotenoids. The c*is* isomers of lycopene have physical and chemical characteristics that are different to those of all-*trans*-lycopene, e.g. lower melting points and decreased colour intensity (Nguyen & Schwartz, 1999).

In most natural matriceos, all-*trans*-lycopene is thus quantitatively the most important isomer.[1] Among the naturally-occurring *cis* isomers, 5-*cis*-, 9-*cis*- and 13-*cis*-lycopene usually predominate. For illustrative purposes, the isomer composition of lycopene typically found in unprocessed, raw (red) tomatoes and in tomato-based foodstuffs is provided in Table 1 (Schierle et al., 1997). It is to be noted that in other fruits and vegetables, including tangerine-type tomatoes, other *cis* isomers and/or *cis*/*trans* isomer ratios may be prevalent. Processing and storage do not generally affect the lycopene content (Nguyen & Schwartz, 1999; Agarwal et al., 2001), but heat treatment has sometimes been reported to result in *trans* to *cis* isomerization, leading to a higher content of *cis* isomers in processed foods. Table 1 also contains data on lycopene in human plasma, in which lycopene is one of the most abundant carotenoids. A significant proportion of lycopene in human plasma is present as *cis* isomers (Khachik et al., 1997a; Schierle et al., 1997).

### *2.1.2 Specifications*

The Committee received one application for lycopene from *B. trispora* from Vitatene SA.

Lycopene crystals from *B. trispora* contain at least 95% total lycopene (of which at least 90% is all-*trans*-lycopene) and up to 5% other carotenoids (β- and γ- carotene). The extraction solvents isopropanol and isobutyl acetate may be present in the final product at concentrations of less than 0.1% and 1%, respectively. Imidazole used during fermentation may be found in lycopene at a concentration of less than 0.0001%.

The purity of lycopene from *B. trispora* is several times greater than that of lycopene extracted from tomatoes, which contains approximately 5% or more of total lycopene, while the *cis*/*trans* isomer ratio is comparable in lycopene from both sources.

Lycopene from *B. trispora* is a red crystalline powder. It is freely soluble in chloroform and tetrahydrofuran, sparingly soluble in vegetable oils, ether and hexane, and is insoluble in water. Lycopene is sensitive to light, heat and oxygen, but is stable when stored under inert gas in light-proof containers in a cool place.

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[1] Before 1992 it was not possible to separate 5-*cis*-lycopene analytically from all-*trans*-lycopene. Since the separation of the two stereoisomers became a standard only after 1998, earlier reported all-*trans*-lycopene contents may have been overestimated.

***Table 1. Isomer composition of lycopene in different samples***

| Sample | Isomer (as a percentage of total lycopene) | | | | |
|---|---|---|---|---|---|
| | All-*trans*-lycopene | 5-*cis*-lycopene | 9-*cis*-lycopene | 13-*cis*- + 15-*cis*-lycopene | Other *cis* isomers of lycopene |
| Raw tomatoes | 94–96 | 3–5 | 0–1 | 1 | < 1 |
| Cooked tomato-based foodstuffs | 35–96 | 4–27 | < 1–14 | < 1–7 | < 1–22 |
| Human blood plasma | 32–46 | 20–31 | 1–4 | 8–19 | 11–28 |

From Schierle et al. (1997)

Since it is not possible to get crystalline lycopene into an aqueous solution, and because of its susceptibility to oxidative degradation in the presence of light and oxygen, lycopene crystals are not suitable for commercial use. Only formulated material is marketed for use in food. Lycopene crystals from *B. trispora* are formulated as suspensions in edible oils or as water dispersible powders, and are stabilized with antioxidants. The other substances present in the marketed formulations (such as sunflower-seed oil and α-tocopherol) are common food ingredients and do not raise safety concerns.

### *2.1.3 Absorption, distribution and excretion*

#### *Rats*

Using mesenteric lymph-duct cannulated male albino rats as an animal model, Clark et al. (1998) investigated the absorption of purified lycopene (from tomato paste) after continuous infusion with a lipid emulsion (olive oil) containing 20 µmol/l of lycopene (2.5 ml/h) via a feeding tube placed into the duodenum. Lymph samples were collected for analysis at 2-h intervals up to 12 h after the start of infusion from three animals. In a second experiment, emulsions with four different concentrations of lycopene (5, 10, 15, 20 µmol/l) were intraduodenally infused into three rats per treatment, and samples of lymph collected at between 6 to 12 h of infusion were analysed (representing absorption under steady-state conditions). In a third experiment the possible interactions of lycopene (20 µmol/l) with canthaxanthin (20 µmol/l) were studied in four animals per treatment group Absorption, calculated by dividing the concentration of lycopene recovered in the lymph per hour by the concentration of lycopene infused into the duodenum per hour, ranged from 2 to 8%, with an average recovery of 6%. Lycopene was absorbed intact in a dose-dependent manner, with a steady-state reached in the lymph after 6 h of continuous intraduodenal infusion. Lycopene and canthaxanthin did not significantly affect each other's absorption (Clark et al., 1998). In an

additional study, similar to the second experiment, the effect of different oils (olive oil and corn oil) on the absorption of lycopene was investigated. Lycopene was less efficiently absorbed from corn oil emulsions (average recovery, 2.5%) than from olive oil emulsions (average recovery, 6%) (Clark et al., 2000).

The colonic absorption and distribution of lycopene was investigated in female Sprague-Dawley rats. Groups of six rats, with or without a single-barreled colostomy at the mid colon) received a single intragastric or intracolonic dose of 12 mg of lycopene (56 mg/kg bw; source not specified) in corn oil or plain corn oil (controls), and were euthanized 24 h after exposure for determination of lycopene content in the faeces and tissues (jejunum, colon, liver). Lycopene was detected in the blood in trace amounts within 4 to 8 h after the single dose, and was subsequently deposited in the liver, suggesting that lycopene is absorbed from the colon as well as the small intestine of rats. A large amount of lycopene was recovered in the faeces. After intragastric lycopene treatment, lycopene was detected in the mucosae of the proximal and distal colon of the colostomized rats (Oshima et al., 1999).

Groups of F344 rats received diets containing lycopene at a concentration of 0, 0.005, 0.012, 0.024, 0.05, or 0.124% for 10 weeks (equivalent to 0, 2.5, 6, 12, 25, or 60 mg of lycopene/kg bw per day, respectively) in a study investigating the uptake and tissue disposition of lycopene. There were 10 and 20 animals of each sex in the treated and control groups, respectively, and lycopene was administered as a tomato oleoresin (Betatene) containing 5.7% carotenoids (3.7% lycopene, 0.04% 2,6-cyclolycopene-1,5-diol and almost 2% others) in medium-chain triglyceride. Approximately 55% of the administered lycopene was recovered in the faeces. In males and in females, concentrations of lycopene were highest in the liver (42–120 µg/g of tissue), followed by mammary gland (174–309 ng/g of tissue), serum (145–313 ng/g of tissue), lung (134–227 ng/g of tissue), and prostate (47–97 ng/g of tissue). Tissue concentrations were not related to the dose administered (Zhao et al., 1998).

Other publications on the kinetics of lycopene in rats have also been provided (Mathews-Roth et al., 1990; Zaripheh et al., 2003; Zaripheh & Erdman, 2005), as well as publications on the kinetics of lycopene in dogs (Koryotko et al., 2003) and monkeys (Mathews-Roth et al., 1990). These studies have been summarized in section 2.1.3 of the monograph 'Lycopene (synthetic)', in this volume.

*Calves*

In a 2-week feeding study in preruminant calves, an evaluation was made of differences in lycopene absorption from a synthetic lycopene preparation (LycoVit 10%; 10% lycopene) versus lycopene from a natural tomato resin (Lyc-O-Mato Beads 5%; 5% lycopene). Groups of eight calves received lycopene as daily doses at 15 mg for 14 days in milk-replacer. Plasma was analysed for carotenoids before, directly after and at several time-points during a 10-day post-treatment period. In plasma, all-*trans*- and 5-*cis*-lycopene were present, as well as three lycopene metabolites not previously found. These metabolites (probably hydrogenation products of all-*trans*- and 5-*cis*-lycopene) contributed 52% to the total lycopene content measured at the end of the intervention period. On days 14 to 21, the concentration of total lycopene in the plasma in calves receiving lycopene from the

synthetic preparation (216–286 nmol/l) was more than three-fold that in calves fed lycopene from the tomato resin (46–72 nmol/l); however, no differences were observed in the distribution of the isomers and metabolites of lycopene (Sicilia et al., 2005).

*Humans*

No specific studies with lycopene from *B. trispora* in humans were available, but a number of publications were provided on the kinetics of lycopene in general. These have been summarized below.

Lycopene, like all carotenoids, is fat-soluble and follows the same digestion and intestinal absorption pathways as dietary fat (Rao & Agarwal, 1999). Absorption of dietary carotenoids begins with their release from the food matrix and dissolution in the lipid phase, followed by incorporation into lipid micelles in the small intestine, which is required for mucosal uptake, and finally, transport to the lymphatic and/or portal circulation (Erdman et al., 1993; Parker, 1996; Holloway et al., 2000). Movement of carotenoids from the mixed lipid micelle into the mucosal cells of the duodenum appears to occur via passive diffusion, and subsequent transport from the enterocytes to the blood stream involves incorporation into chylomicrons (with lycopene in the hydrophobic core), and secretion into the lymphatics. There is no evidence for significant portal absorption of carotenoids in humans (Parker, 1996). Carotenoids are transported in plasma exclusively by lipoprotein, with lycopene being transported primarily by low density lipoprotein (LDL) (Erdman et al., 1993; Parker, 1996; Holloway et al., 2000). Lycopene accumulates in tissues rich in LDL receptors such as liver, adrenals and testes (Holloway et al., 2000). Other human tissues containing detectable concentrations of lycopene include blood plasma, adipose tissue, prostate, and lung (Schmitz et al., 1991; Redlich et al., 1996; Rao & Agarwal, 1999). Reported tissue concentrations of lycopene vary between individuals by about 100-fold (Rao & Agarwal, 1999).

Lycopene is the most predominant carotenoid in human plasma. Lycopene reaches its maximum concentration in the plasma 24–48 h after dosing (Stahl & Sies, 1992) but with repeated dosing the blood concentration continues to rise until a steady state is reached. Rao & Agarwal (1999) reported a half-life of lycopene in plasma in the order of 2–3 days, while Cohn et al. (2004) reported estimated half-lives of 5 and 9 days for all-*trans*- and 5-*cis*-lycopene, respectively. When subjects were given a diet low in or without lycopene, their plasma lycopene concentrations were significantly lower after 1–2 weeks (Böhm & Bitsch, 1999; Porrini & Riso, 2000; Cohn et al., 2004); half-lives were then estimated to be between 12 and 33 days (Rock et al., 1992; Burri et al., 2001). Besides the effect of dietary fat (and dietary fibre content) on lycopene bioavailability, a number of conditions have been suggested to affect the absorption, metabolism or clearance of carotenoids, e.g. smoking and alcohol consumption (Bowen et al., 1993; Gärtner et al., 1997). Smokers have been reported to have lower plasma concentrations of most carotenoids than non-smokers. This was also demonstrated for lycopene by Pamuk et al. (1994). However, other studies in smokers (Peng et al., 1995; Brady et al., 1996; Driskell et al., 1996; Mayne et al., 1999) showed no effect of smoking on plasma lycopene concentrations. Chronic alcohol consumption has been reported

to decrease dietary absorption of carotenoids (Leo & Lieber, 1999). However, Brady et al., (1996) found no interaction between alcohol and plasma lycopene.

Plasma lycopene concentrations reported for controls in several studies ranged from 0.2 to 1.9 μmol/l (Schierle et al., 1997; Paetau et al., 1998; Mayne et al., 1999; Porrini & Riso, 2000; Hoppe et al., 2003; Cohn et al., 2004). The most abundant geometrical isomers in human plasma are all-*trans*-lycopene and 5-*cis*-lycopene, with all the *cis* isomers contributing to more than 50% of total lycopene (see also Table 1; Schierle et al., 1997; Rao & Agarwal, 1999). The most prominent geometric isomer that occurs in plant sources is all-*trans*-lycopene. While heat treatment and processing may result in *trans*- to *cis*-isomerization, leading to increases (< 10%) in the *cis*-lycopene content of foods (Schierle et al., 1997; Boileau et al., 2002), this cannot fully explain the higher concentrations of *cis* isomers found in human blood (and tissues) when compared with those in the foods consumed. Hence, biological conversions may take place in humans after consumption (Holloway et al., 2000). Indeed, exposure to low pH in the stomach has been shown to result in a small increase in *cis* isomers of lycopene (Boileau et al., 2002). It is not known whether the proportion of *cis* isomers is increased in plasma because of their greater intrinsic bioavailability or because of a faster catabolism of the all-*trans* isomer in the body, or both. It was speculated that, probably owing to the shorter length of the molecule, their greater solubility in mixed micelles and lower tendency to aggregate, *cis* isomers are more readily bioavailable (Boileau et al., 1999, 2002).

The bioavailability of carotenoids is affected by food preparation in the presence of lipids, and the functional status of the intestine. In humans, depending on the presence of fat in the meal, appreciable quantities of carotenoids are absorbed and can be found in circulating plasma and later in adipose tissue (Su et al., 1998). Studies have demonstrated that the absorption of lycopene is increased when it is ingested with a high-fat diet. The addition of oil to tomato juice before heating also improves lycopene bioavailability (Stahl & Sies, 1992; Fielding et al., 2005). Heat treatment in the processing of raw tomatoes results in the release of lycopene from the cellular matrix, making it more bioavailable (Gärtner et al., 1997).

Studies on the bioavailability of synthetic lycopene and lycopene from natural sources have reported conflicting results. Some studies did not find a difference in bioavailability between supplements containing natural lycopene extracts and lycopene from tomato products (Paetau et al., 1998; Rao & Agarwal, 1998), while others reported that lycopene from tomato juice or lycopene in the form of a tomato oleoresin was clearly better absorbed than lycopene from fresh tomatoes (Böhm & Bitsch, 1999). Tang et al. (2005) reported that lycopene from cooked tomatoes was about three times less bioavailable than synthetic lycopene dissolved in corn oil. However, no oil was added to the lycopene extracted from tomatoes, which might have had a certain effect on the bioavailability, as was reported by Stahl & Sies (1992) and Fielding et al. (2005). Other studies reported that the bioavailability of synthetic lycopene was comparable to that of lycopene extracted from tomatoes in the form of an oleoresin (Hoppe et al., 2003) or from tomato juice (Paetau et al., 1998). While the absorption of lycopene can be affected by other carotenoids (Wahlqvist et al., 1994; Gaziano et al., 1995), lycopene did not affect the absorption

of other carotenoids like α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, and lutein (Hoppe et al., 2003).

### *2.1.4 Metabolism*

The metabolic pathway of lycopene has not been fully described. A number of oxidative metabolites of lycopene have been identified in tomato paste, tomato juice and in human serum (Khachik et al., 1998a). The major metabolites were identified as lycopene 1,2-epoxide and lycopene 5,6-epoxide. Other minor metabolites were also identified, including 2,6-cyclolycopene-1,5-diol I and 2,6-cyclolycopene-1,5-diol II. A few of the metabolites are naturally found in tomato products at low concentrations. In human serum and human milk only 2,6-cyclolycopene-1,5-diol I and 2,6-cyclolycopene-1,5-diol II were found (Khachik et al., 1997a, 1998a). These two epimeric isomers are dihydroxylycopenes with a five-member ring end-group. Apparently, lycopene undergoes oxidation to yield lycopene 5,6-oxide followed by cyclization and enzymatic reduction to form the two epimeric isomers of 2,6-cyclolycopene-1,5-diol (Khachik et al., 1997b, 1998b). None of the products formed by cleavage in the respective 11 conjugated double bonds of lycopene (as described by Kim et al., 2001) were detected in the blood of humans.

### *2.1.5 Effects on enzymes and other biochemical parameters*

The effect of the administration of lycopene (purity, 98.7%) in corn oil on drug-metabolizing enzyme capacity (PROD, EROD, BROD, MROD, QR, UDPGT, and GST)[2], antioxidant enzyme activities and the ability to modulate PhIP[3]-induced DNA adducts in liver and colon and oxidative stress in blood was investigated in rats. Groups of four female Wistar rats received lycopene at a dose of 0 (corn oil only), 0.001, 0.005, 0.05 or 0.1 g/kg bw per day for 14 days. Blood was collected on day 14 before administration of the last dose in order to determine plasma lycopene concentration. The last dose was given in conjunction with tritium-labelled PhIP, and 24 h later, blood was taken, animals were killed and liver was removed for preparation of subcellular fractions. Plasma lycopene concentrations in the lycopene-supplemented groups were low (16–71 nmol/l), indicating poor absorption. BROD activity in the liver was induced in a dose-dependent fashion (up to and including 0.05 g/kg bw per day) at all doses investigated. EROD activity was induced only at the two highest concentrations tested. The extent of induction, however, was relatively minor for both enzymes (two-fold or less). Neither PROD nor MROD were affected at any dose. An investigation of selected phase II detoxification enzymes showed that lycopene is capable of inducing hepatic QR, approximately two-fold, at doses between 0.001 and 0.05 g/kg bw per day, while at the highest dose of 0.1 g/kg bw per day the activity was comparable to the control level. UDPGT and GST activity were only slightly increased (less than two-fold) at

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[2] EROD: ethoxyresorufin *O*-deethylase; MROD: methoxyresorufin *O*-demethylase; PROD: pentoxyresorufin *O*-dealkylase; BROD: benzyloxyresorufin *O*-dealkylase; QR: quinone reductase; UDPGT: uridine 5'-diphosphate glucuronosyltransferase; GST: glutathione transferase.

[3] PhIP: 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine

0.005 and 0.1 g/kg bw per day, respectively. Investigation of antioxidant enzymes showed slight induction of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) at the lower doses, while at the higher doses the (still slightly increased) activities returned to control levels. PhIP-induced oxidative stress in plasma was not affected by lycopene at any dose, nor was the level of PhIP-DNA adducts in liver or colon (Breinholt et al., 2000).

The minor inducing effects of lycopene on drug-metabolizing enzymes as observed in the above study were not observed in other studies. Gradelet et al. (1996) investigated phase I (PROD, EROD, BROD, MROD, NDMAD, ERDM)[4] and phase II xenobiotic-metabolizing enzyme activities (QR, GST, 4NP-UGT, 4-HBP-UGT, ALDH1, ALDH3)[5] in liver microsomes and cytosol of five male Wistar rats given diets containing lycopene at 300 mg/kg (as 5% lycopene oleoresin from tomato, mixed with corn oil) for 15 days. In this study, lycopene only affected the activity of NDMAD, which was reduced to 60% of the control value. In another study, eight male Wistar rats given 15 g per day of a diet containing 300 mg/kg lycopene (as 3.6% lycopene oleoresin from tomato, mixed with maize oil) for 16 days, after which the effect on xenobiotic metabolizing enzyme activities in the liver, lung, kidney and small intestine was investigated. BROD activity in the lung was reduced to 41% of control value, while other activities (EROD, PROD, MROD, GST) were not affected by lycopene treatment (Jewell & O'Brien, 1999). With the exception of hepatic QR, Zaripheh et al. (2005) did not observe changes in the activity of phase I (EROD, BROD, MROD) and phase II enzymes (QR, GST) in liver and selected extra-hepatic tissues of F344 rats fed lycopene at a concentration of 0.25 g/kg diet for periods of 0, 3, 7, 30, or 37 days, or for 30 days followed by a basal diet period for 7 days. Hepatic QR was increased in rats receiving the lycopene-supplemented diet for 3 and 7 days, but not in rats treated for 0, 30, or 37 days. Withdrawal of lycopene from the diet significantly reduced hepatic QR activity compared to the enzyme activity observed at 30 days of treatment.

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

No studies of acute toxicity were available with lycopene from *B. trispora*. Data were provided on the acute toxicity of lycopene from other natural sources. The acute toxicity of lycopene from a natural tomato oleoresin extract was tested in two studies stated to be certified in compliance with good laboratory practice (GLP), and which resembled OECD test guidelines 401 (limit test; 1987) and 402 (limit test; 1987). Three other studies of acute toxicity have been performed with lycopene extracted from tomatoes. Details on the conduct of these studies, however, were not available. The results are summarized in Table 2.

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[4] NDMAD: *N*-nitrosodimethylamine *N*-demethylase; ERDM: erythromycin *N*-demethylase.

[5] 4NP-UGT: *p*-nitrophenol UDP glucuronosyltransferase; 4-HBP-UGT: 4-hydroxybiphenyl UDP glucuronosyl-transferase; ALDH: aldehyde dehydrogenase.

### *2.2.2 Short-term studies of toxicity*

*Rats*

The possible toxicity of lycopene biomass (biomass of *B. trispora*; batch No. LC-411; 4.4% lycopene), extracted from the fermentation manufacturing process of lycopene, was examined in a short-term study in male and female Wistar rats. Based on the results of a 21-day range-finding study, groups of 20 rats of each sex were given diets containing the dried lycopene biomass at 0, 0.1, 0.3, or 1.0% (w/w) for 28 days, corresponding to mean daily intakes of 0, 90, 272, and 906 mg/kg bw in males, and 0, 87, 260, and 868 mg/kg bw in females. The study followed OECD guideline 407 (1995), and was certified for compliance with GLP and quality assurance (QA). Observations included clinical signs, neurobehavioural observations and motor activity assessment, growth, food consumption, food conversion efficiency, haematology, clinical chemistry, organ weights, gross necropsy, and histopathology.

Haematological measurements revealed statistically significant decreases in mean corpuscular volume and prothrombin time in male rats of the group at 1.0%. The decreases were, however, very small (3% and 6%, respectively), and other erythrocyte or coagulation variables were not significantly affected. Therefore, these findings were considered to be of no toxicological relevance. The only finding upon histopathology was a statistically significantly decreased incidence of 'increased hyaline droplet nephropathy' in males of the group at 1.0% (0 out of 20 versus 5 out of 20 in the control group), which is of no toxicological relevance. All other observations did not reveal treatment-related changes (Jonker, 2000).

Groups of 20 male and 20 female Wistar rats were given diets containing lycopene, as a suspension in sunflower-seed oil (20% w/w), at concentrations of 0, 0.25, 0.50, and 1.0% for 90 days. The lycopene examined in this study was derived from *B. trispora*. The sunflower-seed oil was used to balance the level of added fat in the experimental diets, i.e. about 4–5%. The corresponding mean intake of lycopene was calculated to be 0, 145, 291, and 586 mg/kg bw per day for males and 0, 156, 312, and 616 mg/kg bw per day for females. The study followed OECD guideline 408 (1998), and was stated to be certified for compliance with GLP. Plasma concentrations of lycopene were not determined in this study.

Clinical signs related to treatment with lycopene were limited to pink discoloration of the fur of all animals at the highest dose and many at the intermediate dose, resulting from direct contact with the red-staining lycopene present in the dietary admixture. Neurobehavioural testing, including functional observational battery and motor activity assessment, and ophthalmological examinations revealed no treatment-related effects.

There were no statistically significant or consistent differences in body weights, food or water consumption, organ weights, or in parameters of haematology, clinical chemistry or urine analysis between the treated and control groups.

The only treatment-related finding upon gross necropsy was a reddish discoloration of the contents of the gastrointestinal tract of all treated rats. This discoloration did not extend to any tissue. Histopathological examination did

***Table 2. Studies of acute toxicity with natural lycopene***

| Lycopene preparation | Species | Sex | Route | $LD_{50}$ (mg/kg bw) | Reference |
|---|---|---|---|---|---|
| Lyc-o-mato 6%[a] (natural tomato oleoresin extract) | Rat | M, F | Oral | > 5000 | Matulka et al. (2004) |
| Lyc-o-mato 6% (natural tomato oleoresin extract) | Rat | M, F | Dermal | > 2000 | Matulka et al. (2004) |
| Lycopene from tomatoes | Mouse | M | Oral | > 3000 | Milani et al. (1970) |
| Lycopene from tomatoes | Mouse | M | Subcutaneous | > 3000 | Milani et al. (1970) |
| Lycopene from tomatoes | Mouse | M | Intraperitoneal | > 3000 | Milani et al. (1970) |

M: male; F: female

[a] A 6% lycopene solution in tomato oleoresins, derived from food-processing tomatoes.

not reveal lycopene-related lesions. In the absence of toxicologically relevant findings, the no-observed-effect level (NOEL) was a dietary concentration of 1.0% lycopene, the highest dose tested, equal to 586 and 616 mg/kg bw per day for males and females, respectively (Jonker et al., 2003).

The short-term toxicity of a natural tomato oleoresin extract (NTOE) derived from processing tomatoes was investigated in a 13-week study of toxicity in CD rats. Groups of 20 male and 20 female rats were given the NTOE (Lyc-o-mato 6%, containing 6% lycopene) at dose of 0, 45, 450, or 4500 mg/kg bw per day diluted in peroxide-free corn oil and administered by gavage. These doses correspond to intakes of lycopene of 0, 2.7, 27, and 270 mg /kg bw per day, respectively. Body weight, food consumption, and clinical signs were observed throughout the study. Analyses of haematology, clinical chemistry, ophthalmoscopy, and urine analysis were conducted at various time-points through the study. At the end of the study, plasma concentration determination, organ weight measurements, and macroscopic and microscopic evaluation of a number of tissues were conducted. The study was stated to be certified for compliance with GLP.

Absorption was demonstrated by the presence of lycopene in plasma, with similar levels for the groups receiving the intermediate or highest dose and a lower level for the group at the lowest dose. Treatment-related signs were restricted to staining of the faeces (orange-coloured at the lowest dose (males only) and intermediate dose, red-coloured at the highest dose). Body-weight gain, food intake, and food conversion efficiency of treated animals were similar to the controls. There were neither ocular lesions nor significant treatment-related differences between groups in urine analysis parameters, organ weights, macroscopic and microscopic findings. Plasma alkaline phosphatase activities of treated animals were lower (not

statistically significantly) than those of the controls, which was thought to have been the consequence of the smaller volume of corn oil used in the treatment groups. Slightly higher blood urea concentration and leukocyte count and lower bilirubin concentration in females at the highest dose, and slightly increased platelet values in all treated females (no dose–response relationship) were not considered to be toxicologically relevant. The highest dose tested, 4500 mg/kg bw per day, equivalent to 270 mg of lycopene/kg bw per day, was reported to be the NOEL for NTOE (Matulka et al., 2004).

F344 rats received diets containing lycopene at a concentration of 0, 0.005, 0.012, 0.024, 0.05, or 0.124% (equivalent to lycopene at 0, 2.5, 6, 12, 25, or 60 mg/kg bw per day, respectively) for 10 weeks in a study investigating the uptake and tissue disposition of lycopene. There were 10 and 20 animals of each sex in the treated and control groups, respectively, and lycopene was administered as a tomato oleoresin (Betatene) containing 5.7% carotenoids (3.7% lycopene and 2% others) in medium-chain triglyceride. No adverse effects of lycopene treatment were noted on weight gain, behaviour, or coat appearance, with the exception of a brownish discoloration of the tail in a few experimental animals (Zhao et al., 1998).

In studies evaluating the potential effect of lycopene on liver and other tissue enzyme activity, no significant variations in comparison to controls were observed in food intake, body weight, or liver weight in a group of five male Wistar rats given diets containing lycopene at a concentration of 300 mg/kg (as 5% lycopene oleoresin from tomato, mixed with corn oil) for 15 days (Gradelet et al., 1996) or groups of four female Wistar rats receiving gavage administrations of lycopene (purity, 98.7%) in corn oil at doses ranging from 1 to 100 mg/kg bw per day for 14 days (Breinholt et al., 2000). Likewise, Jewell & O'Brien (1999) did not observe any differences in food intake, body weight, or organ weights (liver, lung, kidney, small intestine) of eight male Wistar rats receiving 15 g per day of a diet containing lycopene at 300 mg/kg (as 3.6% lycopene oleoresin from tomato, mixed with maize oil) for 16 days.

Publications on short-term studies of toxicity performed with synthetic lycopene have also been provided. McClain & Bausch (2003) described a 4- and a 14-week feeding study in rats, and Mellert et al. (2002) a 3-month study in rats treated by gavage. Other studies with synthetic lycopene not of classical toxicity design included a 30-day and 100-day feeding study in rats (Zaripheh & Erdman, 2005; Zbinden & Studer, 1958), and a 28-day and 192-day study in dogs given capsules (Koryotko et al., 2003; Zbinden & Studer, 1958). All these studies have been summarized in section 2.2.2 of the monograph 'Lycopene (synthetic)', in this volume. Synthetic lycopene was well tolerated in these studies. The only treatment-related findings were a red discoloration of the faeces and, in one study, also of contents of the gastrointestinal tract, owing to ingestion and excretion of the red-staining test substance. The liver appeared to be the target organ in the feeding studies, the discoloration observed being associated with orange-brown pigment deposits but not with histopathological liver damage. The Committee considered that the observations in the short-term studies of toxicity did not represent adverse effects. The NOELs for lycopene in the full studies of toxicity were 1000, 500 and

300 mg/kg bw per day for the 4-week, 14-week and 3-month study, respectively, corresponding to the highest doses tested in these studies.

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

No long-term studies of toxicity or carcinogenicity have been conducted with lycopene from *B. trispora*. Several studies were provided that examined the potential chemopreventive effects of lycopene on experimentally-induced or spontaneous (one study) tumorigenesis. In these studies, mice were given drinking-water containing lycopene at a concentration of up to 50 mg/l for up to 21 weeks (Kim et al., 1997; Kim et al., 1998), or a diet containing lycopene at a concentration of 0.5 or 700 mg/kg for 8–13 months or 28 weeks, respectively (Nagasawa et al., 1995; Black, 1998). Rats received lycopene at doses of up to 6 mg per animal twice in one week by gavage (Narisawa et al., 1996), in drinking-water at up to 25 mg/l for up to 35 weeks (Narisawa et al., 1998; Okajima et al., 1997), in the diet at up to 500 mg/kg for up to 19 weeks (Cohen et al., 1999; Jain et al., 1999; Wargovich et al., 2000), or up to 10 mg/kg bw via intraperitoneal injection, for 5 days (Wang et al., 1989) or twice per week for 18 weeks (Sharoni et al., 1997). These studies were however not designed to examine adverse health effects, so mostly only body weights, intakes (food and/or water) and sometimes some organs (weights or histopathology) were monitored. In general, these parameters did not show intergroup differences, but it must be noted that in almost all studies no appropriate negative control group (i.e. no carcinogen, and no lycopene) was included. Besides, the duration of treatment in the studies was short-term rather than long-term.

### *2.2.4 Genotoxicity*

The results of two studies of genotoxicity in vitro with lycopene from *B. trispora*[6] are summarized in Table 3 (CTBR, 2003a, 2003b). The studies followed OECD test guidelines 471 (1997) and 473 (1997), and were certified for compliance with GLP and QA. The results of some studies of genotoxicity with lycopene from other natural sources, as reported in literature, have also been summarized in Table 3.

A review on studies performed with synthetic lycopene has also been provided, in which several studies on genotoxicity in vitro and in vivo have been described (McClain & Bausch, 2003). The studies in vitro concerned gene mutation tests in bacteria (*Salmonella typhimurium*, *Escherichia coli*) and mammalian cells (mouse lymphoma L1578Y $Tk^{+/-}$ cells) and a chromosomal aberration test in human lymphocytes. The studies in vivo included tests for micronucleus formation in mice (bone marrow and peripheral blood) and a test for unscheduled DNA synthesis in rats (liver). All these studies are summarized in Table 2 of the monograph 'Lycopene (synthetic)', in this volume. Unformulated synthetic lycopene is sensitive to air and light, thereby forming mutagenic degradation products. When formulated and as such protected against oxidative processes, synthetic lycopene gave predominantly negative results in the studies on genotoxicity.

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[6] According to the applicant the lycopene tested in the CTBR studies was from *Blakeslea trispora*. The CTBR reports do not specifically mention the source of lycopene.

### *2.2.5 Reproductive toxicity*

No studies of reproductive or developmental toxicity have been conducted with lycopene from *B. trispora*. Three publications were provided, describing a two-generation study of toxicity in rats (Zbinden & Studer, 1958) and studies of developmental toxicity in rats and rabbits (Christian et al., 2003; McClain & Bausch, 2003) given synthetic lycopene. These studies have been summarized in section 2.2.5 of the monograph 'Lycopene (synthetic)', in this volume. Synthetic lycopene was reported to have not affected normal reproduction. In the studies of developmental toxicity, synthetic lycopene was generally tolerated well, without adverse effects on reproductive parameters or on the growth, survival and development of offspring. Synthetic lycopene was not teratogenic. As observed in short-term studies of toxicity, the only treatment-related finding was a red discoloration of the faeces and of the contents of the gastrointestinal tract. The NOEL for maternal and developmental toxicity was approximately 300 mg/kg bw per day for rats and approximately 200 mg/kg bw per day for rabbits, corresponding to the highest doses tested in the studies.

## *2.3 Observations in humans*

### *2.3.1 Case studies*

Reich et al. (1960) reported a case of lycopenodermia occurring in a female aged 61 years who consumed 2 l of tomato juice per day for several years (exact duration not specified). The subject suffered from recurrent bouts of abdominal pain, associated with nausea, vomiting and diarrhoea, and presented with orange-yellow discoloration of the skin on the hands, forearms, face, and soles of feet. Clinical and chemical investigation revealed unusually high serum lycopene concentrations and hepatic storage of lycopene pigments, as evidenced by large, round vacuolated parenchymal cells, and the presence of fatty cysts and fine yellow masses. The authors concluded that the subject suffered from lycopenodermia, caused by high intakes of lycopene from tomato juice (Reich et al., 1960).

Similar symptoms were reported for a female aged 19 years who consumed four to five large tomatoes plus pasta with tomato sauce daily for 3 years. A yellow-orange pigmentation was observed on the forehead, nasolabial folds, palms of the hands, and soles of the feet, and recurrent abdominal pain was reported. Hepatic echographia revealed liver alterations caused by deposits of lycopene, and when dietary intake of tomatoes was restricted, there was a complete regression of pigmentation and the abdominal pain disappeared. Based on clinical features and dietary history, the authors diagnosed the subject with lycopenodermia (La Placa et al., 2000).

Additional case studies documenting incidences of orange-yellow skin discoloration (e.g. carotenodermia) in individuals consuming diets rich in tomatoes or tomato products (e.g. tomato soup) have been reported (Bonnetblanc et al., 1987; Gandhi et al., 1988), and both have identified the lycopene content of the tomatoes/tomato products as the probable cause of the discoloration.

***Table 3. Studies of genotoxicity with lycopene from* Blakeslea trispora *and from other natural sources***

| End-point | Test system | Test substance | Concentration | Result | Reference |
|---|---|---|---|---|---|
| *In vitro* | | | | | |
| Reverse mutation | *S. typhimurium* TA98, TA100 | Lycopene from tomato paste (in DMSO) | 100 µg/plate, ± S9 | Negative[a] | He & Campbell (1990) |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA* | Lycopene from tomato paste (in DMSO) | 0.05–5000 µg/plate, ± S9 | Negative[a] | Aizawa at al. (2000) |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *E. coli* WP2 *uvrA* | Lyc-o-mato 6%[b] (natural tomato oleoresin extract; in water) | 312.5–5000 µg/plate, ± S9 | Negative[c] | Matulka et al. (2004) |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA* | Lycopene 20% CWD[d] (in water) | Up to 5000 µg/plate, ± S9 | Negative[e] | CTBR (2003a) |
| Chromosomal aberration | Human lymphocytes | Lycopene 20% CWD (in water) | 1280, 2560, or 5000 µg/ml, ± S9 | Negative[f] | CTBR (2003b) |
| *In vivo* | | | | | |
| Micronucleus formation | Mouse peripheral blood | Lycopene in tomato juice (1:1 diluted with water) | 54 mg lycopene/l, ad libitum for 14 days[g] | Negative | McClain & Bausch (2003) |
| DNA damage (Comet assay) | Human lymphocytes | Lycopene in tomato juice | 40 mg lycopene/day, for 2 weeks | Negative[h] | Pool-Zobel et al. (1997) |
| DNA damage (Comet assay) | Human lymphocytes | Lycopene in tomato purée | 16.5 mg lycopene/day, for 21 days | Negative[i] | Riso et al. (1999) |

***Table 1.*** (contd.)

| | | | | | |
|---|---|---|---|---|---|
| Spontaneous mutation | LacZ mouse DNA from lung, colon and prostate | Lycopene-rich tomato oleoresin[j] | 7 or 14 g/kg diet, for 9 months | Negative | Guttenplan et al. (2001) |

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rat liver

[a] With and without metabolic activation from S9. No further details available.

[b] A 6% lycopene solution in tomato oleoresins, derived from food-processing tomatoes.

[c] With and without metabolic activation from S9, using the plate incorporation method. No cytotoxicity was observed. Precipitation was seen at 5000 µg/plate.

[d] Batch no. 154LC1, containing 21.2% lycopene in an octenyl succinic anhydride starch matrix with α-tocopherol.

[e] With and without metabolic activation from S9, using both the plate incorporation method and the preincubation method. No cytotoxicity was observed. Red precipitate was present at all doses but did not interfere with scoring.

[f] With and without metabolic activation from S9. The cells were exposed for 4 h without and with S9 and were harvested 17 h later, or for 21 h without S9 and harvested immediately thereafter. No effects on relative mitotic index were observed.

[g] Equivalent to 3.7–12.4 mg of lycopene/kg bw per day, estimated based on an average (water) intake of 3-10 ml/day for growing mice and an average body weight of 43.5 g. No toxic effects were observed.

[h] Lycopene suppressed the level of DNA strand breaks, but not the level of oxidized pyrimidine bases.

[i] Lycopene reduced lymphocyte DNA damage as induced by hydrogen peroxide.

[j] Betatene, a 5.7% carotenoid suspension (3.7% lycopene and 2% others) in medium-chain triglycerides.

Hence, prolonged high intakes of lycopene-containing food accompanied by high serum concentrations of lycopene can result in a cutaneous disease referred to as lycopenodermia. Lycopenodermia can be accompanied by the presence of lycopene deposits in focal areas of the liver, often resulting in the formation of fatty cysts, and by abdominal pain. It is reversible upon cessation of lycopene ingestion.

### *2.3.2 Clinical trials*

A number of clinical trials were provided, mostly with a focus on investigating the antioxidant properties of lycopene supplementation. Safety-related end-points were only addressed in few of these studies (see short summaries below), but were limited to reporting of adverse symptoms and/or measurement of some haematology and clinical chemistry parameters.

In a dietary intervention study in 23 healthy male volunteers, administration of 40 mg of lycopene (as tomato juice) for 2 weeks was tolerated well and did not significantly affect blood haemoglobin, leukocyte count, serum electrolytes, or serum cholesterol (Müller et al., 1999).

In a cross-over dietary intervention study, 19 healthy subjects (10 males and 9 females) received daily 39.2 mg of lycopene from spaghetti sauce, 50.4 mg of lycopene from tomato juice, 75 mg of lycopene from tomato oleoresin capsules, and a placebo. All four treatments lasted 1 week, followed by a 1-week washout phase. Treatment with lycopene had no effect on plasma lipid (total, HDL- and LDL-cholesterol, and triglyceride) concentrations, and no adverse symptoms were reported throughout the duration of the study (Agarwal & Rao, 1998).

No adverse effects were reported in groups of 12 men and 12 women consuming daily 15 mg of encapsulated synthetic (LycoVit 10%) or tomato-derived lycopene (Lyc-O-Mato Beads 5%) in a 28-day study (Hoppe et al., 2003).

After placing 34 healthy female volunteers on a lycopene-rich diet for 1 week (providing approximately 40 mg of lycopene per day), no changes were observed in leukocyte count, lipid profile, liver function tests, and serum creatine kinase activity (Chopra et al., 2000).

No effects were found on concentrations of total and HDL-cholesterol, and triglycerides in groups of female volunteers (six to eight per group) treated for 6 weeks with a daily dose of 5 mg of lycopene obtained from three different sources (i.e. tomato oleoresin soft gel capsules, tomato juice, or raw tomatoes) (Böhm & Bitsch, 1999).

In a multi-cohort study in healthy French (38 males and 37 females), Irish (72 males and 66 females), Dutch (33 males and 39 females), and Spanish volunteers (32 males and 32 females), serum responses to carotenoids and tocopherols were measured upon supplementation with capsules containing 15 mg of carotenoids (carotene-rich palm oil, lutein, or lycopene) in corn oil or corn oil only (placebo), with or without 100 mg of α-tocopherol. The volunteers, divided into four groups of each sex per cohort, received supplements over 20 weeks. In the first 4 weeks, they received α-tocopherol only, in the next 12 weeks either carotene-rich palm oil, lutein, lycopene, or placebo, and in the final 4 weeks the

carotenoids/placebo with α-tocopherol. Only in the Spanish cohort was monitoring carried out for biochemical and haematological indices, plasma total, HDL- and LDL-cholesterol concentrations, and any incidences of adverse events. Results of haematological analysis and clinical chemistry in this cohort were unremarkable and no significant changes were observed in plasma lipid concentrations. Carotenodermia was reported in 25% of the subjects in the Spanish cohort supplemented with lycopene, compared with 95% of those supplemented with carotene and 40% of those supplemented with lutein (Olmedilla et al., 2002).

In a double-blind study conducted by Postaire et al. (1997), healthy subjects (10 per group) received either 26 mg of β-carotene plus 4 mg of lycopene per day or 6 mg of β-carotene plus 6 mg of lycopene in capsules for 8 weeks. A slight change in the yellow, but not red, pigmentation of the skin (i.e. carotenodermia) was observed in the group receiving β-carotene at a high dose. A significant increase in skin melanin concentration was found in both treatment groups.

Prostate cancer patients ($n = 32$) received approximately 30 mg of lycopene per day from tomato sauce-based pasta dishes during a 3-week intervention segment of a clinical trial. Patients were asked to record any gastrointestinal adverse effects (i.e. constipation, burping, gas and/or flatulence, nausea, bloating, diarrhoea, cramping, and heartburn). With the exception of three patients who reported minor gastrointestinal disturbances, which resolved within a few days, the dietary intervention was well accepted (Chen et al., 2001).

In a randomized clinical trial in 15 patients with prostate cancer, subjects were treated with 15 mg of lycopene (as Lyc-O-Mato capsules) twice per day for 3 weeks, during which time subjects were asked to report any adverse events, and were subjected to full physical examinations and a complete blood count and chemistry profile assessment. Another group of 11 patients that did not receive lycopene was also monitored. None of the subjects reported any adverse events and no abnormalities were observed in blood counts or clinical chemistry (Kucuk et al., 2001).

Taken together, these studies show that administration of dietary lycopene is generally well tolerated, with adverse effects limited to minor gastrointestinal disturbances in prostate cancer patients and dermal discoloration in healthy people treated with lycopene for several weeks.

## *3. DIETARY INTAKE*

### *3.1 Introduction*

Lycopene is a naturally-occurring pigment found in a number of fruits and vegetables, notably tomato, watermelon, guava, pink grapefruit, and apricot. It is also produced by a number of algae and fungi. These foods are common to most cultures; therefore, lycopene is already part of the diet. Because of evidence that the consumption of lycopene is associated with some potential health benefits, food processors are seeking to add lycopene to processed foods. Such foods include: flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings,

sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. Additionally, lycopene is available directly as a dietary supplement. The Committee considered the additional exposure to lycopene from its proposed food uses in context with the naturally-occurring background intake from food.

The Committee received dossiers concerning lycopene exposure from three food-ingredient processors and obtained additional information from the published literature and national government sources (BASF, 2006; DSM, 2006; Vitatene, 2006). Lycopene can be obtained from a number of sources: extracted from fruits, such as tomatoes or watermelons, extracted from fungi, such as *Blakeslea trispora*, or chemically synthesized from smaller organic starting materials. Although the Committee was asked to evaluate both synthetic lycopene and lycopene derived from *B. trispora*, the proposed food uses/use levels for lycopene from *B. trispora* encompass those of synthetic lycopene (with the single exception of cereal bars), therefore, the estimated exposure was considered together. Although purified lycopene is unstable when exposed to light and temperatures above room temperature, it is known to be stable in a food matrix (processed tomato products) (Agarwal et al., 2001).

### *3.2 Background exposure to lycopene from food*

Concentrations of lycopene in fruit and vegetables typically vary from approximately 1 mg/100 g of fresh fruit to 20 mg/100 g, with some specially-bred tomatoes having concentrations reaching 40 mg/100 g. In processed tomato products, such as ketchup, juice, and pastes, the lycopene can be concentrated to up to 100 mg/100 g food (Lugasi et al., 2003). National dietary exposures to lycopene have been estimated and reported in the literature. Table 4 summarizes some of these estimates. The estimates consistently range from approximately 1 to 10 mg/person per day, with the sole exception of a Canadian study that showed background intakes of approximately 25 mg/day. The Committee noted that the concentrations of lycopene measured in foods and used in this exposure study were consistently three to five times higher than those used in the other studies. No explanation of these high levels was reported. An international estimate of lycopene intake from food was prepared using the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets. Based primarily on tomato intake, the estimates ranged from 1 to 7 mg/person per day in the five regional diets (DSM, 2006).

In a submission to the Food and Drug Administration's Generally Recognized As Safe (GRAS) notification programme, a food-ingredient processor estimated that the mean background intake of lycopene from food in the USA was 8.2 mg/day (GRN 156; US Food and Drug Administration, 2002b). According to a second submission, it was stated that an intake of 5 mg/day was comparable to background intake (GRN 119; US Food and Drug Administration, 2002a). These estimates are consistent with the reported national estimates and the GEMS/Food international estimates.

***Table 4. National estimates of intake of lycopene from natural dietary sources***

| Country | Intake(s) (mg/person per day) | Reference |
|---|---|---|
| Canada | 6.3, 25 | Johnson-Down et al. (2002); Agarwal et al. (2001) |
| Finland | 0.8 | Järvinen (1995) |
| France | 2.0 | DSM (2006) |
| Germany | 1.3 | Pelz et al. (1998) |
| Hungary | 4.4 | Lugasi et al. (2003) |
| Netherlands | 1.3 | Goldbohm et al. (1998) |
| United Kingdom | 1.0, 5.0 | Scott et al. (1996); DSM (2006) |
| United States of America | 0.6, 1.6 | Vanden Langenberg et al. (1996) |
| | 3.7, 3.1, 11 | Forman et al. (1993); Yong et al. (1994); McClain & Bausch (2003) |

### *3.3 Exposure to added lycopene from proposed food uses*

The Committee considered the proposed uses for lycopene in numerous food categories, including flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings, sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. Table 5 contains food categories and associated proposed use levels for lycopene from *B. trispora*. The food category system (Annex B) of the General Standard for Food Additives (GSFA) of the Codex Alimentarius Commission [CODEX STAN 192-1995 (Rev. 6-2005)] was used to classify the uses. The highest use level proposed (either for the USA or the EU) appears in the table. These food category/use level combinations were used with food intake information from the US Continuing Survey of Food Intake by Individuals (1994–6, 1998 supplement) and the UK National Diet and Nutrition Survey (1992–3, 1997) to derive estimates of added lycopene intake for the USA and European populations (Table 6 and Table 7, respectively). Food intakes from a Dutch Survey (DNFCS-3, 1997–8) were combined with food category/use level combinations (Table 8) from the dossier concerning synthetic lycopene to estimate intake from that source (Table 9). Additional analyses using French and UK food intake data were provided; estimated intakes were less than those presented in Table 9, and are not presented in this monograph (DSM, 2006).

Each of the analyses suggests that an intake of approximately 30 mg/day could be expected for a consumer at or above the 95th percentile of the intake distribution. This high-percentile intake is approximately 10 times greater than mean background intakes of lycopene (it would be expected that high-percentile background intake might be two or more times greater than the mean). The conservatisms in each analysis have been noted: all foods that might contain added lycopene would do so, and at the proposed maximum use level. Because the concentrations for food additive use are approximately four times higher than those needed to colour food, the estimated intake of 30 mg/person per day covers uses of lycopene as a food colour.

***Table 5. Proposed food uses for lycopene from* Blakeslea trispora**

| Food category | Use level (mg/kg) |
|---|---|
| *01.0 Dairy products and analogues* | |
| 01.1.2 Cultured dairy drinks | 20 |
| 01.1.2 Dairy-based fruit drinks | 20 |
| 01.7 Gelatin desserts, puddings, and custards | 25 |
| *02.0 Fats, oils, and fat emulsions* | |
| 0.2.2.2 Table fat spreads | 5 |
| *05.0 Confectionery* | |
| 05.2.1 Hard candy | 25 |
| 05.2.2 Jelly products | 25 |
| 05.4 Decorations, fillings, and icings | 25 |
| *06.0 Cereals and cereal products* | |
| 06.3 Ready-to-eat cereals | 50 |
| *07.0 Bakery wares* | |
| 07.1.2 Crackers | 30 |
| *12.0 Salts, spices, soups, salads, protein products, and fermented soybean products* | |
| 12.5.1 Prepared and condensed soups | 7 |
| 12.5.2 Dry soup mixes | 575 |
| 12.6.1 Low-fat salad dressings | 20 |
| 12.6.2 Tomato-based gravies and specialty sauces | 50 |
| 12.6.2 Non-emulsified sauces, seasonings, relishes, and pickles | 50 |
| 12.9 Meat substitutes | 50 |
| *13.0 Foodstuffs intended for particular nutritional uses* | |
| 13.4 Dairy- and non-dairy-based meal replacement beverages | 25 |
| *14.0 Non-dairy beverages* | |
| 14.1.2.1 Fruit juices | 25 |
| 14.1.3.1 Fruit nectars | 25 |
| 14.1.4 Energy, sport, and isotonic Drinks | 25 |
| 14.1.4.2 Fruit-flavoured drinks | 25 |
| *15.0 Ready-to-eat savouries* | |
| 15.1 Nutrient bars | 50 |
| 15.1 Salty snacks | 30 |

From General Standard for Food Additives of the Codex Alimentarius Commission (CODEX STAN 192-1995, Rev. 6, 2005).

***Table 6. Summary of the estimated daily intake of lycopene from* Blakeslea trispora*, USA 1994–1996***

| Population group | Age group (years) | % Users | Actual no. of total users | Consumption (mg/person per day) | | | |
|---|---|---|---|---|---|---|---|
| | | | | All-person | | All-user | |
| | | | | Mean | 90th percentile | Mean | 90th percentile |
| Infants | 0 to 2 | 78.7 | 2,591 | 6.7 | 15.4 | 8.5 | 17.0 |
| Children | 3 to 11 | 99.6 | 6,132 | 10.7 | 19.5 | 10.8 | 19.5 |
| Female teenagers | 12 to 19 | 98.8 | 707 | 10.6 | 21.3 | 10.7 | 21.6 |
| Male teenagers | 12 to 19 | 98.1 | 714 | 13.1 | 27.1 | 13.3 | 27.1 |
| Female adults | 20 and up | 97.2 | 4,534 | 7.1 | 15.2 | 7.3 | 15.3 |
| Male adults | 20 and up | 96.5 | 4,834 | 9.6 | 20.4 | 10.0 | 20.9 |

From 1998 USDA CSFII Data

***Table 7. Summary of the estimated daily intake of lycopene from* Blakeslea trispora, *UK, 1992–1993, 1997***

| Population group | Age group (years) | % Users | No. of total users | Consumption (mg/person per day) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | All-person | | | All-user | | |
| | | | | Mean | 90th %ile | 95th %ile | Mean | 90th %ile | 95th %ile |
| Children | 1.5–4.5 | 95.5 | 1574 | 7.6 | 14.6 | 18.7 | 7.9 | 14.7 | 19.0 |
| Young people | 4–10 | 97.5 | 816 | 8.3 | 15.8 | 18.3 | 8.5 | 15.9 | 18.6 |
| Female teenagers | 11–18 | 93.5 | 417 | 6.4 | 13.6 | 15.9 | 6.7 | 14.1 | 16.5 |
| Male teenagers | 11–18 | 92.3 | 384 | 7.6 | 16.6 | 20.8 | 8.3 | 17.0 | 21.5 |
| Female adults | 16–64 | 72.4 | 694 | 3.1 | 7.7 | 10.6 | 4.0 | 8.6 | 12.0 |
| Male adults | 16–64 | 71.5 | 548 | 3.5 | 9.0 | 11.5 | 4.5 | 10.0 | 13.2 |

%ile: percentile; From UK National Diet and Nutrition Survey data, 1992–1993, 1997.

***Table 8. Proposed food uses for synthetic lycopene***

| Food category | Use level (mg/kg) |
|---|---|
| Fruit and vegetable juice | 25 |
| Fruit juice drinks | 25 |
| Soft drinks | 25 |
| Squash | 25 |
| Sports and energy drinks | 25 |
| Dairy fruit drinks | 25 |
| Yogurt, yogurt drinks | 50 |
| Cereals | 80 |
| Cereal bars | 80 |
| Crackers | 100 |
| Margarine, low-fat spreads | 40 |
| Hard candy | 70 |

***Table 9. Estimated daily intake of synthetic lycopene, Netherlands, 1997–1998***

| Population group (years) | Intake (mg/person per day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All-person | | | | All-user | | | |
| | N | Mean | 50th %tile | 95th %tile | N | Mean | 50th %tile | 95th %tile |
| Child aged 1–3 | 254 | 13.2 | 11.8 | 29.2 | 253 | 13.2 | 11.8 | 29.2 |
| Boys aged 4–9 | 242 | 15.9 | 15.0 | 29.6 | 241 | 16.0 | 15.0 | 29.6 |
| Girls aged 4–9 | 272 | 15.0 | 14.1 | 28.4 | 272 | 15.0 | 14.1 | 28.4 |
| Males aged 10–18 | 391 | 18.9 | 17.7 | 36.8 | 390 | 18.9 | 17.7 | 36.8 |
| Females aged 10–18 | 380 | 16.5 | 14.9 | 32.7 | 380 | 16.5 | 14.9 | 32.7 |
| Males aged 19 and older | 2117 | 8.8 | 6.5 | 24.9 | 2042 | 9.1 | 6.7 | 25.2 |
| Females aged 19 and older | 2544 | 8.3 | 6.2 | 22.7 | 2431 | 8.7 | 6.6 | 22.9 |

%tile: percentile
From Dutch DNFCS-3, 1997–1998 data

## *4. COMMENTS*

*Toxicological data*

The Committee considered the results of short-term studies of toxicity and studies of genotoxicity that had been performed with formulations of lycopene from *B. trispora* complying with the specifications as prepared at the present meeting, and that met appropriate standards for study protocol and conduct.

In a short-term study of toxicity, rats received diets mixed with a suspension of 20% (w/w) lycopene in sunflower-seed oil, resulting in dietary concentrations of lycopene of 0, 0.25, 0.50, or 1.0%, equal to approximately 0, 150, 300, and 600 mg/kg bw per day respectively, for 90 days. Lycopene from *B. trispora* was well tolerated, and there were no adverse effects. The only treatment-related finding was a red discoloration of the contents of the gastrointestinal tract, caused by ingestion of the red-staining test substance. The NOEL for lycopene was approximately 600 mg/kg bw per day, the highest dose tested.

Lycopene from *B. trispora* has been tested in vitro for its capacity to induce reverse mutations in *S. typhimurium* and *E. coli* and chromosomal aberrations in human lymphocytes. In those studies, lycopene was formulated as 20% cold water-dispersible product. Lycopene gave negative results in both studies.

No studies of acute toxicity, long-term studies of toxicity or studies of reproductive and developmental toxicity have been conducted with lycopene from *B. trispora*. No data were available on the bioavailability of formulated lycopene from *B. trispora*, but it is expected that after ingestion lycopene from *B. trispora* is equivalent to natural dietary lycopene, because the other components in the final formulations are also present in food.

The Committee also considered a number of published studies of pharmacokinetics and metabolism, tolerance, acute toxicity, genotoxicity, and short-term studies of toxicity with lycopene derived from other natural sources. The materials tested in those studies (e.g. tomato-derived [oleoresin] extracts, tomato paste, tomato juice) did not comply with the food-additive specifications for lycopene from *B. trispora*, and several studies were not aimed at examining adverse health effects. Nonetheless, the Committee was able to conclude that there is evidence for a similar kinetic profile indicating low absorption of orally administered lycopene in laboratory species and humans, that little is known about the metabolism of lycopene and that, taken as a whole, the results are consistent with low toxicity, show no evidence for genotoxicity, and generally reveal no adverse effects in humans after administration of dietary lycopene. There is also evidence for a common feature in the alteration of the isomeric ratio to favour *cis* isomers after consumption of lycopene, given that all-*trans*-lycopene is less abundant in plasma of humans and animals than it is in lycopene in foods. This is also likely to be the case for lycopene from *B. trispora*.

On the basis of the observed phenomenon of *trans*- to *cis*-isomerization after ingestion, the Committee concluded that differences in *trans* and *cis* isomer ratio of lycopene from *B. trispora* and other lycopenes (whether from other natural sources

or chemically synthesized) are not toxicologically relevant. The Committee thus considered lycopene from *B. trispora* to be toxicologically equivalent to chemically synthesized lycopene.

*Dietary exposure assessment*

Lycopene is a normal constituent of the human diet owing to its presence in a number of vegetables and fruits. Dietary intakes of lycopene range from 1 to 10 mg/person per day, based on published estimates from eight countries. Additional exposure to lycopene would result from its proposed uses in a variety of food types, including flavoured dairy beverages, yogurts, candies, cereals, soups, salad dressings, sauces, fruit and vegetable juices, sports drinks, carbonated beverages, and cereal and energy bars. An estimate of high exposure (greater than 95th percentile), which includes intake from fruits and vegetables, is 30 mg/person per day. This estimate is based on food intake data from a number of national surveys, combined with proposed maximum levels for use of lycopene in food. This estimate is conservatively high in that it is assumed that lycopene would be present in all foods within a food type, at the maximum use level.

## 5. EVALUATION

Lycopene from *B. trispora* is considered to be toxicologically equivalent to chemically synthesized lycopene, for which an ADI of 0–0.5 mg/kg bw was established by the Committee at its present meeting. This was given further credence by the negative results obtained for lycopene from *B. trispora* in two tests for genotoxicity, and the absence of adverse effects in a short-term study of toxicity considered at the present meeting. The ADI for synthetic lycopene was therefore made into a group ADI of 0–0.5 mg/kg bw to include lycopene from *B. trispora*.

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## PROPYL PARABEN (addendum)

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### *1. EXPLANATION*

The parabens (methyl, ethyl, and propyl *p*-hydroxybenzoate) having a functional use as preservatives in food were evaluated by the Committee at its sixth, ninth, tenth and seventeenth meetings (Annex 1, references *6*, *11*, *13* and *32*). At its seventeenth meeting, the Committee established a group acceptable daily intake (ADI) of 0–10 mg/kg bw (expressed as the sum of methyl, ethyl, and propyl esters of *p*-hydroxybenzoic acid). Additional information subsequently became available concerning the estrogenic and reproductive effects of the parabens, which led the European Food Safety Authority (European Food Safety Authority, 2004) to exclude propyl paraben from the group ADI for the parabens. At its Thirty-seventh Session (Codex Alimentarius Commission, 2005), the *Codex Committee on Food Additives and Contaminants,* placed propyl paraben on the priority list for toxicological re-evaluation by JECFA.

## 2. BIOLOGICAL DATA

### 2.1 Biochemical aspects

#### 2.1.1 Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism and excretion of the parabens were studied in rats, rabbits and dogs some years ago and have been described earlier by this Committee (Annex 1, reference *33*). In summary, the methyl, ethyl, and propyl esters of *p*-hydroxybenzoic acid are well absorbed and the ester linkage is readily hydrolysed by liver esterases, as indicated by rapid excretion and high urinary levels of *p*-hydroxybenzoic acid and conjugated products of hydrolysis.

### 2.2 Toxicological studies

#### 2.2.1 Reproductive toxicity

Several of the parabens have been tested for effects on reproductive function and for estrogenic activity, in vitro and in vivo. In order to compare the results for propyl paraben with those for other parabens, relevant studies on all the parabens are described below.

*(a) Effects on juvenile and adult males*

Groups of eight male Wistar rats aged 3 weeks were given diets containing methyl or ethyl *p*-hydroxybenzoate at a dose of 0.00%, 0.1%, or 1.0% for 8 weeks, corresponding to average intakes of methyl *p*-hydroxybenzoate of 103 and 1030 mg/kg bw per day and ethyl *p*-hydroxybenzoate of 103 and 1043 mg /kg bw per day, respectively. After 8 weeks, the rats were killed, blood was taken for hormone assays, testes, epididymides, prostate, seminal vesicles and preputial glands were weighed, sperm counts in testes and epididymides were determined and the testes were examined morphologically. Neither compound had an effect on weights of the reproductive organs, on sperm counts in the testes and epididymides, or on the morphological examinations of spermatogonia, spermatocytes, round spermatids and elongated spermatids. Serum concentrations of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were not affected (Oishi, 2004).

Groups of eight male Wistar rats aged 3 weeks were given diets containing propyl *p*-hydroxybenzoate at a dose of 0.00%, 0.01%, 0.10% or 1.00% for 4 weeks, corresponding to approximate intakes of propyl-*p*-hydroxybenzoate of 10, 100 and 1000 mg/kg bw per day, respectively. The basal diet used was a modified AIN93G diet devoid of soya-based phytoestrogens. After 4 weeks, the rats were killed, blood was taken for hormone assays, testes, epididymides, prostate, seminal vesicles and preputial glands were weighed and sperm counts in testes and epididymides were determined. There were no effects on the weights of the reproductive organs. The cauda epididymal sperm reserves and concentrations were significantly decreased in rats in the groups receiving the intermediate and highest doses. Sperm reserves were 43.6, 31.1, 25.7 and 22.5 x $10^7$/cauda in control group and in the

groups receiving the lowest, intermediate and highest dose, respectively. Sperm concentrations were 108, 70.8, 63.1 and 48.8 x $10^7$/g, respectively. The daily sperm production (DSP) and its efficiency (DSP/g) in the testes were also significantly lower in all treated groups compared with controls. DSP in treated groups was about 70% of the control value; however, no dose–response relationship was observed (37.5, 26.2, 27.0 and 25.9 x $10^6$ in the control, group and in the groups receiving the lowest, intermediate or highest dose, respectively). Serum testosterone was significantly decreased in a dose-dependent manner, the reduction being significant at the highest dose (9.08, 8.20, 7.17 and 5.86 ng/ml in the control group, and in the groups receiving the lowest, intermediate and highest dose, respectively) (Oishi, 2002a).

Groups of eight male Crj:CD-1 mice aged 4 weeks were given diets containing butyl *p*-hydroxybenzoate at a dose of 0.00%, 0.01%, 0.10% or 1.00% for 10 weeks, corresponding to average intakes of butyl *p*-hydroxybenzoate (calculated from food consumption) of 14.4, 146 and 1504 mg/kg bw per day, respectively. After 10 weeks, the mice were killed, blood was taken for testosterone assay, testes, epididymides, ventral prostate, seminal vesicles and preputial glands were weighed, sperm counts in testes and epididymides were determined and the testes were examined morphologically. Dose-dependent reductions were observed in spermatid counts in the seminiferous tubules; the reduction in round spermatids was statistically significant at the highest dose and the reductions in elongated spermatids were statistically significant at all three doses. Serum testosterone was significantly decreased by about 45% at the highest dose (Oishi, 2002b).

Groups of eight Wistar rats aged 3 weeks were given diets containing butyl *p*-hydroxybenzoate at a dose of 0.00%, 0.01%, 0.10% and 1.00% for 8 weeks, corresponding to average butyl p-hydroxybenzoate intakes of 10, 100 and 1000 mg/kg bw per day, respectively. At the end of 8 weeks, the rats were killed, blood was taken for testosterone assay, testes, epididymides, ventral prostate, seminal vesicles and preputial glands were weighed, and sperm counts in testes and epididymides were determined. The absolute weight of the seminal vesicles was significantly reduced at the highest dose and there was a dose-related reduction in absolute weights of the epididymides, reaching statistical significance at the highest dose. The cauda epididymal sperm reserve of all treated groups was significantly decreased. The sperm count of the highest dose group was 58% of the control value. The DSP and efficiency in the testis was also significantly lower in all treated groups, in a dose-related manner. Serum testosterone concentrations also showed dose-related reductions that were significant at the intermediate and highest doses (Oishi, 2001).

Butyl *p*-hydroxybenzoate has been reported to show potent spermaticidal activity in human spermatozoa in vitro as a result of impairment of the sperm membrane function. In this respect, butyl *p*-hydroxybenzoate was three and eight times more potent than propyl or methyl *p*-hydroxybenzoate, respectively (Song et al., 1989, 1992). In human sperm in vitro, propyl-*p*-hydroxybenzoate was an effective spermicide at a concentration of 3 mg/ml (Bao-Liang et al., 1989).

*(b) Effects on offspring*

Neonatal Wistar rats were given butyl *p*-hydroxybenzoate subcutaneously at a dose of 2 mg/kg bw per day on postnatal days 2–18. Diethylstilbestrol and ethinylestradiol were used as positive controls. Testes were weighed at age 18, 25, 35 and 75 days. Both positive-control substances caused significant reductions in testes weight at each time-point, but butyl *p*-hydroxybenzoate had no effect on testis weight or other aspects of testis development (distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, immunoexpression of the water channel aquaporin-1) (Fisher et al.,1999).

Pregnant Sprague-Dawley rats were injected subcutaneously with butyl-*p*-hydroxybenzoate at a daily dose of 100 or 200 mg/kg bw from day 6 of gestation to postnatal day 20 and the offspring were examined at postnatal day 21, 49, 70 and 90. In the group exposed to 200 mg/kg bw per day, the proportion of pups born alive and of pups surviving to weaning were significantly decreased. In both treatment groups, the body weights of female offspring were significantly decreased at postnatal day 49, 70 and 90. The weights of testes, seminal vesicles and prostate glands and the days to vaginal opening were significantly decreased in male and female offspring, respectively on postnatal day 49, but not on postnatal day 70 or 90, in rats exposed to 100 mg/kg bw per day. No such effects were observed after 200 mg/kg bw per day. The weights of female reproductive organs were not affected. The sperm count and the sperm motile activity in the epididymis were decreased at postnatal day 90 after 100 and 200 mg/kg bw per day. Testicular expression of estrogen receptor mRNAs was increased in the 200 mg/kg bw per day group at postnatal day 90 (Kang et al., 2002).

*(c) Studies on estrogenic effects*

*In vitro*

Methyl, ethyl, propyl and butyl *p*-hydroxybenzoate, 4-*n*-dodecyl-*p*-hydroxybenzoate acid and *p*-hydroxybenzoic acid were tested in the in-vitro recombinant yeast estrogen screen (YES), in which yeast cells are transfected with the human estrogen receptor alpha (ERα) gene. Weak positive reactions were observed with the butyl, propyl, ethyl, and methyl esters (butyl > propyl > ethyl > methyl), while 4-*n*-dodecyl-*p*-hydroxybenzoate and *p*-hydroxybenzoic acid were without activity. The relative potency of 17β-estradiol (E2) was approximately 2 500 000 times greater than that of methyl *p*-hydroxybenzoate. The magnitude of the estrogenic response increased with alkyl group size, with 17β-estradiol having a relative potency of 150 000, 30 000 and 10 000 times greater than the ethyl, propyl and butyl esters, respectively. The effect of propyl *p*-hydroxybenzoate and butyl *p*-hydroxybenzoate was inhibited by addition of the anti-estrogen, 4-hydroxy-tamoxifen, demonstrating that these compounds had to interact with the estrogen receptor in order to show estrogenic activity. In a competitive binding assay it was also found that non-metabolized butyl *p*-hydroxybenzoate was able to compete with $^{3}$H-17β-estradiol for binding to the rat estrogen receptor with an affinity approximately five orders of magnitude lower than diethylstilbestrol and between one and two orders of magnitude less than 4-nonylphenol (Routledge et al., 1998).

Methyl, ethyl, propyl, butyl, benzyl and dodecyl *p*-hydroxybenzoate were tested in the YES assay in another study. Relative to 17β-estradiol, estrogenic activity was detected for the propyl (1/30 000), butyl (1/8 000) and benzyl *p*-hydroxybenzoates (1/4 000). The relative potencies of the methyl and ethyl esters were insignificant at 1/3 000 000 and 1/200 000, respectively and no activity was detected for the dodecyl ester. A total of 73 phenolic compounds were assayed in this study and the authors concluded that the presence of an unhindered -OH group in the *para* position and a relative molecular mass in the range of 140 to 250 were necessary for activity (Miller et al., 2001).

Using a standardized estrogen receptor competitive-binding assay, seven parabens were tested for their ability to displace $^{3}$H-17β-estradiol from the estrogen receptor (obtained from uteri from ovariectomized Sprague-Dawley rats) and their relative binding affinity (RBA) was calculated. 2-Ethylhexyl *p*-hydroxybenzoate (RBA = 0.018%) was the most potent paraben tested, followed by heptyl (0.008%), benzyl (0.003%), butyl (0.0009%), propyl (0.0006%), ethyl (0.0006%), and methyl *p*-hydroxybenzoate (0.0004%). For comparison, RBAs for 4-nonylphenol and bisphenol A in this assay were 0.035% and 0.008%, respectively (Blair et al., 2000).

The estrogenic activity of parabens in vitro was found to increase in the order methyl, ethyl, propyl, butyl, isopropyl and isobutyl *p*-hydroxybenzoate by assaying estrogen receptor-dependent proliferation of human MCF7 breast cancer cells. Their potencies were $10^{5}$ to $10^{7}$ times lower than that of 17β-estradiol. Using a competitive binding assay it was also shown that the parabens had similar relative (to diethylstilboestrol) binding affinities (RBA) to the human estrogen receptors α (ERα) and β (ERβ). The RBA values for the parabens ranged from 0.01% to 0.1% of that of diethylstilbestrol (Okubo et al., 2001).

Another study on the estrogenic effects of methyl, ethyl, propyl and butyl *p*-hydroxybenzoate in estrogen-sensitive human MCF7 breast cancer cells had also been reported. Competitive inhibition of $^{3}$H-17β-estradiol binding to MCF7 cell estrogen receptors could be detected at $10^{6}$-fold molar excess of butyl (86% inhibition), propyl (77%), ethyl (54%) and methyl *p*-hydroxybenzoate (21% inhibition). At concentrations of $10^{-6}$ mol/l the parabens also increased the expression of estrogen-regulated genes in the MCF7 cells and increased the proliferation of the cells in monolayer culture. Molecular modelling showed that the parabens bind to the ligand-binding domain of the ERα-receptor (Byford et al., 2002).

Using similar experimental methods, isobutyl and benzyl *p*-hydroxybenzoate were also shown to be estrogenic in MCF7 cells and in another estrogen-sensitive human breast-cancer cell line, ZR-75-1, but to have no effect on the estrogen-insensitive MDA-MB-231 human breast-cancer cell line. Competitive inhibition of $^{3}$H-17β-estradiol binding was detected at 100 000-fold molar excess of isobutyl (81% inhibition) and benzyl *p*-hydroxybenzoate (57% inhibition). At concentrations of $10^{-5}$ mol/l, these parabens maximally increased the expression of estrogen-regulated genes in the MCF7 cells and increased the proliferation of the MCF7 and ZR-75-1 cells in monolayer culture. The proliferation induced in MCF7 cells by both parabens could be inhibited by the anti-estrogen, ICI 182 780 (Darbre et al., 2002, 2003).

The expression profiling of 120 estrogen-responsive genes in MCF7 cells treated with methyl, ethyl, propyl or butyl *p*-hydroxybenzoate has been investigated. Significant correlations were obtained for the propyl (R = 0.74) and butyl (R = 0.60) esters, but not for the methyl (R = -0.21) and ethyl (R = 0.19) esters (Terasaka et al., 2006).

In MCF7 cells, the common metabolite of the parabens, *p*-hydroxybenzoic acid, is reported to have weak estrogenic activity, It displaced 17β-estradiol from the estrogen receptor by 54% at $5 \times 10^6$-fold molar excess and by 99% at $10^7$-fold molar excess. It also increased the expression of a stably transfected estrogen-responsive chloramphenicol acetyltransferase reporter gene (ERE-CAT) at $5 \times 10^{-4}$ mol/l in MCF7 cells after 24 h and 7 days and this could be inhibited by the anti-estrogen, ICI 182 780. Proliferation of MCF7 and ZR-75-1 cells was also increased by *p*-hydroxybenzoic acid at $10^{-5}$ mol/l. Methyl *p*-hydroxybenzoate was reported to have a relative binding activity similar to that of *p*-hydroxybenzoic acid, but to have a greater activity on gene expression and cell proliferation in these test systems (Pugazhendhi et al., 2005).

The estrogenic activity of methyl, ethyl, propyl and butyl *p*-hydroxybenzoate and *p*-hydroxybenzoic acid itself was investigated using three reporter cell lines derived from the human cervical epithelioid carcinoma HeLa cell line, transfected with either the human ERα or the human ERβ gene. Neither *p*-hydroxybenzoic acid nor the methyl ester was active in either cell line at a concentration of up to $10^{-5}$ mol/l. Estrogenic activity increased with increasing alky chain length, with the ethyl, propyl and butyl esters showing activity in both cell lines at concentrations of $10^{-6}$ mol/l (propyl and butyl esters) or > $10^{-6}$ mol/l (ethyl ester) (Gomez et al., 2005).

Combinations of xenoestrogens, which included benzyl *p*-hydroxybenzoate, have been shown to produce an estrogenic effect in vitro in the YES assay, although the concentrations of each chemical were below their individual thresholds for effect in the assay (Rajapakse et al., 2002).

*In vivo*

Methyl and butyl *p*-hydroxybenzoate have been tested in uterotrophic assays using both immature and ovariectomized (OVX) rats treated by oral gavage and/or subcutaneous administration. OVX rats were only dosed subcutaneously. Methyl *p*-hydroxybenzoate at oral or subcutaneous doses up to 800 mg/kg bw per day for 3 days did not increase the uterus weight in immature rats and failed to increase uterus weight or vaginal cornification in OVX rats. Butyl *p*-hydroxybenzoate did not produce a statistically significant increase in uterus weight in immature rats treated by oral administration at a dose of up to 1200 mg/kg bw per day for 3 days. However, subcutaneous administration of butyl *p*-hydroxybenzoate at doses of 400–800 mg/kg bw per day significantly increased wet weights of the uterus in immature rats. The relative potency of the butyl ester was at least 10 000-fold less than that of subcutaneously administered 17β-estradiol. In the OVX rats, subcutaneous doses of 1000–1200 mg/kg bw per day were needed to produce statistically significant uterotrophic effects (Routledge et al., 1998).

Methyl, ethyl, propyl and butyl *p*-hydroxybenzoate have been tested in an uterotrophic assay in mature OVX CD1 mice treated by subcutaneous administration. The parabens were given for 3 days at two different equimolar doses of 362 and 1086 µmol/kg bw per day, corresponding to 55 and 165, 60 and 180, 65 and 195 and 70 and 210 mg/kg bw per day, for the methyl, ethyl, propyl and butyl *p*-hydroxybenzoate, respectively. Both doses of all the parabens tested caused a significant increase in uterine wet weight compared with controls, accompanied by hypertrophy of the uterine luminal and glandular epithelium and myometrium. Relative potencies, compared with 10 µg of 17β-estradiol (100), were in a similar range (0.02–0.009) for all four parabens, i.e. 5 000 to 25 000 times less potent than estradiol. The luminal epithelial hyperplasia response suggested that the estrogenic activity increased with increasing alkyl chain length (Lemini et al., 2004).

In mice, subcutaneous administration of isobutyl *p*-hydroxybenzoate at a dose of 12 mg/day (approximately 720 mg/kg bw per day) for 3 days and topical administration of three daily doses of benzyl *p*-hydroxybenzoate at 33 mg/animal (approximately 2000 mg/kg bw per day) applied to the dorsal skin, increased the uterine weight in immature female CD1 mice. Relative potencies, compared with 17β-estradiol, were 2 400 000 and 330 000 times lower for the isobutyl and butyl parabens, respectively. Topical administration of benzyl *p*-hydroxybenzoate as three daily doses at 10 mg/animal (approximately 750 mg/kg bw per day) to the dorsal skin of immature mice did not significantly increase uterine weight (Darbre et al., 2002, 2003).

The estrogenic activity of *p*-hydroxybenzoic acid (the main metabolite of the *p*-hydroxybenzoic acid esters in mammals) was investigated in CD1 mice. A dose-dependent response on vaginal cornification and uterotrophic activities in both immature and adult OVX mice was seen after subcutaneous administration of *p*-hydroxybenzoic acid at 5 mg/kg bw per day for 3 consecutive days. The potency was calculated to be approximately 1000 times less than that of 17β-estradiol (Lemini et al., 1997).

The estrogenic activity of methyl, ethyl, propyl and butyl *p*-hydroxybenzoate and their shared main metabolite, *p*-hydroxybenzoic acid, was investigated in mouse and rat uterotrophic assays. Immature B6D2F[1] mice were treated by oral gavage or subcutaneously with the test compounds at a dose of 1, 10 or 100 mg/kg bw per day for 3 consecutive days; none of the compounds tested produced any estrogenic responses and, for ethyl *p*-hydroxybenzoate, there was no response even at an oral dose of 1000 mg/kg bw per day. In immature Wistar rats, *p*-hydroxybenzoic acid was inactive when administered subcutaneously at a dose of 5 mg/kg bw per day. Butyl *p*-hydroxybenzoate produced no response at a dose of 200 or 400 mg/kg bw per day, and only a weak estrogenic response at 600 mg/kg bw per day; the paraben was at least 6000 times less potent than the positive control, estradiol benzoate at 0.1 mg/kg bw per day (Hossaini et al., 2000). Thus the estrogenic effect of *p*-hydroxybenzoic acid given subcutaneously at 5 mg/kg bw per day, as reported by Lemini et al. (1997), was not confirmed in this study, even at a subcutaneous dose of up to 100 mg/kg bw per day.

*(d) Studies on androgenic and anti-androgenic effects*

*In vitro*

Isobutyl, butyl, isopropyl, propyl, ethyl and methyl *p*-hydroxybenzoate have been studied for androgenic and anti-androgenic activity in two different types of stably transfected CHO-K1 cell lines. None of the compounds had androgenic activity. Isobutyl, butyl, isopropyl, propyl *p*-hydroxybenzoate all showed anti-androgenic activity. They showed weak inhibitory effects on dihydrotestosterone (DHT)-induced luciferase activity, with $IC^{50}$ values ranging from 4.2 to $8.6 \times 10^{-5}$, compared with $IC_{50}$ values of $3.0 \times 10^{-7}$ and $7.0 \times 10^{-7}$ for the known anti-androgens, cyproterone acetate and flutamide, respectively. The ethyl and methyl *p*-hydroxybenzoate did not show any significant activity, with $IC_{50}$ values of $> 1.0 \times 10^{-4}$ (Satoh et al., 2005).

### 2.3 Observations in humans

The presence of small amounts of parabens in tissue samples from human breast tumours has been reported. Samples of breast tumours were obtained from 20 patients and analysed by high-performance liquid chromatography (HPLC) and tandem mass spectrometry for methyl, ethyl, propyl, butyl, isobutyl, and benzyl *p*-hydroxybenzoate. The reported mean total concentration of parabens was 20.6 ng/g tissue (blank value of 33.8 ng/g subtracted). Methyl *p*-hydroxybenzoate was the paraben present in the greatest amount (12.8 ng/g) followed by propyl *p*-hydroxybenzoate (2.6 ng/g). Benzyl *p*-hydroxybenzoate could not be detected. The authors calculated a corrected (for 50% recovery) average total paraben concentration of 100 ng/g, and compared this with the concentration of approximately 150 ng/ml ($10^{-6}$ mol/l) of propyl, butyl, and isopropyl *p*-hydroxybenzoate known to stimulate estrogen-dependent growth in MCF7 human breast cancer cells (Darbre et al., 2004).

## 3. DIETARY INTAKE

No exposure data were provided to the Committee for this assessment and no specific information on the intake of propyl paraben was available. No information on the food categories in which the parabens can be used or on maximum permitted use levels were available in the GSFA online Food Additives Group details for methyl, ethyl, propyl parabens and their sodium salts.[1]

In the European Union (EU), methyl, ethyl-, and propyl *p*-hydroxybenzoates and their salts have been permitted as food additives in six categories of processed foods, as shown in Table 1.

In the United States of America (USA), the parabens are used as an ingredient in food at levels that should not exceed those consistent with good manufacturing practices (GMP). Current GMP allows for a maximum level of 0.1%

[1] See United States Code of Federal Regulations, Title 21: Food and Drugs at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm.

***Table 1. Permitted use levels of parabens in the European Union***

| EU food category | Maximum permitted level (mg/kg)[a] | Codex GSFA food category |
|---|---|---|
| Snacks – potato, cereal, flour or starch-based and processed nuts, including coated nuts and nut mixtures | 300 | 15.1<br>15.2 |
| Confectionery (excluding chocolate) | 300 | 5.2 |
| Liquid dietary food supplements | 2000 | 13.6 |
| Jelly coatings of meat products (cooked, cured or dried) | 1000 | 8.2 and 8.3 |
| Surface treatment of dried meat products | *Quantum satis*[b] | 8.2.1.2 |

EU, European Union; GSFA, General Standard for Food Additives
[a] Maximum permitted level in the EU, singly or in combination for the group
[b] 'Sufficient' quantity

in food (Title 21 of the US Code of Federal Regulations, Sections 172.145, 184.1490 and 184.1670, see also section 172.[1]

An exposure assessment was performed for parabens in a report from the European Commission on dietary food additive intake in the EU according to the guidelines on first conservative estimates (Tier 1). Intakes were below the EU temporary group ADI for the parabens of 10 mg/kg bw per day (European Commission, 2001).

An estimate of intake was made for this meeting of the Committee based on maximum permitted use levels and from food consumption data available from an EU report (European Commission, 2004). Results, as shown in Table 2, indicate that, even if it was assumed that all four food categories consumed by high consumers contain parabens at the EU maximum permitted level, the sum of these exposures together would not exceed the group ADI of 10 mg/kg in any subgroup of the population, including young children.

*Heptylparaben*—Part 172 Food additives permitted for direct addition to food for human consumption, Subpart B – Food Preservatives, Section 172.145; *Methylparaben*-—Part 184 Direct food substances affirmed as generally recognized as safe, Subpart B – Listing of Specific Substances Affirmed as GRAS, Section 184.1490;

*Propylparaben*-—Part 184 Direct food substances affirmed as generally recognized as safe, Subpart B – Listing of Specific Substances Affirmed as GRAS, Section 184.1670; and Part 172 Food additives permitted for direct addition to food for human consumption, Subpart F – Flavoring Agents and Related Substances - Section 172.510 - Natural flavoring substances and natural substances used in conjunction with flavours.

***Table 2. Estimates of total intakes of parabens used in food***

| Geographical area, date | Estimated intakes (mg/day) | Estimated intakes in average to high quantiles (mg/kg bw per day) |
|---|---|---|
| USA, 2002 | 222 to 466 [a] | 3.7 to 7.8 [c] |
| EU, 2004 | 72 to 318 [b] | 1.2 to 5.3 [c] |
| | 60 to 140 [b] | 2 to 9.3 [d] |

[a] Sum of the average and 90th percentiles of potential intake based reported for six food categories
[b] Sum of the average and 95th percentiles of potential intake reported for four food categories
[c] Assuming an average body weight of 60 kg for adults
[d] Assuming an average body weight of 30 kg for children and 15 kg for small children

A publication from Soni et al. (2002) has assessed exposure to parabens in food in the USA in the population aged between 2 and 65 years. Based on similar conservative assumptions to those used for the EU estimates, dietary intake as shown in Table 2 varied from an average of 222 mg/day to 466 mg/kg for consumers at the 90th percentile, equivalent to about 4–8 mg/kg bw per day for an adult, assuming, a body weight of 60 kg.

## *4. COMMENTS*

*Toxicological data*

Data on endocrine and reproductive effects are available from studies in vitro and in vivo with various parabens, including the three parabens used in food, and on their common metabolite, *p*-hydroxybenzoic acid. They show that the likelihood of such effects is related to the length of the alkyl chain, with occurrence and potency increasing with increasing chain length. The three parabens used as food additives (methyl, ethyl and propyl *p*-hydroxybenzoate) are those with the shortest chain length.

The parabens have been shown to exhibit weak estrogenic activity in a number of test systems in vitro. They are able to bind to the estrogen receptors ERα and ERβ and to stimulate proliferation in estrogen-dependent mammalian cell lines. In these test systems, estrogenic potency increases with increasing length and branching of the alkyl chain in the following order: methyl < ethyl < propyl < butyl < isopropyl < isobutyl < benzyl < heptyl < 2-ethylhexyl *p*-hydroxybenzoate. For example, in assays screening for estrogenic activity in recombinant yeast (using yeast cells transfected with the human ERα gene), the relative potency of 17β-estradiol was around 3 million-fold that of methyl *p*-hydroxybenzoate; and that of 17β-estradiol was 150 000–200 000-fold that of ethyl *p*-hydroxybenzoate. The relative potency of 17β-estradiol was 30 000-fold and 10 000-fold that of the propyl and butyl esters, respectively. One study has reported that the common metabolite of the parabens, *p*-hydroxybenzoic acid, shows estrogenic activity by several

measures in estrogen-dependent mammalian cell lines, with relative binding affinity to the estrogen receptor being 500 000 times lower than that of 17β-estradiol. Two other studies on *p*-hydroxybenzoic acid have reported that it is inactive in vitro. The Committee considered that the relevance for human health, if any, of very weak estrogenic activity in vitro is unclear at present.

The estrogenic activity of the parabens and their common metabolite, *p*-hydroxybenzoic acid, has been tested in vivo in uterotrophic assays in immature or ovariectomized mice or rats treated by oral, subcutaneous or topical dermal administration. While methyl, ethyl and propyl parabens showed uterotrophic activity after dosing by the subcutaneous route, none of those were active in the uterotrophic assay when given orally by gavage at doses of up to 800 mg/kg bw per day for the methyl paraben, up to 1000 mg/kg bw per day for the ethyl paraben and up to 100 mg/kg bw per day for the propyl paraben. For *p*-hydroxybenzoic acid, one study reported an uterotrophic effect in mice after subcutaneous administration, but this was not confirmed in a subsequent study in which it was given orally or subcutaneously at higher doses than in the first study.

Several studies have investigated the effects of parabens on male reproductive parameters in rodents. Juvenile rats given diets containing propyl paraben at doses equivalent to about 10, 100 or 1000 mg/kg bw per day for 4 weeks showed dose-related reductions in epididymal sperm reserves and sperm concentrations at the intermediate and highest doses, reductions in daily sperm production in the testis and reductions in serum concentrations of testosterone in all treated groups. In a similar study, in which diets containing butyl paraben at the same doses were given for 8 weeks, similar effects were observed but they were more marked than those with propyl paraben and, in addition, epididymal and seminal vesicle weights were reduced. Similar effects on sperm counts and serum concentrations of testosterone were observed in juvenile mice given butyl paraben at dietary doses of 15–1500 mg/kg bw per day for 10 weeks. In contrast to butyl and propyl parabens, neither methyl paraben nor ethyl paraben showed any effects on male reproductive organs, sperm parameters or sex hormones in juvenile rats given dietary doses of up to 1000 mg/kg bw per day for 8 weeks. There are insufficient data to conclude whether the effects observed with parabens of higher alkyl chain length in males are mediated via an estrogenic, anti-androgenic or some other mechanism.

*Dietary exposure assessment*

No specific information on the intake of propyl paraben was available to the Committee. Estimates of total dietary intake of parabens by consumers have been calculated, using the respective use levels from the USA and the EU and assuming an average adult body weight of 60 kg. In the USA, average to 90th-percentile intakes range from 3.7 to 7.8 mg/kg bw per day. In the EU, average to 95th-percentile intakes range from 1.2 to 5.3 mg/kg bw per day. The estimates are highly conservative, being based on the assumption that parabens are used in all possible foods at the highest maximum permitted levels.

## 5. EVALUATION

The Committee concluded that, in view of the adverse effects in male rats, propyl paraben (propyl *p*-hydroxybenzoate) should be excluded from the group ADI for the parabens used in food. This conclusion was reached on the grounds that the group ADI was originally set on a no-observed-effect level (NOEL) of 1000 mg/kg bw per day for a different toxicological end-point—growth depression—taken from the range of studies then available for the methyl, ethyl and propyl parabens. Propyl paraben has shown adverse effects in tissues of reproductive organs in male rats at dietary doses of down to 10 mg/kg bw per day, which is within the range of the group ADI (0–10 mg/kg bw), with no NOEL yet identified.

The Committee maintained the group ADI of 0–10 mg/kg bw for the sum of methyl and ethyl esters of *p*-hydroxybenzoic acid.

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# FOOD CONTAMINANTS

## *ALUMINIUM FROM ALL SOURCES, INCLUDING FOOD ADDITIVES (addendum)*


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## 1. EXPLANATION

### 1.1 Introduction

Various aluminium compounds were evaluated by the Committee at its thirteenth, twenty-first, twenty-sixth, twenty-ninth, thirtieth and thirty-third meetings (Annex 1, references *20*, *44*, *59*, *70*, *73* and *83*). At its thirteenth meeting, an acceptable daily intake (ADI) 'not specified' was established for sodium aluminosilicate and aluminium calcium silicate (Annex 1, reference *20*). At its thirtieth meeting, the Committee noted concerns about a lack of precise information on the aluminium content of the diet and a need for additional safety data. The Committee set a temporary ADI of 0–0.6 mg/kg bw expressed as aluminium for all aluminium salts added to food, and recommended that aluminium in all its forms should be reviewed at a future meeting.

In the evaluation made by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*), emphasis was placed on estimates of consumer exposure, absorption and distribution of dietary aluminium and possible neurotoxicity, particularly the relationship between exposure to aluminium and Alzheimer disease. The Committee set a provisional tolerable weekly intake (PTWI) of 0–7.0 mg/kg bw for aluminium, including food additive uses. This was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing sodium aluminium phosphate (SALP) acidic at a concentration of 3% for 189 days, equivalent to approximately 110 mg/kg bw aluminium. A consolidated monograph was produced (Annex 1, reference *84*).

Aluminium was re-evaluated by the Committee at its present meeting, as requested by the Codex Commission on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005).

The Committee was asked to consider all data relevant to the evaluation of the toxicity and intake (including bioavailability) of aluminium used in food additives and from other sources, including SALP. CCFAC asked that the exposure assessment cover all compounds included in the Codex General Standard for Food Additives (GSFA).

Two documents were particularly important in the evaluation made by the Committee at its present meeting: the International Programme on Chemical Safety (IPCS) *Environmental Health Criteria* document on aluminium (WHO, 1997) and a report by the United Kingdom (UK) Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) on a water pollution incident that occurred in Cornwall, England in 1988 (COT, 2005). The Committee used those assessments as the starting point for its evaluation and also evaluated other data in the scientific literature relating to aluminium compounds. No original toxicological data on aluminium-containing food additives were submitted.

### *1.1.1 General considerations on exposure*

Aluminium is the third most abundant element and a major constituent of the earth's crust, where it is present as $Al^{3+}$ in combination with oxygen, fluorine, silicon and other constituents, and not in the metallic elemental state. It is released to the environment both by natural processes and from anthropogenic sources. It is naturally present in varying amounts in most foodstuffs, and concentrations in food crops are influenced by geographical region. Use of aluminium and aluminium compounds in processing, packaging and storage of food products, and as flocculants in the treatment of drinking-water may contribute to its presence in drinking-water and foods. A number of aluminium salts are used as food additives (see section 6.1.4). In general, the foods that contain the highest concentration of aluminium are those that contain aluminium additives (WHO, 1997).

## *2. BIOLOGICAL DATA*

## *2.1 Biochemical aspects*

### *2.1.1 Absorption, distribution and excretion*

#### *(a) Absorption*

The mechanism of gastrointestinal absorption of aluminium is complex and has not yet been fully elucidated (WHO, 1997). The extent to which aluminium is absorbed depends upon the amount of the chemical species present in the gut lumen, in the blood and in the interstitial fluid. Aluminium species may be modified in the gut before absorption. Absorption will also be influenced by complexing ligands (e.g. citrate, lactate) and competing ions (e.g. iron, silicon). Some authors have suggested that acid digestion in the stomach would solubilize the majority of ingested aluminium to the monomolecular species. This would then be converted to the aluminium hydroxide as pH is neutralized in the duodenum. The solubility of $Al^{3+}$ is lowest at neutral pH. Most of the substance is expected to precipitate in the

intestine, making it unavailable for uptake, with subsequent faecal excretion. Aluminium complexes, particularly in the presence of carboxylic acids such as citrate, are thought to improve solubility in the intestine and hence increase aluminium available for intestinal uptake (Reiber et al., 1995; Yokel & McNamara, 2001).

There are indications that the toxicokinetics of aluminium are dose-dependent and since high doses have been administered in many studies, the results of these studies, with respect to their relevance to humans, should be interpreted with caution. It should also be considered that accurate quantification of aluminium absorption has proved difficult. One reviewer highlighted the fact that, in the past, the absence of an appropriate radioisotope compromised the reliability of studies. Measurement of blood concentrations appears to be a poor indicator of aluminium absorption and, while urinary excretion appears to provide a better estimation of aluminium absorption, it offers no information about retention in tissues such as bone. It has been suggested that measurement of the $^{26}Al$ radioisotope by high-energy accelerator mass spectrometry (AMS) offers a more accurate measurement of aluminium levels, since the lower limit of detection ($10^{-18}$ g) allows physiological concentrations of aluminium to be quantified (Drueke, 2002).

A study investigated the influence of the chemical species of aluminium on uptake using the Caco-2 model of gastrointestinal absorption in vitro. Flux across and uptake into Caco-2 cells was investigated for the aluminium ion, and for aluminium citrate, maltolate, hydroxide and fluoride, at a concentration of 8 mmol/l. The flux of aluminium fluoride was dramatically increased at 2 h compared with that of the other aluminium species. This was associated with a reduced transepithelial electrical resistance (TEER) which indicates opening of tight junctions between cells resulting in increased paracellular flux, possibly as a result of toxicity caused by fluoride and/or aluminium. The permeability of all aluminium species highly correlated with a marker of paracellular diffusion, with the exception of aluminium hydroxide (possibly owing to its poor solubility). Calcium (1.25 mmol/l) is required in the culture medium to maintain tight junction integrity and its absence greatly increased aluminium flux across the monolayer. Kinetic studies indicated that uptake of aluminium species into cells was probably the result of passive diffusion. The use of the $^{26}Al$ radioisotope at 2 µmol/l, a concentration more relevant to drinking-water, showed that uptake and flux of aluminium were not significantly different among the aluminium species. Approximately 0.015% of the aluminium in the uptake medium fluxed across the monolayer, while about 0.75% remained associated with the cells, corresponding to an intracellular aluminium concentration of 5 µmol/l (Zhou & Yokel, 2005).

Rat small intestine was perfused with a buffered (pH 7) solution of aluminium sulfate (93 µmol/l). Of the total aluminium perfused, only 62.2 ± 6.1 (standard deviation, SD) % was recovered from the effluent and 35.1 ± 5.8 (SD) % was recovered from the mucus and mucosa, predominantly in the distal third. Since ultrafiltration experiments had indicated that only 14.3 ± 1.3 (SD)% of a freshly prepared perfusate was able to pass through an ultrafilter, the authors suggested that a proportion of the mucosal aluminium is likely to be colloidal/particulate (Powell et al., 1994).

In studies of rat intestinal perfusion, it was found that aluminium uptake was reduced by various paracellular pathway blockers and a sodium transport blocker. Aluminium uptake from calcium-supplemented medium was increased and the authors suggested this might be owing to decreased resistance to paracellular flux in the absence of calcium (Provan & Yokel, 1988a). Follow-up work suggested aluminium might interact with the calcium uptake pathway since various calcium-channel blockers reduced aluminium uptake. Conversely calcium-channel activators increased aluminium uptake (Provan & Yokel, 1988b).

Studies of rat intestinal perfusion with aluminium chloride (20 mmol/l) found that increasing concentrations of sodium chloride (0–120 mmol/l) did not affect aluminium uptake. However, increasing concentrations of calcium chloride (0–10 mmol/l) were associated with a reduction in aluminium absorption (van der Voet & de Wolff, 1998). The effect of aluminium on radiolabelled calcium ($^{45}Ca$) uptake was investigated in cultured chicken enterocytes. Increasing concentrations of aluminium lactate (0–150 µmol/l) resulted in approximately 50% reduction in $^{45}Ca$ uptake, although the effect of aluminium does not appear to be sensitive to calcium-channel activators. Similarly, an isolated intestinal loop experiment in rats showed that the presence of aluminium chloride at 50 mg Al/kg bw[1] resulted in a significant reduction in calcium uptake (Orihuela et al., 2005a).

Aluminium was detected in samples of whole blood, urine and tissue from rats treated with aluminium lactate at 12 mg/kg bw, but not 1 mg/kg bw, by oral gavage (Wilhelm et al., 1992).

Groups of 10 male Wistar rats received either deionized water or drinking-water supplemented with aluminium chloride (5 or 20 mgAl/kg bw per day) for 6 months. The animals were placed in metabolic cages for 6 days before, during (third month) and at the end of the study, for measurement of water consumption and diuresis (balance study). Absorption was reported to be 6.1 and 5.8 µg/kg bw per day, in the groups receiving doses of 5 and 20 mg Al/kg bw per day respectively (Somova & Khan, 1996).

In studies reported by the World Health Organization (WHO, 1997), the relationship between solubility of various aluminium compounds and absorption was examined in Sprague-Dawley rats given aluminium compounds orally at a dose of 1.2 mmol (35 mg Al) per kg bw. Aluminium absorption, measured by urinary excretion, largely mirrored solubility. Urinary excretion of 0.015% of the administered dose was detected with aluminium hydroxide, with excretion being twice that for aluminium chloride and aluminium lactate. Inclusion of citrate resulted in an increase in excretion of 50–100 times. The validity of using urinary excretion as a marker of absorption was assessed in isolated intestinal loop experiments, which supported the excretion data (Froment et al., 1989a). Further work indicated that absorption was likely to occur in the proximal small intestine. A mechanism was proposed whereby potent calcium chelation by aluminium citrate compromises tight

[1] The abbreviation 'Al' is used in expressing dietary concentrations or doses as aluminium rather than as the administered aluminium compound, and for total aluminium content in food, or its associated dietary exposure when the nature of the aluminium compounds present is unknown.

junction integrity, leading to enhanced paracellular absorption (Froment et al., 1989b).

The $^{26}Al$ radioisotope was used to assess gastrointestinal absorption, tissue retention and urinary excretion of dietary aluminium in the presence and absence of citrate in rats. Groups of 20 rats were given 3.8 ng of $^{26}Al$ and 63 ng of $^{27}Al$ by oral gavage in 400 µl of deionized water, with either 20 mg of citric acid or weak hydrochloric acid of similar pH. Urine was collected throughout the study and two animals per dose group were killed at 0.5, 1, 1.5, 2, 4, 6, 8, 120, 360 and 720 h after gavage. $^{26}Al$ present in plasma reached a peak of 0.01% of the administered dose 1 h after gavage, with a modest but statistically significant increase in aluminium absorption in the presence of citrate (Jouhanneau et al., 1997).

A contemporary study also using the $^{26}Al$ radioisotope, given to fasted rats as either aluminium hydroxide or citrate, found fractional absorption to be 0.1% and 5% respectively (Schonholzer et al., 1997).

Groups of at least four guinea pigs were fed one of six diets twice per week for 3 weeks: sponge cake and orange juice (1 : 1); sponge cake and water (1 : 1); bread and jam and tea (2 : 1 : 1); bread and jam and water (2 : 1 : 1); orange juice; or tea (available instead of drinking-water and in the absence of diet for 2 h per day). The total amount of aluminium ingested from these test diets was 44 000, 37 000, 300, 230, 5, and 240 µg respectively. Each 24 h test-diet period was preceded and followed by an 8 h fast, with guinea-pig chow being consumed ad libitum at all other times. Control animals ate guinea-pig chow only, consuming approximately 2800 µg of aluminium. The sponge cake contained SALP acidic, and the test diets with sponge cake contained substantially more aluminium than the other test diets.

Aluminium concentrations were measured in brain, kidney and bone (femur) by inductively coupled plasma-mass spectrometry (ICP-MS), and in upper intestinal contents by size exclusion chromatography (SEC) coupled to ICP-MS. Aluminium concentrations in the femurs of animals fed sponge cake, with or without orange juice, were significantly higher than in animals fed any other diet. Femur concentrations of aluminium were higher in the animals fed orange juice and sponge cake than in those fed cake without orange juice, although the amount of aluminium ingested was similar in the two groups. In the kidney, concentrations of aluminium were below the detection limit in animals fed guinea-pig chow, bread, tea and jam, but aluminium was detectable in animals fed the diets containing sponge cake. Concentrations were significantly higher in the animals fed sponge cake and orange juice compared with controls, but not in animals fed sponge cake and water. None of the diets produced elevated concentrations of aluminium in the brain. Less than 1% of aluminium in the upper intestinal contents was found in the soluble fraction, and characterization by SEC-ICP-MS indicated that this aluminium was not present as citrate (Owen et al., 1994).

An 8-week feeding study in rats examined the absorption of aluminium (1.5– 2 g/kg diet) either as hydroxide or complexed with organic anions—citrate, malate, lactate or tartrate. All the organic anions significantly increased plasma aluminium concentrations compared with those in the group treated with aluminium

hydroxide. There was no significant difference in plasma aluminium concentrations between the organic anion treated groups (Testolin et al., 1996).

Rats were given $^{26}Al$ (3.8 ng in $^{27}Al$, 63 ng) by oral gavage (300 µl), in water with a low, medium or high concentration of silicon (< 0.1, 6 or 14 mg Si/l respectively) in the presence or absence of citrate (26 g/l). While citrate significantly increased fractional intestinal absorption of $^{26}Al$ by a factor of 6–7, silicon had no significant effect, either in the presence or absence of citrate. The same study also found a significant 15-fold increase in $^{26}Al$ absorption in animals subjected to a 24 h fast compared with non-fasted animals (Drueke et al., 1997).

A small study examining aluminium concentrations in the blood 60 min after administration of a drink containing aluminium citrate (Al, 0.3 g; citrate, 4.8 g) found larger increases in blood concentrations in elderly people (aged > 77 years) compared with people aged 20–70 years. This study also found that increases in blood aluminium concentrations in younger patients with Alzheimer disease were similar to those in elderly sufferers and in controls. The authors suggested that aluminium exposure in these groups may be increased twofold (Taylor et al., 1992).

In a two-part study, the authors initially assessed the speciation of aluminium in vitro in infusions of black tea. Tea samples were incubated alone (pH 4.5) or with an equal volume of human gastric juice (pH 2.2) for 1 h at 37 °C, then centrifuged through micro-concentrators with relative molecular mass cut-offs of 3, 10 and 30 kDa. Further acid-digested samples were adjusted to pH 6.5 and then centrifuged through 3000 Da filters. Of the aluminium in the tea, 78% passed through the 3 kDa filter, and this percentage rose to over 90% with the addition of gastric juice. However, when the gastric juice-digested infusion was adjusted to pH 6.5, just 5% of the aluminium passed through the 3 kDa ultrafilter. These findings suggested that when digested tea passes from the stomach into the duodenum, the pH change would be expected to cause a rapid re-association of aluminium with species with a high relative molecular mass, such as polyphenols.

In the second part of the study, one healthy volunteer drank 2 l of tea over 4 h while commencing a 24 h urine collection. Urine collection continued for a further 24 h in which no tea was consumed, but deionized water was allowed ad libitum. There was little difference in the concentration of aluminium in urine during the two 24 h collection periods. However, urinary volume and total aluminium excretion were greater after drinking tea than during the second 24 h collection period. The authors suggested that only a small proportion of the aluminium in tea is potentially available for absorption throughout the small bowel (Powell et al., 1993).

Aluminium uptake was determined in a single human volunteer given a single oral dose of $^{26}Al$ (1.1 µg) in sodium citrate. Plasma concentrations of the isotope were measured 6 h after administration and uptake was determined by extrapolation. Uptake was estimated to be 1% of the administered dose (Priest et al., 1995; Priest, 2004).

In a later study, patients with Down syndrome and controls were given orange juice containing $^{26}Al$ and the effect of added silicate was assessed. Gastrointestinal absorption factors were calculated (aluminium absorbed :

aluminium administered). Control values ranged from 0.04 to $1.5 \times 10^{-4}$. The addition of silicate reduced absorption by a factor of approximately 7, while uptake was five times higher in patients with Down syndrome (Priest, 2004).

The uptake of various forms of aluminium was assessed in human volunteers dosed with 100 mg of aluminium via a paediatric feeding tube. Absorption fractions were calculated for aluminium citrate, aluminium hydroxide and aluminium hydroxide with sodium citrate ($5 \times 10^{-3}$, $1.04 \times 10^{-4}$ and $1.36 \times 10^{-3}$ respectively). These results demonstrated the greater bioavailability of the citrate complex and the ability of citrate to enhance the bioavailability of aluminium in another chemical form. This study also noted that variability between the two subjects appeared to be caused by longer retention of the $^{26}Al$ in the intestine, before defaecation. The $^{26}Al$ remained in the intestine for approximately 1 day longer in one subject and this was associated with higher blood concentrations and protracted excretion (Priest et al., 1998).

In another study, a fruit drink containing $^{26}Al$ (27 ng) was given to 13 patients with Alzheimer disease (aged 63–76 years) and 13 age-matched controls after an overnight fast. This study found that gut absorption ranged from 0.06–0.1% of the administered dose with a 1.6-fold increase in absorption by Alzheimer patients (Moore et al., 2000).

A fractional aluminium absorption of 0.22% was determined by comparing $^{26}Al$ urinary concentrations in human male volunteers given $^{26}Al$ either by intravenous injection or in drinking-water (Priest et al., 1995b; Priest et al., 1998a cited in COT, 2005).

Three human male volunteers were given aluminium (280 mg, 104 mmol) as aluminium hydroxide with citrate (3.2 g, 1.67 mmol) in 100 ml of fruit juice, after an overnight fast. The authors suggested that it is unlikely that the aluminium was absorbed as aluminium citrate, since the blood citrate peak preceded the aluminium peak by 45–60 min. Therefore, they favoured the hypothesis proposed by Froment et al. (1989b) whereby citrate facilitates aluminium absorption by opening tight junctions in the gut epithelium (Taylor, 1998).

*(b) Distribution*

Groups of 10 male Wistar rats received either deionized water or aluminium chloride (5 or 20 mg Al/kg bw per day) for 6 months. Aluminium was measured in plasma, brain, liver, bone and kidney and showed dose-related significant increases in concentration when compared with the control animals (Somova & Khan, 1996).

Groups of 20 male Wistar rats were given aluminium chloride at a dose of 5 mg Al/kg bw per day by intravenous injection for 3 consecutive days. Half of the animals were sacrificed on day 4 and the other half on day 22. Haematological parameters and aluminium and iron concentrations in brain, liver, kidney and bone were studied. It was noted that aluminium had accumulated in the brain, bone and kidney of the animals sacrificed after 4 days. These levels had returned to normal after 22 days, when increased concentrations of aluminium in the liver were noted (Somova et al., 1995).

Groups of six rats were given aluminium hydroxide ($Al(OH)_3$) or aluminium chloride at a dose of 0.1, 2.0 or 100 mg Al/l (equivalent to 0, 0.01, 0.2 and 5.5 mg Al/kg bw per day) with either water, citrate or acetate for 10 weeks. Aluminium concentrations were determined in tibia, brain, liver, intestine, blood and kidney by flameless atomic absorption. These did not differ between the treatment groups, with the exception of the intestine, where intestinal cell aluminium concentrations increased in a dose-dependent manner in the presence of citrate (Fulton, 1989).

Rats were given a single oral dose of aluminium at 0, 0.2, 0.4, or 0.8 mmol as aluminium lactate by gavage in 1 ml of 16% citrate (equivalent to 0, 0.04, 0.08 and 0.16 mg Al/kg bw per day). The diet used in this study contained 7.79 mg Al/kg. Tissue aluminium concentrations were determined after 7 h by atomic absorption spectrophotometry. Significant increases above values for controls were observed in all tibia samples. Serum and kidney concentrations of aluminium in the groups at 0.2 and 0.4 mmol were significantly increased above those of the controls, with a significantly greater increase at 0.8 mmol. Significant increases in the liver and spleen were only observed at 0.8 mmol. Rats at 0.8 mmol retained significantly greater amounts of aluminium in soft tissues than those at 0.2 or 0.4 mmol. The authors suggested this might indicate that physiological mechanisms were unable to prevent the tissue accumulation of aluminium in the rats given the highest dose (Sutherland et al., 1996).

In rats dosed orally with $^{26}Al$ (3.8 ng with 63 ng $^{27}Al$) uptake of aluminium into bone was found to be rapid (approximately 1 h) and it remained in the skeleton for the duration of a 30 day study. These authors suggested a minimum residence time of approximately 500 days (Jouhanneau et al., 1997).

Groups of growing (age 2 months), mature (age 8 months) and ageing (age 19 months) male Sprague-Dawley rats were given aluminium lactate at a concentration of 0.8 mmol by oral gavage. Rats were sacrificed on days 1, 9, 15, 21, 27, 36 and 44 (minimum of seven per age group). One day after dosing, growing rats had higher concentrations of aluminium in bone (tibia) than did mature and ageing rats, which had similar concentrations. Ageing rats had higher concentrations in the kidneys on day 1, and lower concentrations on day 9 than growing and mature rats. The half-life (time taken for tissue concentration to halve) of aluminium in the kidneys and tibias increased with age. Multiple stepwise regression analysis indicated that several factors that change with age (including animal size, kidney function, bone turnover and metabolism of other minerals), but not age itself, were predictive of tibia aluminium concentration. Age was also a predictor of liver and spleen aluminium concentrations. However, the measured changes in gut, kidney, bone and mineral metabolism were less predictive of soft tissue aluminium concentration than of bone aluminium concentration (Greger & Radzanowski, 1995).

Microdialysis was used to measure aluminium in extracellular fluid of frontal cortex, lateral ventricle and blood in rats (species not reported). The concentration of aluminium in the dialysate from the frontal cortex reached a maximal steady value within 5 min after the administration of aluminium citrate (0.5 mmol/kg bw) as an intravenous bolus. Also, there was a higher concentration of aluminium, and

higher brain : blood ratio, in the frontal cortex than in cerebrospinal fluid. The authors stated that this supports the suggestion that aluminium enters the brain from blood, through the blood–brain barrier, rather than through the choroid plexus. The concentration ratio of aluminium in extracellular fluid in the brain : blood was 0.15 at constant blood and brain extracellular-fluid aluminium concentrations, suggesting that the transfer of aluminium citrate across the blood–brain barrier is mediated by carriers, rather than by diffusion. Various substrates were included in the dialysate of the microdialysis probe implanted in the frontal cortex of rats. Addition of $CN^-$ or 2,4-dinitrophenol as metabolic inhibitors, pyruvate as a substrate for the carrier monocarboxylate transporter (MCT), or other factors to reduce proton availability and proton gradients significantly increased the brain : blood ratio to approximately 1. These results are consistent with the hypothesis for MCT-mediated transport across the blood–brain barrier. However, lack of aluminium citrate uptake in rat erythrocytes expressing MCT1 and the band 3 anion exchange transporter suggests it is not an effective substrate for either of these transporters. Uptake of aluminium citrate into murine-derived endothelial cells appeared to be independent of sodium and pH, and dependent on energy. Uptake was inhibited by substrates and/or inhibitors of the MCT and organic anion transporter families. Determination of $^{26}Al$ concentrations in rat brain indicated a prolonged brain half-life (approximately 150 days). The authors noted that this is difficult to extrapolate to humans owing to insufficient insight into allometric scaling for metals between rats and humans (Yokel, 2005).

Rats aged 2 months received intraperitoneal injections of aluminium gluconate (0.667 mg Al/250 µl) three times per week for 2 months. The concentration of aluminium was estimated in brain regions and liver. Liver concentration was reported to be 44-times higher in treated rats than in controls, while a 3.5-fold increase was observed in the brain, with some regions appearing to be more vulnerable to aluminium accumulation. The highest concentrations were reported in the temporal cortex, hippocampus and anterior olfactory nuclei. The impact of aluminium exposure on distribution of glutamate, aspartate and glutamine was also studied. Of the three amino acids assayed, the distribution pattern of glutamine in the brain was distinctly different to that in controls (Struys-Ponsar et al., 1997).

Lactating rats with a litter size of 11 were injected subcutaneously with a solution containing 20 dpm $^{26}Al$ ( $^{26}AlCl_3$) and 0.009 mg $^{27}Al$ ( $^{27}AlCl_3$) daily from postnatal days 1 to 20. Incorporation of $^{26}Al$ into the brain, liver, kidneys and bone of suckling rats was measured by mass spectrometry and shown to increase significantly from days 5 to 20. After weaning, the amount of aluminium in the liver and kidneys decreased remarkably. However, in the brain the amount of $^{26}Al$ had only diminished slightly up to 140 days after weaning (Yumoto et al., 2003).

In a review of published studies, papers were identified in which aluminium was administered to pregnant rats, mice or rabbits and accumulation of aluminium was measured in dams, fetus or offspring. Seven studies were identified in which aluminium was administered during gestation and fetal accumulation was determined. In another seven studies, aluminium was administered at least until birth and evaluated the accumulation in the dams and/or pups. These fourteen

studies included four different aluminium compounds (hydroxide, chloride, lactate and citrate) administered by four routes (gavage, feed, intraperitoneal injection and subcutaneous injection) with total doses ranging from 14 to 8400 mg/kg bw. Fetal aluminium concentrations were not increased in six of the seven studies and pup aluminium concentrations were not increased in four of the five studies in which they were measured. Maternal aluminium concentrations were increased in some studies, but there was no consistent pattern of organ-specific accumulation and it was reported that the positive results of several of the studies were contradicted by subsequent reports from the same laboratory. Placental concentrations were increased in six out of nine studies and were greater than corresponding fetal concentrations (Borak & Pierce, 1998).

In a human volunteer, blood samples were taken at 6, 12 and 24 h after ingestion of $^{26}Al$ (100 ng, 70 Bq) with $^{27}Al$ (1 µg) in sodium citrate. The highest plasma $^{26}Al$ concentration (0.3 ng/l) was found in the sample collected at 6 h. Assuming a plasma volume of 3 l, 1% of the administered dose (1 ng) would have been in the circulation. Of this, 5% appeared in a fraction with low relative molecular mass. The remainder was associated with the fraction with high relative molecular mass, specifically, 80% with transferrin, 10% with albumin and 5% in other species with high relative molecular mass (Day et al., 1991). Consistent with this, studies of aluminium binding indicate that that 90% of the aluminium in blood is associated with transferrin, with the remaining 10% existing as aluminium citrate (Ohman & Martin, 1994). While binding studies have shown that transferrin is the strongest aluminium-binding protein in blood, a difference of nearly 10 log units in transferrin binding between aluminium and iron (Martin et al., 1987), indicates that aluminium is unlikely to compete with iron for transferrin binding.

*(c) Excretion*

Rats dosed orally with $^{26}Al$ (3.8 ng with 63 ng $^{27}Al$) were found to have excreted approximately 90% of the aluminium in the urine within 48 h after dosing (Jouhanneau et al., 1997).

The importance of bile as an excretory route for ingested aluminium has been explored. Bile ducts of 30 male Sprague-Dawley rats were cannulated to allow both bile collection and re-infusion of bile acids. Five days after surgery, rats (average body weight, 191 ± 4 g) were given a single oral dose of aluminium (0, 0.2, 0.4, or 0.8 mmol, equivalent to 0, 0.04, 0.08 and 0.16 mg Al/kg bw per day) as aluminium lactate given by gavage in 1 ml of 16% citrate. Bile was collected from unanaesthetized rats 1–7 h after dosing. Biliary aluminium secretion was highest during the first hour of bile collection. All rats dosed with aluminium secreted significantly greater amounts of aluminium in bile than did rats in the control group. However, biliary aluminium secretion did not vary among animals given aluminium at different doses, suggesting that biliary secretion of aluminium was saturated at these doses (Sutherland et al., 1996).

A human male volunteer was given a solution of $^{26}Al$ (0.7 µg, 574 Bq) in trisodium citrate (35 mg) intravenously. Urinary and faecal excretion were 83% and 1.8%, respectively, of the initial dose over 13 days after administration. Whole body

retention of $^{26}$Al was 15% 13 days after administration, declining to 4% at 1178 days, corresponding to a biological half-life of 7 years (Priest et al., 1995). In a second study, six human male volunteers were each given a solution of $^{26}$Al (84 ng, 60 Bq) with citrate (25 mg) intravenously. On average, 59 ± 10 (SD) % was excreted in the first 24 h and by 5 days 72 ± 7 (SD) % had been excreted in the urine. The urinary excretion of $^{26}$Al did not correlate with either the mass of voided urine, or excretion of sodium, potassium, calcium, magnesium or phosphorus. Faecal excretion was 1.2% ± 0.3 (SD) over the 5 days after administration. On the fifth day, whole-body retention ranged from 16% to 36%, with a mean of 27 ± 7 (SD) %. The authors suggested that the substantial interindividual variation probably reflected genuine differences in the clearance patterns, which may have implications for whole-body concentrations in the long term (Talbot et al., 1995).

### *2.1.2 Effects on enzymes and other parameters*

Aluminium has been reported to modify the absorption of essential minerals (WHO, 1997).

In a study conducted in vitro, isolated chick duodenum enterocytes were incubated for 1 h with aluminium lactate at 100 µmol/l. In the presence of aluminium, the maximum uptake of calcium and the affinity constant ($k_m$) were significantly decreased. This reduction was not reversed in cells in which the aluminium-containing media was replaced by aluminium-free media before the measurement of uptake of aluminium. The effect of aluminium on calcium uptake was concentration-dependent (measured range of concentrations of aluminium: 10, 20, 50, 100, 125 or 150 µmol/l) exhibiting an inhibitory saturation-type phenomenon. Calcium uptake was lower at pH 6.5 than at pH 7.4, differences being statistically significant in the range of 20 to 50 µmol/l. Calcium channel activators A23187 and capsaicin did not modify the effects of aluminium (Orihuela et al., 2005a).

The influence of intestinal glutathione (GSH) concentrations on the effects of aluminium on calbindin-D9k-related calcium transport was assessed in adult male Wistar rats given aluminium chloride by oral gavage daily for 7 days (50 mg Al/kg bw). This treatment significantly increased the tissue aluminium content in the small intestine (as measured at the end of the experimental period) compared with control animals. At 24 h, intestinal calcium absorption was significantly decreased in rats given aluminium, or aluminium plus GSH (5 and 10 mmol/kg bw). After 7 days, the inhibitory effect of aluminium on calcium absorption was prevented by simultaneous administration of aluminium with GSH (10 mmol/kg bw). Depletion of GSH by intraperitoneal injection of buthionine sulfoximine (2 mmol/kg bw,) decreased calcium absorption in control animals, and further enhanced the inhibition of calcium absorption by aluminium. Aluminium decreased the duodenal expression of calbindin-D9k, this was prevented by co-administration of GSH at 7 days, but not at 24 h (Orihuela et al., 2005a).

The same authors carried out further studies on the effect of aluminium on GSH metabolism in the small intestine. Adult male Wistar rats were given aluminium chloride at a dose of 30, 60, 120 and 200 mg/kg bw per day by oral gavage for 7 days. The authors commented that exposure to aluminium from the diet and

drinking-water was negligible, although data were not provided. It is unclear whether the doses were expressed as aluminium or as aluminium chloride. There was a dose-related decrease of GSH in the small intestine that was statistically significant at doses of 60 mg/kg bw and above. A 71% increase in the GSH concentration was measured at the highest aluminium dose assayed. The ratio of oxidized : reduced glutathione (GSSG : GSH) increased as the aluminium dose increased becoming statistically significant at 200 mg/kg bw. Specific activities of glutathione-synthase (from 60 mg /kg bw per day) and glutathione-reductase (from 120 mg /kg bw per day) were significantly reduced (26 and 31% respectively) compared with the controls, while glutathione *S*-transferase activity was shown to only be slightly modified by treatment with aluminium. A positive linear correlation between the intestine GSH depletion and a reduction of in-situ calcium absorption, both produced by aluminium, was reported. The authors commented that the results taken as a whole indicate that aluminium alters GSH metabolism in the small intestine by decreasing its turnover, leading to an unbalanced redox state in the epithelial cells, thus contributing to deterioration in GSH-dependent absorptive functions (Orihuela et al., 2005b).

Intragastric administration of aluminium lactate at 0 or 10 mg Al/kg bw per day to six male Wistar rats for 12 weeks resulted in significant increases in intrasynaptosomal calcium concentrations, decreased $Ca^{2+}$ ATPase, increased calcium uptake and increased calpain activity in the brain, indicating alterations in calcium homeostasis. No information on the aluminium content of the diet was provided (Kaur & Gill, 2005).

Aluminium chloride has been investigated for effects on enzymes and other parameters associated with oxidative damage. Groups of seven male Sprague-Dawley rats were treated orally at a dose of 34 mg/kg bw every other day for 30 days, equivalent to 17 mg/kg bw per day. This dose was stated to be 1/25 of the rat oral median lethal dose ($LD_{50}$) for aluminium and the comparison indicates that the dose is expressed as aluminium rather than aluminium chloride, although this is not clear from the paper. No other details on dosing and no information on aluminium content of the diet are provided. Treatment with aluminium chloride resulted in changes in a large number of parameters, including significantly increased thiobarbituric acid reactive substances and decreased glutathione *S*-transferase activity and levels of sulfydryl groups in plasma, liver, brain, testes and kidney. A range of aminotransferase and similar enzymes were decreased in liver and testes and increased in plasma, acetylcholinesterase decreased in brain and plasma. Concomitant administration of vitamin E (100 mg/kg bw) or selenium (200 µg/kg bw) partially or totally alleviated the effects of aluminium chloride on these parameters (El-Demerdash, 2004).

A similar study was conducted in rabbits at the same laboratory. Groups of six male New Zealand White rabbits were treated orally with aluminium chloride at a dose of 34 mg/kg bw every other day for 16 weeks, equivalent to 17 mg/kg bw per day. This dose was stated to be 1/25 of the rabbit oral $LD_{50}$ for aluminium and the comparison indicates that the dose is expressed as aluminium rather than aluminium chloride, although this is not clear from the paper. No other details on dosing and no information on aluminium content of the diet were provided. The

effects of aluminium chloride were similar to those in the study of El-Demerdash (2004). Vitamin E and selenium were not investigated but amelioration by ascorbic acid (40 mg/kg bw) was reported (Yousef, 2004).

In a study to investigate the possible effects of aluminium exposure on various aspects of calcium homeostasis, three male rhesus monkeys (body weight, 3–4 kg) received aluminium lactate at at dose of 25 mg Al/kg bw by gastric intubation on alternate days for 52 weeks, equivalent to 13 mg Al/kg bw per day. There was no information on the aluminium content of the diet. Aluminium exposure caused a decline in the activity of $Ca^{2+}$ ATPase in the brain. The total calcium content was also significantly raised. Concomitant to this, the levels of lipid peroxidation were increased in the treated animals, suggesting aluminium-induced neuronal change. The authors suggest that the results indicate that the toxic effects of aluminium could be mediated through modifications in the intracellular calcium homeostasis with resultant altered neuronal function (Sarin et al., 1997a).

Three male rhesus monkeys (body weight, 3–4 kg) received aluminium lactate at a dose of 25 mg Al/kg bw on alternate days for 52 weeks, equivalent to 13 mg Al/kg bw per day. There was no information on the aluminium content of the diet. Aluminium administration caused a significant decrease in the total lipid, glycolipid and phospholipid in the brains of these primates. Phospholipid to cholesterol ratios were markedly increased, indicating a loss of membrane integrity, supported by the observation that aluminium had a significant effect on the various membrane-bound enzymes in terms of decreased activities of Na+K+ ATPase and acetylcholinesterase, along with a decrease in the myelin-specific enzyme, 2'3'-cyclic nucleotide phosphohydrolase. The authors considered the latter decrease was suggestive of possible demyelination, which in turn can be attributed to aluminium-induced lipid peroxidation and resultant loss of lipids (Sarin et al., 1997b).

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

The oral $LD_{50}$ of aluminium chloride was 3630 ± 400 mg/kg bw, equal to 737 ± 81 mg Al/kg bw in male Wistar rats. Effects observed in rats after dosing at and above 520 mg Al/kg bw included lethargy, reduced spontaneous movement, lacrimation and breathing difficulties. No effects were observed in the animals receiving the lowest dose (325 mg Al/kg bw) (Kumar, 2001).

### *2.2.2 Short-term studies of toxicity*

#### *(a) Rats*

Groups of 15 male albino rats (strain not reported) were given aluminium sulfate at 0, 17, 22, 29, 43, 86 or 170 mg Al/kg bw or potassium aluminium sulfate at 29 or 43 mg Al/kg bw by oral gavage for 21 days. No information was provided on the aluminium content of the diet. The effects of both compounds were similar at comparable doses of aluminium. The end-points were histopathological examination of heart, liver, kidney, brain, testes, stomach and femur. Mild

histopathological effects were reported in the kidney and liver at the lowest dose 17 mg Al/kg bw per day (as aluminium sulfate). Severity of effects increased with dose and effects on nerve cells, testes, bone and stomach were also reported at higher doses. WHO (1997) stated that the data presented were inadequate to verify the reported effects (Roy et al., 1991; WHO, 1997).

Groups of 10 male Wistar rats received aluminium chloride in deionized water as drinking-water for 6 months at doses stated to be 5 and 20 mg Al/kg bw, although it is unclear how these doses were achieved. Control animals consumed deionized water. All animals consumed 'standard pellet food' ad libitum. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. After 6 months, the body weights of animals at the lowest dose (5 mg Al/kg bw per day) and at the highest dose (20 mg Al/kg bw per day) were 80% and 84% of control, respectively. Interim body weights were not reported. Erythrocyte count in the groups at the lowest and highest dose was reduced by 31% and 23% respectively; haemoglobin by 27% and 28% respectively; erythrocyte glucose-6-phosphate dehydrogenase by 14 % and 11% respectively; erythrocyte acetylcholinesterase was reduced by 29% and 20% respectively; erythrocyte volume fraction was reduced by 10% in both groups. No significant changes in leukocyte count were found in either group (Somova & Khan, 1996).

Results of histopathological observations in the above study were reported separately. At 20 mg Al/kg bw, there were spongiform changes and neurofibrillary degeneration in the hippocampus of the brain and atrophy and fibrosis in the kidney (Somova et al., 1997).

Groups of 16 male Sprague-Dawley rats were fed diets containing aluminium hydroxide for 29 days. Groups received 1079 mg Al/kg diet, 1012 mg Al/kg diet plus 4% citrate, or 2688 mg Al/kg diet plus 4% citrate, equivalent to approximately 100, 100 and 270 mg Al/kg bw per day respectively. Concentrations of aluminium in tibia, liver and serum and urinary excretion of aluminium were highly correlated with oral exposure. Ingestion of citrate had small but significant effects on aluminium retention. Erythrocyte volume fractions were inversely correlated with tissue concentrations of aluminium (Greger & Powers, 1992; cited in WHO, 1997).

Aluminium tissue concentrations and body and organ weight changes were measured in a $2 \times 2 \times 2 \times 2$ factorial design study exposing groups of rats to diets containing 13 or 1100 mg Al/kg as hydroxide or citrate and calcium (2.7 or 10 g/kg diet) for 30 days. Further groups of six animals in a $4 \times 2$ factorial design experiment were exposed to 14 or 900 mg Al/kg diet per day and one of four levels of citrate for 28 days. Ingestion of citrate was reported to increase the retention of aluminium in bone of rats fed the highest dose (100 mg Al/kg diet per day) and on the high-calcium diet, aluminium concentrations were reported to decrease without a change in growth of the animals. In a third experiment, of $2 \times 2 \times 2$ factorial design, groups of seven rats were exposed to 9 or 1000 mg Al/kg diet per day and citrate. 'Sham' operations were carried out on these animals, or they had one kidney removed. Reduction in kidney function was insufficient to alter growth, but aluminium retention was increased in bone by 13% (Ecelbarger & Greger, 1991; cited in WHO, 1997).

*Dogs*

Groups of four male and four female beagle dogs were fed SALP basic at dietary concentrations of 0, 3000, 10 000 or 30 000 mg/kg for 26 weeks. The measured aluminium concentrations averaged 94, 284, 702 and 1922 mg Al/kg diet, providing average doses equal to 4, 10, 27 or 75 and 3, 10, 22 or 80 mg Al/kg bw per day for male and female dogs, respectively. Toxicity was reported to be limited to a sharp transient decrease in food consumption and a concomitant decrease in body weight in males at the highest dose, but these data are not shown in the publication. No treatment-related effects on serum chemistry, haematology or urine analysis were observed. A decrease in testes weight was seen in males at the highest dose and two animals had moderate seminiferous tubule germination epithelial cell degeneration and atrophy. Mild to moderate hepatocyte vacuolation accompanied by hypertrophy and mild bile stasis was also seen in the animals at this dose. The authors considered that the effects on the testes and liver were likely to be caused by the decreased food consumption. Very mild to mild tubular-glomerular nephritis was also reported in the males at the highest dose. There were no significant changes in bone aluminium content. A 60% increase in concentration of aluminium in the brain was recorded in the female, but not male, dogs at the highest dose (Pettersen et al., 1990). WHO (1997) concluded that the lowest-observed effect level (LOEL) from this study was 75–80 mg Al/kg bw per day.

A similar study with SALP acidic, as described in an unpublished report, was used by the Committee at its thirty-third meeting in deriving the PTWI of 7.0 mg /kg bw for aluminium (Annex 1, references *83*, *84*). The published paper describing this study was not cited in the monograph, but is available and is described here for comparison. Groups of six male and six female dogs were fed diets containing SALP acidic at concentrations at 0, 0.3, 1.0 or 3.0% for 6 months. The authors stated that the basal diet was reanalysed for contaminants and the concentration and homogeneity of SALP in the blended diets was verified analytically; however, data were not provided on aluminium content of the control diet. Groups of males and females given test diet consumed less food most weeks than controls; intake was noted sporadically to be significantly decreased in all the treated groups of females. The authors noted that the differences in food consumption were not considered to be toxicologically significant, in the absence of any correlating loss in body weight. The average daily food intake of SALP was calculated on the basis of food consumption data and body weight. For males given 0.3, 1.0 or 3.0%, the mean intakes were reported to be 120, 320 and 1030 mg/kg bw per day, and for females they were 110, 360 and 1090 mg/kg bw per day, respectively, corresponding to 10, 27 and 88 mg Al/kg bw per day and 9, 31 and 93 mg Al/kg bw per day. The Committee at its thirty-third meeting (Annex 1, reference *84*) concluded that 3% in the diet was equivalent to a dose of SALP of 1250 mg/kg bw, which was equivalent to approximately 110 mg Al/kg bw.

The nature and frequency of the adverse signs observed were such that none were considered to be related to treatment. No effect on haematological or clinical chemistry parameters was observed in treated dogs. All the measured parameters were considered to be within the normal range for dogs of this age and strain. Ophthalmological examinations, conducted before the start of the test and

at terminations, revealed no adverse ocular changes. No treatment-related changes were evident in the results of urine analysis or faecal occult blood tests. None of the organ weights (absolute or relative values) of treated animals differed significantly from those of control animals. At autopsy and upon histopathological examination the variations observed were within the normal range for dogs of this age and strain (Katz et al., 1984).

Comparison of the above two studies indicates that the basic form of SALP may be more toxic than the acidic form. The FAO specification monograph for SALP acidic (Annex 1, reference *178*) specifies not less than 95% of $NaAl_3H_{14}(PO_4)_8.4H_2O$, and 'insoluble in water', while SALP basic is a mixture of 70% of a complex of SALP (sparingly soluble) and 30% of disodium phosphate (very soluble). Both are cited as soluble in hydrochloric acid, but it is possible that differences in bioavailability resulting from differing solubilities could offer an explanation for these results.

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

No new long-term studies of toxicity or carcinogenicity were identified.

### *2.2.4 Genotoxicity*

No new studies of genotoxicity conducted according to standard protocols were identified. Studies reported in WHO (1997) suggest that aluminium is able to form complexes with DNA and can cross-link chromosomal proteins and DNA. A number of mechanistic studies have investigated DNA damage and cell cycling.

Human peripheral blood lymphocytes were treated with aluminium chloride at 1, 2, 5, 10 and 25 µg/ml at different stages of the cell cycle, and micronucleus formation and apoptosis were assessed. The frequency of micronucleus formation increased initially, but decreased at high concentrations (10 and/or 25 µg/ml), correlating with an increase in apoptosis. The G0/G1 phase of cell cycle was found to be more sensitive than the S/G2 phases. The authors concluded that this indicates oxidative stress or liberation of DNase as a major source of DNA damage induced by aluminium (Banasik et al., 2005).

Human peripheral blood lymphocytes were treated with aluminium chloride at concentrations of 1, 2, 5, 10 and 25 µg/ml (corresponding to 4, 8, 21, 40 and 104 µmol/l) for 72 h. The level of DNA damage and apoptosis was assessed by comet assay, and apoptosis was confirmed by flow cytometry. Aluminium induced DNA damage in a concentration-dependent manner at concentrations of up to 10 µg/ml. At 25 µg/ml, DNA damage declined, accompanied by a high level of apoptosis, indicating selective elimination of damaged cells. In addition, cells were pre-treated with aluminium chloride (10 µg/ml for 72 h) and then irradiated with 2 Gy to examine effect of aluminium on DNA repair. Cells pre-treated with aluminium chloride showed a decreased DNA repair capacity (Lankoff et al., 2006).

Lymphocytes or skin fibroblasts of patients with sporadic ($n$ = 14) or familial ($n$ = 8) Alzheimer disease were assessed. The frequency of spontaneous micronucleus formation in cells from patients with sporadic or familial Alzheimer

disease was significantly higher than in controls. Treatment with aluminium sulfate [$Al_2(SO_4)_3$] did not increase the frequency of micronucleus formation in lymphocytes or fibroblasts of Alzheimer patients, but did induce micronucleus formation at a concentration of 1 mmol/l in cells from control subjects (Trippi et al., 2001).

The effect of aluminium ions on DNA synthesis, assessed by $^3H$ thymidine incorporation, was studied in normal human dermal fibroblasts in vitro using concentrations of 1.85–74 µmol Al/l (aluminium nitrate) and incubation periods of 1, 2, 3, 4 and 5 days. At 1.85 µmol/l, aluminium salts exerted a slight positive, but not significant, effect on DNA synthesis after day 3 or 5 of incubation. This effect was seen to be statistically significant at concentrations of 3.7 µmol/l and 2 days exposure onward. At 74 µmol/l and 5 days exposure, synthesis increased by 322% over control. Human dermal fibroblast proliferation was also studied. Aluminium salts moderately increased fibroblast division in a continuous manner from 7.4–74 µmol/l after 3 days incubation (Dominguez et al., 2002).

### *2.2.5 Reproductive toxicity*

#### *(a) Multigeneration studies*

Groups of 40 Swiss Webster mice were fed diets containing 7 (control), 500 or 1000 mg Al/kg diet as aluminium lactate either from conception until weaning or from conception to age 150–170 days. According to later studies by these authors, these dietary concentrations were expected to be equivalent to < 1, 50 or 100 mg Al/kg bw per day in adult mice. A battery of six neurobehavioural tests was applied at 150–170 days. There were no treatment-related effects on the body weight of the dams of offspring or on litter size. A higher incidence of cagemate aggression was reported in the offspring at the highest dose as adults. At the conclusion of the study, grip strength was reduced in mice of both treatment groups, but this was not dose-dependent or increased by post-weaning exposure. Brain, spinal cord and liver aluminium concentrations were elevated in adults with continuous exposure after weaning, again with no clear dose–response relationship (Golub et al., 1995).

Swiss Webster mice received diets containing 7 (control), 100, 500 or 1000 mg Al/kg diet as aluminium lactate throughout development (conception to age 35 days). The authors stated that these dietary concentrations provided doses of < 1, 10, 50 or 100 mg Al/kg bw per day in adult mice. The basal diet used in this study was 'sub-optimal', intended to mimic the daily intake of nutrients by young women, which while not necessarily deficient, represents a normal deviation from recommended intakes. Data were drawn from a pool of 30 to 40 pregnancies per treatment group. There were no differences in number of dams completing pregnancy, duration of gestation, pregnancy body-weight gain, litter size at birth or birth weight. By weaning, both males and females in the groups at 500 or 1000 mg Al/kg (50 and 100 mg/kg bw per day) weighed significantly less than did the controls (Golub & Germann, 2001).

Male CD-1 mice aged 8–9 weeks were given aluminium chloride by subcutaneous injection at doses of 0, 7 or 13 mg Al/kg bw per day for 14 days before

mating. Females were not dosed at any time during this study. The doses determined for use in this study were designed to reach serum concentrations comparable to those reported in haemodialysis patients. Male mice were mated with three randomly assigned female mice daily for 9 weeks. Mean mating frequencies for the aluminium-treated groups reduced significantly from weeks 4 to 6 and a marked reduction in male fertility was also observed. Mating was reported to have returned to near normal control levels as the experiment terminated. Significantly higher numbers of postimplantion losses, fetal mortality and induced petechial haemorrhage, but no significant fetal abnormalities were observed in the groups treated with aluminium. The dominant lethal assay showed no difference in the number of implantations between aluminium-treated males and controls. Similar implantation losses were observed in all the groups except the group at the highest dose of aluminium at weeks 3 and 5. Further groups of 25 male mice were treated as before, at weeks 3, 5 and 11, and 8 animals of each group were examined for serum and testicular aluminium. The weights of the reproductive organs of the aluminium-treated animals decreased significantly as aluminium accumulated in the testes. Spermatogenic impairment in the testes within the seminiferous tubules was also apparent, but these disturbances disappeared at the end of the experiment. The authors concluded that aluminium exerted substantial negative effects on male reproductive function and produced genetic toxicity. However, these effects were found to be reversible (Guo et al., 2005).

Female Sprague-Dawley rats were given drinking-water containing aluminium (as aluminium nitrate nonahydrate) at doses of 0, 50 and 100 mg Al/kg bw per day for 15 days before mating and then throughout gestation, lactation and post-weaning. The aluminium content of the feed was 42 mg/kg. In order to enhance the gastrointestinal absorption, doses of 355 and 710 mg/kg per day of citric acid were added to the drinking-water of the groups exposed to 50 and 100 mg Al/kg bw per day, respectively. Controls received water supplemented with 710 mg/kg per day of citric acid. It is noted that doses were adjusted to maintain a constant uptake of aluminium. Body weight was decreased relative to controls on postnatal days 12–21 in pups treated with 100 mg Al/kg bw per day. Sexual maturation was delayed in all aluminium-treated females and in aluminium-treated males at 100 mg/kg bw per day. Forelimb grip strength was reduced in males at 100 mg Al/kg bw per day (Colomina et al., 2005).

*(b) Developmental toxicity*

Oral administration of aluminium has been reported to result in developmental effects, including growth retardation and skeletal anomalies, with the severity of effects being highly dependent on the form of aluminium and the presence of organic chelators that influence bioavailability (WHO, 1997). These data are summarized in Table 1, and indicate that the lowest-observed-adverse-effect level (LOAEL) for developmental effects was 13 mg Al/kg bw per day after treatment with aluminium nitrate, a soluble form of aluminium, administered by gavage (derived from the study of Paternain et al., 1988). Dose-related maternal toxicity (reduced body-weight gain) was also reported.

Groups of 10 pregnant Sprague-Dawley rats were given aluminium nitrate as a daily dose at 180, 360 or 720 mg/kg by oral gavage on days 6 to 14 of gestation, equivalent to 13, 26 or 52 mg Al/kg bw per day. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. Number of corpus lutea, total implantions, number of dead and live fetuses and number of resorptions were recorded and there were no significant adverse effects on these parameters. However, there was a dose-dependent increase in the number of stunted fetuses and the number of litters with runt fetuses in the groups treated with aluminium. Dams given aluminium gained significantly less body weight throughout gestation (non-dose-related) and their placentas weighed significantly less. Fetal weight, body length and tail length from the treated groups showed significant decreases, with fetal body weights being significantly lower in all treated groups in a dose-related manner. Treatment with aluminium resulted in a significantly increased incidence in skeletal variations in all the treated groups (rib and sternebral variations and reduced ossification and a significant increase in haematomas at the highest dose) (Paternain et al., 1988).

Groups of 10 pregnant Sprague-Dawley rats were given aluminium nitrate at a dose of 180, 360 or 720 mg/kg, equivalent to 13, 26 or 52 mg Al/kg bw per day. by oral gavage from day 14 of gestation until day 21 of lactation The diet was reported to contain 60 mg Al/kg, which would have provided a dose equivalent to 6 mg Al/kg bw per day. No mention was made of maternal toxicity. The dosing regime did not produce overt fetotoxic effects, other than a decrease in birth weight at the highest dose. However, the number of litters was significantly lower in the treated groups than in the controls. The growth of the offspring was significantly less from birth and throughout lactation for the group at the highest dose (52 mg Al/kg bw per day). The animals in all the aluminium-treated groups weighed significantly less than controls at day 21. Relative organ weights (heart, lungs, spleen, liver, kidneys, brain) were reported and in many cases were significantly increased in treated animals relative to controls. The effects were not dose-dependent and in some cases (especially for the brains) would simply be caused by the growth retardation of the animals. The authors concluded that very few toxic effects were observed in the group at the lowest dose (Domingo et al., 1987).

A total of 31 time-mated Charles River CD dams were fed a solution of aluminium lactate at a dose of 0, 5, 25, 50, 250, 500 or 1000 mg Al/kg bw per day by daily gastric gavage from days 5 to 15 of gestation. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. The 390 offspring were evaluated for morphological and physiological parameters of reproductive functioning, including birth weight, anogenital distance, timing of vaginal opening, regularity of estrous cycles, duration of pseudopregnancy, number of superovulated oocytes and gonadal weight. No consistent or reproducible findings were reported in these parameters, with the exception of the regularity of estrous cycles. A temporary increase in the proportion of aberrant estrous cycles was detected in the first four cycles after vaginal opening in the group at 250 mg/kg bw per day, with none by the fifth consecutive cycle. The authors suggested that aluminium does not have a developmental reproductive toxic effect (Agarwal et al., 1996).

***Table 1. Studies of developmental toxicity with aluminium salts administered orally, published since the previous evaluation performed by the Committee***

| Species | Route | Compound | Dose | Duration (days of gestation) | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Mouse (Swiss, 20 per group) | Gavage | $Al(OH)_3$ | 66.5, 133, 266 mg/kg bw per day [a,b] | Days 6–15 | No evidence of maternal toxicity, embryo/fetal toxicity or teratogenicity reported | Domingo et al. (1989) |
| Mouse (Swiss Webster) | Diet | Aluminium lactate | 25 and 1000 mg Al/kg diet, equivalent to 4 and 100 mg Al/kg bw per day | Day 0 to weaning | LOAEL: 100 mg/kg bw per day (gestation and lactation exposure: growth retardation in offspring beginning on postnatal day 10) | Golub et al. (1992) |
| Mouse (Swiss CD1, 10–13 per group) | Gavage | $Al(OH)_3$<br>Aluminium lactate<br>$Al(OH)_3$ + lactic acid | 57.5 mg/kg bw per day [a,b]<br>166 mg/kg bw per day [a,b]<br>627 mg/kg bw per day [a,b] | Days 6–15 | $Al(OH)_3$: No toxicity reported<br>Aluminium lactate: LOAEL: 166 mg/kg bw per day (poor ossification, skeletal variations, cleft palate)<br>$Al(OH)_3$ + lactic acid: no toxicity reported | Colomina et al. (1992) |
| Mouse (CBA) | Water | $Al_2(SO_4)_3$ | 750 mg/l [a,b] | Days 10–17 | No toxicity reported | Clayton et al. (1992), cited in WHO (1997) |

***Table 1.*** (contd)

| Species | Route | Compound | Dose | Duration (days of gestation) | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Rat (Sprague-Dawley) | Gavage | $Al(NO_3)_3$ | 13, 26 or 52 mg Al/kg bw per day (+ equivalent of 6 mg/kg bw per day from diet) | Days 14–21 | LOAEL: 13 mg Al/kg bw per day (decreased survival ratios) | Domingo et al. (1987) |
| Rat (Sprague-Dawley, 10 per group) | Gavage | $Al(NO_3)_3$ | 13, 26 or 52 mg Al/kg bw per day [a] | Days 6–14 | LOAEL: 13 mg Al/kg bw per day (fetal malformations and variations) | Paternain et al. (1988) |
| Rat (Sprague-Dawley) | Diet | $AlCl_3$ with accompanying parathyroid hormone injection | 50 mg/kg bw per day [a,b] | Days 6–19 | LOAEL: 50 mg/kg bw per day (reduced skeletal ossification and increased incidence of skeletal varitions) | McCormack et al. (1979), cited in WHO (1997) |
| Wistar rats (13–14 per group) | Diet | $AlCl_3$ | 160 or 200 mg Al/kg bw per day [a] | Day 8 to parturition | LOAEL: 160 mg/kg/ per day (pre-weaning mortality) | Bernuzzi et al. (1986) |
| Rat (Sprague-Dawley, 15–19 per group) | Gavage | $Al(OH)_3$<br>Aluminium citrate<br>$Al(OH)_3$ + citric acid | 384 mg/kg bw per day [a,b]<br>1064 mg/kg bw per day [a,b]<br>384 mg/kg bw per day + 62 mg citric acid/kg bw per day [a,b] | Days 6–15 | LOAELs (reduced fetal body weight; increased skeletal variations) | Gómez et al. (1991), cited in WHO (1997) |

***Table 1.*** (contd)

| Species | Route | Compound | Dose | Duration (days of gestation) | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Rat (Wistar, 18–19 per group) | Gavage | $Al(OH)_3$ | 192, 384, 768 mg/kg bw per day [a,b] | Days 6–15 | NOEL: 768 mg/kg bw per day | Gómez et al. (1990), cited in WHO (1997) |
| Rat (CD, approx. three per group) | Gavage | Aluminium lactate | 0, 5, 25, 50, 250, 500 or 1000 mg Al/kg bw per day [a] | Days 5–15 | No consistent neuroendocrine or reproductive effects | Agarwal et al. (1996) |

Adapted from WHO (1997)

$AlCl_3$ : aluminium chloride; $Al(OH)_3$: aluminium hydroxide; $Al(NO_3)_3$: aluminium nitrate; $Al_2(SO_4)_3$: aluminium sulfate; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.

[a] No information on aluminium content of diet; [b] Unclear if dose is expressed as aluminium or as administered substance.

Groups of 20 pregnant mice were given $Al(OH)_3$ at a daily dose of 0, 66.5, 133 or 266 mg/kg by oral gavage on days 6 to 15 of gestation and killed on day 18 of gestation. Females were evaluated for body-weight gain, food consumption, appearance and behaviour, survival rates and reproduction data. No significant effects attributable to treatment were seen in any of these parameters. No treatment-related changes were recorded in the number of total implants, resorptions, number of live and dead fetuses, fetal size parameters and fetal sex distribution. Examination of the fetuses did not reveal any external, skeletal or soft tissue differences in comparison with the controls. Thus, the authors concluded that no maternal, embryo/fetal or teratogenicity was observed with the doses of aluminium administered to mice in this study (Domingo et al., 1989).

### *2.2.6 Special studies*

#### *(a) Studies of neurotoxicity and neurobehaviour*

There is considerable evidence that aluminium is neurotoxic in experimental animals, but species variation exists. In susceptible species (rabbit, cat, guinea-pig, ferret), the toxicity is characterized by progressive encephalopathy resulting in death associated with status epilepticus. The progressive neurological impairment is associated with neurofibrillary pathology in large and medium size neurons predominantly in the spinal cord, brain stem and selected areas of the cortex. These fibrils are morphologically and biochemically different from those that occur in Alzheimer disease. In addition, aluminium has been found to induce epileptic seizures in all species studied (e.g. primates, rodents and fish). These effects have been observed after parenteral injection (e.g. intrathecal, intracerebral and subcutaneous) and there have been no reports of progressive encephalopathy or epilepsy when aluminium compounds were given orally (WHO, 1997).

Behavioural impairment has been observed in the absence of overt encephalopathy or neurohistopathology in rats and mice given diets or drinking-water containing soluble aluminium salts (e.g. lactate, chloride) generally at doses of 200 mg Al/kg bw per day or more, as summarized in Table 2. Effects involved impairment of performance on passive and conditioned avoidance responses (COT, 2005). Because these studies were designed specifically to investigate behavioural effects and other potential end-points were incompletely evaluated, a possible role of organ damage (kidney, liver, immunological) cannot be discounted (WHO, 1997).

The effects of oral exposure to aluminium on brain development have been studied in mice. Effects recorded in more than one study in immature animals included impaired performance of reflexes and simple behaviours. Postnatal mortality and growth were also affected at the higher doses in some of these studies. Adult rats and mice have also been assessed for brain function after development exposures. Reduced grip strength and startle responsiveness were found to persist up to age 150 days. There was no effect on reactions to the light avoidance task in rats after gestational or postnatal exposure (WHO, 1997).

Swiss Webster mice were fed diets containing aluminium at 25 (control), 500 or 1000 mg Al/kg (as aluminium lactate) from conception through weaning. Maternal intakes were reported to be 5, 100 and 200 mg Al/kg bw, respectively at the

beginning of pregnancy and 10, 210 and 420 mg Al/kg bw, respectively near the end of lactation. Weights, food intake and toxic signs were recorded at regular intervals and pregnancy outcome evaluated. Pups were assessed for growth, neurobehavioural development and toxic signs before weaning. They were then assessed immediately after weaning and 2 weeks after weaning during which time they were maintained on control (25 mg Al/kg) diet. No maternal or reproductive toxicity was detected and there were no group differences in pup mortality, growth, toxic signs or neurobehavioural development before weaning. In general, dietary aluminium was associated with dose-related greater foot splay, decreased sensitivity to heat and greater forelimb and hindlimb grip strength shortly after weaning and, to some extent, after a 2-week recovery period on control diet (Donald et al., 1989).

Male Swiss Webster mice were fed diets containing 7 (control, with and without citrate), 100, 500, 750 or 1000 mg Al/kg diet as aluminium lactate (with 3.2% citrate to promote aluminium absorption) from the beginning of puberty (45 days of age) for either 4 or 8 weeks. There was no effect of aluminium content on food intake in any of the treatment groups, or on liver, spleen and tibia weights. A decrease in brain weight was recorded in the animals that received 1000 mg Al/kg diet (which the authors considered provided 100 mg Al/kg bw per day), for 4 weeks but not in the same group treated for the longer duration. A dose-related effect of aluminium on forelimb grip strength was recorded in the groups exposed for 4 weeks (i.e. in pubertal mice) but this effect disappeared in young adulthood, despite continued administration of aluminium (Golub & Keen, 1999).

Groups of 18 male and female Swiss Webster mice were fed diets containing aluminium at a dose of 1000 mg Al/kg diet in the form of aluminium lactate, from conception and throughout their lifespan. The authors considered this diet to provide a dose to adult mice of 100 mg Al/kg bw per day, control diet provided less than 1 mg Al/kg bw per day. Animals in the control and treated groups had a similar mortality rate and no evidence of gross neurodegeneration was seen. There were no consistent differences in neurobehavioural tests based on grip strength, temperature sensitivity or negotiating a maze. The only toxic signs reported were red eyes, fur loss and circling (motor stereotypy) all with a low incidence (no group incidences reported) (Golub et al., 2000).

In the study described in section 2.2.5.1, Swiss Webster mice received diets containing 7 (control), 100, 500 or 1000 mg Al/kg diet as aluminium lactate, throughout development (conception to age 35 days) and were subjected to behavioural tests as adults (aged more than 90 days). The authors considered these dietary doses to be equivalent to less than 1, 10, 50 and 100 mg Al/kg bw per day in adult mice. By weaning, both males and females in the groups at 500 or 1000 mg Al/kg weighed significantly less than controls. One offspring from each litter was used for behavioural testing. Subtle deficits in several neuroparameters, including impaired learning in a maze, were observed in the animals that received diet containing 1000 mg Al/kg, but not at the lower doses. A reduction in hindgrip strength was reported in approximately 15% of animals receiving the highest dose, this was no longer significant after adjustment for body weight (Golub & Germann 2001).

***Table 2. Studies of developmental neurotoxicity with aluminium salts administered orally, published since the previous evaluation by the Committee***

| Species | Route | Compound | Dose | Duration | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Mouse (Swiss Webster; males and females, eight per group) | Diet | Aluminium lactate | 7 (control), 500 or 1000 mg Al/kg diet 50 or 100 mgAl/kg bw per day | Conception through weaning, or; conception through adulthood | LOAEL: 50 mg/kg bw per day (reduced grip strength) | Golub et al. (1995) |
| Mouse (Swiss Webster; 20 per group) | Diet | Aluminium lactate | 7 (control), 100, 500 or 1000 mg/kg diet < 1, 10, 50 or 100 mg/kg bw per day | Conception to age 35 days | LOAEL: 50 mg/kg bw per day (weighed significantly less than controls) 100 mg/kg bw per day (neuroparameters) | Golub & Germann (2001) |
| Mouse (Swiss Webster) | Diet | Aluminium lactate | 25 (control), 500 or 1000 mg Al/kg diet, equivalent to 4, 75 or150 mg/kg bw per day | Conception through weaning | LOAEL 75 mg/kg per day (foot splay, forelimb and hind limb grip strengths, thermal sensitivity) | Donald et al. (1989)* |
| Mouse (Swiss Webster) | Diet | Aluminium lactate | 25 (control) or 1000 mg Al/kg 100 mg/kg bw per day | Conception through lactation | LOAEL: 100 mg/kg bw per day (growth retardation, forelimb grasp strength) | Golub et al. (1992) |
| Mouse (Swiss CD1, 10–13 per group) | Diet | Aluminium lactate | 25 (control), 500 and 1000 mg Al/kg diet equivalent to 4, 75 and 100 mg Al/kg bw per day | Day 0 of gestation to weaning | LOAEL: 100 mg/kg bw per day (increased landing foot splay, increased hindlimb grip strength, decreased temperature sensitivity in 21 day old mice) | Donald et al. (1989) |

***Table 2.*** (contd)

| Species | Route | Compound | Dose | Duration | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Mouse (Swiss Webster) | Diet | Aluminium lactate with and without citrate | 7 (control),100, 500, 750 or 1000 mg/kg diet<br><1, 10, 50, 75 or 100 mg/kg bw per day | 4 or 8 weeks from beginning of puberty (45 days) | NOAEL: 100 mg/kg bw per day<br>(no consistent toxic effects recorded) | Golub & Keen (1999) |
| Mouse (Swiss Webster;<br>18 per group) | Diet | Aluminium lactate | 7 (control) and 1000 mg Al/kg diet<br>< 1 and 100 mg/kg bw per day in adults | Conception through lifespan | LOAEL: 100 mg/kg (red eyes, fur loss, circling) | Golub et al. (2000) |
| Mouse (Swiss Webster) | Diet | Aluminium lactate | 7 (control) or 1000 mg Al/kg diet<br>200 mg Al/kg bw per day in pregnant mice<br>420 mg Al/kg bw per day in lactating mice<br>130 mg Al/kg bw per day in adult offspring | Conception to weaning or; conception to age 52 days | LOAEL: 1000 mg Al/kg diet (lower response amplitudes, reduced auditory startle) | Golub et al. (1994) |
| Rat (Wistar; (13–14 per group) | Diet | $AlCl_3$ | 160 or 200 mg Al/kg bw per day [a] | GD 8 to parturition | LOAEL: 160 mg/kg per day (pre-weaning mortality, delay in neuromotor development) | Bernuzzi et al. (1986) |

***Table 2.*** (contd)

| Species | Route | Compound | Dose | Duration | NOAEL/LOAEL | Reference |
|---|---|---|---|---|---|---|
| Rat (Wistar; 6–12 per group) | Diet | Aluminium lactate or $AlCl_3$ | 100, 200 or 300 mg Al/kg bw per day ($AlCl_3$) [a]<br>100, 200 or 400 mg Al/kg bw per day (Allactate) [a] | Days 1–21 of gestation | LOAEL: 200 mg Al/kg bw per day as $AlCl_3$ (grip strength)<br>100 mg Al/kg bw per day as Allactate (grip strength)x | Bernuzzi et al. (1989a) |
| Rat (Wistar; 25–38 per group) | Oral gavage | Aluminium lactate | 100, 200 or 300 mg Al/kg bw per day [a] | Postnatal days 5–14 | LOAEL: 100 mg Al/kg bw per day (negative geotaxis test) | Bernuzzi et al. (1989b) |
| Rat (Wistar; four per group; multiple groups per dose) | Oral gavage | Aluminium lactate | 100 or 200 mg Al/kg bw per day [a] | Postnatal days 5–14 | LOAEL: 200 mg/kg bw per day (increased brain Al, decreased choline acetyltransferase & general activity) | Cherroret et al. (1992) |
| Rat (Wistar; 6–9 per group) | Diet | Aluminium lactate | 400 mg Al/kg bw per day [a] | Days 1–7 of gestation; or days 1–14 of gestation; or conception to parturition | LOAEL: 400 mg Al/kg bw per day (locomotor co-ordination) | Muller et al.(1990) |

$AlCl_3$: aluminium chloride; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.

[a] No information on aluminium content of diet

Soluble (aluminium chloride, 30 and 100 mg/kg bw) and insoluble (aluminium hydroxide, 100 and 300 mg/kg bw) aluminium were administered orally once per day for 90 days to Long-Evans rats (groups of 10 males and 10 females). It is unclear if these doses related to the content of aluminium or of the substance tested. No information was provided on aluminium content of the chow. No relevant differences in body weight or general condition were observed between treatment groups. Performance in learning to negotiate a maze was significantly impaired in all of the aluminium-treated groups, with the performance of those receiving the highest dose of aluminium chloride being worst. The aluminium content of the brains was significantly elevated in each treatment group. The elevation was highest in those animals treated at 100 mg/kg bw (336% of control values). Brain acetylcholinesterase activity was significantly elevated by 65–83% in the two groups receiving the highest dose. Brain choline acetyltransferase activity was significantly lowered to 58% of control in the group treated with aluminium chloride at 100 mg/kg bw . (Bilkei-Gorzo, 1993).

Male Wistar rats (age 2 months, $n$ = 19 test, $n$ = 10 control) were given aluminium gluconate daily by intraperitoneal injection three times per week for 2 months or 3 months, or 2 months with 1 months rest. The test animals received 0.667 mg Al/250 µl and controls received an equal volume of sodium gluconate by intraperitoneal injection. Treatment began 2 months before behavioural testing and was maintained throughout the maze learning to avoid any decrease in tissue aluminium concentrations. No significant difference in body weight was observed at the end of the 2 months of treatment. Before the maze experiment the body weight of the rats was reduced by food deprivation and maintained at 80% of their free-feeding value. Rats were submitted to a radial maze test to determine the influence of aluminium on cognitive and non-cognitive behavioural processes. Both learning abilities (working memory and reference memory) and rapidity (time spent to respond to and master a trial) were recorded. Aluminium concentration was evaluated in the brain, serum and liver, significant increases were recorded in all tissue measurements. In the brain, aluminium accumulation was area-specific; the highest levels being observed in the temporal cortex, anterior olfactory nucleus and hippocampus. Despite the accumulation in the brain, no decrease in learning ability was observed, the only behavioural difference observed was a decrease in rapidity (Struys-Ponsar et al., 1997).

Pregnant rats received diets containing aluminium lactate at 400 mg Al/kg bw per day for either the first week (days 1–7 of gestation); first and second (days 1–14 of gestation); or from day 1 of gestation to parturition. Maternal body weight was significantly decreased on days 16 and 19 of gestation by 26% and 35%, respectively, for the group treated from day 1 of gestation to parturition, but not at the other doses. No effect of treatment on litter size, mortality rate or body-weight gain of pups was noted. Performance of the pups was impaired in a negative geotaxis test for those receiving the second two dosing regimes, and in locomotor co-ordination and operant conditioning tests for all three treatment groups. No differences were apparent in grasping and righting reflexes (Muller et al., 1990)

Pregnant Wistar rats received diets containing either aluminium chloride (100, 300 or 400 mg Al/kg bw per day) or aluminium lactate (100, 200 or 400 mg

Al/kg bw per day) from day 1 of gestation to parturition. Maternal food and water consumption was not affected by treatment. A 5–10% deficit in maternal body weight was reported at day 18 of gestation in the groups receiving the intermediate and highest dose of aluminium chloride and the highest dose of aluminium lactate, but not at earlier times. No effect of treatment on litter size was detected, but increased mortality was reported during the first week. This effect was significant in the groups receiving 300 mg Al/kg bw per day as aluminium chloride or 400 mg Al/kg bw per day as aluminium lactate. The neuromotor maturation of surviving pups treated with aluminium showed impairment during the first 2 weeks of life, with grasping reflex being significantly affected in all three groups receiving aluminium lactate and in all treated groups except that receiving the lowest dose of aluminium chloride (Bernuzzi et al., 1989a).

Astrocytes were prepared from cerebral cortex of rats aged 1–3 days. A 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test gave an $IC_{50}$ of approximately 343 µmol Al/l. In subsequent experiments, cultures were exposed to aluminium chloride at concentrations of 200, 400 or 800 µmol/l. Lactate dehyrogenase release showed a significant increase at 800 µmol/l. Cellular toxicity measured by vacuolation of cytoplasmic compartment and swollen appearance was measured in all but the control cultures. Exposure of cultures to 200, 400 or 800 µmol/l specifically increased glutamine synthetase at all three doses. In parallel with this increase, a higher rate of disappearance of glutamate from culture media was observed, as well as an accumulation of glutamine in the cellular extract. The authors suggest that these results indicate that the astrocyte population is a potential target for toxic action of aluminium that could mediate the pathogenesis of this metal (Struys-Ponsar et al., 2000).

*(b) Hormonal activity/effects*

Groups of six ICR mice (CD-1 derived) were given aluminium chloride at a dose of 35 mg Al/kg bw per day by intraperitoneal injection for 12 days to study effect on nitric oxide production in serum and testis. Serum and testicular aluminium concentrations increased significantly compared with controls. Aluminium administration significantly increased the production of nitric oxide and decreased testicular adenosine 3',5'-cyclic monophosphate (cAMP). As a consequence of the decreased cAMP activity, the observed transport of cholesterol into the mitochondria of Leydig cells, and thus the secretion of testosterone, was reduced (Guo et al., 2005).

*(c) Effects on bone*

Excessive deposits of aluminium in the skeleton may result in a syndrome referred to as 'aluminium-induced bone disease', and a number of animal models of osteomalacia involve intraperitoneal or intravenous injection of aluminium. Osteomalacia occurs when aluminium concentrations in bone reach 100 µg/g bone ash, which is more than 10 times the normal human bone aluminium concentration. No studies of oral administration were identified (WHO, 1997).

## 3. OBSERVATIONS IN HUMANS

### 3.1 Biomarkers of exposure

Concentrations of aluminium in blood, urine and faeces can be measured in humans, but these measurements are not directly related to oral exposure to aluminium (ATSDR, 1999). One reason is that the gastrointestinal tract is a barrier to aluminium uptake (Priest, 2004), and a second reason is that the composition and acidity of the diet affect how much aluminium will be absorbed (Becaria et al., 2002). ATSDR (1999) pointed out that high levels of exposure to aluminium can be reflected by aluminium concentrations in urine, but noted that the rapid excretion of aluminium in urine affects the validity of this parameter as a measurement of bioavailability.

Priest (2004; p. 375) estimated that "…most aluminium that enters the blood is excreted in urine within a few days or weeks." Aluminium concentrations in faeces can also be measured and used to estimate a part of the oral exposure to aluminium, but not the portion that is absorbed by the body (ATSDR, 1999).

#### *3.1.1 Biomarkers of effects*

At the current time, no simple non-invasive test was available to measure the effects of oral exposure to aluminium in humans (ATSDR, 1999).

#### *3.1.2 Clinical observations*

##### *(a) Dialysis encephalopathy and other disorders in patients with chronic renal failure*

In the early to mid 1970s, reports were published describing a cluster of symptoms observed in patients from different dialysis units (Alfrey et al., 1972; Mahurkar et al., 1973; Barratt & Lawrence, 1975; Rosenbek et al., 1975). According to Alfrey et al. (1976), the majority of patients described in the reports had been on intermittent haemodialysis for several years, and the clinical findings included speech difficulty, asterixis, myoclonus, dementia, focal seizures and an abnormal electroencephalogram. The symptoms often progressed to coma and death. This cluster of symptoms became defined as dialysis encephalopathy syndrome (DES) and the cause of the syndrome was investigated. Initially, clinicians suggested a number of possible causes: viral infections, vitamins, amino acid or dopa deficiency, hypertension, drug intoxication, toxic metal deposition or aluminium accumulation (Starkey, 1987). Over the past 30 years, a substantial amount of evidence on this disorder has been collected and aluminium is now widely considered to be a primary cause of DES (Kerr et al., 1992; WHO, 1997; Flaten, 2001; Goyer & Clarkson, 2001). This evidence included findings of elevated concentrations of aluminium in blood, bone, muscle, and brain tissue in patients with DES (Starkey, 1987; Goyer & Clarkson, 2001). Kerr et al. (1992) discussed four factors involved in aluminium intoxication of patients with chronic renal failure: "1) exposure to large volumes of contaminated fluid during haemodialysis, peritoneal dialysis, haemofiltration and occasionally, intravenous therapy; 2) ingestion of grams of aluminium daily as a

phosphate binder; 3) loss of the renal excretory pathway for aluminium; and 4) increased aluminium absorption from the gut in uraemia" [p. 123]. In addition to DES, other disorders associated with aluminium have been observed in patients with chronic renal failure on dialysis, including osteomalacia, extraskeletal calcification, microcytic anaemia and cardiac arrest (Starkey, 1987; Drüeke, 2002).

Among the early studies on DES and concentrations of aluminium in water used to make dialysis fluids, one study found that DES rarely affected patients in those centres in the UK that used water with aluminium concentrations of less than 50 µg/l (Parkinson et al., 1979, 1981). Another study in the Trent region in England found that the average water concentration for patients on dialysis that developed encephalopathy was 328 µg/l (average aluminium concentration in water for patients experiencing multiple bone fractures was 160 µg/l, for patients on dialysis without either disorder, 80µg/l) (Platts et al.,1977). When Parkinson et al. (1981) summarized the early clinical studies or outbreaks of DES that included measures of the concentration of aluminium in water used to make dialysis fluid, they found that the concentrations of aluminium in water associated with DES were usually reported to be greater than 200 µg/l. Treating water used for dialysis with various methods such as filtration, carbon adsorption, reverse osmosis and de-ionization, depending on the water supply, has been found to reduce the incidence of DES (Parkinson et al., 1981; Kerr et al., 1992).

*(b) Osteomalacia*

In addition to bone changes observed in patients on dialysis, osteomalacia has also been observed in several patients on long-term parenteral nutrition (TPN) who had a variety of gastrointestinal illnesses with malabsorption but who had not been taking large amounts of antacids (Klein et al., 1982; Ott, 1985). Klein et al. (1982) found a substantial quantity of aluminium delivered intravenously in the TPN when casein hydrolysate was used as the protein source. They found that the patients had elevation of serum aluminium content, increased urinary excretion of aluminium and a high content of aluminium in trabecular bone. While suspicious, the researchers state that the "…data do not prove a pathogenic relationship between Al and bone disease" (Klein et al., 1982; p. 1425).

There have also been a few case reports of adults, infants and a child with normal renal function who experienced skeletal changes from frequent use of aluminium-containing antacids for the treatment of gastrointestinal illness (Neumann & Jensen, 1989; Foldes et al., 1991; Pivnick et al., 1995; Shetty et al., 1998; Woodson, 1998; ATSDR, 1999). The antacids in these cases were considered to induce phosphate depletion that resulted in alteration of bone. One example of such a case was described by Woodson (1998). A woman aged 39 years, taking large doses of an antacid containing a high concentration of aluminium and magnesium hydroxide for peptic ulcer and gastritis, reported pain in the right foot. X-ray examination of the foot revealed a callous around a stress fracture of the calcaneous. Bone biopsy found that 27.6% of the bone surface had aluminium deposits. The amount of intake of elemental aluminium in the antacid was estimated to be 6.3 g/day and 18 kg over 8 years. Woodson suggested that the antacid had bound phosphate in the gut causing its malabsorption and that profound

phosphate depletion had occurred that resulted in osteomalacia. When the patient stopped intake of the antacid, she had improvement in symptoms and objective findings.

*(c) A case of severe cerebral congophilic angiopathy, an Alzheimer-related disease*

In July 1988, a water authority inadvertently discharged 20 tonnes of aluminium sulfate into the drinking-water supplied to Camelford, UK and its vicinity. The drinking-water was considered to be heavily polluted for 3 days, not only with the increased concentration of aluminium but also with copper, lead and zinc that had leached from pipes, owing to the increased acidity of the water (Coggon, 1991; Owen et al., 2002). The highest aluminium concentration measured in water for this accident was 620 mg/l (Owen et al., 2002). The highest concentration considered palatable for drinking was 100 mg/l (WHO, 1997 cites Clayton, 1989). Initial acute effects reported in this population were gastrointestinal problems and oral ulceration (Coggon, 1991). Since January 1989, an advisory group of independent experts has met on several occasions to evaluate possible long-term health effects from the accident. Other epidemiology studies from this setting are discussed in section 3.1.3, but a recently published case study (Exley & Esiri, 2006) will be considered here.

Exley & Esiri (2006) report postmortem findings on brain tissues from a resident of Camelford who was referred for a neurological examination in 2003 at age 58 years and died in 2004 of an unspecified neurological condition. Examination of brain tissue revealed "a rare form of sporadic early-onset ß amyloid angiopathy in cerebral cortical and leptomeningeal vessels, and in leptomeningeal vessels over the cerebellum" (Exley & Esiri, 2006, p.1). DNA testing of brain tissue detected APOE genotype ε4/4. During the analysis of brain tissue for concentration of aluminium, the examiner was masked to whose tissue sample was under study and conducted analysis on tissue from this patient along with three other patients. A range of aluminium concentrations in tissues were found for the present case, from a low in the range of 3–7 µg/g dry weight (exact measurement not presented in paper) to a high of 23.0 µg/g dry weight. The authors report these concentrations as coincident with the severely affected areas of the cortex and find them to be high in comparison to what they consider to be usual aluminium concentration in brain tissue, 0–2 µg/g dry weight. One of the three other individuals had neuropathology similar to the case but was 22 years older; and the highest aluminium concentration in the tissue of this person was 25.16 µg/g. The paper does not state that this person was exposed in the Lowermoor incident so presumably he/she was not exposed. The researchers also discuss findings from other studies regarding APOE genotype ε4/4 as a risk factor for early age onset of Alzheimer disease and for deposition of ß-amyloid angiopathy in walls of cortical and leptomeningeal blood vessels. They were not aware of examination of brain tissue for aluminium concentrations in other studies of similar cases. They conclude by indicating that it is not yet understood what role aluminium has, if any, in the initiation and progression of this rare disease.

### *3.1.3 Epidemiological studies*

#### *(a) Alzheimer disease, dementia or cognitive impairment*

##### *(i) Exposure to aluminium in drinking-water*

There have been several reviews of the epidemiology studies of aluminium in water in relation to Alzheimer disease, dementia or cognitive impairment (e.g. Doll, 1993; WHO, 1997; Flaten, 2001; Jansson, 2001 & COT, 2005). A table summarizing the epidemiology studies reviewed in the WHO report (1997) updated by COT (2005) is shown at the end of this section (Table 3). Studies reviewed in this document include additional studies not listed in the original table and, for the purpose of the discussion below, have been inserted in the table (indicated by asterisks). The studies are listed by publishing year in the table, but they are grouped according to study design in the text for the purpose of comparison.

An epidemiology study was included in this review only if information on exposure assessment and disease definition were provided and if the study was adjusted for one or more of the possible risk factors for Alzheimer disease. The one exception to this definition was the inclusion of a brain autopsy case–control study (McLachlan et al., 1996). Potential confounders of the relationship between aluminium and Alzheimer disease were not collected in the study, but classification of cases was meticulous at the tissue level and information on exposure to aluminium in water 10 years before death was ascertained, warranting its inclusion in this review.

One comment by investigators that recurs throughout these studies is the issue regarding bioavailability of aluminium. They recognize that the concentration of aluminium is much higher in food than in water and that only a small portion of the daily intake of aluminium is from water. One investigator postulated that "aluminium in drinking-water is either dissolved or readily brought into solution and its bioavailability may therefore be much higher than aluminium from other sources" (Martyn, 1989; p. 59); however, the bioavailability of aluminium from water versus foods is not yet fully understood and the relation of different species of aluminium in human absorption requires further study. Only one of the epidemiology studies below examined any form of aluminium other than total aluminium concentrations in water.

*Ecological study*

Flaten (1990) conducted an exploratory ecological study to determine the association of age-adjusted death rates from dementia, Parkinson disease and amyotrophic lateral sclerosis (ALS) for 193 municipality aggregates in Norway with the weighted mean aluminium values in drinking-water for each of these aggregates (weighted by the number of persons served by the individual water works). Death certificates containing ICD-8 codes for dementia, Parkinson disease and ALS as the underlying or contributory cause of death were used to ascertain disease. Death rates were calculated for 10 year and for 5 year intervals for the years, 1969–1983. Aluminium content in finished water was obtained across four seasons in 1982–1983 from each of the Norwegian waterworks and ranged from: not

detectable (< 0.008 mg/l) to 4.10 mg/l (the value at the 90th percentile was 0.238 mg/l). For dementia, the death rates for the period 1974–1983 increased for both men and women across low, medium and high categories of aluminium concentration in water, with no overlap in the 95% confidence intervals. For Parkinson disease, there were somewhat higher death rates at higher aluminium concentration in water but the 95% confidence intervals overlapped. No significant association between ALS death rates and aluminium concentrations in water was observed.

Flaten (1990) emphasized the exploratory nature of his ecological study and noted that increased dementia rates might be explained by other factors, such as socioeconomic variables. He found, for example, that dementia rates also correlated strongly with population density although "Al concentration and the percentage living in densely populated areas were not strongly inter-correlated" [p. 165]. Furthermore, while a patient may have dementia, the disease may not be considered by a physician as an underlying or contributory cause of death and reporting of this disorder on a death certificate may vary widely; however, unless under-reporting varied systematically by area of aluminium concentration, it is difficult to perceive how this would strongly influence the findings.

Flaten (1990) mentioned that aluminium levels in Norway may be changing in water over time owing to increased acidification of rain which helps mobilize aluminium from the soil. This change should be distributed throughout the country and not in a specific region. Because death rates were ascertained over a 10-year period and aluminium concentrations in water were ascertained over four seasons in a single year, exposure levels of aluminium before onset of disease are not known on an ecological or individual level.

*Prevalence (cross-sectional) studies*

Martyn et al. (1989) studied prevalence of dementia and epilepsy in 88 county districts in England for the age group 40–69 years. Dementia and epilepsy were defined by results on a computer tomography (CT) scan and by clinical information supplied on a request form for the CT scan. Dementia was detected in 1203 persons and categorized as probable Alzheimer disease, possible Alzheimer disease, cerebrovascular dementia or other causes of dementia. Age-adjusted rates of disease for each county district were calculated and adjusted for differences from nearest CT scan as well as size of the population served by the CT scan units.

Exposure to aluminium was based on the residual concentration of aluminium for each water source across the 88 county districts over a 10 year period before diagnosis of patients. A mean concentration of aluminium in the water was determined for each county district. Five categories of water exposure were created: 0–0.01 mg/l, 0.02–0.04 mg/l, 0.05–0.07 mg/l, 0.08–0.11 mg/l, and > 0.11 mg/l. Risk of Alzheimer disease and epilepsy in patients in county districts with higher mean aluminium concentration were calculated relative to the lowest category of aluminium concentration (0 to 0.01 mg/l). Significant relative risks were found for the 'probable Alzheimer disease' category, but not for other dementia disease categories or epilepsy. Table 3 indicates this finding, but a clear dose–response

trend of increasing risk of probable Alzheimer disease with increasing aluminium concentration in water was not observed.

Michel et al. (1991) studied a sample of persons residing in France (they used a three-stage approach to sampling: they randomly sampled cantons (districts) in the department of Gironde, then communes (parishes) from the cantons and finally, study participants from the parishes); 4050 subjects were obtained through sampling, but 1258 (31%) persons declined to participate in the study. The final sample size was 2792 persons.

Alzheimer disease was determined in a two-stage process. For the first stage, a screening was done by psychologists (Diagnostic Statistical Manual (DSM)-III criteria for dementia and psychometric tests). Patients who tested positive by the criteria in the first stage went to a second stage. In the second stage, senior neurologists interviewed patients and performed a clinical examination (using the criteria for Alzheimer disease specified by the Joint Working Group of the US National Institute of Neurological and Communicable Disorders and Stroke and Alzheimer Disease and Related Disorders (NINCDS-ARDA)) (McKhann et al., 1984). Exposure to aluminium was based on the concentration of aluminium in the well water for a parish. Aluminium concentration in well water ranged from 0.01 to 0.16 mg/l. The researchers evaluated whether increasing levels (across four categories) of aluminium in well water across parishes correlated with increasing prevalence of Alzheimer disease across parishes. They found a statistically significant association between prevalence of probable Alzheimer disease and category of aluminium concentration. This association continued to be observed after adjusting for age, education, and urban/rural. They found a relative risk of 1.16 for an increase of 0.01 mg/l and relative risk of 4.53 for an increase in 0.1 mg/l (95% confidence interval (CI), 3.36–6.10).

Wettstein et al. (1991) conducted a prevalence study in Switzerland to test the mnestic and naming performance of 800 persons aged 81 to 84 years, who had lived for more than 15 years in either of two districts: in one of these districts the water supply contained a high concentration of aluminium and in the other the concentration of aluminium in the water supply was low (98 µg/l versus 4 µg/l). The researchers also examined the serum and urinary aluminium concentration of 40 nursing-home patients living in either of the two districts to examine differences in intake of metal from water; 20 of the nursing home patients (10 patients in each district) were diagnosed as having senile dementia.

The two city-districts selected for the study had socioeconomically similar populations. Four hundred study participants from the low-aluminium district and 405 study participants from the high-aluminium district were enrolled in the study, using the same method for selecting individuals for contact in each district. The participants were interviewed and given the mnestic and naming subtest of the Mini Mental Status test.

No significant differences in test scores for the participants in the two districts were found. Age and education affected these scores but these factors were equally distributed in the two districts and did not appear to confound the findings. Serum aluminium concentrations in the nursing-home patients with Alzheimer disease

were somewhat higher in the low-aluminium district than the high-aluminium district, the opposite of postulated results. For the 10 control patients in each district there was no significant difference in mean serum aluminium concentrations. Urinary excretion of aluminium was similar in the two groups of nursing-home patients with Alzheimer disease; however, the urinary aluminium : creatinine ratio was significantly higher in control patients from the low-aluminium district than from the high-aluminium district. Researchers concluded that they could not demonstrate a consistent effect of residence in nursing homes with high or low aluminium in drinking-water (Wettstein et al., 1991, p.100). This study is interesting because it attempted to evaluate biomarkers for exposure to aluminium while determining whether poorer mnestic and naming performance are associated with higher concentrations of aluminium in water.

Forbes et al. (1992; 1994a; 1994b; 1995a; 1997) studied participants from a cohort **o**f men enrolled in the Ontario Longitudinal Study of Aging; this cohort initially enrolled 2000men at age 45 years and has been followed for more than 30 years. Forbes et al. evaluated the association between impaired mental function and various chemicals in drinking-water. In 1990–1991, the researchers administered a questionnaire by telephone to the remaining participants. Participants responded directly to the questionnaire ($n$ = 513) or the questionnaire was administered to proxy persons if participants were deceased (usually to a relative of a deceased cohort member, $n$ = 224) or unable to respond for other reasons ($n$ = 45). A total of 782 interviews were conducted (Forbes et al., 1992; 1994b). In later papers, the number of interviews increased to 870 (545 participants, 276 relatives, and 49 proxies) (Forbes et al., 1994a; 1997). The questionnaire contained about 100 questions, including a modified mental status test and nine questions which tested short-term memory. When all 100 questions were answered correctly, the study participant was characterized as having no impairment of mental function; otherwise, a study participant was considered to have some impairment of mental function (Forbes et al., 1992; 1994b).

Information on the residence of these men over the past 30 years was known. The researchers estimated exposure to aluminium for each individual based on the concentration of aluminium currently in the water supply of the city in which the individual had lived the longest. For the analysis, there was information available on both the questionnaire results and water exposure information for 485 men from the cohort (Forbes et al., 1992); in later papers, information for a somewhat higher number of men was available. The odds ratio for the association of some impairment of mental function with high concentrations of aluminium (≥0.085 mg/l) in drinking-water compared with lower concentrations was 1.14, which was not statistically significant ($p$ > 0.05) (Forbes et al., 1992). When only the data from the study participants who were directly interviewed were analysed, the odds ratio increased to 1.53, but was not statistically significant (95% CI, 0.94–2.51) (Forbes et al., 1994b).

Forbes et al. (1992; 1994a; 1994b; 1995a; 1997) also evaluated a number of other water constituents or characteristics, including fluoride, pH, organic matter (DOC), turbidity, silica, and iron, and found the association between aluminium and impairment of mental function to be somewhat more complicated (data from all study

participants used). For example, a significant association of aluminium and fluoride concentrations with impairment of mental function was found when persons consuming water with high aluminium concentrations (≥0.085 mg/l) and low fluoride levels (< 0.13 mg/l) were compared with persons consuming water with low aluminium concentrations and high fluoride concentrations (OR = 2.72, $p$ = .01) (Forbes et al., 1992). Low or high iron concentrations in the water did not appear to affect this association (Forbes et al., 1997). As another example, at a pH of greater than 8.05, the association between high aluminium concentrations and impairment of mental function was in a positive direction (OR = 1.30 (95% CI, 0.85–2.04), while at medium and low pH, the association was in the opposite direction (at pH range 7.85–8.05, OR = 0.68 (95% CI, 0.21–2.19); at pH < 7.85, OR = 0.76 (95% CI, 0.28–2.06), although none of the findings were statistically significant. A logistic regression model that accounted for various water constituents or characteristics and other factors (that is, fluoride, pH, turbidity, silica, iron, source of water, level of education, health at age 62 years, income at age 45 years, total number of moves, and age) found a significant association between high aluminium concentrations and impairment of mental function (OR = 2.35, 95% CI, 1.32–4.18) (Forbes et al. 1995a). In regard to their findings, Forbes et al. (1994a, 1994b) comment that the results must be considered in light of the problem of substantial drop-out of participants from Ontario Longitudinal Study of Aging over 30 years, which potentially affects whether the results from the remaining participants are representative of the study population. They also comment that the presence of any impairment of mental function on screening tests is not the same as dementia, and comment that they may not have considered other possible important confounding factors.

Jacqmin et al. (1994) studied 3777 French men and women aged 65 years and older enrolled at baseline for a population-based cohort study (the Paquid study). These participants were randomly selected from electoral rolls from one of 75 randomly selected rural or urban parishes in the administrative areas of Gironde or Dordogne in south-western France (more sampling detail described above, Michel et al., 1991, Gironde only). A study participant who scored less than 24 on the Mini-Mental State Examination (MMSE) was considered to have cognitive impairment.

Exposure to aluminium was based on information collected in two surveys in 1991 of 75 drinking-water areas that supplied the cohort participants. In addition to aluminium, these surveys measured pH and various chemicals in the water (aluminium, calcium, and fluorine). The researchers also collected historical information on chemical concentrations in water for the period 1981–1991. For each parish, the researchers calculated a weighted mean of all measures for each chemical.

The researchers analysed prevalence rates for cognitive impairment across increasing concentrations of aluminium, calcium, fluorine and increasing pH. The main finding of the study was a significant protective effect of high calcium concentrations for cognitive impairment before and after adjusting for the effects of age, sex, level of educationa and occupation of the study subjects. No significant association of aluminium concentrations with cognitive impairment was observed

(highest category of aluminium in this study was greater than or equal to 18 µg/l), except when the logistic regression model incorporated an interactive term for aluminium and pH. The researchers further evaluated this relationship. A stratified analysis of pH and aluminium demonstrated that the odds ratio for the association of aluminium with cognitive impairment mildly increased in magnitude with increasing doses of aluminium when the pH was 7.0 but decreased in magnitude with increasing doses of aluminium when the pH was 8.0. It was not clear to the researchers how to interpret this finding. The study did not demonstrate an effect of calcium on the aluminium-cognitive impairment relationship. Researchers also analysed exposure to aluminium cooking vessels, but did not find an association of this exposure with cognitive impairment.

The results of this study conflict with those of the study conducted by Michel et al. (1991), athough the participants in the Michel study are a sub-sample (from Gironde) of the participants in this study. Jacqmin et al. (1994; p. 49) consider that the difference in the two studies is related to problems with the historical assessment of aluminium in drinking-water: "A previous report on the Paquid study supported the hypothesis of an association between the risk of Alzheimer's disease and high levels of aluminium in the drinking water. However, this was based on retrospective measures of the concentrations of aluminium that were not reliable: in particular, some of these measures were old and sampling and dosage techniques have changed in recent years" There were other differences between the two studies, including a difference in the health end-point (probable Alzheimer disease versus cognitive impairment).

Forbes et al. (1995b) used Ontario death certificate data from Statistics Canada for a cross-sectional mortality study. Researchers searched for ICD-9 codes for Alzheimer disease (ICD-9 code 331.0), presenile dementia (ICD-9 code 290.1) or bronchopneumonia (ICD-9 code 485) when they examined records. A total of 3161 persons who died with Alzheimer disease or presenile dementia as the underlying cause of death were enrolled in the study. It is difficult to determine from the paper how the researchers calculated death rates for Alzheimer disease or presenile dementia, but presumably all other deaths in the same age category as those from Alzheimer disease were used as the denominator for the rates. Bronchopneumonia deaths were considered only briefly in the paper in relation to water concentrations of chemicals because bronchopneumonia may be the actual cause of death in many patients with Alzheimer disease.

Presumably, the researchers estimated exposure to aluminium in water for each individual based on the concentration of aluminium currently in the water supply for the residence where the individual lived at the time of death. They had used residence water-supply information for living individuals when evaluating exposure in their earlier studies (Forbes et al., 1992, 1994a, 1994b), but the method of assigning exposure for persons who died was not explicitly stated.

Researchers categorized aluminium concentrations in water according to three categories: 0.067 mg/l, 0.068–0.20 mg/l and ≥ 0.336 mg/l. There were no values between 0.21 and 0.335 mg/l. More than 75% of the individuals with Alzheimer disease were exposed at the intermediate level, 0.068–0.20 mg/l, while

less than 1% ($n = 14$) of individuals were exposed at the highest level, > 0.336 mg/l. It is not clear from the methods why the researchers selected these particular ranges of aluminium concentration for each category. Results of the analysis showed a significantly greater than 1.0 rate ratio for the association of death from Alzheimer disease with the highest category compared with the lowest (all ages of Alzheimer disease: rate ratio = 2.42 (95% CI, 1.42–4.11); this rate ratio increased when higher ages were used to identify deaths from Alzheimer disease (i.e. at 75 years and older, at 85 years and older). However, there was a less than 1.0 rate ratio for the association of death from Alzheimer disease with the intermediate category of values compared with the lowest (all ages of Alzheimer disease: rate ratio, 0.92, 95% CI, 0.84–1.01). From these findings, the researchers proposed that there may be a J-shaped rather than linear dose–response relationship when aluminium concentrations in water reach the levels found in this study. When only two categories were used in analysing the data, above or below 0.075 mg/l (or 0.068 or 0.085 mg/l), the rate ratios for the association of Alzheimer disease death with the higher category compared with the lower category were all below 1.0 (Forbes et al., 1995b; p. 646).

The researchers also modeled the effects of fluoride, pH and silica ($SiO_2$) on the aluminium–Alzheimer disease association, finding possible interactive effects of some of these chemicals. For example, when high concentrations of fluoride (> 0.5 mg/l) and pH (≥7.96) are present, the risk ratio for high concentrations of aluminium associated with Alzheimer disease is reduced. In a later paper, Forbes et al. (1997) added iron concentrations in water to the multivariate analysis (poisson regression model) and continued to observe the J-shaped association between aluminium concentrations and death from Alzheimer disease for persons aged 85 years or older.

Gillette-Guyonnet et al. (2005) analysed baseline data from an ongoing cohort study, the Epidemiology of Osteoporosis (EPIDOS) study, that follows women aged more than 75 years residing in five cities in France ($n = 7598$ women) to determine risk factors for fracture of the femoral neck. Gillette-Guyonnet et al. (2005), however, used data from this study to compare exposures to concentrations of chemicals in water in women with cognitive impairment at baseline of the study to exposures in women without cognitive impairment at baseline.

Cognitive impairment at baseline was assessed by the Short Portable Mental Status Questionnaire (SPMSQ) and women with a Pfeiffer score of < 8 were considered to have cognitive impairment. Exposure to chemicals in water was assessed from questioning women about water intake, from the tap or from bottled mineral water, and from obtaining data about the concentrations of aluminium, silica and calcium concentrations in brands of bottled water or the city water supply. Aluminium in water varied from undetectable to 0.032 mg/l in bottled water and from 0.01 to 0.063 mg/l in the five cities in the study.

The researchers did not find an association between cognitive impairment and aluminium in the baseline study. They were able to adjust for a number of potential confounders in their analyses, including age, education and income. They

did find that cognitive impairment was associated with lower silica concentrations in drinking-water. No effect of calcium in water was observed.

Only one of the seven prevalence studies presented here examined daily ingestion of water at the individual level; most used average concentration of aluminium in the water supply to residences as an estimate of aluminium exposure. For three of the seven studies, an association of Alzheimer disease with aluminium was found, although the measurement of historical aluminium concentrations was called into question for one of these studies. Of the other four studies, all of which examined cognitive impairment, only one found an association between cognitive impairment and aluminium. The issue of the possible effect of other chemicals in drinking-water on the relationship between Alzheimer disease and aluminium was evaluated in two studies, but little information on potential confounding factors was available overall. A difficulty for several of these studies is determining an estimate of exposure before development of disease.

*Case–control studies*

Neri & Hewitt (1991) conducted a cross-sectional case–control study in Ontario, Canada in 1986. Cases were 2344 individuals aged 55 years or older who had a diagnosis of Alzheimer disease or presenile dementia by ICD-9 code (331.0 or 290.0) on their hospital summary record. Controls were 2232 individuals matched by age and sex to cases that had non-psychiatric diagnoses on their hospital record. Aluminium concentration of finished water in the municipal water supply for the area of the patient's current residence was obtained (if water information was not available for the patient's locality, the individual was not enrolled in the study). The unmatched analysis indicated that the estimated relative risk for Alzheimer disease increased with increasing concentrations of aluminium in the water supply (from 0.01 mg/l to above 0.20 mg/l), although the statistical significance of this finding was not shown.

Forster et al. (1995) conducted a case–control study of presenile dementia of the Alzheimer type (PDAT) in northern England to determine risk factors for this disease. Cases ($n$ = 109) were ascertained through hospital admission and other medical records; they were individuals who were aged less than 65 years when they were diagnosed as having dementia by clinical algorithm criteria for Alzheimer disease in the period 1981–1989 and confirmed at the study interview. Controls ($n$ = 109) were randomly selected from the same northern regional health authority (although not the same general practice) as the cases and pair-matched to cases on age and sex. Close relatives were used as informants for both cases and controls to respond to questions about exposure to risk factors in the period before the onset of PDAT in the cases. Historical data on aluminium concentration in drinking-water was obtained from the local water authority in the mid to late 1980s where each case lived the longest in the 10 years before onset of PDAT (the same time period and residence criteria was used for the matched control). Aluminium concentration in water was analysed and no significant association of Alzheimer disease with this exposure was seen across four categories of increasing concentration of aluminium (< 50 µg/l, > 50 µg/l, > 99 µg/l, > 149 µg/l). Taylor et al. (1995) used the same study participants and used exposure to current water supply levels of aluminium and

silicon to examine if dissolved silicon in drinking-water was related to PDAT. The reason for studying this relationship was based on the possibility that silicon might affect the bioavailability of aluminium. They found that soluble silicon concentrations for the cases did not differ significantly from those for the controls, but did find a significant inverse relationship between silicon and aluminium concentrations (Spearman's rank order correlation coefficient 0.43, $p < 0.001$).

McLachlan et al. (1996) conducted a case–control study in Canada. Definition of cases and controls was based on postmortem histopathological examination of brains of individuals who were residents of Ontario at time of death. Cases were categorized as having definitive pathological diagnosis of Alzheimer disease ($n = 296$) with an absence of any other degenerative process, or Alzheimer disease pathology with other neuropathologic changes and clinical dementia ($n = 89$). Controls were categorized as having either no histopathology on postmortem ($n = 125$) or having histopathological changes for several other diseases, none for which an association with aluminium has been found ($n = 340$).

Exposure to aluminium in water was based on water source for residence at time of death and also on a 10-year residential history obtained for a subset of the cases ($n = 119$) and controls ($n = 51$) from a telephone interview of next of kin of these individuals. The 10-year exposure was calculated using water supply data for each residence of an individual weighted by duration of residence in that location. In the analysis, the cases from the first category were compared with controls and then all cases were combined and compared with controls (all controls and a subset of controls). A significant association was found between Alzheimer disease and aluminium concentrations in water > 100 µg/l for residence at time of death or 10-year exposure history (all cases and controls, current: OR = 1.7 (95% CI, 1.2–2.5); 0-year: OR = 2.6; 95% CI, 1.2–5.3). Similar results were found for other configurations of case–control comparisons. Other potential confounders of the relationship between Alzheimer disease and aluminium were not available in this study, including age. The 10-year residential history may not be sufficient to detect exposures that cause disease.

Martyn et al. (1997) conducted a case–control study that involved men from eight regions of England and Wales who were aged between 42 and 75 years. Study participants were identified from the CT records of eight neuroradiology centres. Men with a possible diagnosis of dementia on the CT record, a normal CT scan or a CT scan which showed only cerebral atrophy without evidence of infarction, and hospital notes indicating a clinical diagnosis of Alzheimer disease were enrolled as cases ($n = 106$). Men were enrolled into one of three control groups when: (a) the CT scan indicated a diagnosis of dementia without other evidence indicating Alzheimer disease ('other dementia' control group, $n = 99$); (b) the CT scan indicated a diagnosis of primary brain cancer ('brain cancer' control group, $n = 226$); or (c) the CT scan indicated another diagnosis other than dementia, such as malignant brain tumour, epilepsy or chronic disabling disease ('other' control group, $n = 441$).

Exposure to aluminium in drinking-water was ascertained through questionnaire information given to the study participant or the next of kin (if participant had died or was unable to fill out questionnaire). The questionnaire elicited the

addresses of all places of residence of 3 years or more since the study participant was aged 25 years. The investigators collected information on concentrations of aluminium and molybdate-reactive silica in drinking-water for these addresses and estimated an average concentration of the chemicals in drinking-water over time for each individual. The exposure for aluminium in water ranged from less than 0.015 mg/l to greater than 0.109 mg/l. In a comparison of the cases with each control group, no increased risk of Alzheimer disease with aluminium in drinking-water was observed, nor was a protective effect of silicon in drinking-water found as hypothesized. The use of three control groups aided in providing evidence that the lack of association was consistently found and less likely to be a result of study bias from control selection or control response to questionnaire.

Gauthier et al. (2000) conducted a case–control study in Quebec, Canada, enrolling 68 cases of Alzheimer disease and 68 controls matched pair-wise to cases on age and sex. These study participants were a subset of a larger random sample of persons aged 70 years or older selected from the Quebec health plan files. Alzheimer disease diagnosis occurred after a three-phase assessment of sampled individuals. A screening test, the Modified Mini-Mental State Examination (3MS), determined if sampled subjects were considered cognitively impaired or not. If they were considered cognitively impaired, they underwent a second phase of assessment for dementia (standardized clinical interview with proxy respondent and a series of neuropsychological tests with subject), and if considered to have dementia, underwent a standardized medical examination with a neurologist. The cases were selected from those persons diagnosed as possible or probable Alzheimer disease without other co-morbid anomalies by the neurologist, and controls were selected from among persons without dementia. Individual exposure of the cases and controls to aluminium in water was determined in two ways using data from a water sampling campaign in 1995–1996: (a) concentrations in the water supply to the participant's current residence; and (b) estimated long-term exposure to aluminium since 1945 based on the study participant's residential history (constructed with current water data from municipalities). During the laboratory analysis of water a total of 19 physicochemical variables were assessed. Speciation of aluminium was also performed, including concentrations of total aluminum, total dissolved aluminium, total monomeric aluminium, organic monomeric aluminium, and inorganic monomeric aluminium. Other information collected about the cases and controls included occupational history, highest level of education, family history, medical history and presence or absence of ApoE ε4 allele (from the participants' blood samples).

The results of the study showed no significant association between long-term exposure to forms of aluminium found in drinking-water and Alzheimer disease. However, for current exposure, a significant association was found between organic monomeric aluminium and Alzheimer disease (OR = 2.67, 95% CI, 1.04–6.90), adjusted for education, presence of family cases of Alzheimer disease, and presence of at least one ApoE ε4 allele. The authors suggest that there is biological plausibility for this finding, citing research about how this form of aluminium complexes with organic acids of low relative molecular mass and how complexes are absorbed into the gastrointestinal tract, circulate in blood and cross the

blood–brain barrier (p. 239). They suggest that the reason for not observing this association for long-term exposure may be related to the imprecision of the long-term exposure estimates.

Gillette-Guyonnet et al. (2005) studied women in a single city (Toulouse) of a five-city cohort study who agreed 7 years after enrolling in the Epidemiology of Osteoporosis (EPIDOS) study to be enrolled in another prospective study; this other prospective study aims to determine risk factors for Alzheimer disease. The researchers compared women who enrolled in this additional study and were diagnosed with Alzheimer disease (cases, $n = 60$) to women enrolled in the study of Alzheimer disease who had normal cognitive function (controls, $n = 323$). They described the design of this study a nested case–control study (nested within the larger cohort). They retrospectively used the information on exposure to chemicals in water collected 7 years earlier at baseline of EPIDOS (1992–1994) and again in 1999–2000. Exposure to chemicals in water was determined by questioning women about water intake, from the tap or from bottled mineral water, and obtaining data about the concentrations of aluminium, silica and calcium concentrations in brands of bottled water or the city water supply. Aluminium concentration in the water supply of Toulouse was measured as 0.063 in 1992–1994 and 0.060 mg/l in 1999–2000.

To determine the status of the women with respect to Alzheimer disease, researchers began by employing the SPMSQ, the MMSE and the Grober and Buschke test to assess cognitive function. These tests, an assessment in the home of the study participant's independence in instrumental activities of daily living and computed tomography reports or scans were evaluated by a geriatrician and neurologist to determine if the woman had normal cognitive function, mild cognitive impairment, Alzheimer disease or other types of dementia. The clinicians used NINCDS-ADRDA criteria in the diagnosis of Alzheimer disease.

The researchers did not find an association between Alzheimer disease and aluminium in the study. They were able to adjust for a number of potential confounders in their analyses, including age, education and income. They did, however, find that Alzheimer disease was associated with lower silica concentrations in drinking-water. No effect of calcium in water was observed.

An additional case–control study conducted by Altmann et al. (1999) that examined association of aluminium in water from the Camelford water pollution incident with disease differs from the above case–control studies in that it aimed to measure disturbance of cerebral function, rather than Alzheimer disease, using psychological tests and visual evoked potentials (VEP). A difficulty with this study is that selection of cases ($n = 55$) was not investigator-initiated but was initiated by lawyers on behalf of persons who were considering litigation on account of alleged effects from the water pollution incident. The cases complained about short-term memory loss and impaired concentration. Therefore, the cases had an idea about both the exposure and possible health effects before the study was begun. The investigators selected as controls 15 siblings of the cases (nearest in age to the cases of his or her siblings) who had not lived in the area of water contamination since before the incident. Because of the likelihood that controls were aware that

cases were considering litigation, it is possible that this awareness had some affect on the control's performance on tests. The least subjective test in the battery of tests used in the study was VEP. A significant difference between the 15 pairs of cases and their siblings was detected on VEP, with the siblings having a better flash pattern. Potential confounders of this finding, if any, are not discussed in the paper. Due to issues with the study design and differences in end-points, this study will not be included in the comparison with the other case–control studies.

In summary, two of the six case–control studies presented here showed a statistically significant association of Alzheimer disease with aluminium. Exposure assessment varied in the six studies, from ascertaining aluminium concentration in the current residential water supply in a cross-sectional case–control study (Neri & Hewitt, 1991) to questioning individuals directly about their ingestion of tap or bottled mineral water in a nested case–control study (Gillette-Guyonnet et al., 2005). Three of the studies depended on informants to recall the past residential history for cases or controls, which was used to construct historical exposure to water. The use of informants, rather than direct questioning of study participants, are often required in retrospective studies of patients with a disease involving memory loss but may result in inaccurate exposure assessment.

Another issue to consider when examining the results of these studies is disease definition. Disease definition varied from identification of cognitive impairment in women (Gillette-Guyonnet et al., 2005) to Alzheimer disease identification using postmortem histopathological examination of brain tissue (McLachlan et al., 1996). If aluminium has a role only in the pathogenesis of Alzheimer disease and not cognitive impairment or other forms of dementia then careful attention to criteria for evaluating the form of the disease is warranted.

Of the two studies showing an association of Alzheimer disease with aluminium, the study by Gauthier et al. (2000) evaluated the speciation of aluminium in water and the association of Alzheimer disease was found for only one of these species, organic monomeric aluminium. This finding leads to questions about bioavailability of different species of aluminium and the effect, if any, on pathogenesis of Alzheimer disease.

*Prospective cohort study*

Rondeau et al. (2000) analysed data from a prospective cohort study (the Paquid cohort) of 3777 persons who were aged 65 years or older and lived at home at the onset of the study. These participants were randomly selected from electoral rolls from one of 75 randomly selected rural or urban parishes in the administrative areas of Gironde or Dordogne in south-western France (more sampling detail described above (Michel et al., (1991), Gironde only). Alzheimer disease was determined in a two-stage process. For the first stage, a screening was done by psychologists (DSM-III criteria for dementia and psychometric tests). Patients who tested positive by the criteria in the first stage went to a second stage. In the second stage, senior neurologists interviewed patients and performed a clinical examination (NINCDS-ADRDA criteria for Alzheimer disease and Hachinski score for vascular dementia). Study participants were re-examined at specific intervals after

the start of the study with the same criteria as above to detect new cases of dementia. An additional test for dementia, the MMSE, was added after the baseline exams.

Exposure to aluminium was based on information collected in two surveys in 1991 of 77 drinking-water areas that supplied the cohort participants. These surveys measured pH and various chemicals in the water (aluminium, calcium, and fluorine). Information on chemical analyses of drinking-water (aluminium and silicon) conducted by the sanitary administration between 1991 and 1994 was also used. The researchers additionally collected historical information on chemical concentrations in water for the period 1981–1991. For each parish, the researchers calculated a weighted mean of all measures for each chemical (for the 70 of 77 drinking-water areas that had all available information). For the analysis, aluminium was characterized in three ways: (1) ≥ 0.1 vs < 0.1; (2) as a continuous variable; and (3) grouped into four categories (< 0.0038 mg/l, ≥ 0.0038 to < 0.0110 mg/l, ≥ 0.0110 to < 0.1000 mg/l, ≥ 0.1000). Aluminium concentrations in water ranged from 0.001 to 0.459 mg/l, with a median value of 0.009 mg/l.

For analysis of the data, the researchers evaluated 2698 participants of the original cohort. The reason for the drop in sample size from the baseline number was because of the following reasons: the exclusion of persons who were demented at baseline ($n$ = 102) in order to ascertain incident cases during 8 years of follow-up, the lack of participation of study subjects in follow-up visits (due to death or refusal ($n$ = 703)), and the lack of water measurement and adjustment co-variates for some participants ($n$ = 274).

Results of the analysis showed that the risk associated with dementia (incident cases, $n$ = 253) or the risk associated with Alzheimer disease (incident cases, n = 182) was significantly elevated at aluminium concentrations in drinking-water of 0.1 mg/l compared with the lowest aluminium concentrations (respectively, relative risk, RR = 1.99, 95% CI, 1.20–3.28; RR = 2.14, 95% CI, 1.21–3.80, adjusted for age, gender, level of education, place of residence, and wine consumption). There was no linear dose–response observed across the four aluminium categories with dementia or Alzheimer disease, and the researchers suggest the possibility of a threshold effect at the highest category of aluminium concentration. Concentrations of silicon in the water of > 11.25 mg/l were associated with a reduced risk for developing dementia or Alzheimer disease, but there did not appear to be an interaction between aluminium and silicon associated with outcome of disease. No effect of pH was observed.

This study found an association of Alzheimer disease and dementia with aluminium concentrations ≥ 0.1 mg/l in water, although no dose–response was observed. The study had several strengths. Exposure was assessed before onset of disease and information about potential confounders of the relationship between Alzheimer disease and aluminium was obtained directly from study participants rather than informants; disease was diagnosed carefully with standard tests and clinical evaluation; and researchers examined whether loss to follow-up of cohort participants explained findings. The researchers also analysed water for other chemicals and could examine the effect of these chemicals on the relationship

between Alzheimer disease and aluminium. Findings from the study conflicted with findings from another study using the full cohort and the same exposure assessment (Jacqmin et al., 1994), but the latter study evaluated study participants at baseline in the cohort and used a different health end-point, cognitive impairment (scoring below 24 on the MMSE).

*Conclusions about studies of exposure to aluminium in drinking-water*

Some of the epidemiology studies suggest the possibility of an association of Alzheimer disease with aluminium in water, but other studies do not confirm this association. Many of the methodological issues concerning the studies are discussed above. All studies lack information on ingestion of aluminium from food and how concentrations of aluminium in food affect the association between aluminium in water and Alzheimer disease. Some of the studies have examined the effect of other chemicals in water but more information is needed in this area.

*(ii) Exposure to aluminium in food*

There are very few studies that incorporate information about dietary intake in epidemiological studies of Alzheimer disease, and all studies used a case–control design.

Rogers & Simon (1999) conducted a pilot case–control study to determine whether intake of food containing aluminium additives differs in individuals with and without newly diagnosed Alzheimer disease. The cases and the matched controls were selected from a nursing home in New York, USA from March to November 1993. The cases were defined as persons with newly-diagnosed Alzheimer disease from 1990 to 1993. The diagnosis of Alzheimer disease was ascertained using criteria specified by NINCDS-ADRDA. A total of 46 participants composing 23 matched pairs were enrolled in the study. Next-of-kin or spouse responded to questions on the participants' medical history, lifestyle behaviour and dietary intake before admission to the Centre.

The crude ORs for the association between categories of aluminium-containing foods and Alzheimer disease were generally low and non-significant. A statistically significant association between food intake and disease was only found for one category "pancakes, waffles, biscuits, muffins, cornbread, corn tortillas" based on five discordant matched case–control pairs (OR = undefined; $p = 0.025$).

Several of the ORs markedly increased when adjusted for other possible factors that may affect the food-disease relationship, thus indicating the instability of several of these estimates. The large difference between the crude and adjusted ORs after adjusting for up to six covariates in the conditional logistic regression model also indicates the difficulty of using complex models to analyse a small number of matched case–control pairs. The authors did not discuss or show the confidence interval around each OR, and therefore, the reader could not examine the extent of variability of the estimates. Other difficulties with the study included: (1) the long time to recall of dietary intake information—surrogates had to recall diet in a time period up to 8 years before the interview; (3) the potential for differential recall between case versus control surrogates owing to the likely differences in

intensity of care of study participants and the influence that intensity of care may have on knowledge of diet; (4) the potential for a control to change usual dietary patterns when diagnosed with heart disease or high blood pressure, especially if he or she reduces intake of fatty foods such as biscuits containing aluminium; (5) the lack of validation of dietary intake questions developed for this study; and (6) the problems with incomplete information about the amount of aluminium in different brands of foods. Although the findings in this pilot study should be interpreted cautiously and considered exploratory, they are intriguing enough to suggest that future epidemiological studies of intake of food containing aluminium and Alzheimer disease are warranted.

A few epidemiology studies have also examined the relation between tea drinking and Alzheimer disease. Pennington (1987) summarized the literature on aluminium content in individual foods and found that the concentration of aluminium in tea leaves and powder ranged from 67.0 to 14.0 mg/100 g, but that aluminium concentration in a cup of brewed tea (8 fl oz) ranged from 0.05 to 1.07 mg/100 g. Findings from the epidemiology studies on tea are presented below.

Broe et al. (1990) conducted a case–control study in Australia in 1986–1988 that enrolled 170 newly diagnosed cases of Alzheimer disease and 170 controls matched to cases on age, sex and, when possible, attendance of the same general practice clinic as the case. Cases were evaluated by neurologists and underwent a standardized battery of tests and examinations for Alzheimer disease and were classified as probable or possible Alzheimer disease by NINCDS-ADRDA criteria. Trained interviewers questioned individuals, usually in their homes, about health history, family history, lifestyle and occupational or domestic exposures. Interviewers asked about tea drinking history. No significant association between tea drinking and Alzheimer disease was detected on analysis of matched pairs, even at levels of “>4 cups of tea daily sometime in life” (odds ratio = 1.42, 95% CI, 0.93–2.17).

In another case–control study in northern England (Forster et al., 1995, see description of study above), the measure “>4 cups of tea daily” was also used, but no significant association between tea drinking and PDAT was detected although, as in the Australian study, the odds ratio was above 1.0 (OR = 1.4; 95% CI, 0.81–2.63).

***Table 3. Epidemiological studies of aluminium in drinking-water and dementia or Alzheimer disease***

| Type of study | Measure of exposure to aluminium in water | Outcome measure/data source | Results | Reference |
|---|---|---|---|---|
| Ecological | Aluminium in drinking-water (concurrent)<br>Four seasonal samples | Mention of dementia ICD9 290, 290.1 (dementia), 342.0 (Parkinson), 348.0 (ALS); sex-adjusted death certificate | AD only:<br>Al (mg/l) Males Females RR<br>< 0.05 1.00 1.00<br>0.05-0.2 1.15 1.19<br>> 0.2 1.32 1.42<br>PD and ALS—no gradient | Flaten (1990) |
| Morbidity prevalence | Aluminium in finished drinking-water; historical | Dementia by diagnostic category (not standard)<br>CT scan centre records age-sex-adjusted | All males and females<br>RR = 1.3–1.5, no dose-response relationship<br>Males and females aged < 65 years<br>RR = 1.4–1.7, dose response | Martyn et al. (1989) |
| Morbidity prevalence case–control | Finished drinking-water aluminium; historical | 'Cases' were hospital discharges of AD (ICD 9 331.0),<br>Presenile dementia (ICD 9 290)<br>Age/sex/residence-matched controls with other diagnoses<br><br>Hospital Medical Records Institute database-Ontario | RR from OR, gradient for AD<br>Al (mg/l) RR from OR<br>< 0.01 1.00<br>0.01–0.099 1.13<br>0.010–0.199 1.26<br>> 0.2 1.46 | Neri & Hewitt (1991) |

***Table 3.*** (contd)

| Type of study | Measure of exposure to aluminium in water | Outcome measure/data source | Results | Reference |
|---|---|---|---|---|
| Morbidity prevalence | Aluminium in finished drinking-water; residence > 15 years; urinary aluminium and serum aluminium; historical and concurrent | Mnemic skills in octogenerarians; sample of 800 residents in high & low aluminium areas; urinary and serum aluminium from 10 AD patients and controls in each area age, sex, education population-based | No difference in mean scores of tests for cognitive function<br>Slightly higher serum aluminium in AD in low aluminium areas; similar urinary excretion in AD and controls; hypothesis of association not supported | Wettstein et al. (1991) |
| Morbidity prevalence | Aluminium in drinking-water, historical | Cognitive function in sample of > 65 year olds by test battery (DSM III), population-based (2792); age, sex, education, SES, aluminium in water-many sources for the data | Probable AD, gradient-adjusted for age, education, residence, RR = 4.53/100 µg/l aluminium; RR corrected to NS with current aluminium measurement [Jacqmin et al. 1994] | Michel et al. (1991) |
| Morbidity prevalence* | Aluminium in drinking-water, historical | Cognitive function; 100 questions including a modified mental test and 9 questions short-term memory | NS/OR = 1.14 (Al ≥ 0.085 mg/l)<br>S/OR = 2.35 (Al ≥ 3.14 µmol/l) adjusted for fluoride, pH, turbidity, silica, iron, source of water, education, health, income, moves, and age | Forbes et al. (1992), (1995a) |
| Morbidity prevalence | Aluminium in water, pH, calcium | Cognitive function | Calcium protective<br>RR = 1.2 with pH < 7.3<br>NS/all other pH values | Jacqmin et al. (1994) |

***Table 3.*** (contd)

| Type of study | Measure of exposure to aluminium in water | Outcome measure/data source | Results | Reference |
|---|---|---|---|---|
| Case-control* | Aluminium in drinking-water, historical | PDAT<br>Persons aged < 65 years;<br>109 cases, 109 controls (matched to cases, age, sex) | OR/NS for aluminium measures at all levels (highest > 149 µg/l) | Forster et al. (1995) |
| Morbidity prevalence | Aluminium in drinking-water | Males, mention of AD or presenile dementia (ICD-9), death certificate<br>No age-education adjustments.<br>Controlled for pH, SiO2, F in water | RR of AD at all ages = 2.42 for aluminium >336 µg/l<br>RR of AD at age 75 + = 3.15 for aluminium > 336 µg/l<br>*sample size = 14 at aluminium > 336 µg/l<br>No linear dose-response (J-shaped response curve) | Forbes et al. (1995b) |
| Case-control | Aluminium in drinking-water; residence-weighted; historical | Pathological confirmation of diagnosis in all cases and controls; no age-sex-education adjustment | RR = 1.7 for aluminium > 100 µg/l<br>RR = 2.5 for aluminium > 100 µg/l when based adjustment for 10-year weighted exposure history | McLachlan et al. (1996) |
| Morbidity incidence (Case-control) | Aluminium in drinking-water, exposure as years before diagnosis | 106 males clinical diagnosis AD, Controls = other dementia, brain cancer, other neurodegeneration, *no disease, all aged 45–75.<br>Adjusted for age, SiO2 | No increased risk, highest aluminium > 109 µg/l vs lowest aluminium< 16 µg/l | Martyn et al. (1997) |

***Table 3.*** (contd)

| Type of study | Measure of exposure to aluminium in water | Outcome measure/data source | Results | Reference |
|---|---|---|---|---|
| Case-control | Aluminium in drinking-water, estimates (historical) of long term exposure, variety of aluminium species | 68 elderly clinically diagnosed age and sex-matched controls adjusted for education, ApoE ε4 allele, AD or dementia family history | NS for all aluminium measures except monomeric organic aluminium at time of diagnosis, OR = 2.67 | Gauthier et al. (2000) |
| Prospective cohort | Aluminium in drinking-water, time-weighted historical | 2698 males and females aged > 65 at baseline, 8 year follow-up dementia (DSM-IIIR, MMSE), AD (NINCDS-ARDRA) population-based (3401), adjusted for age, sex, education, place of residence, wine consumption | RR of dementia = 1.99 for aluminium > 0.1 mg/l<br>RR of AD = 2.20 for aluminium > 0.1 mg/l<br>High silica levels may be protective | Rondeau et al. (2000) |
| Morbidity prevalence* | Aluminium in drinking-water and questionnaire response about bottled and tap water | 5691 women aged > 75 years at baseline, 5 cities, Epidemiology of Osteoporosis study, cognitive impairment, Pfeiffer score < 8 | NS for aluminium<br>Tap water range: 0.01-0.063 mg/l, five cities<br>Bottled mineral water range: non-detectable to 0.032 mg/l | Gilette-Guyonnet et al. (2005) |

***Table 3.*** (contd)

| Type of study | Measure of exposure to aluminium in water | Outcome measure/data source | Results | Reference |
|---|---|---|---|---|
| Nested case–control* | Aluminium in drinking-water and questionnaire response about bottled and tap water | 60 cases diagnosed with SPMSQ, MMSE, Grober and Buschke test, assessment of daily living, NINCDS-ADRA (AD); 323 controls, normal cognitive function adjusted for age, education, income | NS for aluminium<br>Tap water range: 0.060-0.063 mg/l, one city<br>Bottled mineral water range: non-detectable to 0.032 mg/l | Gilette-Guyonnet et al. (2005) |

From ICPS (1997), updated from COT (2005)
*Updated or inserted in this document)
MMSE: Mini-Mental State Examination; AD: Alzheimer disease; ALS: amyotrophic lateral sclerosis; NINCDS-ADRA: US National Institute of Neurological and Communicable Disorders and Stroke and Alzheimer Disease and Related Disorders; NS: non-significant; OR: odds ratio; PD: Parkinson disease; PDAT: presenile dementia of the Alzheimer type; RR: relative risk; SES, socioeconomic status; SPMSQ: Short Portable Mental Status Questionnaire.

A third case–control was conducted in Canada (The Canadian Study of Health and Aging, 1994). This study enrolled study participants aged 65 years or older from communities and institutions across Canada (except Ontario), using health insurance plan information. Sampled individuals from the community were screened for cognitive impairment with the 3MS. If the 3MS suggested impairment, individuals underwent clinical examination. Diagnosis of dementia was based on DSM-III-R, while possible or probable Alzheimer disease was based on the findings of dementia plus NINCDS-ADRDA criteria. Persons in institutions were examined clinically without first undergoing screening. Cases were selected from the possible and probable Alzheimer disease groups of patients ($n = 258$) and controls ($n = 535$) were selected from individuals assessed to be cognitively normal. Controls were frequency-matched to cases on age group, study centre, and residence in a community or institution. Proxy respondents for cases and controls answered a risk-factor questionnaire that included a limited dietary history, including a question about tea intake. The researchers did not find an association between Alzheimer disease and tea (OR = 1.40; 95% CI, 0.86–2.28, adjusting for age, sex, education and residence.

A fourth pilot case–control study conducted by Rogers & Simon (1999) in the USA and described immediately above also found no association between Alzheimer disease and tea (OR = O.6, $p = 0.69$). The OR was calculated from a very small number of discordant pairs ($n = 11$ pairs).

*(iii) Exposure to aluminium in antacids*

Aluminium concentrations in antacids, when present, are at a much higher concentration than are found in water. Daily intake of aluminium in antacids has been estimated as 1 g or more by Anke et al. (2001) and up to 5 g by Lione (1985). Case-reports of the effects of aluminium-containing antacids related to skeletal changes are described earlier in this document. Flaten (2001) reviewed 13 epidemiological studies that were germane to evaluating antacid use and Alzheimer disease, including three studies that indirectly evaluated this relationship by studying groups of patients with peptic ulcer or who were regular users of the $H_2$ blocker cimetidine. The 13 studies were published from 1984 to 1999, and the majority of the studies had a case–control design (9 of the 13). There was no prospective study design among these studies that could have allowed for ascertainment of antacid use directly from the patient before onset of disease. None of the estimates of risk (OR, RR or standardized mortality ratio, SMR) achieved statistical significance, and in 11 of the 13 studies, the risk estimate was below 1.0, tending in the direction of a protective association of the use of antacids with Alzheimer disease. However, sample size in most of these studies is very small, dose and frequency of use information for the antacid was not obtained, and information about whether antacids contain aluminium is sometimes not presented.

Case-reports of frequent use of antacids and its effect on bone are discussed above in the section on osteomalacia.

*(b) Other neurological conditions: ALS, parkinsonism–dementia*

Studies of patients and sampling of drinking-water and garden soil have been conducted in three areas of the western Pacific with high incidence rates of amyotrophic lateral sclerosis (ALS) and parkinsonism–dementia (Gajdusek & Salazar, 1982; Perl et al., 1982). These three areas include Guam, the Kii Peninsula of Japan and southern West New Guinea. Unusually low concentrations of calcium and magnesium were found in the drinking-water and soil from these areas, while relatively high concentrations of other elements were found, including aluminium. Gajdusek & Salazar (1982) observed that villages with a high incidence of ALS or parkinsonism–dementia had different geographical terrain compared with other villages in the same general regions with low incidence of disease; however, investigators only obtained samples of water and soil from the high but not the low incidence villages. Perl et al. (1982) examined accumulation of aluminium within the brain tissue of eight Guamanian persons, three of whom had died of ALS or parkinsonism–dementia and five of whom (the controls) had died of non-neurological disorders and did not show signs of neurological disease before death. The brain tissue of all eight persons contained aluminium, but concentrations were higher in the patients with ALS and parkinsonism–dementia and in one control compared with the remaining four controls. Perl et al. (1982) found the presence of neurofibrillary tangle-bearing hippopcampal neurons in the persons with the higher concentrations of aluminium, but did not detect neurofibrillary tangle-bearing neurons in the four remaining controls. The role of aluminium, if any, in the initiation and development of the disease is not elucidated in these studies since the effect of the other factors potentially associated with the disease or their interactions are not yet fully understood.

In Italy, Bergomi et al. (2002) conducted a population-based case–control study to evaluate the association between exposure to trace elements, including aluminium, and sporadic ALS. They enrolled patients from five provinces of Italy whose first diagnosis of ALS occurred in 1998–1999. A neurologist diagnosed ALS and determined if the disease was possible, probable or definite, based on the El Escorial criteria (Brooks, 1994). A patient with possible or probable ALS was followed until a conclusive diagnosis was made and then defined as a case in the study. Controls were selected from the same population as the cases, employing random sampling from the National Health Service directory (all citizens in Italy are included in the National Health Service) and matching by same birth year and gender as the cases. The sample size for the study was 62 persons, 22 cases (10 women and 12 men) and 40 controls (18 women and 22 men). Investigators administered a questionnaire on clinical, life-style and dietary factors and sampled toenail specimens and blood. Toenails were analysed for a number of trace elements, including cadmium, lead, copper, manganese, selenium, chromium, cobalt, iron and aluminium. No association between ALS and aluminium was found. The meaning of this result is difficult to determine since toenail concentration of aluminium as a biomarker for chronic environmental exposure to aluminium has yet to be validated.

*(c) All-cause mortality*

As a follow-up to the water pollution incident in Camelford, UK and its vicinity (see section 3.1.2, case of severe cerebral congophilic angiopathy, for a fuller description of this incident), Owen et al. (2002) compared mortality in the area with water pollution ($n$ = 11 114 residents) to mortality in an adjacent area free of pollution ($n$ = 5359 residents). They collected information on deaths from July 1988 to December 1997 and corrected death rates for differences in age distribution and sex between the two populations. They calculated an SMR for the exposed population and a SMR for the unexposed population, standardized to England and Wales or standardized to Cornwall and the Isles of Scilly. The ratio of the SMR for the exposed population to the SMR for the unexposed population was 1.08 (95% CI, 0.97–1.21), standardized to England and Wales. The ratio of SMRs, standardized to Cornwall and the Isles of Scilly was closely similar. These results suggest a very small but not statistically significant excess of mortality in the exposed group. No other factors were considered in this study. The authors noted that not all deaths were accounted for if individuals moved out of either area before the recommendation in 1991 to ascertain deaths. It is not clear how much influence under-reporting of deaths had on the estimates.

*(d) Other disorders*

At the present time, oral exposure to aluminium in humans has not been associated with cancer, genotoxicity or reproductive toxicity.

## 4. ANALYTICAL METHODS

### 4.1 Food additives

Each specification monograph for food additives containing aluminium has methods of analysis for identity and purity (qualitative methods for identification, and quantitative methods for assessing the purity of the additive). The methods are either included in the monographs or refer to methods in common for two or more substances in Volume 4 of the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*).

### 4.2 Food samples.

Food samples would normally be rendered into soluble samples, e.g. by microwave-assisted acid digestion, before instrumental measurement of aluminium. Graphite-furnace atomic absorption spectrometry, inductively coupled plasma-optical/atomic emission spectrometry (ICP-OES/AES) and inductively coupled plasma mass spectrometry (ICP-MS) are commonly used methods for measuring aluminium in foods. All offer low detection limits, which are typically 0.1 mg/kg sample or lower, depending on the sample type.

Graphite-furnace atomic absorption spectrometry can be affected by high-chloride matrices and this can be a problem especially for biological samples. ICP-AES and ICP-MS are relatively free from interference, although Ti and Ca can give

high background signals in ICP-AES and ICP-MS can suffer interference from beryllium oxide, boron oxide and cyanide.

In proficiency testing schemes, errant results that deviate from the mode cannot be ascribed to any particular analytical method. All the methods described above are capable of returning reliable results for aluminium in food.

Aluminium is an abundant element in the environment so background levels in laboratory analysis can be a problem, particularly when measuring biological samples (blood, plasma etc) where concentrations are low (low parts per billion, µg/kg). Concentrations in food are generally higher (low parts per million, mg/kg) and so analytical background contamination is not such a problem but it always needs to be guarded against.

A large proportion of errant results in proficiency testing schemes are submitted by laboratories using non-accredited procedures. These errant results tend to be too high compared with the norm, and this may be owing to insufficient care taken to exclude high background levels of aluminium in reagents and glass/plastic ware or other contamination issues.

## *5. SAMPLING PROTOCOLS*

There is no specific Codex method of sampling of food to be analysed for aluminium, but there are Codex Alimentarius Committee Guidelines CAC/GL 50 (2004) 'General guidelines in sampling' which are helpful.

## *6. EXPOSURE TO ALUMINIUM IN THE DIET AND OTHER SOURCES*

Only consumer exposure to aluminium in the diet and other routes or commodities was considered by the Committee, without consideration for occupational exposure. Previous reviews by the Committee (WHO, 1989a; WHO, 1989b) IPCS (WHO, 1997) & COT (COT, 2005) and recent literature data were considered.

Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact material and food additives. When dietary exposure was expressed on a kg body weight basis, a standard 60 kg adult was considered, unless otherwise specified.

### *6.1 Dietary exposure (including drinking-water)*

In the last evaluation made by the Committee, dietary exposure, particularly through foods containing aluminium compounds used as food additives, was found to represent the major route of aluminium exposure by the general public excluding persons who regularly ingest aluminium-containing drugs (WHO, 1989a; 1989b). The review by IPCS in 1997 confirmed that non-occupational human exposure to aluminium in the environment is primarily through ingestion of food and water (WHO, 1997). Of these, food appeared as the principal contributor.

The three dietary sources of aluminium are natural sources (foods and beverages, drinking-water), packaging and utensils used during food preparation and storage and food additives.

The geological origins of the soil and its conditions, especially its pH, have a significant influence on the aluminium content of the food chain. The solubility of aluminium compounds may increase when acid rain decreases the pH of the soil, as a consequence aluminium content increases in surface water, plants and animals (Anke, 2001).

### *6.1.1 Drinking-water*

In the last evaluation by the Committee (Annex 1, reference *84*), although water was not found to contribute significantly to the total aluminium exposure from all sources for most individuals, elevated aluminium concentrations were reported in water from certain areas and resultant aluminium exposure could be as high as the dietary contribution.

Aluminium in natural waters is mainly derived from weathering of rocks and minerals. Analytical data from drinking-water in the USA suggest that the aluminium content of raw surface water is higher than that of raw ground water. Thus 55% of the raw surface waters had a concentration of greater than 50 µg/l vs only 4% of the raw ground waters (Miller, 1984).

Concentration of dissolved aluminium in raw water near pH 7 is typically between 1 and 50 µg/l, but can increase to 500–1000 µg/l in acidified water (Yokel, 2004, cited in Schafer). Based on the consumption of 2 l of water per day, exposure through this source is therefore up to 2 mg/day, corresponding to 0.03 mg Al/kg bw per day

Aluminium may also be present in drinking-water owing to the use of salts of aluminium as a chemical coagulation-based treatment of surface waters, which is the most common approach for treatment of surface waters (WHO, 2004). Chemical coagulants are usually salts of aluminium or iron. Typical coagulant doses are 2–5 mg Al/l. Coagulation is used for removal of microorganisms, turbidity and colour and can also remove certain heavy metals and low-solubility organic chemicals, such as certain organochlorine pesticides. No health-based guideline value for aluminium in drinking-water has been established by WHO. However, practical levels were derived which minimize concentrations of aluminium in finished water: 0.1 mg/l or less in large water treatment facilities, and 0.2 mg/l or less in small facilities (WHO, 2004). These recommendations provide a compromise between the beneficial effects of the use of aluminium salts as coagulants in water treatment on the one hand, and discoloration and health concerns about aluminium as a potential neurotoxicant, on the other hand. The presence of aluminium at concentrations in excess of 0.1–0.2 mg/l is unlikely since it often leads to consumer complaints as a result of deposition of aluminium hydroxide floc in distribution systems and the exacerbation of discoloration of water by iron.

Based on a daily consumption of 2 l per day, dietary exposure to aluminium from treated drinking-water may be up to 0.4 mg/day, corresponding to 0.007 mg/kg bw per day.

### *6.1.2 Aluminium from natural dietary sources*

The aluminium content of the flora also depends on the variety, part and age of the plant. The concentration of aluminium is high in leaves, medium in blossoms, ears, fruits and seeds, and low in stalks (Anke et al., 2001).

Müller et al. (1998) reported analytical data on 128 foods and drinks in Germany in 1988 and 1992, including non-processed foods. The highest concentrations of aluminium (mg/kg fresh matter) were found in spices (mean, 145 mg/kg fresh matter; range, 6.5–695), cocoa and cocoa products (mean, 33 mg/kg; range, 9–103) and herbs (mean, 19 mg/kg; range, 8–26). Intermediate concentrations were found in vegetables (mean, 5.7 mg/kg; range, 0.7–33) and in meat, sausage, offal (mean, 5.4 mg/kg; range, 2.5–10). Lowest concentrations were found in fruit (mean, 1.5 mg/kg; range, 0.4–2.6). Generally, a relatively large variation in concentration was found within all categories of foodstuffs. Most foodstuffs contained less than 5 mg/kg.

Tea leaves contain high concentrations of aluminium, but only a small proportion of it remains in the tea decoction, providing around 0.4 mg Al/cup (Neelam, 2000).

According to Greger (1992), most unprocessed foods in the USA contain aluminium at less than 5 mg/kg and most individuals consume aluminium at 1 to 10 mg/day from natural dietary sources. The average Swedish daily diet from unprocessed foods was calculated to contain about 0.6 mg aluminium with three food items providing 80%: coffee, wheat flour and tea (Jorhem & Haegglund, 1992).

### *6.1.3 Aluminium migrating from food-contact material (food containers, cookware, utensils and packaging)*

Because of its lightness, malleability, tensile strength and corrosion resistance, aluminium is used extensively in structural materials in the packaging of foodstuffs and beverages (cans, cartons, laminated paperboard packages, tubs, foil), in kitchen utensils (knives and forks, pots and pans, baking trays, mocha-type coffee pot). The use of aluminium has increased in recent years owing to the widespread use of precooked or frozen foods sold in disposable trays or wrapped in aluminium foil. Aluminium dissolves in non-oxidizing acids and can therefore be released from aluminium-containing packaging into the foodstuff in presence of an acidic medium. A number of studies have been conducted to estimate potential exposure from this source.

In Sweden, the aluminium content of beverages packed in glass bottles was not found to be different from that of aluminiumcans, indicating that the release of aluminium from the cans to the contents is small (Jorhem & Haegglund, 1992). A number of studies show that migration of aluminium from aluminium-containing cookware and utensils into food was found to be high if acidic foods (tomato sauces,

sauerkraut) are cooked in uncoated aluminium containers. The highest rate of migration is found when aluminium utensils are used for acid foods (AFSA/AFSSPS/AVS, 2003; Scancar, 2003).

In the Netherlands, aluminium in duplicate diets of 18 subjects cooking in aluminium pans was found to be similar to that of the other subjects (Ellen et al., 1990). A study performed in Italy showed that aluminium does migrate from containers to foods and beverages in conditions representative of actual use, with the highest release into acidic and salty foods (pickles and tomatoes); the overall increase in dietary exposure through this source could reach 6 mg/day under theoretical worst-case assumptions (Gramiccioni et al., 1996). On the other hand, aluminium in duplicate diets of Swedish women using regularly aluminium utensils and foils was found to be 2 mg/day higher than that of women who did not use them (Jorhem & Haegglund, 1992).

A higher contribution from aluminium migration was reported in a study conducted in India (Neelam, 2000). Food was prepared according to traditional recipes in stainless steel, old aluminium vessels (age 10 years) and new aluminium vessels (age 1 to 15 days) and analysed for aluminium. Based on food consumption data, exposure in the urban population was estimated to be 9.6 (range, 5.6–16.2), 14.2 (range, 8.3–23.2) and 18.2 (range, 10.5–32) mg/day, respectively. These data suggest that daily use of an aluminium vessel may lead to an increased exposure of around 7 mg/day.

### *6.1.4 Aluminium present in food additives*

Table 4 presents the provisions made for aluminium compounds in the current draft Codex GSFA.

Some aluminium-containing additives are listed in the current draft versions of Table 1 and 2 of the Codex GSFA and for these additives reference is made to the PTWI for aluminium established in 1988 by the JECFA Committee. This is the case for aluminium ammonium sulfate and SALP, acidic and basic. These aluminium compounds may be used according to good manufacturing practices (GMP) in a large number of products and at maximum levels in other products. The Committee noted that maximum levels are generally expressed as aluminium (e.g. 35 000 mg/kg expressed as Al for SALP used in processed cheese) but that in some cases the reporting basis is not specified (aluminium ammonium sulfate, up to 10 000 mg/kg in bakery products).

Some additives containing aluminium are listed in Tables 1, 2 and 3 of the current draft Codex GSFA. In Table 3, reference is made to an ADI 'not specified' for aluminium, and sodium aluminium silicate, calcium aluminium silicate and aluminium silicate are allowed at GMP in food in general. Specifications are available in the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*) for other aluminium compounds, but no provision has yet been made for them in the Codex GSFA. This is the case for aluminium lakes of dyes and colours, aluminium sulfate and potassium aluminium sulfate. Other aluminium compounds are used in a number of countries but are not reported in the Codex

GSFA or in the *Combined Compendium*. This is the case for aluminium powder, aluminium oxide, potassium aluminium silicate and SALP.

The Committee was provided with an assessment of exposure to SALP in Europe (EFPA, 2005). In this assessment, the total annual sales of SALP in the countries of the European Union (EU) in 2004 (852 tonnes) was divided by 380 million (the estimated number of residents) to calculate the average exposure per capita: 2.24 g of SALP per year. Based on a standard body weight of 60 kg, the average exposure was estimated to be about 0.1 mg/kg bw per day, expressed as SALP. This exposure corresponds to an intake of less than 0.01 mg Al/kg bw per day, based on the 8.5% aluminium content of the tetra hydrate SALP acidic.

The Committee was also provided with disappearance data for the USA, collected by the International Food Additives Council, for a number of aluminium compounds used as food additives (O'Brien Nabors, 2006). Overall, aluminium present in SALP, basic and acidic; aluminium sodium sulfate; sodium aluminium silicate and aluminium lakes intended for human consumption, sold in the USA in 2003 and 2004 amounted to respectively 5921 pounds (x 0.45 = 2664 kg) and 5961 pounds (x 0.45 = 2682 kg), equivalent to 9 mg Al/capita per year (based on populations of 290 850 005 and 293 656 842 in 2003 and 2004 respectively).

These data may provide an estimate of average exposure to aluminium through additives in two very large populations (the EU and USA). However, the consumption is not homogeneously distributed among EU countries with the UK being the largest consumer of SALP) and may not be homogeneously distributed among consumers. Thus, from the use of approximately four million pounds in weight of aluminium in food additives in the USA in 1982, it has been estimated that the average citizen of the USA theoretically consumes 21.5 mg Al/day from food additives. However, further information allows it to be estimated that about 5% of adults in the USA were exposed to more than 95 mg Al/day from additives while 50% of them were exposed to 24 mg or less. These data indicate that individual use of aluminium-containing food additives varies greatly among consumers (Greger, 1992).

Further data are available to estimate exposure in the population of interest i.e. regular consumers of products containing aluminium compounds.

According to Greger (1992), the most commonly used aluminium-containing food additives in the USA are acidic SALP (leavening agent in baked goods); the basic form of SALP (emulsifying agent in processed cheese); aluminium phosphates (acidifying agents); bentonite (materials-handlings aid), aluminium lakes of various food dyes and colours, aluminium silicates (anti-caking agents). Although aluminium-containing additives were found to be present in only a limited number of foods, some processed foods have very high contents. Greger (1992) reports concentrations of 297 mg/kg in processed cheese, 400 mg/kg in home made corn bread, 128 mg/kg in muffins, 2300 mg/kg in baking powder and 164 mg/kg in salt.

***Table 4. Aluminium compounds used as food additives present in the current draft GSFA***

| Name | Function | Applications | Levels of use (expressed as aluminium) | INS No. | JECFA evaluationa |
|---|---|---|---|---|---|
| SALP, acidic & basic | Acidity regulator, emulsifier in processed cheeses, raising agent in bakery products, stabilizer, thickener | Baking powder, flours, bakery products, cheese, cocoa powders, desserts, bakery wares, confectionery, mixes for soups and sauces, concentrates for water-based flavoured drinks | Up to 35 000 mg/kg in processed cheese and 45 000 mg/kg in flours | 541(i), 541(ii) | PTWI for aluminium powder (GSFA Tables 1 and 2) |
| Aluminium ammonium sulfate | Firming agent, raising agent, stabilizer | Bakery products (including ordinary bakery products), egg products, herbs and spices, soya-bean products, snacks, processed fish, processed vegetables, candied fruit | Up to 10 000 mg/kg in bakery products<br>GMP in starch and soya-bean products | 523 | PTWI for aluminium powder (GSFA Tables 1 and 2) |
| Sodium aluminium silicate | Anti-caking agent | Salt and salt substitutes, sugar, grain<br>Permitted for use in food in general | Up to 20 000 mg/kg in salt<br>GMP in grain and food in general | 554 | ADI 'not specified' |

***Table 4*** (contd)

| Name | Function | Applications | Levels of use (expressed as aluminium) | INS No. | JECFA evaluationa |
|---|---|---|---|---|---|
| Calcium aluminium silicate | Anti-caking agent | Salt and salt substitutes, sugar,<br>Grape wines, grain<br>Permitted for use in food in general | Up to 20 000 mg/kg in salt<br>GMP in grain, grape wine and food in general | 556 | ADI 'not specified' (GSFA Tables 1, 2 and 3) |
| Aluminium silicate | Anti-caking agent | Salt and salt substitutes<br>Grain, herbs and spices<br>Permitted for use in food in general | Up to 10 000 mg/kg in salt<br>GMP in grain, herbs and spices and in food in general | 559 | ADI 'not specified' (GSFA Tables 1, 2 and 3) |

ADI: acceptable daily intake; GMP: good manufacturing practice; GSFA: General Standard for Food Additives; SALP: sodium aluminium phosphate
[a]As reported in current draft Codex GSFA.

Also, the Swedish study of Jorhem & Haegglund (1992) clearly demonstrated that aluminium compounds used as food additives increase the daily intake of aluminium by one order of magnitude in consumers of the foods which contain such compounds. The major contributor of aluminium to the Swedish duplicate diets was found to be a chocolate/mint cake. The high concentration of aluminium in this cake may derive from its known ingredients cocoa, mint and/or baking soda. The six diets including this cake contained on average 72 mg Al/day while the mean content of the remaining 99 diets was 9.7 mg Al/day.

In Germany, the highest aluminium content in processed foods was found in biscuits (22 mg/kg) and in soft cheese (8 to 16 mg/kg) Müller et al., 1998).

In the 2000 UK TDS, the miscellaneous cereals group was reported with the highest mean concentration of aluminium (19 mg/kg fresh weight) (FSA, 2004).

In the second Chinese Total Diet Study (Junquan Gao, 2006) the highest content was found in cereal products (50 mg/kg), owing to the use of leavening agents containing aluminium. The maximum concentration of aluminium given in Chinese standards was 100 mg/kg in cereals and cereal products.

The potential high aluminium content of cereal products and in particular of ordinary baked goods may be of special importance in a number of countries since they constitute staple food and may therefore be consumed regularly in large quantity.

### *6.1.5 Assessment of total dietary exposure*

Duplicate diet studies have been performed in a number of countries (Table 5). Mean values varied between 3 and 13 mg/day. The highest single reported value was 100 mg/day in a sample from Sweden. Data reported in Germany suggest that aluminium progressively decreased in the diet by about half from 1988 to 1996, probably owing to efforts to reduce the acidity of rain (Anke et al., 2001).

Moreover, duplicate diet studies were collected and analysed in nine developed and developing countries involved in a multicentre study published in 1991 (Parr et al., (1991). The 75th percentile of exposure to aluminium was estimated after normalizing for a 10 MJ daily energy intake. The lowest values were observed in Japan and Norway (2 mg/day). Increasing values were observed in Italy, Spain, Thailand, Brazil, China, Iran and Turkey reaching 18 mg in Sudan.

A number of market-basket studies have been performed, allowing estimation of exposure in different population groups by calculation (Table 6). These results are based on mean content of aluminium in food groups and mean consumption.

In the adult population, mean exposure to aluminium estimated by model diet or market basket varied from approximately 2 mg in the French survey to more than 40 mg/day in China.

***Table 5. Intake of aluminium determined by the duplicate portion technique in several countries***

| Country | Year of investigation | Mean (range) in mg/day | Reference | Remarks |
|---|---|---|---|---|
| Netherlands | 1978 | 4.6 (1.4–33.3) | Ellen et al. (1990) | 101 adults (26 females and 75 males), one 24 h sample each |
| Netherlands | 1984–1985 | 3.1 (0.6–12.9) | Ellen et al. (1990) | 110 adults (53 females and 57 males) 1 week sample each |
| Hungary | 1989–1990 | 3.3 (0.3–19.4) | Gergely et al. (1991) | 84 samples |
| Japan | 1981 | 4.0 (1.3–10.3) | Shiraishi et al. (1989) | 31 males, 62 24 h samples |
| Germany | 1988<br>1991/2<br>1996 | 5.4/6.5<br>4.6/4.9<br>3.1/3.2 | Anke et al. (2001) | Females/males mixed diet |
| Germany | 1996 | 4.1/4.1 | Anke et al. (2001) | Females/males; ovo-lacto-vegetarian diet |
| Italy | Not reported | 2.5/3.1/4.3/6.3 | Gramiccioni et al. (1996) | Four different regions (overall 19 24 h samples) |
| India | 2000–2001 | 6.4 (1.9–12.1) | Tripathi et al. (2002) | 45 24 h samples |
| Taiwan (China) | 1989, 1990 | 5.2/4.9 | Liu & Chung (1992) | 15 subjects, three 24 h samples, females/males |
| Sweden | Not reported | 13.0 (1.2–100) | Jorhem & Haegglund (1992) | 105 duplicate diets in 15 non-smoking females |

Exposure to aluminium was found to be lower in the 1993 US Food and Drug Administration (FDA) Total Diet Study (TDS) when compared with that conducted in 1984 (Pennington & Schoen, 1995). The highest mean exposure per kg bw was found in small children: 6 mg/day for children aged 2 years, which corresponds to approximately 0.5 mg/kg bw per day based on a standard 12 kg bw.

***Table 7. Intake of aluminium (mg/day) calculated with the market basket method or a model diet in several countries***

| Country | Year of investigation | Mean or range, in mg/day; Males/ females | Remarks | Reference |
|---|---|---|---|---|
| China | 1992–1993 | 17.8<br>31.5<br>43.4/41.5 | Young children (2–7 years)<br>Older children (8–12 years)<br>Adults (20–50 years) | Junquan Gao (2006) |
| Japan | 1986 | 3.8/3.5<br>4.1/3.0<br>2.3/2.3 | Children (3 years)<br>Teenagers (16 years)<br>Adult (40 years) males | Shiraishi et al. (1988) |
| UK | 1997<br>2000 | 3.4<br>4.7 | | MAFF (1999)<br>MAFF (2004) |
| Finland | 1975–1978 | 6.7 | | Varo & Koivistoinen (1980) |
| USA | 1993 | 0.7<br>11.5<br>7<br>8–9 | 6—11 months<br>14–16 years, males<br>Adult females<br>Adult males | Pennington & Schoen (1995) |
| USA | 1984 | 1.8<br>6.3<br>8.6<br>12.7<br>8.7<br>13.7<br>8.9<br>11.8 | 6–11 months<br>2 years<br>14–16 years females<br>14–16 years males<br>25–30 years females<br>25–30 years males<br>60–65 years females<br>60–65 years males | Pennington & Jones (1989) |
| USA | 1985 | 14.3 | 25–30 years males | Iyengar et al. (1987) |
| France | 2000 | 1.3<br>1.6 | 3–15 years<br>15 years and above | Leblanc et al. (2005) |

MAFF: Ministry of Agriculture, Fisheries and Food

In contrast, the 2000 UK TDS revealed that dietary exposure to aluminium has increased by about one third, reaching 4.7 mg/day (FSA, 2004) versus 3.4 mg/day in the previous UK TDS conducted in 1997 (MAFF, 1999). In the more recent study, miscellaneous cereals, which contained aluminium at a mean concentration of 19 mg /kg, were the most significant (45%) contributor to the dietary exposure of the population, probably owing to the use of aluminium-containing food additives. On the other hand, bread contained aluminium at an average concentration of 3 mg/kg and contributed only 7% of the overall exposure. Exposure expressed per kg bw varied from 0.06 mg/kg bw per day in the elderly to 0.16 mg/kg bw per day in toddlers (1.5–4.5 years), based on measured body weight. High levels of exposure, estimated on the basis of high level of consumption, was estimated to vary from 0.13 mg/kg bw per day in the elderly to 0.33 mg/kg bw per day in toddlers.

In the second Chinese Total Diet Study conducted in 1992–93, high exposure to aluminium was estimated owing to the high mean aluminium content of cereal products. In children, mean estimated dietary exposure was around 1 mg/kg bw per day in both age class 2–7 years and 8–12 years: considering as standard body weight 16.5 kg and 29.4 kg respectively. Exposure in high consumers of these products or in regular consumers of products would be higher.

*Infants*

Since the 1990s, there has been some concern about the aluminium content of infant formulae (Greger, 1992). The aluminium content of human milk and cows' milk was found to be negligible (< 0.05 mg/l) (Koo et al., 1989, cited by Greger, 1992) while high levels of aluminium were found in milk-based formulae and soya-based formulae leading to the presence of aluminium at of 0.01–0.36 and 0.4–6.4 mg/l respectively in the products ready for consumption (Greger, 1992). A high concentration of aluminium was also found in soya-based powder infant formula present on the Swedish market (Jorhem & Haegglund, 1992): 14 mg/kg which, based on a typical dilution factor of 1 : 7, corresponds to 2 mg/l in the reconstituted milk.

Based on the German DONALD study (Kersting, 1998), in a 3 month infant weighing on average 6.1 kg, average and 95th percentile consumption of dry infant formula are respectively 105 and 144 g/day, which, based on a 1:7 dilution factor correspond to respectively 0.7 and 1 l/day of reconstituted formulae.

Thus, infants aged 3 months consuming a soya-based formula containing aluminium at a concentration of 6 mg/l (the highest concentration reported) once reconstituted could be exposed to approximately 4 mg/day on average and 6 mg/day for high percentile consumption. In the case of milk-based formulae containing aluminium at 0.4 mg/l (highest concentration reported), once reconstituted, potential exposure to aluminium would be up to 0.3 mg/day for average consumption and 0.4 mg/day for high consumption. Infants fed the same quantities of human or cows' milk would be exposed only to less than 0.03 mg/day for average consumption and less than 0.05 mg/day for high consumption.

Expressed on a kg body weight basis these values correspond to 1 mg/kg bw and 0.06 mg/kg bw for high consumption in infants fed soya-based formulae and milk-based formulae, respectively. In the case of infants fed human or cows' milk, high consumption would lead to an aluminium exposure of less than 0.01 mg/kg bw.

### *6.2 Other sources of exposure*

#### *6.2.1 Inhalation*

When aluminium was reviewed by the Committee in 1988, exposure to aluminium from air, even in industrial areas, was found to be minor relative to that from food (Annex 1, reference *84*).

Atmospheric concentrations of aluminium in non-industrial rural and urban areas range from 0.05 to 0.5 and 0.1 to 5 μg/m$^3$ and are typically 0.2 and 1 μg/m$^3$, respectively. In industrial areas, the concentration of aluminium may rise to 25–2500 μg/m$^3$. The main source in remote locations is weathering of aluminosilicate rocks and soils. Anthropogenic sources are coal combustion, iron, pumice stone, cement, kaolin and chalk works as well as waste incineration. Atmospheric particulate aluminium consists of silicates, oxides and hydroxides (Yokel & McNamara, 2001; Yokel, 2004, cited in Schafer, 2005).

Consumer exposure through air is a minor source of exposure. According to *Environmental Health Criteria 194*, pulmonary exposure may contribute up to 0.04 mg/day (WHO, 1997).

#### *6.2.2 Dermal exposure to consumer products containing aluminium*

Aluminium chlorohydrate in antiperspirants produces insoluble aluminium hydroxide on the skin to form an obstructive plug in the sweat gland duct. Many deodorant stones contain aluminium sulfate. In dental rinses and toothpastes, aluminium is used to reduce dentinal hypersensitivity. Aluminium is found in some acne cleaning preparations as an abrasive (Yokel, 2004, cited in Schafer, 2005). Results of a preliminary study on dermal absorption of aluminium chlorohydrate used as active ingredient of antiperspirant suggest that about 4 μg of aluminium is absorbed from a single use on both underarms (Flarend, 2001).

#### *6.2.3 Consumption of medicines containing aluminium*

Aluminium hydroxides administered orally are used as antacids and in phosphate binders. Aluminium is also an auxiliary in diarrhoeal remedy preparations and buffered analgesics, in anorectic preparations (as a keratolytic) and vaginal douches, in products for dermatitis (as an astringent), in first-aid antibiotics, and antiseptics. Furthermore, aluminium salts are added as adjuvant to vaccines and allergy immunotherapeutics in order to increase their antigenic properties (Yokel, 2004, cited in Schafer, 2005; Anke et al., 2001). According to Anke et al. (2001), daily intake of aluminium in e.g. antacids may be 1 g and more. According to Lione (2005), if taken as directed, the daily intake of aluminium from antacids can be as much as 5 g, while aluminium-buffered aspirin used for rheumatoid arthritis can contribute 0.7 g/day.

*Bioavailability of aluminium*

Available data are not sufficient to correct the exposure assessment on the basis of bioavailability. Aluminium contained in some additives such as silicates may have a low bioavailability, but the main sources of exposure are sulfates and phosphates used in cereal products. Experimental data suggest that absorption of aluminium increases in presence of citric acid through chelation (Fulton & Jeffery, 1990). Very few data are available on the content of citric acid in the diet. Citric acid is one of the main organic acids present in fruit, with amounts varying from 0.05 to 3.2 g/l in juices (Chinnici et al., 2005). Citric acid in cheese was shown to vary from 0.07 g/kg in brie to 1.5 g/kg in cheddar cheese (Mullin & Emmons, 1997). Citric acid may also be added to fruit based products (fruit juices, jam, cocoa products) and to cheese as an additive. A diet high in fruit and fruit based products could eventually lead to a higher bioavailability of aluminium.

## *7. EFFECTS OF PROCESSING*

The Committee found no information on reduction of the aluminium content of foodstuffs by processing.

## *8. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF RISK OF CARCINOGENICITY/TOXICITY*

### *8.1 Contribution of data to assessment of risk*

#### *8.1.1 Pivotal data from biochemical and toxicological studies*

Assessment of the bioavailability of aluminium compounds is confounded by limitations in the analytical methodology, particularly for older studies, by concurrent exposure to modifying factors and by dose-dependency. Speciation appears to be an important factor in absorption and it is widely assumed that soluble aluminium compounds, such as the chloride and lactate salts, are more bioavailable than insoluble compounds, such as aluminium hydroxide or silicates. Studies in laboratory animals and in human volunteers generally show that absorption of aluminium is less than 1%. However, because of the differences in methodology, it is not possible to draw precise conclusions on the rate and extent of absorption of different aluminium compounds. Concurrent intake of organic anions (particularly citrate) increases the absorption of aluminium, while other food components, such as silicates and phosphate, may reduce the absorption of aluminium.

Studies reviewed by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*) showed no detectable aluminium in the urine of normal subjects given aluminium hydroxide gel (2.5 g Al/day, equivalent to 42 mg/kg bw per day assuming body weights of 60 kg) for 28 days. In contrast, faecal excretion of aluminium in patients with chronic renal disease given aluminium hydroxide (1.5–3.5 g Al/day, equivalent to 25–57 mg/kg bw per day, assuming body weights of 60 kg) for 20–32 days indicated a daily absorption of 100–568 mg of aluminium.

Slight increases in concentrations of aluminium in plasma were reported over the study period.

Oral dosing of rats with aluminium compounds has been shown to result in increased concentrations of aluminium in blood, bone, brain, liver and kidney. Studies with $^{26}Al$ administered intravenously to a small number of human volunteers indicate a biological half-life of about 7 years (in one individual) and interindividual variation in clearance patterns.

Aluminium compounds have been reported to interfere with the absorption of essential minerals such as calcium and phosphate, although the extent to which this occurs at dietary exposure levels is unclear.

The available toxicological studies were from the published literature and were not designed to assess the safety of food additives. Most were conducted to investigate specific effects or mechanisms of action, and many do not provide information on the dose–response relationship. Some do not make clear whether the stated dose relates to aluminium or to the aluminium compound tested. A further complication is that many studies do not appear to have taken into account the basal aluminium content of the animal feed before addition of the test material. Some studies refer to a basal aluminium content of about 7 mg/kg, which would not add significantly to the doses of aluminium under investigation. However, ATSDR (1999) reported that there are diverse concentrations ranging from 60 to 8300 mg/kg feed and that substantial variation between brands and between lots occurs. For chow containing aluminium at a concentration of 200 mg/kg, applying the default JECFA conversion factors indicates doses equivalent to 30 mg Al/kg bw for mice and 20 mg Al/kg bw for rats. The toxicological data are influenced by the solubility, and hence the bioavailability, of the tested aluminium compounds, and the dose–response relationship will be influenced by the aluminium content of the basal animal feed.

Recent studies have identified effects of aluminium compounds at doses lower than those reviewed previously by the Committee. Studies in rats, rabbits and monkeys have indicated effects on enzyme activity and other parameters associated with oxidative damage and calcium homeostasis in short-term studies with aluminium compounds administered at oral doses of 10–17 mg Al/kg bw per day. These studies involved administration at a single dose and did not take into account the aluminium content of the diet. The functional relevance of the observations is unclear and since the total exposure is unknown, they are not suitable for the dose–response analysis.

Mild histopathological changes were identified in the kidney and liver of rats given aluminium sulfate by gavage at a dose of 17 mg Al/kg bw per day for 21 days. Rats given drinking-water containing aluminium chloride at a dose of 5 and 20 mg Al/kg bw per day for 6 months showed non-dose-dependent decreases in body weight and changes in haematological parameters and acetylcholine-associated enzymes in the brain. Histopathological changes were observed in the kidney and brain at doses of 20 mg Al/kg bw per day in the latter study. These effects have not been observed in other studies and total exposure is unknown since aluminium content of the diet was not taken into account.

Beagle dogs given diets containing SALP (basic) for 6 months showed decreased food intake and body weight and histopathological changes in the testes, liver and kidneys in the males at the highest concentration tested, 1922 mg Al/kg of diet, equal to 75 mg Al/kg bw per day. No effects were seen in female dogs at this dietary concentration, equal to 80 mg Al/kg bw per day. The no-observed-effect level (NOEL) in this study was a dietary concentration of 702 mg Al/kg, equal to 27 mg Al/kg bw per day. This study is similar to that providing the basis for the previously established PTWI, which used SALP acidic. The Committee noted that there was no explanation for the observed sex difference, and limitations in the reporting made interpretation of this study difficult.

Special studies have highlighted a potential for effects on reproduction, on the nervous system and on bone. Few of these studies are adequate to serve as a basis for the determination of no-effect levels, as they were designed to address specific aspects and only a very limited range of toxicological end-points were examined.

Soluble aluminium compounds have demonstrated reproductive toxicity, with LOELs in the region of 13–200 mg Al/kg bw per day for reproductive and developmental effects with aluminium nitrate. None of these studies identified NOELs. The lowest LOELs were obtained in studies in which aluminium compounds were administered by gavage; taking into account the aluminium content of the diet, the total dose may have been in the region of 20 mg Al/kg bw per day or more.

Neurotoxicity potential has received particular attention because of a speculated association of aluminium with Alzheimer disease. Many of the studies in laboratory animals have been conducted using parenteral administration and are of uncertain relevance for dietary exposure because of the limited bioavailability of aluminium compounds likely to be present in food. In contrast to studies with other routes of administration, the available data from studies using oral administration do not demonstrate definite neuropathological effects. Some studies indicate that certain aluminium compounds, especially the more soluble forms, have the potential to cause neurobehavioural effects, at doses in the region of 50 to 200 mg Al/kg bw per day administered in the diet. The studies indicating the lowest LOELs took account of the basal diet content of aluminium and one of these studies also indicated a NOEL of 10 mg Al/kg bw per day.

The previously established PTWI of 0–7.0 mg/kg bw for aluminium was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing SALP acidic at a dietary concentration of 3% for 189 days, equivalent to approximately 110 mg Al/kg bw.

The new data reviewed at the present meeting indicated that soluble forms of aluminium may cause reproductive and developmental effects at doses lower than that used to establish the previous PTWI. Although insoluble aluminium compounds may be less bioavailable, the evidence that other dietary components, such as citrate, can increase uptake of insoluble aluminium suggests that data from studies with soluble forms of aluminium can be used as a basis for deriving the PTWI.

*8.1.2 Pivotal data from human clinical/epidemiological studies*

The previous evaluation of aluminium made by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*) did not include epidemiology studies. Since then, a number of epidemiology studies have been conducted, with most focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some epidemiology studies suggest an association of consumption of aluminium in water with Alzheimer disease, but this was not confirmed in others. None of the studies accounted for ingestion of aluminium in food, a potentially important confounding factor. The studies relied on concentrations of aluminium in the residential water supply as a measure of exposure, with the one exception of a study that also assessed ingestion of bottled water.

There is minimal information from the epidemiology literature about the association between intake of aluminium in food and neurological conditions, and the current information from a pilot case–control study evaluating Alzheimer disease is considered to be preliminary. Epidemiology studies of the use of antacids did not capture dose information and did not demonstrate an association with neurological conditions. In the literature there have been a few case reports of adults, infants and a child with normal kidney function who experienced skeletal changes attributable to frequent use of aluminium-containing antacids considered to induce phosphate depletion.

In summary, no pivotal epidemiology studies were available for the risk assessment.

## *9. COMMENTS*

*Absorption, distribution, metabolism and excretion*

Assessment of the bioavailability of aluminium compounds is confounded by limitations in the analytical methodology, particularly for older studies, by concurrent exposure to modifying factors and by dose-dependency. Speciation appears to be an important factor in absorption and it is widely assumed that soluble aluminium compounds, such as the chloride and lactate salts, are more bioavailable than insoluble compounds, such as aluminium hydroxide or silicates. Studies in laboratory animals and in human volunteers generally show that absorption of aluminium is less than 1%. However, because of the differences in methodology, it is not possible to draw precise conclusions on the rate and extent of absorption of different aluminium compounds. Concurrent intake of organic anions (particularly citrate) increases the absorption of aluminium, while other food components, such as silicates and phosphate, may reduce the absorption of aluminium.

Studies reviewed by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*) showed no detectable aluminium in the urine of normal subjects given aluminium hydroxide gel (2.5 g Al/day, equivalent to 42 mg Al/kg bw per day assuming body weights of 60 kg) for 28 days. In contrast, faecal excretion of aluminium in patients with chronic renal disease given aluminium hydroxide

(1.5–3.5 g Al/day, equivalent to 25–57 mg Al/kg bw per day, assuming body weights of 60 kg) for 20–32 days indicated a daily absorption of 100–568 mg of aluminium. Slight increases in concentrations of aluminium in plasma were reported over the study period.

Oral dosing of rats with aluminium compounds has been shown to result in increased concentrations of aluminium in blood, bone, brain, liver and kidney. Studies with $^{26}Al$ administered intravenously to a small number of human volunteers indicate a biological half-life of about 7 years (in one individual) and interindividual variation in clearance patterns.

Aluminium compounds have been reported to interfere with the absorption of essential minerals such as calcium and phosphate, although the extent to which this occurs at dietary exposure levels is unclear.

*Toxicological data*

The available studies were from the published literature and were not designed to assess the safety of food additives. Most were conducted to investigate specific effects or mechanisms of action, and many do not provide information on the dose–response relationship. Some do not make clear whether the stated dose relates to elemental aluminium or to the aluminium compound tested. A further complication is that many studies do not appear to have taken into account the basal aluminium content of the animal feed before addition of the test material. Some studies refer to basal aluminium content in the region of 7 mg/kg, which would not add significantly to the doses of aluminium under investigation. However, it has been reported that there are diverse concentrations ranging from 60 to 8300 mg/kg feed and that substantial brand-to-brand and lot-to-lot variation occurs. For chow containing aluminium at a concentration of 200 mg/kg, applying the default JECFA conversion factors indicates doses equivalent to 30 mg Al/kg bw for mice and 20 mg Al/kg bw for rats.

The toxicological data are influenced by the solubility, and hence the bioavailability, of the tested aluminium compounds, and the dose–response relationship will be influenced by the aluminium content of the basal animal feed.

Recent studies have identified effects of aluminium compounds at doses lower than those reviewed previously by the Committee. Studies in rats, rabbits and monkeys have indicated effects on enzyme activity and other parameters associated with oxidative damage and calcium homeostasis in short-term studies with aluminium at oral doses in the region of 10–17 mg/kg bw per day. Those studies involved administration at a single dose and did not take into account the aluminium content of the diet. The functional relevance of the observations is unclear and since the total exposure is unknown, they are not suitable for the dose–response analysis.

Mild histopathological changes were identified in the kidney and liver of rats given aluminium sulfate by gavage at a dose of 17 mg Al/kg bw per day, for 21 days. Rats given drinking-water containing aluminium chloride at a dose of 5 or 20 mg Al/kg bw per day, for 6 months showed non-dose-dependent decreases in body weight and changes in haematological parameters and acetylcholine-associated enzymes

in the brain. Histopathological changes were observed in the kidney and brain at doses of 20 mg Al/kg bw per day, in the latter study. Such effects had not been observed in other studies and total exposure was unknown since the aluminium content of the diet was not taken into account.

Beagle dogs given diets containing SALP basic for 6 months showed decreased food intake and body weight and histopathological changes in the testes, liver and kidneys in the males at the highest tested concentration of aluminium, 1922 mg/kg of diet, equal to 75 mg/kg bw per day. No effects were seen in female dogs at this dietary concentration, equal to 80 mg Al/kg bw per day. The NOEL in this study was a dietary concentration of 702 mg/kg, equal to 27 mg Al/kg bw per day. This study is similar to that providing the basis for the previously established PTWI, which used SALP acidic. The Committee noted that there was no explanation for the observed sex difference, and limitations in the reporting made interpretation of this study difficult.

Special studies have highlighted a potential for effects on reproduction, on the nervous system and on bone. Few of those studies are adequate to serve as a basis for the determination of no-effect levels, as they were designed to address specific aspects and only a very limited range of toxicological end-points were examined.

Soluble aluminium compounds have demonstrated reproductive toxicity, with LOELs in the region of 13–200 mg Al/kg bw per day, for reproductive and developmental effects with aluminium nitrate. None of those studies identified NOELs. The lowest LOELs were obtained in studies in which aluminium compounds were administered by gavage; taking into account the aluminium content of the diet, the total dose may have been in the region of 20 mg Al/kg bw per day or more.

Neurotoxicity potential has received particular attention because of a speculated association of aluminium with Alzheimer disease. Many of the studies in laboratory animals have been conducted using parenteral administration and are of uncertain relevance for dietary exposure because of the limited bioavailability of aluminium compounds likely to be present in food. In contrast to studies with other routes of administration, the available data from studies using oral administration do not demonstrate definite neuropathological effects. Some studies indicate that certain aluminium compounds, especially the more soluble forms, have the potential to cause neurobehavioural effects at doses in the region of 50 to 200 mg Al/kg bw per day, administered in the diet. The studies indicating the lowest LOELs took account of the basal diet content of aluminium and one of those studies also indicated a NOEL of 10 mg Al/kg bw per day.

The previously established PTWI of 0–7.0 mg/kg bw for aluminium was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing SALP acidic at a dietary concentration of 3% for 189 days, equivalent to approximately 110 mg Al/kg bw.

The new data reviewed at the present meeting indicated that soluble forms of aluminium may cause reproductive and developmental effects at a dose lower than that used to establish the previous PTWI. Although insoluble aluminium

compounds may be less bioavailable, the evidence that other dietary components, such as citrate, can increase uptake of insoluble aluminium suggests that data from studies with soluble forms of aluminium can be used as a basis for deriving the PTWI.

*Observations in humans*

The previous evaluation of aluminium made by the Committee at its thirty-third meeting did not include epidemiology studies. Since then a number of epidemiology studies had been conducted, with most focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some epidemiology studies of aluminium in water suggested an association of consumption of aluminium in water with Alzheimer disease, but such an association was not confirmed in others. None of the studies accounted for ingestion of aluminium in foods, a potentially important confounding factor. The studies relied on concentrations of aluminium in the residential water supply as a measure of exposure, with the one exception of a study that also assessed ingestion of bottled water.

There was minimal information from the epidemiology literature about the association between intake of aluminium in food and neurological conditions, and the current information from a pilot case–control study evaluating Alzheimer disease was considered to be preliminary. The epidemiology studies of the use of antacids did not capture dose information and did not demonstrate an association with neurological conditions. In the literature there have been a few case reports of adults, infants and a child with normal kidney function who experienced skeletal changes attributable to frequent use of aluminium-containing antacids considered to induce phosphate depletion.

In summary, no pivotal epidemiology studies were available for the risk assessment.

*Exposure to aluminium from the diet and other sources*

Only consumer exposure to aluminium in the diet and via other routes or commodities were considered by the Committee; occupational exposure was not taken into account. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food-contact material and food additives. When dietary exposure was expressed on a kg body weight basis, a standard body weight of 60 kg for an adult was considered by the Committee, unless otherwise specified.

Soil composition has a significant influence on the aluminium content of the food chain. The solubility of aluminium compounds may increase when acid rain decreases the pH of the soil; as a consequence, aluminium content increases in surface water, plants and animals. Most foods contain aluminium at concentrations of less than 5 mg/kg. It is estimated that quantities of about 1–10 mg/day per person generally derive from natural dietary sources of aluminium, corresponding to up to 0.16 mg Al/kg bw per day. The concentration of dissolved aluminium in untreated water at near pH 7 is typically 1–50 µg/l, but this can increase to 1000 µg/l in acidic water. Exposure through this source is therefore up to 2 mg/day, corresponding to

0.03 mg/kg bw per day based on the consumption of 2 l of water per day. Aluminium may also be present in drinking-water owing to the use of aluminium salts as flocculants in the treatment of surface waters. The concentration of aluminium in finished water is usually less than 0.2 mg/l. Based on a daily consumption of 2 l per day, dietary exposure to aluminium from treated drinking-water may be up to 0.4 mg/day, corresponding to 0.007 mg/kg bw per day.

Aluminium is used extensively in structural materials used in food-contact materials, including kitchen utensils. Aluminium can be released into the foodstuff in the presence of an acidic medium. Conservative assessments suggest that mean potential dietary exposure through this source may be up to 7 mg Al/day. Such dietary exposure corresponds to 0.1 mg Al/kg bw per day.

The current draft provisions made for aluminium compounds in the Codex GSFA are reported in Table 4. Some aluminium-containing additives are listed only in the current versions of Table 1 and 2 of the Codex GSFA, and for those additives reference is made to the PTWI for aluminium established in 1988 by JECFA. It is the case for aluminium ammonium sulfate and SALP, acidic and basic. Those aluminium compounds may be used according to GMP in a large number of products and at maximum levels in other products. The Committee noted that maximum levels are generally expressed as aluminium (e.g. 35 000 mg Al/kg, for SALP used in processed cheese) but that in some cases the reporting basis is not specified (up to 10 000 mg/kg in bakery products containing aluminium ammonium sulfate).

The Committee also noted that some food additives containing aluminium are listed in Tables 1, 2 and 3 of the current draft Codex GSFA. In Table 3, reference is made to an ADI 'not specified', and sodium aluminium silicate, calcium aluminium silicate and aluminium silicate are allowed at concentrations consistent with GMP in food in general. Specifications for other aluminium compounds are available in the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*), but no provision had yet been made for them in Codex GSFA. This is the case for aluminium lakes of colouring matters, aluminium sulfate, aluminium powder and potassium aluminium sulfate. Other aluminium compounds are used in a number of countries but are not reported in the Codex GSFA or in the *Combined Compendium of Food Additive Specifications*. This was the case for aluminium oxide and potassium aluminium silicate.

The Committee was provided with an exposure assessment based on annual sales of SALP in Europe suggesting that the average exposure in the general population is about 0.1 mg/kg bw per day, corresponding to less than 0.01 mg Al/kg bw per day, based on the fact that tetrahydrate SALP acidic has an aluminium content of 8.5%. The Committee was also provided with disappearance data from the USA for a number of aluminium compounds used as food additives. Overall, aluminium present in SALP, basic and acidic; aluminium sodium sulfate; sodium aluminium silicate and aluminium lakes intended for human consumption sold in the USA in 2003 and 2004 would provide 9 mg of aluminium per capita per year, corresponding to 0.0004 mg/kg bw per day. Other data provided to the Committee suggest that there is a large range of exposure among consumers. A survey

conducted in 1979 suggests that 5% of adults in the USA were exposed to more than 1.5 mg Al/kg bw per day, from food additives.

Additional data were available to estimate exposure in the population of interest i.e. regular consumers of products containing food additives containing aluminium. In the USA, although aluminium-containing additives were found to be present in only a limited number of foods, some processed foods have a very high aluminium content: processed cheese, 300 mg/kg; home-made corn bread, 400 mg/kg (owing to the use of aluminium-containing leavening agents); muffins, 130 mg/kg; baking powder, 2300 mg/kg; and table salt, 164 mg/kg. In Germany, the processed foods found to have the highest aluminium content were biscuits (22 mg/kg) and soft cheese (8–16 mg/kg). In the 2000 UK Total Diet Study, the miscellaneous cereals group was reported to have the highest mean concentration of aluminium (19 mg/kg). In the 1992–1993 Chinese Total Diet Study, cereal products were also found to have the highest aluminium content (50 mg/kg) owing to the use of leavening agents containing aluminium. The potentially high aluminium content of cereal products and, in particular, of ordinary baked goods may be of special importance in a number of countries where they constitute staple food and may therefore be consumed regularly in large quantities by a significant proportion of the population.

Total dietary exposure to aluminium from all sources has been estimated through duplicate diet studies performed in adults in a number of countries. Mean values varied between 3 and 13 mg/day. The highest single reported value was 100 mg/day. In a multicentre study, exposure at the 75th percentile ranged from 3 to 26 mg/day, according to country. Data reported in Germany suggest that the amount of aluminium in the diet decreased by about half between 1988 and 1996.

A number of market-basket studies have also been performed, allowing estimation of exposure in different population groups based on mean content of aluminium in food groups, and on mean consumption. Exposure for consumers with a high consumption of cereal products or in regular consumers of products that contain higher-than-mean concentrations of aluminium will therefore be higher than estimated in those studies. In the adult population, mean exposure to aluminium estimated by model diet or market basket varied from 2 mg/day in the most recent French survey to more than 40 mg/day in China.

The highest mean exposure to aluminium per kg bw was found in young children: 0.16 mg/kg bw per day in the 1.5–4.5 years age group in the UK, based on measured body weight; approximately 0.5 mg/kg bw per day in the USA in children aged 2 years, considering a standard body weight of 12 kg; approximately 1 mg/kg bw per day in China in age groups 2–7 years and 8–12 years, considering as standard body weight 16.5 kg and 29.4 kg, respectively.

Values for high levels of exposure, estimated on the basis of high levels of consumption, were available for UK children aged 1.5–4.5 years (0.33 mg/kg bw per day).

The issue of bioavailability was considered by the Committee, but available data were not sufficient to correct the exposure assessment on the basis of

bioavailability. Aluminium contained in some food additives such as silicates may have a low bioavailability, but the main sources of exposure are sulfates and phosphates used in cereal products. A diet high in fruit and fruit-based products could lead to higher bioavailability owing to the increased absorption of aluminium in the presence of citric acid. Citric acid is one of the main organic acids present in fruit and may also be added to fruit-based products and to cheese.

The aluminium content of milk and formulae was considered when estimating exposure for infants. The aluminium content of human and cows' milk was found to be negligible (less than 0.05 mg/l), while cows' milk-based and soya-based formulae were found to contain high levels of aluminium, leading to concentrations of 0.01–0.4 and 0.4–6 mg/l, respectively, in the ready-to-drink product. The Committee estimated dietary exposure to aluminium based on the highest of those values in an infant aged 3 months weighing an average of 6 kg, considering 1 l of reconstituted formula per day as consumption at the 95th percentile. Expressed on a kg body weight basis, dietary exposure to aluminium was estimated to be up to 1 mg/kg bw per day and 0.06 mg/kg bw per day in infants fed soya-based formulae and milk-based formulae respectively. In the case of infants fed human or cows' milk, high consumption would lead to aluminium exposures of less than 0.01 mg/kg bw per day.

Sources of exposure to aluminium other than in the diet that were considered by the Committee were air, cosmetic and toiletry products and medicines. aluminium from air, in industrial areas, contributes up to 0.04 mg/day and therefore constitutes a minor source of exposure. Estimates of dermal absorption of aluminium chlorohydrate used as an active ingredient of antiperspirant suggest that only about 4 µg of aluminium is absorbed from a single use on both underarms. Some medical applications of aluminium may lead to long-term exposure: aluminium hydroxides in antacids, phosphate-binders and buffered analgesics. If taken as directed, the daily intake of aluminium from antacids could be as much as 5 g, while aluminium-buffered aspirin used for rheumatoid arthritis could contribute 0.7 g of aluminium per day.

In conclusion, the present assessment confirms previous evaluations made by the Committee in which dietary exposure, particularly through foods containing aluminium compounds used as food additives, was found to represent the major route of aluminium exposure for the general population, excluding persons who regularly ingest aluminium-containing drugs.

## *10. EVALUATION*

The Committee concluded that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than those used in establishing the previous PTWI and therefore the PTWI should be revised. However, the available studies have many limitations and are not adequate for defining the dose–response relationships. The Committee therefore based its evaluation on the combined evidence from several studies. The relevance of studies involving administration of aluminium compounds by gavage was unclear because the toxicokinetics after gavage were expected to differ from toxicokinetics after

***Table 8. Estimated ranges of mean exposure of the adult population to aluminium from different dietary sources***

| Mean exposure | Natural dietary sources | Water (assuming a consumption of 2 l/day) | Food-contact materials | Overall diet, including additives |
|---|---|---|---|---|
| Expressed as mg Al/week | 7–70 | < 0.7 (typical untreated water)<br>1.4–2.8 (water treated with aluminium salts)<br>14 (acidic untreated water) | 0–49[a] | 14–280 |
| Expressed as percentage of PTWI (assuming a body weight of 60 kg) | 2–120 | 1–20 | < 80[a] | 20–500 |

Al: total aluminium
[a]Theoretical exposure using conservative assumptions
PTWI: provisional tolerable weekly intake

dietary administration, and the gavage studies generally did not report total aluminium exposure including basal levels in the feed. The studies conducted with dietary administration of aluminium compounds were considered most appropriate for the evaluation. The lowest LOELs for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50–75 mg Al/kg bw per day.

The Committee applied an uncertainty factor of 100 to the lower end of this range of LOELs (50 mg Al/kg bw per day) to allow for inter- and intraspecies differences. There are deficiencies in the database, notably the absence of NOELs in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The deficiencies are counterbalanced by the probable lower bioavailability of the less soluble aluminium species present in food. Overall, an additional uncertainty factor of three was considered to be appropriate. The Committee confirmed that the resulting health-based guidance value should be expressed as a PTWI, because of the potential for bioaccumulation. The Committee established a PTWI of 1 mg Al/kg bw, which applies to all aluminium compounds in food, including additives. The previously established ADIs and PTWI for aluminium compounds were withdrawn.

The potential range of exposure from dietary sources is summarized in Table 8.

The Committee noted that the PTWI is likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing additives. The Committee also noted that dietary

exposure to aluminium is expected to be very high for infants fed on soya-based formula.

Further data on the bioavailability of different aluminium-containing food additives are required.

There is a need for an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioural end-points, to be conducted on a relevant aluminium compound(s).

Studies to identify the forms of aluminium present in soya formulae, and their bioavailability, are needed before an evaluation of the potential risk for infants fed on soya formulae can be considered.

*Recommendations to Codex*

The Committee recommended that provisions for aluminium-containing additives included in the Codex GSFA should be compatible with the newly established PTWI for aluminium compounds of 1 mg Al/kg bw The Committee noted in particular that provisions for such additives used at levels consistent with GMP in staple foods may lead to high exposure in the general population and in particular in children.

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## *1,3-DICHLORO-2-PROPANOL (addendum)*

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## 1. EXPLANATION

1,3-Dichloro-2-propanol is formed when chloride ions react with lipid components in foods under a variety of conditions, including food processing, cooking, and storage. The compound has been found as a contaminant in various foods and food ingredients, most notably in acid-hydrolysed vegetable protein (acid-HVP) and soy sauces.[1] This compound was first evaluated by the Committee at its forty-first meeting (Annex 1, reference *107*), when it concluded that it is an undesirable contaminant in food and expressed the opinion that its concentration in acid-HVP should be reduced as far as technically achievable.

1,3-Dichloro-2-propanol was re-evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*). Although only a few studies of kinetics and metabolism and few short- and long-term studies of toxicity and of reproductive toxicity were available for evaluation, they clearly indicated that 1,3-dichloro-2-propanol was hepatotoxic and nephrotoxic, induced a variety of tumours in various organs in rats, and was genotoxic in vitro. The Committee therefore concluded that it would be inappropriate to estimate a tolerable intake of 1,3-dichloro-2-propanol. The Committee noted that the dose that caused tumours in rats (19 mg/kg bw per day) was about 20 000 times greater than the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soy sauce (1 µg/kg bw per day), and that the available evidence suggested that in soy sauces 1,3-dichloro-2-propanol was associated with high concentrations of 3-chloro-1,2-propanediol, concentrations of the latter being approximately 50 times higher than those of 1,3-dichloro-2-propanol. Therefore, in the opinion of the Committee, regulatory control of the latter would obviate the need for specific controls on 1,3-dichloro-2-propanol.

The present re-evaluation was conducted in response to a request from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005) that JECFA review and summarize all new data on the toxicology (including new studies of genotoxicity in vivo) and occurrence of 1,3-dichloro-2-propanol. In particular, the Committee was asked to carry out an exposure assessment to readdress 1,3-dichloro-2-propanol as a separate issue from 3-chloro-1,2-propanediol.

Since the last evaluation of 1,3-dichloro-2-propanol by the Committee (Annex 1, references *154*, *155*) evaluations have been produced by other authorities (Committee on Mutagenicity, 2003; FSANZ, 2003; Committee on Carcinogenicity, 2004; National Toxicology Program, 2005).

[1] The term 'soy sauce' is used to encompass liquid seasonings made from soya beans by a range of methods including acid-hydrolysis and traditional fermentation, possibly with the addition of acid-HVP. In some countries, the term 'soy sauce' is reserved solely for fermented products.

## 2. BIOLOGICAL DATA

### 2.1 Biochemical aspects

No new data were available.

### 2.2 Toxicological studies

#### 2.2.1 Short-term studies of toxicity

No new data were available.

#### 2.2.2 Long-term studies of toxicity and carcinogenicity

No new studies were available.

*(a) Dose-response modelling*

Cancer dose–response data were analysed by dose–response modelling, in accordance with the International Programme on Chemical Safety (IPCS) document *Principles for modelling dose–response for the risk assessment of chemicals* (IPCS, 2004). Modelling was performed of data for neoplastic changes reported in the study by Research & Consulting Co. (1986). In this long-term study of carcinogenicity, male and female rats were given drinking-water containing 1,3-dichloro-2-propanol for up to 2 years. Treatment-related increases in the incidence of tumours were observed in the kidney of male rats (Table 1) and in the liver, thyroid gland and the tongue of both sexes (Tables 1 and 2).

Incidence data for individual tumour sites and for animals with treatment-related tumours were compiled for both sexes and used for dose–response modelling. For this purpose, only data for the groups exposed for 2 years were used; interim sacrifice groups were not considered.

*Modelling*

Eight models were fitted to the dose–response data. The benchmark dose was set at a 10% increase in the incidence of tumours above background ($BMD_{10}$) (background adjustment: extra risk). The lower confidence limit for the benchmark dose ($BMDL_{10}$) was 95%. Datasets for the two sexes were modelled independently. All datasets were found to be suitable for modelling, when models were applied using standard options as provided in the software package, without the necessity to implement additional restrictions and/or modifications of the models.

Table 3 shows the results for all treatment-related tumours in male rats. Reasonable fits, judged on the basis of visual examination of curve fits and on statistical evaluation ($p$ values of suitable models were > 0.2), and consistent values for $BMD_{10}$ and $BMDL_{10}$ were obtained for the eight models.

Akaike's information criterion (AIC) values were similar for all models. The logistic, quantal-quadratic and the probit model gave somewhat worse fits, as can be seen from residual values and chi-squared values. The $BMDL_{10}$s from these

***Table 1. Number of male rats bearing tumours after receiving drinking-water containing 1,3-dichloro-2-propanol for 2 years***

| Tumour site | Concentration (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 2.1 | 6.3 | 19 |
| *Kidney* | | | | |
| Tubular adenoma | 0/50 | 0/50 | 3/50 | 9/50 |
| Tubular carcinoma | 0/50 | 0/50 | 0/50 | 1/50 |
| Combined | 0/50 | 0/50 | 3/50 | 9/50 [a] |
| *Liver* | | | | |
| Hepatocellular adenoma | 1/50 | 0/50 | 0/50 | 0/50 |
| Hepatocellular carcinoma | 0/50 | 0/50 | 2/50 | 8/50 |
| Combined | 1/50 | 0/50 | 2/50 | 8/50 |
| *Thyroid gland* | | | | |
| Follicular adenoma | 0/50 | 0/50 | 2/50 | 3/48 |
| Follicular carcinoma | 0/50 | 0/50 | 2/50 | 1/48 |
| Combined | 0/50 | 0/50 | 4/50 | 4/48 |
| *Tongue* | | | | |
| Papilloma | 0/50 | 1/50 | 0/49 | 6/50 |
| Carcinoma | 0/50 | 0/50 | 0/49 | 6/50 |
| Combined | 0/50 | 1/50 | 0/49 | 12/50 |
| *All treatment-affected sites: kidney tubular adenoma and carcinoma, hepatocellular adenoma and carcinoma, follicular adenoma and carcinoma of the thyroid, papilloma and carcinoma of the tongue* | | | | |
| Benign tumours | 1/50 | 1/50 | 5/50 | 17/50 |
| Malign tumours | 0/50 | 0/50 | 4/50 | 14/50 |
| Combined | 1/50 | 1/50 | 8/50 [b] | 29/50 [c] |

From Research & Consulting Co. (1986)

[a] Male No. 274 had an adenoma and a carcinoma and was counted only once

[b] Male No. 201 had a benign and a malign tumour and was counted only once

[c] Males Nos 269 and 274 each had a benign and a malign tumour; both animals were counted only once

models were somewhat higher than from other models, but were still in a reasonable range.

$BMD_{10}$s and $BMDL_{10}$s varied within the range of 5.4 to 7.5 and 3.3 to 6.1 mg/kg bw per day, respectively. This proves the required independency of results from the chosen model. Modelling for malignant tumours only gave similar results (not shown) with $BMD_{10}$ and $BMDL_{10}$ ranges of 8.3 to 10.1 mg/kg bw per day and 5.6 to

***Table 2. Number of female rats bearing tumours after receiving drinking-water containing 1,3-dichloro-2-propanol for 2 years***

| Tumour site | Concentration (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 3.4 | 9.6 | 30 |
| *Liver* | | | | |
| Hepatocellular adenoma | 1/50 | 1/50 | 1/50 | 5/50 |
| Hepatocellular carcinoma | 0/50 | 0/50 | 1/50 | 36/50 |
| Combined | 1/50 | 1/50 | 2/50 | 41/50 |
| *Thyroid gland* | | | | |
| Follicular adenoma | 1/50 | 0/50 | 3/50 | 3/49 |
| Follicular carcinoma | 0/50 | 0/50 | 0/50 | 2/49 |
| Combined | 1/50 | 0/50 | 3/50 | 5/49 |
| *Tongue* | | | | |
| Papilloma | 0/50 | 0/50 | 0/50 | 7/49 |
| Carcinoma | 0/50 | 1/50 | 1/50 | 4/49 |
| Combined | 0/50 | 1/50 | 1/50 | 11/49 |
| *All treatment-affected sites: hepatocellular adenoma and carcinoma, follicular adenoma and carcinoma of the thyroid, papilloma and carcinoma of the tongue* | | | | |
| Benign tumours | 2/50 | 1/50 | 4/50 | 12/50 |
| Malign tumours | 0/50 | 1/50 | 2/50 | 37/50 |
| Combined | 2/50 | 2/50 | 6/50 | 42/50 [a] |

From Research & Consulting Co. (1986)

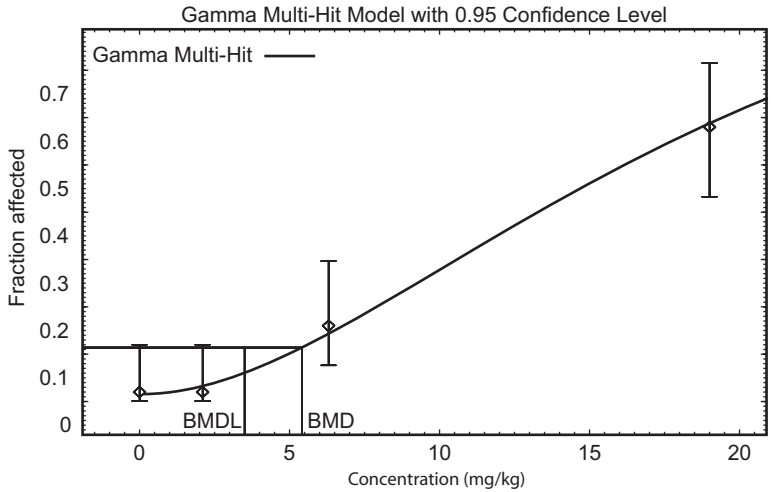
[a] Several animals had both benign and malign tumours and were counted only once.

8.4 mg/kg per day, respectively. Figure 1 shows the dose–response data and the curve fit for the gamma model, as an example.

Modelling results for female rats (animals with treatment-related tumours) are shown in Table 4. As for male rats, several models gave reasonable fits and $BMD_{10}$s and $BMDL_{10}$s varied only within a narrow range: the ranges obtained were 7.6 to 10.3 mg/kg bw per day for $BMD_{10}$ and 6.6 to 7.7 mg/kg bw per day for $BMDL_{10}$. Figure 2 shows the dose–response data for female animals and the curve fit for the Weibull model as an example.

Table 5 gives a summary of the modelling of data for individual tumour sites. Modelling was performed on incidence data for tumour-bearing animals for benign and malign kidney tumours (males only), hepatocellular tumours (males and females) as well as tumours of the tongue (males and females) (see Table 1 and 2 for incidence data for males and females, respectively). Incidence data for thyroid

***Figure 1. Dose–response data (incidence of animals with treatment-related tumours) and adjusted gamma model for male rats***




tumours showed no clear dose–response relationship and were unsuitable for modelling.

***Table 3. $BMD_{10}$, $BMDL_{10}$ and statistical parameters obtained from fitting models to dose–response data for male rats with treatment-related tumours after receiving drinking-water containing 1,3-dichloro-2-propanol for 2 years***

| Model | *p* value | Chi-squared | AIC | $BMD_{10}$ (mg/kg bw per day) | $BMDL_{10}$ (mg/kg bw per day) |
|---|---|---|---|---|---|
| Gamma | 0.507 | 0.44 | 138.1 | 5.4 | 3.5 |
| Logistic | 0.248 | 2.79 | 138.5 | 7.5 | 6.1 |
| Logistic (log dose) | 0.534 | 0.39 | 138.0 | 5.4 | 3.5 |
| Probit | 0.366 | 2.01 | 137.7 | 6.8 | 5.6 |
| Probit (log dose) | 0.731 | 0.12 | 137.7 | 5.4 | 3.6 |
| Quantal-quadratic | 0.493 | 1.42 | 136.9 | 6.5 | 5.6 |
| Weibull | 0.620 | 0.43 | 138.3 | 5.4 | 3.4 |
| Multistage (2nd degree polynomial, betas $\geq 0$) | 0.343 | 0.90 | 138.6 | 5.7 | 3.3 |

AIC: Akaike's information criterion; $BMD_{10}$: benchmark dose for 10% extra risk of tumours; $BMDL_{10}$: 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls. Multistage stands for a specific mathematical model applied.

***Table 4. $BMD_{10}$, $BMDL_{10}$ and statistical parameters obtained from fitting models to dose–response data for female rats with treatment-related tumours after receiving drinking-water containing 1,3-dichloro-2-propanol for 2 years***

| Model | *p* value | Chi-squared | AIC | $BMD_{10}$ (mg/kg bw per day | $BMDL_{10}$ (mg/kg bw per day) |
|---|---|---|---|---|---|
| Gamma | 0.967 | 0.00 | 120.2 | 10.2 | 7.2 |
| Logistic | 0.810 | 0.42 | 118.6 | 9.4 | 7.5 |
| Logistic (log dose) | 0.953 | 0.00 | 120.3 | 10.1 | 7.4 |
| Probit | 0.588 | 1.06 | 119.2 | 8.5 | 6.9 |
| Probit (log dose) | 0.996 | 0.00 | 120.2 | 10.1 | 7.7 |
| Quantal-quadratic | 0.41 | 1.77 | 120.1 | 7.6 | 6.7 |
| Weibull | 0.888 | 0.02 | 120.3 | 10.3 | 7.0 |
| Multistage (3rd degree polynomial, betas $\geq 0$) | 0.854 | 0.03 | 120.3 | 10.3 | 6.6 |

AIC: Akaike's information criterion; $BMD_{10}$: benchmark dose for 10% extra risk of tumours. $BMDL_{10}$: 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

***Figure 2. Dose–response data (incidence of animals with treatment-related tumours) and adjusted Weibull model for female rats***

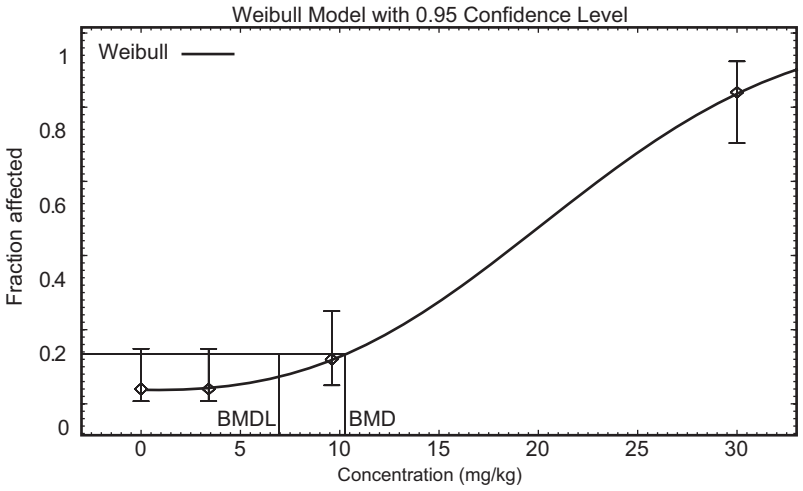


***Table 5. Result of dose–response modelling ($BMD_{10}$, $BMDL_{10}$) for various treatment-affected tumour sites for male and female rats receiving drinking-water containing 1,3-dichloro-2-propanol for 2 years***

| Tumour site and type | Models with acceptable fits | Range of $BMD_{10}$s (mg/kg bw per day) | Range of $BMDL_{10}$s (mg/kg bw per day) |
|---|---|---|---|
| *Males* | | | |
| Renal adenoma and carcinoma | Gamma, log logistic, log probit, Weibull, multistage | 11.1–12.2 | 7.2–7.7 |
| Hepatocellular adenoma and carcinoma | Logistic, probit, log probit, quantal-quadr., multistage | 14.4–16.0 | 10.3–12.3 |
| Tongue papilloma and carcinoma | Gamma, logistic, log logistic, probit, log probit, quantal-quadratic, Weibull, multistage | 12.4–17.9 | 8.7–11.6 |
| *Females* | | | |
| Hepatocellular adenoma and carcinoma | Gamma, logistic, log logistic, probit, log probit, Weibull, multistage | 11.2–14.6 | 9.1–10.1 |
| Tongue papilloma and carcinoma | Gamma, logistic, log logistic, probit, log probit, quantal-quadratic, Weibull, multistage | 17.1–22.8 | 11.5–19.1 |

$BMD_{10}$: benchmark dose for 10% extra risk of tumours. $BMDL_{10}$: 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

*Software*

Modelling was carried out using the US EPA BMDS (benchmark dose software), version 1.3.2.[2]

### *2.2.3 Genotoxicity*

In a test for micronucleus formation in bone marrow of rats in vivo, which was conducted according to OECD guideline No. 474, groups of six male Han Wistar rats were given 1,3-dichloro-2-propanol orally at a dose of 0, 25, 50, or 100 mg/kg

[2] The BMDS is freely available at <http://cfpub.epa.gov/ncea/>.

bw per day for two consecutive days. The high dose produced piloerection, weight loss and lethargy. Bone marrow was harvested 24 h after the final dose. The ratios of polychromatic to normochromatic erythrocytes (PCE/NCE) varied amongst individual animals of the control group, but the mean value was within the range of data from historical control groups. Rats treated with 1,3-dichloro-2-propanol at any dose had group mean PCE/NCE ratios that were comparable to those of the control group and that were within the normal range. Thus toxicity to the bone marrow was not demonstrated. There was no statistically significant increase in the frequency of micronucleus formation with 1,3-dichloro-2-propanol at any dose, while the positive-control agent, cyclophosphamide, was clearly active (Howe, 2002).

In a study of unscheduled DNA synthesis in rat hepatocytes in vivo, which was conducted according to OECD guideline No. 486, groups of three Han Wistar male rats were given a single oral dose of 1,3-dichloro-2-propanol at 0, 40, or 100 mg/kg bw. In the 2–4 h experiment, piloerection and lethargy were observed. Hepatocytes were isolated at 2–4 h and 12–24 h after dosing and cultured in the presence of [$^3$H]thymidine; the incorporation of [$^3$H]thymidine during unscheduled DNA synthesis was measured autoradiographically. No information on liver toxicity was provided. 1,3-Dichloro-2-propanol did not elicit unscheduled DNA synthesis, while the positive-control compounds, 2-acetylaminofluorene and dimethylnitrosamine, were clearly active (Beevers, 2003).

#### *2.2.4 Reproductive toxicity*

No new data were available.

### *2.3 Observation in humans*

No new data were available.

## *3. ANALYTICAL METHODS*

### *3.1 Chemistry*

Chloropropanols are chemicals formed when glycerol reacts with chlorine under acidic conditions. 1,3-Dichloro-2-propanol (CAS Registry No. 96-23-1) boils at 175 °C at normal pressure and its solubility in water at 20 °C is 15.2 g per 100 g.

### *3.2 Description of analytical methods*

1,3-Dichloro-2-propanol can be measured by using gas chromatographic separation and mass spectrometric detection (GC/MS) with an automated headspace procedure (HS GC/MS). The in-house validation methods developed by the Central Science Laboratory (CSL) of the United Kingdom Government Department for Environment Food and Rural Affairs (Crews et al., 2002) and also by the Center for Food Safety and Applied Nutrition (CFSAN) of the United States Food and Drug Administration (FDA) are available and published. The results from the two-method comparison showed that, in spite of a limit of detection (LOD) that was higher for the United Kingdom (UK) method than the FDA method (3.000 versus

0.055 ng/g), the recoveries for the spike-test portions, as well as the amounts of 1,3-dichloro-2-propanol found in the retail products, were comparable for 1,3-dichloro-2-propanol at concentrations of of ≥ 10 ng/g in soy and related sauces.

The CSL method has the advantage of being faster and simpler than the FDA modified method, which is more labour-intensive, but the FDA method has the advantage that it allows extraction and analysis of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol in the same GC/MS run.

In a paper published in 2006, the Chinese authors also proposed a methodology that allows the simultaneous separation and determination of chloropropanols (1,3-dichloro-2-propanol, 2,3-dichloro-2-propanol, 3-monochloro-propane-1,2-diol and 2-monochloro-1,3-propanediol) in soy sauce and other flavouring, using GC/MS in negative chemical and electron-impact ionization modes, at low concentrations of around 0.6 µg/kg (Xiamin Xu et al., 2006).

## 4. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

### 4.1.1 Surveillance data

Chloropropanols are a group of chemical contaminants formed under certain processing and storage conditions; 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol are primary members of this group. 1,3-Dichloro-2-propanol is formed during the production of acid-HVPs such as soya beans from the reaction of hydrochloric acid and residual vegetable fat from the raw materials used. As a result of the use of acid-HVP, 1,3-dichloro-2-propanol has now been found as a contaminant in various foods and food ingredients, most notably in acid-HVPs and soy sauces[3] (Codex Commission on Food Additives and Contaminants, 2006).

### 4.1.2 *National occurrence of 1,3-dichloro-2-propanol*

Data on the occurrence of 1,3-dichloro-2-propanol were submitted by member states of the European Union (European Union, 2004), Australia (FSANZ, 2003), Hong Kong Special Administrative Region of China (SAR) (FEHD, 2005), and Japan (MAFF, 2005 and 2006). Data on soy sauces and related products were also available for the United States of America (USA) from the literature (Nyman et al., 2003b).

Table 6 describes the summary of the distribution-weighted concentration of 1,3-dichloro-2-propanol in soy sauce and soy-sauce based products, in other foods products and in food ingredients from those countries, from 2001 to 2006.

[3] The term 'soy sauce' is used to encompass liquid seasonings made from soya beans by a range of methods including acid hydrolysis and traditional fermentation, possibly with the addition of acid-HVP. In some countries the term 'soy sauce' is reserved solely for fermented products.

### *4.1.3 Soy sauce and soy sauce-based products*

#### *(a) Australia*

Australia submitted occurrence data for 39 individual samples of different soy and oyster sauces, based on food surveys conducted in 2001 and 2002 (FSANZ, 2003). Sampling was undertaken of selected soy and oyster sauces and a range of other foods available at Australian retail outlets. The method used for quantitative analysis of 1,3-dichloro-2-propanol was based on the Association of Official Analytical Chemists (AOAC) Method No. 2000.01. The limit of quantification (LOQ) was 0.01 mg/kg. The majority of soy and oyster sauces did not contain detectable levels of 1,3-dichloro-2-propanol. 1,3-Dichloro-2-propanol was detected only at less than 1 mg/kg in those sauces that also contained 3-chloro-1,2-propanediol, that is, mainly soy seasoning sauce.

- The highest contamination level was reported for the seasoning soy sauce product, with a maximum of about 0.6 mg/kg.
- The average was calculated by assigning a value of half the reporting limits to the 26% of samples for which the reported value was less than the limit.
- The average concentration of 1,3-dichloro-2-propanol in the soy sauce and soy sauce based products was reported to be 0.07 mg/kg.

#### *(b) Hong Kong SAR*

Hong Kong SAR submitted the results of occurrence data in aggregate samples of different soy sauce and sauce-based products using the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) reporting format. Sampling was performed in 2003 and included the results of the analysis of 84 samples. Based on the very low limits of detection and quantification, 0.5 and 1.5 µg/kg respectively for 1,3-dichloro-2-propanol, analyses were performed using GC/MS. No indicator of analytical quality assurance was described in the submission data (FEHD, 2005).

- The average was calculated by assigning a value of half the reporting limits to the 84% of samples for which the reported value was less than the limit.
- The average concentration of 1,3-dichloro-2-propanol reported to be present in soy sauce and sauce-based products was less than 1.0 µg/kg.
- Of the 84 samples, two samples were reported to have tested positive for 1,3-dichloro-2-propanol: these were concentrated soup (2.7 µg/kg) and soy-sauce product (2.4 µg/kg).

#### *(c) European Union*

Individual occurrence data for 1,3-dichloro-2-propanol were available from four member states of the European Union (Austria, Finland, Germany and the UK) from the reports on tasks for Scientific Cooperation on levels of 3-chloro-1,2-propanediol and related substances in foodstuffs (European Union, 2004). The Joint Research Centre of the European Commission assisted in the task by providing advice on analytical methodology. Participants agreed that only occurrence data

obtained after 1997 until the year before the publication report in 2004 should be submitted as there was concern about the robustness of data acquired before that date. Some occurrence data provided by participants were from targeted sampling of products suspected of containing high levels of chloropropanols.

Of the 2035 soy sauce and related samples available, 282 were analysed for 1,3-dichloro-2-propanol. These were of the following origins: Austria ($n$ = 10), Finland ($n$ = 35), Germany ($n$ = 114), and UK ($n$ = 123). The limit of quantification for soy sauce and soy-sauce based products was between 0.005 mg/kg and 0.15 mg/kg.

- The average was calculated by assigning a value of half the reporting limits to the 80% of samples for which the reported value was less than the limit.
- The weighted average concentration of 1,3-dichloro-2-propanol in soy sauce and sauce-based products was reported to be 0.092 mg/kg. For the different types of soy sauce, the average concentrations of 1,3-dichloro-2-propanol were reported as follows: mushroom soy sauce, 0.43 mg/kg; soy sauce and seasoning sauce, 0.08 mg/kg; dark soy sauce, 0.036 mg/kg; and light soy sauce, 0.027 mg/kg. Other average concentrations of 1,3-dichloro-2-propanol reported for sauce-based products were less than 0.01 mg/kg.

*(d) Japan*

Using the GEMS/Food reporting format, Japan submitted the results of occurrence data on levels of 1,3-dichloro-2-propanol in aggregate and individual samples of soy sauce containing acid-HVP. Sampling was performed in 2005–2006 and included results of the analysis of 40 samples of soy sauce made with acid-HVPs. The method used for analysis of 1,3-dichloro-2-propanol was validated in-house using GC/MS, and was published in a paper cited in MAFF (2005 and 2006). [4]The LOD and LOQ were 0.002 mg/kg and 0.004 mg/kg, respectively.

- The results of 83% of analyses of samples of soy sauces made with acid-HVP indicated that concentrations of 1,3-dichloro-2-propanol were below the LOQ. Samples containing quantified levels of 1,3-dichloro-2-propanol also contained 3-chloro-1,2-propanediol (at or above 3.4 mg/kg). The highest level reported was 0.022 mg/kg and the average was 0.005 mg/kg.

*(e) USA*

Results of occurrence data for individual samples of 39 different soy sauce and sauce-based products purchased from retail outlets in metropolitan areas in 2002 were published by the FDA (Nyman et al., 2003a). The method used for quantitative analysis of 1,3-dichloro-2-propanol was based on a modified version of AOAC Method No. 2000.01. Based on this modified method, validated in-house, an LOQ of 0.025 mg/kg was established for 1,3-dichloro-2-propanol. The authors concluded that all samples that contained 1,3-dichloro-2-propanol at a concentration of greater than 0.05 mg/kg were from products manufactured in Asia, indicating that at the time of their study some Asian manufacturers had not made

[4] *Journal of Food Hygienic Society of Japan* 1995, 36(3):360–364.

the necessary processing changes to control the formation of chloropropanols in acid-HVP.

1,3-dichloro-2-propanol was detected in 36% of those sauces that also contained 3-chloro-1,2-propanediol, mainly in samples of soy sauce. The highest concentration reported was 9.84 mg/kg, for a soy sauce.

- The average was calculated by assigning a value of half the reporting limits to the 64% of samples for which the reported value was less than the limit.
- The average concentration of 1,3-dichloro-2-propanol was reported to be 0.62 mg/kg.

### *4.1.4 Food ingredients*

#### *(a) European Union*

Results of the analysis of 1,3-dichloro-2-propanol in over 24 samples of food ingredients were available from the reports on Tasks for Scientific Cooperation on levels of 3-chloro-1,2-propanediol and related substances in foodstuffs, but it was not possible to precisely identify from which member states the data were derived (European Union, 2004). 1,3-Dichloro-2-propanol was investigated in malt products only. Of these samples, 13% were found to contain 1,3-dichloro-2-propanol at a concentration that was greater than the LOQ, set at 0.010 mg/kg. The weighted average concentration of 1,3-dichloro-2-propanol for this food category was reported to be 0.009 mg/kg in quantified samples.

#### *(b) Japan*

Using the GEMS/Food reporting format, Japan submitted to the JECFA secretariat the results of occurrence data on the levels of 1,3-dichloro-2-propanol in acid-HVPs from aggregate and individual samples. Sampling was performed in 2005–2006 and included results of the analysis of 40 samples of acid-HVPs. The method used for analysis was validated in-house using GC/MS. The analytical method has been published in a paper cited in MAFF (2005 and 2006).[4]The LOD and LOQ were 0.002 mg/kg and 0.004 mg/kg, respectively.

- Results of the analysis of 1,3-dichloro-2-propanol in more than 30 out of 40 samples of acid-HVPs from other production processes showed that concentrations of 1,3-dichloro-2-propanol were below the LOQ. The average concentration was reported to be 0.008 mg/kg in acid-HVPs.

### *4.1.5 Other food products*

Only Australia and the European Union submitted occurrence data for foods and food groups likely to contain 1,3-dichloro-2-propanol as a result of processing or storage conditions. Analysis of several products included in the diet, e.g. cereals, meat and meat products, dairy products, fish and seafood products, was reported.

*(a) Australia*

Australia submitted occurrence data for 204 individual samples of other foods based on food surveys conducted in 2001 and 2002 (FSANZ, 2003). Sampling was performed in selected other foods and a range of other foods available at Australian retail outlets. The method used for quantitative analysis of 1,3-dichloro-2-propanol was similar that used to analyse this compound in soy and oyster sauces and was based on AOAC Method No. 2000.01. The LOD and LOQ were 0.003 mg/kg and 0.005 mg/kg, respectively. Analyses were performed for the following food categories: meat and meat products (99 samples), fish and fish products (29 samples), chicken eggs (8 samples), cereals (37 samples), infant food (3 samples), infant formulas (3 samples), cheese, cheddar and processed (12 samples), starchy vegetables (3 samples), roasted coffee and cocoa paste (6 samples), vegetable oils and fat (4 samples). Of these samples, 25% were reported to contain 1,3-dichloro-2-propanol at concentrations greater than the LOQ.

- *Fish and fish products.* 1,3-Dichloro-2-propanol was quantified in three samples: two samples of fish fillets, battered and fried, 0.024 mg/kg; and in one sample of battered fish fillet, fried, at a concentration that was slightly greater than the LOQ of 0.006 mg/kg. The weighted average concentration of 1,3-dichloro-2-propanol for this food category was reported to be 0.0025 mg/kg.
- *Meat and meat products, including poultry and game.* 1,3-Dichloro-2-propanol was quantified in 48 out of 99 samples: 25 out of 44 samples of beef, minced products, fried, raw or cooked, from 0.011 to 0.11 mg/kg; in 22 out of 43 samples of sausages, meat products, fried, raw or cooked and leg ham, from 0.015 to 0.069 (sausages, meat products, fried, raw or cooked ) and from 0.014 to 0.021 (leg ham) mg/kg, respectively**;** and in one sample of chops, lamb raw product, 0.091 mg/kg. The weighted average concentration of 1,3-dichloro-2-propanol for this food category was reported to be 0.019 mg/kg.

*(b) European Union*

Results of the analysis of more than 42 samples of products other than soy sauce and soy sauce-based products were available from the European Union report (European Union, 2004). 1,3-Dichloro-2-propanol was investigated in the following food categories: cereals and cereal products, excluding bakery (two samples), meat and meat products (16 samples), fish and fish products (14 samples) and in salts, spices, soups, sauces, salads and protein (10 samples). Only 2 out of 42 samples were found to contain 1,3-dichloro-2-propanol at a concentration greater than the LOQ, set at 0.010 mg/kg; these were two samples of edible sausage casing found to contain 1,3-dichloro-2-propanol at 0.5 and 1.5 mg/kg.

### *4.1.6 Co-occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol*

Few studies have analysed 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in the same food samples. The USA reported a study in 55 samples in 2003. Hong Kong SAR reported 84 samples in 2003, the UK reported 51 samples in 2000/2002, European Union member states reported 232 samples

in 2004, Australia reported 39 samples in 2000 to 2002 and Japan reported 80 samples in 2005–2006.

Analysis of the study of co-occurrence described below includes cases where quantifiable amounts of both 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol were measured in the same sample of soy sauce and soy sauce-based products and in food ingredients such as acid-HVPs. Figure 3 shows a plot of the percentage of 1,3-dichloro-2-propanol/3-chloro-1,2-propanediol contained in samples of different types of soy sauce (53 samples of soy sauce, 11 samples of seasoning soy sauce, 3 samples of oyster sauce, 11 samples of other sauces and 19 samples of mushroom sauce) and 10 samples of acid-HVPs found to contain both substances at quantifiable concentrations greater than 0.4 mg/kg of 3-chloro-1,2-propanediol.

Based on a limited number of data, some trends were apparent:

- Food ingredients such as acid-HVPs contain less 1,3-dichloro-2-propanol/3-chloro-1,2-propanediol than do mushroom soy sauce or seasoning soy sauce—0.1% versus 1.3–1.4%, respectively.
- Other soy sauces and oyster soy sauce contain more 1,3-dichloro-2-propanol/3-chloro-1,2-propanediol the highest percentage compared to than do other types of soy sauce, at 2.4% and 8.3% respectively.

*(a) Soy sauce and soy sauce-based products*

Co-occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in the same food samples was reported in 102 samples of soy sauce and soy sauce-based products, including 14 samples from the USA, 2 samples from Hong Kong SAR, 7 samples from Japan, 12 from the UK, 58 from the European Union and 9 from Australia.

1,3-Dichloro-2-propanol was detected only in those soy sauces that contained 3-chloro-1,2-propanediol, which suggests that, in this product, 1,3-dichloro-2-propanol is derived from 3-chloro-1,2-propanediol. Co-occurrence was found when samples contained 3-chloro-1,2-propanediol at a concentration greater than 0.4 mg/kg (82% of samples). When 3-chloro-1,2-propanediol is present at a concentration of less than 0.4 mg/kg, 1,3-dichloro-2-propanol is generally rarely detected, but this is reliant on the performance of the analytical method (LOD and LOQ) for analysis of 1,3-dichloro-2-propanol.

The statistical analysis of co-occurrence and the relationship between the two substances includes the analysis of 102 results for which quantifiable amounts of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol were measured in the same sample. As shown by the regression linear depicted in Figure 4, the relationship between 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol is highly significant with a very good Pearson correlation (r = 0.9209). The formula given below describes the linear relationship for predicting the concentration of 1,3-dichloro-2-propanol from a quantified level of 3-chloro-1,2-propanediol of greater than 0.4 mg/kg in soy sauce and sauce-based products:

[1,3-dichloro-2-propanol]= 0.00949866 [3-chloro-1,2-propanediol]

***Table 6. Summary of the distribution-weighted concentration of 1,3-dichloro-2-propanol in soy sauce and soy sauce-based products, in other foods and in food ingredients from various countries, 2001 to 2006***

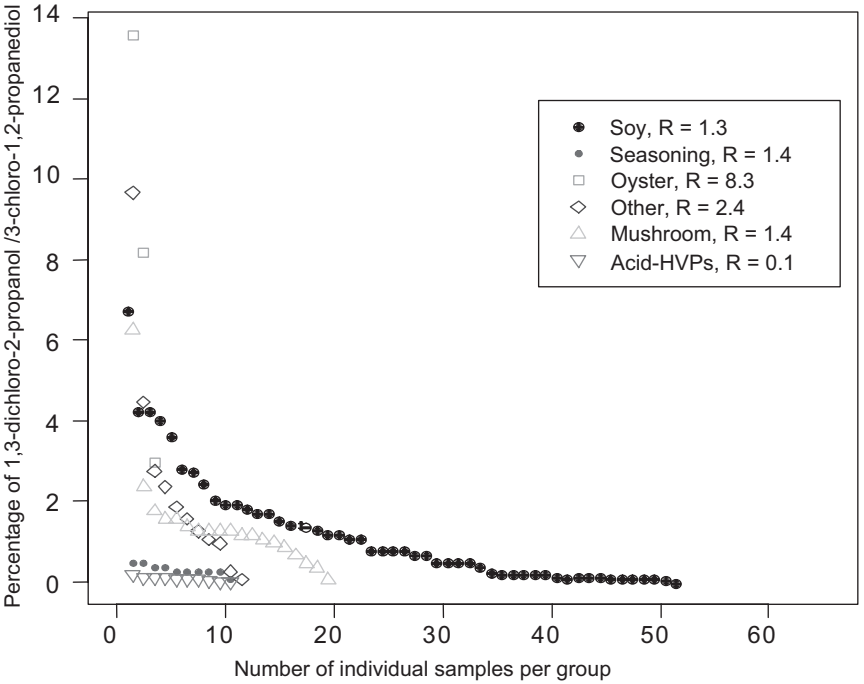
| Products | | Country and date | LOQ (mg/kg) | No. of samples | No. of samples with concentration < LOQ | Mean[a] (mg/kg) | CV[b] (%) | Maximum (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| Soy sauce and soy sauce-based products | | Europe (EC Scoop Task 3.2.9), 2004 | 0.005-0.15 | 282 | 222 | 0.092 | 286 | 1.37 |
| | | Hong Kong SAR, 2003 | 0.0015 | 84 | 82 | 0.001 | NA | 0.003 |
| | | USA, 2000-2002 | 0.025 | 39 | 17 | 0.621 | 309 | 9.84 |
| | | Australia, 2001-2002 | 0.010 | 39 | 17 | 0.070 | 216 | 0.60 |
| | | Japan, 2005-2006 | 0.004 | 40 | 33 | 0.005 | 64 | 0.02 |
| | | All | | 484 | 371 | 0.110 | 332 | 9.84 |
| Meat and meat products | | Australia, 2001-2002 | 0.005 | 99 | 51 | 0.019 | 125 | 0.11 |
| Fish and sea food | | Australia, 2001-2002 | 0.005 | 29 | 26 | 0.0025 | 111 | 0.024 |
| Food ingredients: | Acid HVPs | Japan, 2005-2006 | 0.002 | 40 | 30 | 0.008 | 148 | 0.070 |
| | Malt extract | Europe (EC Scoop task 3.2.9), 2004 | 0.010 | 24 | 21 | 0.008 | 104 | 0.025 |
| | | All | | 56 | 13 | 0.008 | 134 | 0.070 |

CV: coefficient of variation; acid-HVP: acid-hydrolysed vegetable protein; LOQ: limit of quantification; NA: not available; SAR: Special Administrative Region of China.

[a] Concentrations of less than the LOD or LOQ were assumed to be half of those limits and the mean was weighted according to the number of samples from countries.

[b] Coefficient of variation (standard deviation divided by mean, %)

***Figure 3. Percentage of 1,3-dichloro-2-propanol/3-chloro-1,2-propanediol in different soy sauce and soy sauce-based products and acid-HVP***




Acid HVP: acid-hydrolysed vegetable protein.

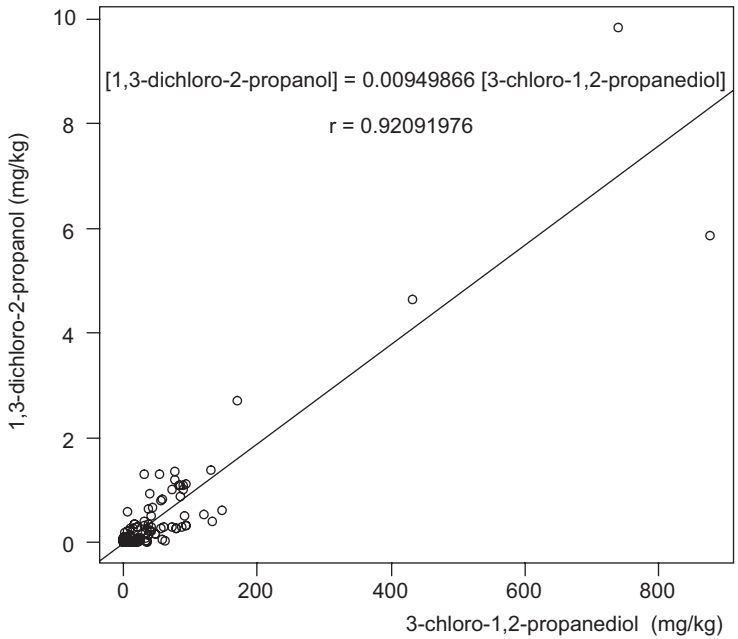
Although to be validated this model needs to be tested with other data sets, it represents at this time the best first approach to considering the relationship between the levels of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in the same kinds of product: soy sauce and related sauce-based products.

Moreover, it could be deduced from data on samples containing 3-chloro-1,2-propanediol at concentrations between greater than 0.4 mg/kg and 1000 mg/kg that such samples would contain 1,3-dichloro-2-propanol at a concentration of about 1%. The data also seem to show that samples containing 3-chloro-1,2-propanediol at between less than 0.4 mg/kg and the LOQ would contain a higher proportion of 1,3-dichloro-2-propanol, at around 34% on average (only four samples, not shown in Figure 2), but this is could also be caused by an analytical artefact whereby the measurement of quantifiable levels of a substance at concentrations close to the LOD and LOQ is subject to some uncertainty.

*(b) Food ingredients*

The co-occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in the same food samples was reported in 10 samples of acid-HVP products provided by Japan. In spite of the few data available, the analyses showed a very

***Figure 4. Co-occurrence of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol at quantifiable concentrations in soy sauce and soy sauce-based products***




good Pearson correlation ($r = 0.8736$). As shown by the linear regression obtained from these data, the relationship between 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol is highly statistically significant and a model of linearity could be proposed. The formula given below describes a linear relationship to predict the concentration of 1,3-dichloro-2-propanol from a quantified level of 3-chloro-1,2-propanediol in acid-HVPs as food ingredients:

[1,3-dichloro-2-propanol] = 0.0017738 [3-chloro-1,2-propanediol]

*(c) Other food products*

Only data from the European Union report and Australia give information on the co-occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in other foods. The levels of 1,3-dichloro-2-propanol in various foods were highly variable and, in main foods, 1,3-dichloro-2-propanol was detected in the absence of detected levels of 3-chloro-1,2-propanediol. 1,3-Dichloro-2-propanol is mainly found at quantifiable level in samples of meat and meat products (50 out of 115 samples) at concentrations that ranged from 0.006 to 0.11 mg/kg. In these products, the concentration of 1,3-dichloro-2-propanol was generally found to be higher than that of 3-chloro-1,2-propanediol. Two samples of fish fillet from the Australia market were found to contain 1,3-dichloro-2-propanol at a concentration of 0.006 and 0.024 mg/kg. 3-Chloro-1,2-propanediol was quantified in 33% of these 50 samples.

Co-occurrence was reported in 16 samples of thick meat sausage (dry-fried), sausage meat (raw or cooked), leg ham and beef (minced cooked), with the average concentration of 1,3-dichloro-2-propanol being 0.034 mg/kg and of 3-chloro-1,2-propanediol being 0.014 mg/kg.

*Summary of national occurrence data and co-occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol*

Data on 1,3-dichloro-2-propanol analysed in food were obtained from several countries. 1,3-Dichloro-2-propanol was only found at quantified levels in samples of soy sauce, of ingredients such as acid-HVPs and malt products, of minced beef (dry-fried, raw or cooked), pork ham, sausage meat (raw or cooked) and in samples of fish fillet (battered and fried). Average levels in samples of soy sauce-based products ranged from 0.09 mg/kg for soy oyster sauce to 0.6 mg/kg for soy mushroom sauce. Average levels were 0.024 mg/kg in samples of fish product, 0.034 mg/kg in samples of meat products and 0.022 mg/kg in samples of malt products.

1,3-Dichloro-2-propanol was detected only in samples that also contained 3-chloro-1,2-propanediol, except in samples of meat and meat products where 1,3-dichloro-2-propanol was detected in the presence (18 samples) and in the absence (32 samples) of detected levels of 3-chloro-1,2-propanediol. In meat products, the concentrations of 1,3-dichloro-2-propanol were generally higher than those of 3-chloro-1,2-propanediol.

The Committee noted that 1,3-dichloro-2-propanol is found in samples of soy sauce and soy sauce-based products when the concentrations of 3-chloro-1,2-propanediol exceed 0.4 mg/kg. Based on limited data, there appears to be a linear relationship between the concentrations of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol, but there was considerable scatter in the data at low concentrations and there was some variation between different types of products. Additional occurrence data would be needed to confirm these relationships, before they could be used to predict the concentrations of 1,3-dichloro-2-propanol based on the concentrations of 3-chloro-1,2-propanediol.

## *5. ESTIMATED DIETARY INTAKE*

### *5.1 National assessments of intake from diet*

Dietary intakes of 1,3-dichloro-2-propanol have been reported for 10 countries (Australia, Denmark, Finland, France, Germany, Ireland, the Netherlands, Sweden, Thailand and the UK). No dietary intakes were available for Latin America and Africa. Mainly, countries have calculated national intake using deterministic modelling by crossing national individual consumption data with national mean occurrence data obtained from surveys conducted in their country.

Consistent with their national food consumption data, all countries except Germany calculated estimates for the adult population and children. Mean occurrence data used in the calculation are those reported in the preceding section

on country occurrence data, where all values classified as being less than the LOQ were assumed to have 1,3-dichloro-2-propanol at a concentration of half the LOQ, except for Thailand where no information was available concerning the treatment of data below the quantification limit. Intake estimates were calculated on a per-kilogram of body weight basis. Estimates reported by each country are described according to the two following variables of intake distribution (mean and high-percentile dietary exposures):

- Average estimates: combination of the average food consumption and mean occurrence data for food consumed
- P95: 95th percentile of the intake distribution

Table 7 summarizes national estimated dietary intakes of 1,3-dichloro-2-propanol from various food sources, including soy sauce and soy sauce-based products.

Table 8 summarizes national estimated consumption of soy sauce and soy sauce-based products by consumers only from various countries, and the corresponding estimated dietary intakes of 1,3-dichloro-2-propanol.

*(a) Australia*

Australia submitted the results of dietary exposure assessment for chloropropanols (FSANZ, 2003). Intake calculations were obtained in a deterministic way using a dietary modelling computer program (Dietary Modelling of Nutritional Data, DIAMOND) crossing mean concentration of 1,3-dichloro-2-propanol in a wide range of foods, including soy sauce and soy sauce-based products consumed by each individual reported in the 1995 National Nutrition Survey based on 24 h-recall methodology. To provide a more refined estimate of dietary exposure, values for concentrations that were below the limit of reporting (LOR = 78%) were assigned a concentration equal to the LOR.

Dietary exposure was conducted using food groupings based on the Codex GSFA classification system for food additives. Foods are classified according to major food types, as raw and processed foods, to which reported analytical concentrations are assigned. Calculations are made in assigning concentrations of 1,3-dichloro-2-propanol to food groups for food eaten “as is”, as well as for food used as ingredients in mixed foods. Where a single food from a food group was analysed, the concentration of 1,3-dichloro-2-propanol was assigned to the whole group, assuming that like foods would contain 1,3-dichloro-2-propanol at a similar concentration. This is likely to result in an overestimation of dietary, but assumes a worst-case scenario.

Table 7 shows that the intake estimates for whole population aged more than 2 years ranged from 0.041 µg/kg bw per day on average to 0.105 µg/kg bw per day for consumers at the 95th percentile. Children aged between 2 and 12 years had exposures that ranged from 0.051 µg/kg bw per day on average to 0.136 µg/kg bw per day for consumers at the 95th percentile. Children aged between 13 and 19 years had exposures that ranged from 0.035 µg/kg bw per day on average to 0.094 µg/kg bw per day for consumers at the 95th percentile. Adults aged 20 years

and older had exposures that ranged from 0.039 µg/kg bw per day on average to 0.101 µg/kg bw per day for consumers at the 95th percentile.

Major contributors to estimated dietary exposures to 1,3-dichloro-2-propanol for all groups were minced meat (45%), sausages (25%), bread (10%) soy and oyster sauce (7%), and ham and bacon (6%).

*(b) European Union*

No national estimates of dietary intake of 1,3-dichloro-2-propanol were available from the European Commission report (European Union, 2004).

As a first approach to estimating exposure to 1,3-dichloro-2-propanol among the European Member States involved in the Scientific Cooperation on levels of 3-chloro-1,2-propanediol and related substances in foodstuffs (European Union, 2004), an assessment of estimated intake was made by the Committee at its present meeting. This assessment was based on data on occurrence and food consumption available for food sources found to contain 1,3-dichloro-2-propanol, which were published in the European Commission report and the Australian report.

This assessment took into account the weighted average concentration of 1,3-dichloro-2-propanol reported in Table 6 for the following food sources: soy sauce products (0.092 mg/kg), meat and meat products (0.019 mg/kg, corresponding to the weighted mean from Australia) and fish products (0.0025 mg/kg, corresponding to the weighted mean from Australia). Average food consumption and consumption at the 95th percentile for these products reported by member states in the EC report was used. Estimated dietary intakes were assessed for eight members of the European community (Denmark, Finland, France, Germany, Ireland, the Netherlands, Sweden and UK) according a mean body weight of 65 kg for adults, 30 kg for children and 15 kg for young children. Owing to differences in the consumption data reported by countries (whole-population, consumers only) and the mode of calculation of intake, estimates of dietary exposure to 1,3-dichloro-2-propanol made by the Committee at its present meeting are likely to be overestimated, as high-percentile (95th) intakes of different products have been summed and it is improbable that a consumer will have a high consumption of all products. Table 6 summarizes estimated intakes for mean and high-percentile consumption from various food sources.

- Estmates of average intake from all sources ranged from 0.008 to 0.048 µg/kg bw per day for the adult population and from 0.011 to 0.035 µg/kg bw per day for children. For consumers at a high percentile (95th), including young children, estimated intakes ranged from 0.025 to 0.133 µg/kg bw per day.
- Estimated intakes from meat and meat products ranged from 0.007 µg/kg bw per day on average to 0.069 µg/kg bw per day for adults at the 95th percentile of consumption. Children have exposures that ranged from 0.009 µg/kg bw per day on average to 0.075 µg/kg bw per day at the 95th percentile of consumption.
- Estimated intakes from soy sauce and related products ranged from 0.001 µg/kg bw per day on average to 0.023 µg/kg bw per day at the 95th percentile for adults. Exposures for children ranged from 0.002 µg/kg bw per day on average to 0.055 µg/kg bw per day at the 95th percentile (Table 8).

Meat and meat products made the greatest contribution to total exposure to 1,3-dichloro-2-propanol in all groups and countries when compared with soy sauce and soy sauce-based products, from 72% to 99% versus <1% to 28%, respectively.

*Consumption of soy sauce and soy sauce-based products by consumers only from various countries and estimated dietary exposure to 1,3-dichloro-2-propanol*

Table 8 summarizes consumption and estimated dietary intakes of 1,3-dichloro-2-propanol from soy sauce and soy sauce-based products in consumers only from various countries. The percentage of consumers of soy sauce and soy sauce-based products was reported to range from 1.4% to 8% in the adult population and from 1% to 8.7% in children. Consumption ranged from 1 to 30 g/day, for various countries, in adults and from 0.6 to 16 g/day in children. An estimate of dietary intakes of 1,3-dichloro-2-propanol was obtained using consumption of soy sauce by consumers only in each country and a fixed weighted concentration of 1,3-dichloro-2-propanol in soy sauce as described in reports by countries (Australia, 0.069 mg/kg; and European countries, 0.092 mg/kg). A standard average body weight of 65 kg for adults, 30 kg for children and 15 kg for young children was used.

For adults, average intakes ranged from 0.001 to 0.011 µg/kg bw per day and intakes at the 95th percentile ranged from 0.006 to 0.032 µg/kg bw per day. In children, average intakes ranged from 0.002 to 0.012 µg/kg bw per day, and intakes at the 95th percentile ranged from 0.007 to 0.055 µg/kg bw per day.

*Summary of national intake estimates*

National estimates of dietary intake of 1,3-dichloro-2-propanol provided by Australia and estimates for European Union member states were assessed by the Committee based on available occurrence data provided both by member states and Australia. Intakes were calculated by linking individual consumption data with mean occurrence data, using the actual body weight of the consumer as reported in consumption surveys.

At the national level, intake estimates from various food sources including soy sauce and soy-sauce products ranged from 0.008 to 0.051 µg/kg bw per day for the average in the general population. For consumers at a high percentile (95th), including young children, intake estimates ranged from 0.025 to 0.136 µg/kg bw per day.

Meat products are the main contributor to intake in all national estimates, ranging from 45% to 99% depending on the country diet. Soy sauce and soy sauce-based products contributed up to 30% in all national estimates. Other food groups contributed up to 10% of the total intake.

The Committee concluded that based on national estimates, an intake of 0.051 µg/kg bw per day of 1,3-dichloro-2-propanol could be taken to represent the average for the general population and that an intake of 1,3-dichloro-2-propanol of 0.136 µg/kg bw per day could be taken to represent consumers with a high intake. In these intake estimates for average to high intake, young children are also included.

***Table 7. Estimated dietary intake of 1,3-dichloro-2-propanol from various food sources including soy sauce and soy sauce-based products***

| Country | Population group | Intake of 1,3-dichloro-2-propanol (μg/kg bw per day)[a] | |
|---|---|---|---|
| | | Mean | 95th Percentile[b] |
| Australia | All (≥ 2 years) | 0.041 | 0.105 |
| | 2–12 years | 0.051 | 0.136 |
| | 13–19 years | 0.035 | 0.094 |
| | ≥ 20 years | 0.039 | 0.101 |
| Denmark | Adult | 0.040 | — |
| Finland | Adult | 0.010 | — |
| France | ≥ 18 years | 0.018 | 0.038 |
| | 3–14 years | 0.031 | 0.064 |
| Germany[c] | Adult | 0.008 | 0.026 |
| | 14 years | 0.011 | 0.036 |
| Ireland | Adult | 0.048 | 0.077 |
| Netherlands | Adult | 0.021 | 0.053 |
| | Children | 0.036 | 0.099 |
| Sweden | Adult | 0.011 | 0.025 |
| United Kingdom[c] | ≥ 18 years | 0.016 | 0.041 |
| | 1.5–4.5 years | 0.035 | 0.133 |

[a] Only 5% of consumers had exposures above this level.

[b] Estimated dietary intakes of 1,3-dichloro-2-propanol for European countries were assessed by the Committee based on available food consumption data and a fixed weighted concentration of 1,3-dichloro-2-propanol for food categories found to contain 1,3-dichloro-2-propanol in the European Union report (European Union, 2004) (soy sauce and soy sauce-based products) and in the Australian report for the other foods group (meat and fish products). Standard average body weights of 65 kg for adults, 30 kg for children and 15 kg for young children were used.

[c] Consumers only

### *5.2 International estimates of intake from GEMS/Food Consumption Cluster Diets*

The new descriptions of the GEMS/Food Consumption Cluster Diets allow a better refinement of intake assessments for chloropropanolsl, e.g. information is provided on soy sauce consumed as is and oil of soya bean for other uses, cheese processed, which makes the diets more relevant than those used for the previous evaluation by the Committee.

***Table 8. Consumption of soy sauce and soy sauce-based products by consumers only from various countries and estimated dietary exposure to 1,3-dichloro-2-propanol***

| Country | Population group | No of consumers (%) | Consumption (g/day) | | Intake of 1,3-dichloro-2-propanol (µg/kg bw per day) [a] | |
|---|---|---|---|---|---|---|
| | | | Mean | P95[b] | Mean | P95 |
| Australia | All (≥ 2 years) | 13 858 (7.6) | 9.3 | 27.9 | — | — |
| | 2–12 years | 2 079 (4.9) | 4.8 | 16.2 | 0.011 | 0.037 |
| | 13–19 years | 1 063 (8.7) | 9.3 | 25.4 | — | — |
| | ≥ 20 years | 10 716 (8.1) | 10.1 | 30.3 | 0.011 | 0.032 |
| France | ≥ 18 years | 1 474 (2.4) | 3.6 | 5.8 | 0.005 | 0.008 |
| | 3–14 years | 1 014 (1) | 3.4 | 5.6 | 0.010 | 0.017 |
| Germany | Adult | 25 000 (5.5) | 0.9 | 4.3 | 0.001 | 0.006 |
| | 4–14 years | 2 614 (6.6) | 0.6 | 2.2 | 0.002 | 0.007 |
| Ireland | Adult | 958 (1.9) | 1.9 | 4.1 | 0.003 | 0.006 |
| Netherlands | Adult | 4 711 (3.7) | 6.1 | 16.0 | 0.009 | 0.023 |
| | Children | 1 383 (7.5) | 4.0 | 14.5 | 0.012 | 0.044 |
| Sweden | Adult | 1 212 (1.4) | 2.3 | 4.8 | 0.003 | 0.007 |
| United Kingdom | Adult | 2 197 (8.7) | 4.0 | 9.0 | 0.006 | 0.013 |
| | 1–4 years | 1 675 (2.4) | 4 | 9.0 | 0.006 | 0.055 |

[a] Estimated dietary intakes of 1,3-dichloro-2-propanol were obtained using consumption of soy sauce by consumers only in each country and a fixed weighted concentration of 1,3-dichloro-2-propanol in soy sauce as described in reports (Australia, 0.069 mg/kg; and European countries, 0.092 mg/kg). Standard average body weights of 65 kg for adults, 30 kg for children and 15 kg for young children were used.

[b] 95th percentile: only 5% of consumers had exposures above this level.

Data on distribution contamination from aggregate food items taking into account the weighting of samples from submitted and published results have been described according to the GEMS/Food categorization of 13 cluster diets (WHO, 2006). Table 6 shows the summary of the distribution-weighted concentration of 1,3-dichloro-2-propanol in soya sauce and soy sauce-based products, in other foods and in food ingredients from various countries from 2001 to 2006 reported as the best available occurrence data.

In general, the food items analysed were well characterized and it was possible to match sources, contamination and consumption to the new GEMS/Food cluster diets. Occurrence data were multiplied by the total mean consumption of the corresponding food category or subcategory reported, to derive mean total intakes

per cluster diet of 1,3-dichloro-2-propanol from all food sources. A summary of the international dietary intake estimates expressed in µg/kg bw per day is presented in Table 9.

International mean intakes from all sources were estimated to range from 0.008 to 0.090 µg/kg bw per day for the 13 GEMS/Food cluster diets, assuming a body weight of 60 kg.

Meat and meat products made the greatest contribution to total exposure in all cluster diets except for clusters C, G, K and L and ranged from 54% to 72%. Soya sauce and oil of soya beans contributed more than 10% of the total exposure in all cluster diets (range, 24–68%; Table 9). Fish products contributed less than 5% of the total exposure in all cluster diets, except clusters A and J.

***Table 9 Summary of estimates of international dietary intake of 1,3-dichloro-2-propanol from all food sources (in µg/kg bw per day) for the 13 GEMS/Food Consumption Cluster Diets***

| Diet | Food category | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Soya [a] | | Meat[b] | | Fish[c] | | |
| | Intake[d] | % Total intake | Intake[a] | % Total intake | Intake[a] | % Total intake | Intake |
| A | 0.003 | 31 | 0.006 | 63 | 0.001 | 6 | 0.009 |
| B | 0.012 | 26 | 0.034 | 72 | 0.001 | 2 | 0.047 |
| C | 0.011 | 57 | 0.008 | 40 | 0.001 | 3 | 0.019 |
| D | 0.007 | 30 | 0.016 | 66 | 0.001 | 4 | 0.024 |
| E | 0.012 | 30 | 0.026 | 67 | 0.001 | 2 | 0.039 |
| F | 0.013 | 24 | 0.040 | 72 | 0.002 | 3 | 0.055 |
| G | 0.020 | 55 | 0.015 | 42 | 0.001 | 3 | 0.036 |
| H | 0.019 | 41 | 0.027 | 58 | 0.001 | 1 | 0.047 |
| I | 0.004 | 31 | 0.008 | 65 | 0.000 | 4 | 0.012 |
| J | 0.003 | 30 | 0.005 | 62 | 0.001 | 8 | 0.008 |
| K | 0.036 | 58 | 0.025 | 41 | 0.000 | 1 | 0.062 |
| L | 0.042 | 68 | 0.017 | 28 | 0.002 | 4 | 0.062 |
| M | 0.041 | 46 | 0.048 | 54 | 0.001 | 1 | 0.090 |

GEMS/Food: Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme

[a] Soya sauce and oil of soya beans.

[b] Meat from cattle, pigs and sheep

[c] Fish, marine and freshwater.

[d] Weighted average concentration as reported in Table 8 for the food category or subcategory considered and food consumption in new 13 GEMS/Food Consumption Cluster Diets.

## 6. COMMENTS

*Toxicological data*

At its present meeting, the Committee reviewed two new studies of genotoxicity, a test for micronucleus formation in rat bone marrow in vivo and an assay for unscheduled DNA synthesis in rat hepatocytes in vivo/in vitro, which met appropriate standards for study protocol and conduct. In those assays, 1,3-dichloro-2-propanol yielded negative results in the tissues assessed. However, toxicity was not demonstrated in the tissues and hence the level of exposure is unclear. No other new data were available.

In the light of the limitations of the negative results for genotoxicity in vivo, the Committee reconsidered the results from the long-term study of carcinogenicity previously evaluated at its fifty-seventh meeting (Annex 1, reference *154*). In that study, rats were given drinking-water containing 1,3-dichloro-2-propanol at a dose of 0, 2.1, 6.3, or 19 mg/kg bw per day in males and 0, 3.4, 9.6, or 30 mg/kg bw per day in females for 104 weeks. Increased incidences of tumours were demonstrated in both sexes at the two higher doses tested. No increase in tumour incidence was seen at the lowest doses tested, 2.1 and 3.4 mg/kg bw per day for male and female rats, respectively. Treatment-related increases in tumour incidence (adenomas and carcinomas) occurred in liver, kidney (males only), the tongue, and thyroid gland. Certain of the tumours (i.e. liver and kidney) might have arisen by non-genotoxic processes, but no clear mode of action was established. Moreover no mode of action was evident for the increased incidence of tongue papillomas and carcinomas in both sexes of rats at the highest dose. In spite of the negative results for genotoxicity in vivo in the tissues assessed (i.e. bone marrow and liver), the Committee could not exclude a genotoxic basis for the neoplastic findings, because of the absence of persuasive negative genotoxicity data in the target organs for carcinogenicity, and the findings that 1,3-dichloro-2-propanol caused point mutations in bacteria and mammalian cells in culture and caused multi-organ carcinogenicity in both sexes. The Committee therefore confirmed that 1,3-dichloro-2-propanol should be regarded as a genotoxic and carcinogenic compound and performed dose–response modelling of the carcinogenicity data from the long-term study in rats to calculate the margin of exposure, according to the recommendations of the Committee at its sixty-first meeting (Annex 1, reference *166*).

The Committee calculated benchmark doses for 10% extra risk of tumours and 95% lower confidence limit for the benchmark dose ($BMDL_{10}$) values for the incidences of treatment-related tumours at each site for each sex. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls. Also, because of the presumed common genotoxic mode of action, the incidences of animals with treatment-related tumours were modelled for each sex. Consistent results were obtained from use of several models for all datasets modelled. The $BMDL_{10}$ values for the individual treatment-related tumours ranged from 7.2 to 19.1 mg/kg bw per day and for the incidence data for tumour-bearing animals from 3.3 to 7.7 mg/kg bw per day, as shown in Table 10.

*Occurrence*

Data on 1,3-dichloro-2-propanol analysed in food were obtained from several countries. 1,3-Dichloro-2-propanol was only found at quantifiable levels in samples of soy sauce, in samples of ingredients such as acid-HVPs and malt products, in samples of minced beef (dry-fried, raw or cooked), pork ham, sausage meat (raw or cooked) and in samples of fish fillet (battered and fried).

Average concentrations in samples of soy sauce-based products ranged from 0.09 mg/kg in soy oyster sauce to 0.6 mg/kg in soy mushroom sauce. Average concentrations were 0.024 mg/kg in samples of fish product, 0.034 mg/kg in samples of meat products and 0.022 mg/kg in samples of malt products.

1,3-Dichloro-2-propanol was detected only in samples that also contained 3-chloro-1,2-propanediol, except in samples of meat and meat products where 1,3-dichloro-2-propanol was detected in the presence (18 samples) and in the absence (32 samples) of detected levels of 3-chloro-1,2-propanediol. In meat products, the concentrations of 1,3-dichloro-2-propanol were generally higher than those of 3-chloro-1,2-propanediol.

The Committee noted that 1,3-dichloro-2-propanol is found in samples of soy sauce and soy sauce-based products when the concentrations of 3-chloro-1,2-propanediol exceed 0.4 mg/kg.

Based on limited data, there appears to be a linear relationship between the concentrations of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol, but there was considerable scatter in the data at low concentrations and there was some variation between different types of products. Additional occurrence data would be needed to confirm the relationships before they could be used to predict the concentrations of 1,3-dichloro-2-propanol based on the concentrations of 3-chloro-1,2-propanediol.

*Dietary exposure assessment*

National estimates of dietary intake of 1,3-dichloro-2-propanol were provided by Australia, and estimates for European Union member states were assessed by the Committee based on available occurrence data provided both by European Union member states and Australia. Intakes were calculated by linking individual consumption data with mean occurrence data, using the actual body weight of the consumer as reported in consumption surveys.

Intake estimates from various food sources including soy sauce and soy-sauce products at the national level ranged from 0.008 to 0.051 µg/kg bw per day for the average in the general population. For consumers at a high percentile (95th), including young children, intake estimates ranged from 0.025 to 0.136 µg/kg bw per day.

Meat products were the main contributor to intake in all national estimates, ranging from 45% to 99% depending on the country diet. Soy sauce and soy sauce-based products contributed up to 30% in all national estimates. Other food groups contributed up to 10% of the total intake.

***Table 10. $BMD_{10}$[a] and $BMDL_{10}$[b] values obtained from fitting models to incidence data for all treatment-related tumours and for individual tumour locations in male and female rats given drinking water containing 1,3-dichloro-2-propanol for 2 years***

| Treatment-affected sites and tumour types | Range of $BMD_{10}$ values (mg/kg bw per day) | Range of $BMDL_{10}$ values (mg/kg bw per day) |
|---|---|---|
| *Males* | | |
| Tumour-bearing animals/all treatment-associated sites | 5.4–7.5 | 3.3–6.1 |
| Renal adenoma and carcinoma | 11.1–12.2 | 7.2–7.7 |
| Hepatocellular adenoma and carcinoma | 14.4–16.0 | 10.3–12.3 |
| Tongue papilloma and carcinoma | 12.4–17.9 | 8.7–11.6 |
| *Females* | | |
| Tumour-bearing animals/all treatment-associated sites | 8.5–10.3 | 6.6–7.7 |
| Hepatocellular adenoma and carcinoma | 11.2–14.6 | 9.1–10.1 |
| Tongue papilloma and carcinoma | 17.1–22.8 | 11.5–19.1 |

[a]$BMD_{10}$: benchmark dose for 10% extra risk of tumours.

[b]$BMDL_{10}$: 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

The Committee concluded that based on national estimates, an intake of 0.051 µg/kg bw per day of 1,3-dichloro-2-propanol could be taken to represent the average for the general population and that an intake of 1,3-dichloro-2-propanol of 0.136 µg/kg bw per day could be taken to represent high consumers. In the intake estimates for average to high intake, young children are also included.

## *7. EVALUATION*

The available evidence suggests that 1,3-dichloro-2-propanol occurs at lower concentrations than 3-chloro-1,2-propanediol in soy sauce and related products, and also in food ingredients containing acid-HVP. However, in meat products the concentrations of 1,3-dichloro-2-propanol are generally higher than those of 3-chloro-1,2-propanediol.

The Committee concluded that the critical effect of 1,3-dichloro-2-propanol is carcinogenicity. The substance yielded negative results in two new studies of genotoxicity, a test for micronucleus formation in bone marrow in vivo and an assay for unscheduled DNA synthesis in vivo/in vitro in rat hepatocytes, but limitations in those studies and positive findings in tests for genotoxicity in vitro as well as lack of

knowledge on the modes of action operative at the various tumour locations led the Committee to the conclusion that a genotoxic mode of action could not be excluded. Accordingly, the cancer dose–response data were analysed by dose–response modelling, and the Committee used eight different models to calculate $BMD_{10}$ and $BMDL_{10}$ values. $BMDL_{10}$ values for the individual tumours ranged from 7.2 to 19.1 mg/kg bw per day and for incidence data on tumour-bearing animals for all treatment-affected locations from 3.3 to 7.7 mg/kg bw per day.

The Committee concluded that a representative mean intake for the general population of 1,3-dichloro-2-propanol of 0.051 µg/kg bw per day and an estimated high-level intake (young children included) of 0.136 µg/kg bw per day could be used in the evaluation. Comparison of the mean and high-levels intakes with the lowest $BMDL_{10}$ of 3.3 mg/kg bw per day, which was the $BMDL_{10}$ for incidence data on tumour-bearing animals for all treatment-affected locations, indicates margins of exposure of approximately 65 000 and 24 000, respectively. Based on those margins of exposure, the Committee concluded that the estimated intakes of 1,3-dichloro-2-propanol were of low concern for human health.

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## *3-CHLORO-1,2-PROPANEDIOL (addendum)*

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### *1. EXPLANATION*

3-Chloro-1,2-propanediol is formed when chloride ions react with lipid components in foods under a variety of conditions, including food processing, cooking, and storage. The compound has been found as a contaminant in various

foods and food ingredients, most notably in acid-hydrolysed vegetable protein (acid-HVP) and soy sauces.[1] 3-Chloro-1,2-propanediol was first evaluated by the Committee at its forty-first meeting (Annex 1, reference 107). The Committee concluded that it is an undesirable contaminant in food and expressed the opinion that its concentration in acid-HVP should be reduced as far as technically achievable.

3-Chloro-1,2-propanediol was re-evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference 154). Short- and long-term studies in rodents showed that 3-chloro-1,2-propanediol is nephrotoxic in both sexes and also affects the male reproductive tract and male fertility. At that meeting, the Committee considered that the kidney was the main target organ and tubule hyperplasia in the kidney the most sensitive end-point for deriving a tolerable intake. This effect was seen in a long-term study of toxicity and carcinogenicity in male and female Fischer 344 rats given drinking-water containing 3-chloro-1,2-propanediol. The Committee concluded that 1.1 mg/kg bw per day, the lowest dose, was a lowest-observed-effect level (LOEL) and that this was close to a no-observed-effect level (NOEL). The Committee established a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw for 3-chloro-1,2-propanediol on the basis of this LOEL, using a safety factor of 500. This factor was considered adequate to allow for the absence of a clear NOEL and to account for the effects on male fertility and for inadequacies in the studies of reproductive toxicity. Data available to the Committee at that time indicated that the estimated mean intake of 3-chloro-1,2-propanediol for consumers of soy sauce would be at or above this PMTDI.

The present re-evaluation was conducted in response to a request from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005a) for the JECFA Committee to review and summarize all new data on the toxicology and occurrence of 3-chloro-1,2-propanediol. In particular, the Committee was asked to carry out an exposure assessment for 3-chloro-1,2-propanediol based on the contributions from all food groups in the diet (not only soy sauce), with particular consideration to population groups that might have higher levels of exposure.

Since the Committee's last evaluation of 3-chloro-1,2-propanediol (Annex 1, references 154, 155) this substance has also been evaluated by the Food Standards Australia New Zealand (FSANZ, 2003).

[1] The term 'soy sauce' is used to encompass liquid seasonings made from soya beans by a range of methods including acid-hydrolysis and traditional fermentation, possibly with the addition of acid-HVP. In some countries, the term 'soy sauce' is reserved solely for fermented products.

## *2. BIOLOGICAL DATA*

### *2.1 Biochemical aspects*

No new information was available.

### *2.2 Toxicological data*

#### *2.2.1 Short-term studies of toxicity*

*Rats*

In a study to investigate effects on neurobehaviour, groups of 10 male and 10 female Sprague-Dawley rats were given 3-chloro-1,2-propanediol as oral doses at 0, 10, 20, or 30 mg/kg bw per day for 11 weeks. Although body-weight gain was significantly decreased in the males at the highest dose, no significant effects on the results of tests for motor activity, landing foot splay, or forelimb grip strength were found in treated rats at weeks 1, 3, 5, 7, 9, or 11 when compared with control rats that received saline. These results suggest that 3-chloro-1,2-propanediol, at the doses used, does not produce neuromotor deficits (Kim et al., 2004).

#### *2.2.2 Long-term studies of toxicity and carcinogenicity*

No new studies were available.

#### *2.2.3 Genotoxicity*

The previously reviewed reports from Fellows (2000) and Marshall (2000) have now been published (Robjohns et al., 2003).

The formation of micronuclei in bone marrow erythrocytes was studied in groups of six male Crl:Han Wist(Glx:BRL)BR rats given 3-chloro-1,2-propanediol as oral doses at 0, 15, 30 or 60 mg/kg bw per day for two consecutive days, based on a preliminary dose range-finding experiment in which a dose of 60 mg/kg bw per day was close to the maximum tolerated dose. Administration of 3-chloro-1,2-propanediol at the highest dose produced signs of toxicity (piloerection) and a reduction in the ratio of polychromatic to normochromatic erythrocytes, indicating toxicity to erythropoietic cells. No increase in micronucleated polychromatic erythrocytes was produced by 3-chloro-1,2-propanediol, while the positive control, cyclophosphamide, was clearly active.

Unscheduled DNA synthesis in hepatocytes was studied in groups of four male Crl:Han Wist(Glx:BRL)BR rats given 3-chloro-1,2-propanediol as single oral doses at 0, 40 or 100 mg/kg bw, based on a preliminary dose range-finding experiment in which 100 mg/kg bw per day was close to the maximum tolerated dosage. In one part of the experiment, hepatocytes were isolated at 2–4 h after dosing and in the second, at 12–14 h after dosing. Hepatocytes were cultured with [$^{3}$H]thymidine, incorporated during unscheduled DNA synthesis, which was measured autoradiographically. The administration of 3-chloro-1,2-propanediol did not elicit any increase in the net number of nuclear grains indicative of

unscheduled DNA synthesis, while the positive controls, 2-acetylaminofluorene and dimethylnitrosamine, were clearly active (Robjohns et al., 2003).

### *2.2.4 Reproductive toxicity*

#### *Rats*

To study mechanisms of antifertility in male rats, groups of 15 male Sprague-Dawley rats aged 8 weeks were given 3-chloro-1,2-propanediol daily by intragastric instillation at a dose of 0, 0.01, 0.05, 0.25, 1 or 5 mg/kg bw per day for 28 days. At the end of the treatment period, male rats were mated overnight with untreated females. Males successfully inducing pregnancy were sacrificed to assess sperm parameters, histopathology of reproductive organs, and spermatogenesis. The pregnant females were sacrificed on day 20 of gestation to evaluate pregnancy outcome. The paternal administration of 3-chloro-1,2-propanediol did not affect body or reproductive-organ weights. The highest dose produced adverse effects on male fertility and pregnancy outcome without inducing histopathological changes in the testes and epididymides of animals that successfully impregnated females. At doses of 0.25 mg/kg bw per day and greater, sperm counts were decreased. The authors applied statistical analysis to the numbers of motile sperm and reported statistically significantly reduced sperm motility at doses of 0.25 mg/kg bw and greater. However, no statistical analysis was performed on the appropriate measure, i.e. motile sperm as percentage of total sperm. There were also errors in the reported percentages of motile sperm; recalculation of percentages yielded the following values: 83%, 80%, 85%, 73%, 73%, and 72% for the control group, and the groups at 0.05, 0.25, 1, and 5 mg/kg bw per day, respectively. There was no apparent effect on the percentage of motile sperm at any dose. The copulation index was unaffected by treatment, while the fertility index was markedly reduced at the highest dose, 14.3% vs 100% in controls. At the highest dose, reductions occurred in the numbers of corpora lutea (10.5 vs 19.8 in controls), total implants (10.5 vs 15.9 in controls) and total live fetuses (1.0 vs 14.5 in controls). 3-Chloro-1,2-propanediol did not affect concentrations of testosterone or luteinizing hormone in the blood of male rats. The NOEL for effects on male fertility and on pregnancy outcome was 1 mg/kg bw per day (Kwack et al., 2004).

Groups of six rats aged 8 weeks were given intraperitoneal doses of 3-chloro-1,2-propanediol at 10 or 100 mg/kg bw, and testes and epididymides were examined 3, 6, 12, and 24 h later. In the testes of animals treated with 3-chloro-1,2-propanediol at a dose of 100 mg/kg bw, apoptosis was not detected by DNA laddering, deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labelling (TUNEL) staining, or caspase-7 protein expression. The level of $H^+$-ATPase as assessed immunohistochemically in the cauda epididymis was reduced. These results indicate that 3-chloro-1,2-propanediol had a spermatotoxic effect, which was mediated by reduced $H^+$-ATPase expression in the cauda epididymis. Reduced $H^+$-ATPase activity is predicted to produce an altered pH in the cauda epididymis, which might lead to a disruption of sperm maturation and motility (Kwack et al., 2004).

To study testicular organogenesis, groups of pregnant rats were given 3-chloro-1,2-propanediol as intragastric doses at 0, 5, 10 or 25 mg/kg bw per day from days 11 to 18 of gestation. On day 19 of gestation, the testes were removed

from fetuses for histological examination, measurement of testosterone production and expression of eight testicular genes was assessed by reverse transcription-polymerase chain reaction (RT-PCR). 3-Chloro-1,2-propanediol and its main metabolite, β-chlorolactic acid, were detected in maternal plasma and in fetal tissues at 0.5–3.0 h after dosing. The mean body-weight gains of pregnant rats treated with 3-chloro-1,2-propanediol at 10 and 25 mg/kg bw were decreased. Testes of exposed fetuses exhibited normal histology and produced testosterone at levels that were similar to controls. In addition, 3-chloro-1,2-propanediol did not alter gene expression in the fetal testes. Thus, 3-chloro-1,2-propanediol had a minimal effect on rat testicular organogenesis (El Ramy et al., 2006).

### *2.3 Observations in humans*

No new information was available.

## *3. ANALYTICAL METHODS*

### *3.1 Chemistry*

3-chloro-1,2-propanediol (CAS Registry No. 96-24-2) is a colourless, slightly oily liquid with a faint and pleasant odour. 3-Chloro-1,2-propanediol boils at 213 °C at atmospheric pressure, does not distill with water vapour, has a density of 1.3204 g/cm$^3$ (20 °C), and is readily soluble in water and ethanol. 3-Chloro-1,2-propanediol is relatively unstable in aqueous alkaline media and are decomposed to glycerol via the intermediate epoxyde glycidiol according to a reaction commonly used to reduce the levels of 3-chloro-1,2-propanediol in commercial hydrolysed vegetable protein (HVP) (Hamlet et al., 2002).

### *3.2 Description of analytical methods*

The application of mass spectrometric detection in recent years has increased sensitivity of analysis. A validated gas chromatography-mass spectrometry (GC/MS) method capable of measuring 3-chloro-1,2-propanediol in food and food ingredients at concentrations as low as 0.010 mg/kg is available and has been accepted as a first action status method by the Association of Official Analytical Chemists (AOAC) (Method No. 2000.01) (Brereton et al., 2001). Results shown that the method is satisfactory when used by analysts not specialized in the determination of 3-chloro-1,2-propanediol and that the method can also be applied to a wide range of foods and ingredients.

The Central Science Laboratory (CSL) of the UK Government Department for Environment Food and Rural Affairs (Crews et al., 2002) and the United States Food and Drug Administration (FDA) (Nyman et al., 2003a) have developed a fully in-house validation method to allow both extraction and analysis of 3-chloro-1,2-propanediol and 1,3-dichloropropanol in the same GC/MS run.

In a paper published in 2006, the Chinese authors also proposed a methodology that allows the simultaneous separation and determination of chloropropanols (1,3-dichloropropanol, 2,3-dichloropropanol, 3-monochloro-

propane-1,2-diol and 2-monochloro-1,2-propanediol) in soy sauce and other flavouring, using GC/MS in negative chemical and electron-impact ionization modes, at concentrations of around 0.6 µg/kg (Xiamin Xu et al., 2006).

## 4. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

### 4.1 Surveillance data

Acid-HVPs are widely used in seasonings and as ingredients in processed savoury food products. They are used to flavour a variety of foods, including many processed and prepared foods such as sauces, soups, snacks, gravy mixes, bouillon cubes, etc. As a result of these uses, 3-chloro-1,2-propanediol has been identified in many foods and food ingredients, most notably in acid-HVP and soy sauces (Codex Commission on Food Additives and Contaminants, 2006)

Recent studies have demonstrated that 3-chloro-1,2-propanediol may also be formed in other processed foods, particularly in meat products (salami and beef burgers), dairy products (processed cheese and cheese alternatives), a range of cereal products subjected to heat treatments such as baking, roasting or toasting (toasted biscuits, doughnuts, malt and malt extract) and some other foods. It seems evident that chloropropanediols and possibly also dichloropropanols in these foods are formed from chlorides and naturally and/or intentionally added lipids (Food Standards Agency, 2005a).

Fatty acid esters of monochloropropanols have recently been identified in a range of processed and unprocessed foods (Svejkovska et al., 2004). To date, only a limited number of analyses have been reported, but the amount of esterified 3-chloro-1,2-propanediol in many of the samples is higher than the amount of free (non-esterified) monochloropropanol in the same samples. The significance of the presence of esterified 3-chloro-1,2-propanediol in food has yet to be determined.

### 4.2 National occurrence of 3-chloro-1,2-propanediol

Data on the occurrence of 3-chloro-1,2-propanediol in food were provided by 14 countries: member states of the European Union (European Union, 2004) (Austria, Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden, the United Kingdom (UK) (Food Standards Agency, 2005b), Australia (FSANZ, 2003), Hong Kong Special Administrative Region of China (SAR) (Food and Environmental Hygiene Department, 2005), Japan (Ministry of Agriculture, Forestry and Fisheries of Japan , 2005 and 2006), Thailand (Ministry of Agriculture and Cooperatives of Thailand, 2006) and by the International Hydrolyzed Protein Council (IPHC; International Hydrolyzed Protein Council, 2005). Data on soy sauces and related products were also available for the United States of America (USA) from the literature (Nyman et al., 2003b).

Since the fifty-seventh meeting of the Committee in 2002 (Annex 1, reference 154) at which only soy sauces and related products were evaluated, more occurrence data have been collected on 3-chloro-1,2-propanediol in heat processed foods like bread and bakery waves, malt, batter, meat and fish products.

Table 1 summarizes the distribution-weighted concentration of 3-chloro-1,2-propanediol in soy sauce and soy sauce-based products, in other foods and in food ingredients from various countries, between 2001 to 2006.

### *4.2.1 Soy sauce and soy sauce-based products*

#### *(a) Australia*

Australia submitted occurrence data for 39 individual samples of different soy and oyster sauces based on food surveys conducted in 2001 and 2002 (FSANZ, 2003). Selected soy and oyster sauces and a range of other foods available at Australian retail outlets were sampled. The method used for the quantitative analysis of 3-chloro-1,2-propanediol was based on AOAC Method No. 2000.01. The limit of quantification (LOQ) for 3-chloro-1,2-propanediol was 0.01 mg/kg. The majority of soy and oyster sauces did not contain 3-chloro-1,2-propanediol at detectable concentrations. A small number of samples contained high concentrations of 3-chloro-1,2-propanediol.

- The average was calculated by assigning a value of half the reporting limits to the 44% of samples for which the reported value was less than the limit.
- The highest average concentration was reported for soy seasoning sauce product, with a maximum at 74.3 mg/kg. For the other sauces, the reported average concentration is 0.16 mg/kg.

#### *(b) Hong Kong SAR*

Hong Kong submitted occurrence data for 3-chloro-1,2-propanediol in aggregate samples of different soy sauce and sauce-based products, using the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) reporting format. Sampling was performed in 2003 and included the analytical results for 84 samples. Based on the very low level of the limit of detection (LOD) and LOQ, respectively 2.5 and 7.5 µg/kg, analysis was carried out using GC/MS. No indicator of analytical quality assurance was described in the submitted data (Food and Environmental Hygiene Department, 2005).

- The average was calculated by assigning a value of half the reporting limit to the 84% of samples for which the reported value was less than the limit
- A higher average concentration of 3-chloro-1,2-propanediol is reported for concentrated sauce (105 µg/kg) and concentrated soup (14.6 µg/kg). Other averages reported for sauce-based products are between about 2.5 and 5.0 µg/kg.

#### *(c) European Union*

Individual data on 3-chloro-1,2-propanediol were submitted for 10 countries of the European community—Austria, Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden and the UK—from the reports on Tasks for Scientific Cooperation on levels of 3-chloro-1,2-propanediol and related substances in foodstuffs (European Union, 2004). The Joint Research Centre of the European

Commission assisted in the task by providing advice on analytical methodology. Participants agreed that only occurrence data obtained after 1997 until the year before the publication of the report in 2004 should be submitted as there was concern about the robustness of data acquired prior to that date. Some occurrence data provided by participants were from targeted sampling of products suspected to contain chloropropanols at high concentrations.

Results of analysis of 3-chloro-1,2-propanediol in over 2035 samples of soy sauce and soy sauce-based products were provided by all 10 member states of the European Union : Austria ($n$ = 316), Denmark ($n$ = 43), Finland ($n$ = 163), France ($n$ = 73), Germany ($n$ = 692), Ireland ($n$ = 178), the Netherlands ($n$ = 273), Norway ($n$ = 51), Sweden ($n$ = 76) and UK ($n$ = 170). The LOQ for soy sauce and soy sauce-based products was mainly between 0.006 mg/kg and 1 mg/kg (98%). A few results (less than 2%) were provided with a LOQ of 2.5 or 5 mg/kg.

- The average was calculated by assigning a value of half the reporting limit to the 65% of samples for which the reported value was less than the limit.
- The weighted average for the group was reported to be 9.16 mg/kg. The highest average concentration of 3-chloro-1,2-propanediol was reported for seasoning sauce (40.9 mg/kg), mushroom soy sauce and light soy sauce (15.5 mg/kg), soy sauce (7.8 mg/kg), dark soy sauce (5.3 mg/kg) and teriyaki sauce (3.4 mg/kg). Others averages reported for sauce-based products were less than 1 mg/kg. The distribution of contamination in products differs according to the country of origin. Products from China (not including Hong Kong), Thailand and Viet Nam had the highest mean concentrations of 3-chloro-1,2-propanediol, with respectively 51%, 61% and 95% of samples with quantifiable levels containing 3-chloro-1,2-propanediol at greater than 10 mg/kg.

*(d) Japan*

Japan submitted occurrence data on the levels of 3-chloro-1,2-propanediol in aggregate and individual samples of acid-HVP containing soy sauce, using the GEMS/Food reporting format. Sampling was performed in 2005–2006 and included analysis results for 3-chloro-1,2-propanediol in 200 samples of soy sauce made with acid-HVP and in 104 samples of soy sauce made by traditional fermentation. The method used for analysis of 3-chloro-1,2-propanediol had been validated in-house using GC/MS. The analytical method has been published published in a paper cited in MAFF (2005 and 2006).[2] The LOD and LOQ were 0.002 mg/kg and 0.004 mg/kg, respectively.

Eighty-nine percent of analytical results from samples of soy sauce made by traditional fermentation are below the LOQ versus (vs) 0.6% of results for samples of soy sauces made with acid-HVP. The highest concentration reported was 33 mg/kg for soy sauces made with acid-HVP and 0.008 mg/kg for soy sauce made by traditional fermentation. The average concentration of 3-chloro-1,2-propanediol was 1.8 mg/kg for soy sauces made with acid-HVP and 0.003 mg/kg for soy sauce made by traditional fermentation.

[2] *Journal of Food Hygienic Society of Japan* 1995, 36(3):360–364.

*(e) International Hydrolyzed Protein Council (IHPC)*

Data based on the annual surveys in 2004 were submitted by IHPC for consideration by the Committee at its present meeting. In all, 26 commercial samples of soy sauce made with acid-HVP were analysed for 3-chloro-1,2-propanediol content by at least two different laboratories. No information on the sampling protocol or analytical methods used was provided, nor were indicators of analytical quality assurance described in the report submitted to JECFA. Differences in the analytical sensitivites between laboratories were between 0.01 and 0.065 mg/kg.

Eighty-nine percent of analytical results from soy sauce were below the LOQ. The highest concentration reported was 4 mg/kg. The average concentration of 3-chloro-1,2-propanediol was 0.18 mg/kg.

*(f) Thailand*

- Thailand submitted occurrence data on the concentrations of 3-chloro-1,2-propanediol in seasoning soy sauces from aggregate samples (212 samples, of which 190 were collected from household surveys and 23 from market surveys) and other foods (56 samples, of which 24 were collected from household surveys and 32 from market surveys) (Ministry of Agriculture and Cooperatives of Thailand, 2006). The method used for quantitative analysis of 3-chloro-1,2-propanediol was based on AOAC Method No. 2000.01. The LOD and LOQ for 3-chloro-1,2-propanediol were 0.001 mg/kg and 0.010 mg/kg respectively. No information was available on the number of samples in which 3-chloro-1,2-propanediol was present at less than the LOQ, nor on how censored data had been treated in the calculation of the average content of 3-chloro-1,2-propanediol in different seasoning products.
- 3-chloro-1,2-propanediol was quantified only in seasoning products from household surveys. The average concentration of 3-chloro-1,2-propanediol in seasoning sauce (acid-HVP) (30 samples) was reported to be 0.454 mg/kg and the highest concentration was 5.12 mg/kg. The average concentration of 3-chloro-1,2-propanediol in fermented soya bean (35 samples) was reported to be 0.087 mg/kg and the highest concentration was 2.64 mg/kg. The average content of 3-chloro-1,2-propanediol in soup powder (seven samples) was reported to be 0.032 mg/kg and the highest concentration was 0.085 mg/kg. The average content of 3-chloro-1,2-propanediol in local dark sauce (38 samples) was reported to be 0.010 mg/kg and the highest concentration was 0.043 mg/kg.
- Average concentrations reported for other seasoning products and other foods were less than 0.01 mg/kg.
- The weighted average for the soy sauce group has been calculated to be 0.09 mg/kg.

*(g) USA*

Occurrence data for individual samples of 55 different soy sauce and sauce-based products purchased from retail outlets in metropolitan areas in 2002 had been published by the FDA (Nyman et al., 2003a). The method used for quantitative analysis of 3-chloro-1,2-propanediol was based on AOAC Method No. 2000.01. An

LOQ of 0.025 mg/kg was established for 3-chloro-1,2-propanediol. Tha authors concluded that all samples that contained 3-chloro-1,2-propanediol at > 1 ppm (I mg/kg) were manufactured in Asia, indicating that at the date of the publication some Asian manufacturers had not made the necessary processing changes to control the formation of chloropropanols in acid-HVPs.

A small number of samples contained 3-chloro-1,2-propanediol at high concentrations (> 6 mg/kg). The highest concentration was reported for a soy sauce, with a maximum concentration of 3-chloro-1,2-propanediol of about 876 mg/kg.

- The average was calculated by assigning a value of half the reporting limit to the 40% of samples for which the reported value was less than the limit
- The average concentration reported for soy sauce and sauce-based products was 44.1 mg/kg.

*4.2.2 Food ingredients*

*(a) European Union*

Results of the analysis of 3-chloro-1,2-propanediol in more than 295 samples of ingredients were available in the European Union report (European Union, 2004). 3-Chloro-1,2-propanediol has been investigated in the following food ingredients: breadcrumbs (6 samples), caramel (5 samples), gelatin (12 samples), HVPs (146 samples), meat extract (16 samples), malts (63 samples), modified starches (9 samples), yeast extract (12 samples) and seasonings (15 samples). Of these samples, 34% contained 3-chloro-1,2-propanediol at a concentration greater than the LOQ set at 0.010–0.020 mg/kg in the following ingredients:

- *HVPs:* results for 146 samples were provided. 3-Chloro-1,2-propanediol was quantified in 39% of samples. The weighted average for this food category was reported to be 0.171 mg/kg.
- *Meat extract:* results for 16 samples were provided. 3-Chloro-1,2-propanediol was quantified in 31% of samples. The weighted average for this food category was reported to be 0.064 mg/kg.
- *Malts:* results for 63 samples were provided. 3-Chloro-1,2-propanediol was quantified in 49% of samples. The weighted average for this food category was reported to be 0.096 mg/kg.
- *Modified starches:* results for nine samples were provided. 3-Chloro-1,2-propanediol was quantified in 22% of samples. The weighted average for this food category was reported to be 0.059 mg/kg.
- *Seasonings:* results for 15 samples were provided. 3-Chloro-1,2-propanediol was quantified in 27% of samples. The weighted average for this food category was reported to be 0.016 mg/kg.

*(b) Japan*

Japan submitted the results of occurrence data in aggregate and individual samples on the concentrations of 3-chloro-1,2-propanediol in acid-HVPs, using the WHO GEMS/Food reporting format. Sampling was performed in 2005–2006 and included results for 3-chloro-1,2-propanediol in acid-HVPs in 148 samples from

well-controlled production processes and 49 from other production processes The published method used for the analysis of 3-chloro-1,2-propanediol had been validated in-house using GC/MS (cited in MAFF, 2005, 2006).[3] The LOD and LOQ were 0.002 mg/kg and 0.004 mg/kg, respectively.

- All the results for 3-chloro-1,2-propanediol in acid-HVPs from well-controlled production processes or from other production processes were less than the LOQ. The highest concentration reported was 44 mg/kg for acid-HVPs from other production processes vs 0.14 mg/kg for acid-HVPs from well-controlled production processes. The average concentration of 3-chloro-1,2-propanediol was 6.5 mg/kg for acid-HVP from other production processes vs 0.047 mg/kg for acid-HVPs from well-controlled production processes.

*(c) IHPC*

Data from the last three annual surveys 2000–2002 were submitted by IHPC for consideration by the Committee at its present meeting. In all, 86 commercial samples of acid-HVP had been analysed for 3-chloro-1,2-propanediol content by at least two different laboratories (21 commercial samples in 2000, 38 in 2001 and 22 in 2002). No information on the preparation of the samples or analytical methods used by the laboratories was presented, and there was no indication that analytical quality assurance had been performed, according to the report submitted to the Committee. Differences in analytical sensitivities observed between laboratories were expressed on a dry-substance basis (in 2000 the LOD ranged from 0.01 to 0.2 mg/kg; in 2001, from 0.007 to 0.025 mg/kg; in 2002, from 0.003 to 0.1 mg/kg).

- 9% of analytical results were less than the LOQ. The highest concentration reported was 2.54 mg/kg. The average concentration of 3-chloro-1,2-propanediol was calculated to be 0.103 mg/kg.

### *4.2.3 Other products*

Few countries have reported data on 3-chloro-1,2-propanediol for products other than soy sauce and sauce-based products. Australia and the European Union submitted occurrence data on foods and food groups likely to contain 3-chloro-1,2-propanediol as a result of processing or storage conditions. Analysis of several products included in the diet, such as cereals, meat and meat products, dairy products, fish and seafood products, was reported.

*(a) Australia*

Australia submitted occurrence data for 204 individual samples of different foods based on food surveys conducted in 2001 and 2002 (FSANZ, 2003). Selected foods and a range of foods available at Australian retail outlets were sampled. The method used for quantitative analysis of 3-chloro-1,2-propanediol was similar to that used for soy and oyster sauces and was based on AOAC Method No. 2000.01. The LOD was 0.005 mg/kg and the LOQ was 0.01 mg/kg. Analyses have been done on the following food categories: meat and meat products (99 samples), fish and fish products (29 samples), chicken eggs (8 samples), cereals (37 samples), infant

food (3 samples), infant formulas (3 samples), cheese, cheddar and processed (12 samples), starchy vegetables (3 samples), roasted coffee and cocoa paste (6 samples), vegetable oils and fat (4 samples).

Only 9% of the samples were found to contain 3-chloro-1,2-propanediol in quantities greater than the LOQ.

- *Cereals:* none of the 37 samples were found to contain 3-chloro-1,2-propanediol at a quantifiable level. The weighted average concentration for this food category was reported to be 0.003 mg/kg.
- *Fish and fish products:* 3-chloro-1,2-propanediol was quantified in 17% of samples, and only in fish portion crumbed-over baked (0.037 mg/kg). The weighted average concentration for this food category was reported to be 0.009mg/kg.
- *Meat and meat products,* including poultry and game: 3-chloro-1,2-propanediol was quantified in 26% of samples (mainly in bacon dried, sausage fried, raw or cooked and leg ham) at concentrations between 0.006 and 0.069 mg/kg. The weighted average concentration for this food category was reported to be 0.007 mg/kg.

*(b) European Union*

Results of the analysis of 3-chloro-1,2-propanediol in more than 1637 samples of products other than soy sauce were available in the European Union report (European Union, 2004): Denmark ($n$ = 19), Finland ($n$ = 345), France ($n$ = 146), Germany ($n$ = 71), Ireland ($n$ = 25), Norway ($n$ = 54), Sweden ($n$ = 151) and UK ($n$ = 547). Of the samples, 35% contained 3-chloro-1,2-propanediol at a concentration greater than the LOQ, mainly set at 0.010 mg/kg. The overall weighted average concentration of 3-chloro-1,2-propanediol in all 'others' products analysed by member states was reported to be 0.013 mg/kg.

- *Dairy*: results were submitted for 137 samples, mainly from the UK. 3-Chloro-1,2-propanediol was quantified in 86% of samples, only in cheese, processed cheese and cheese analogue (68 out of 79 samples), at an average concentration of about 0.008–0.012 mg/kg. The weighted average concentration for this food category was reported to be 0.007 mg/kg.
- *Fat and oils, and fat emulsions*: results were provided for 34 samples. 3-Chloro-1,2-propanediol was quantified in 41% of samples, mainly in mixed and/or flavoured products based on fat emulsion product (14 out of 34 samples) at 0.184 mg/kg. The weighted average concentration for this food category was reported to be 0.09 mg/kg.
- *Fruit and vegetables*: results were provided for 37 samples. 3-Chloro-1,2-propanediol was quantified in 62% of samples, mainly in processed vegetables, products made from nuts and seeds (23 out of 37 samples) at 0.068 mg/kg. The weighted average concentration for this food category was reported to be 0.061 mg/kg.
- *Confectionery*: results were provided for 39 samples. 3-Chloro-1,2- propanediol was quantified in 8% of samples, in confectionery products and in sugar-based confectionery (3 out of 39 samples) at 0.007 mg/kg. The weighted average concentration for this food category was reported to be 0.006 mg/kg.

- *Cereals and cereals products*: results were provided for 203 samples. 3-Chloro-1,2-propanediol was quantified in 26% of samples, mainly in products containing flour and starch at 0.010 mg/kg (7 out of 11 samples) and pasta and noodles (94 out of 143 samples) at 0.032 mg/kg. The weighted average concentration for this food category was reported to be 0.024 mg/kg.
- *Bakery wares*: results were provided for 337 samples. 3-Chloro-1,2- propanediol was quantified in more than half of the samples (51%), mainly in crackers (0.030 mg/kg), cake and cookies (0.029 mg/kg), other ordinary bakery products (0.027 mg/kg), bread and rolls and other fine bakery products (0.016 mg/kg), and biscuits (0.013 mg/kg). The weighted average concentration for this food category was reported to be 0.020 mg/kg.
- *Meat and meat products, including poultry and game*: results were provided for 153 samples. 3-Chloro-1,2-propanediol was quantified in 36% of samples, mainly in sausage casings (13.9 mg/kg), processed comminuted meat, poultry and game products non-heat-treated (0.062 mg/kg), processed meat, poultry and game products comminuted or in whole pieces heat-treated or non-heat- treated or frozen (0.012 mg/kg). The weighted average concentration for this food category was reported to be 1.47 mg/kg.
- *Fish and fish products including molluscs, crustaceans and echinoderms (MCE):* results were provided for 60 samples. 3-Chloro-1,2-propanediol was quantified in 25% of samples, mainly in fully preserved fish and fish products, including MCE hot smoked (0.041 mg/kg) and canned (0.017 mg/kg), and in processed fish and fish products, including MCE cooked/or fried (0.010 mg/kg). The weighted average concentration for this food category was reported to be 0.013 mg/kg.
- *Salts, spices, soup sauces, salads, protein products*: results were provided for 454 samples. 3-Chloro-1,2-propanediol was quantified in 45% of samples, mainly in non-emulsified sauce (e.g ketchup, cheese sauce, cream sauce, brown gravy where 2 out of 59 samples were highly contaminated, containing 3-chloro-1,2-propanediol at a concentration of 2.82 mg/kg), in herbs, spices, seasoning and condiments where 75% of samples contained 3-chloro-1,2-propanediol at quantified levels (166 out of 221 samples, 0.259 mg/kg), in protein products (1 out of 7 samples, 0.210 mg/kg), in sauces and like products (0.048 mg/kg), in mixes for sauces and gravies (0.033 mg/kg) and in mixes for soups and broths (0.011 mg/kg). The weighted average concentration for this food category was reported to be 0.286 mg/kg.
- *Foodstuffs intended for particular nutritional uses:* very few samples were analysed but all of them four were quantified. The weighted average concentration for this food category was reported to be 0.030 mg/kg.
- *Beverages excluding dairy products*: results were provided for 131 samples. 3-Chloro-1,2-propanediol was quantified in 7% of samples (9 out of 131), mainly beer and malt beverages, being found at a low concentration (0.006 mg/kg). The weighted average concentration for this food category was reported to be 0.007 mg/kg.

- *Ready-to-eat savouries*: results were provided for 23 samples. 3-Chloro-1,2-propanediol was quantified in 34% of samples, mainly in snacks—potato, cereal, flour or starch-based foods (0.013 mg/kg). The weighted average concentration for this food category was reported to be 0.010 mg/kg.
- *Composite foods*: results were provided for 24 samples. 3-Chloro-1,2-propanediol was quantified in 21% of samples. The weighted average concentration for this food category was reported to be 0.013 mg/kg.

*(c) Thailand*

Occurrence data on the concentrations of 3-chloro-1,2-propanediol found in aggregate samples of other foods (56 samples: 24 collected by household survey and 32 by market survey) (Ministry of Agriculture and Cooperatives of Thailand, 2006) were available. 3-Chloro-1,2-propanediol has only been found at quantified levels in snack food products (5 samples) from household-survey sampling; the average concentration 3-chloro-1,2-propanediol was < 0.010 mg/kg and the highest value was 0.028 mg/kg.

*Summary of occurrence data for 3-chloro-1,2-propanediol*

Data on the occurrence of 3-chloro-1,2-propanediol in food were provided by 14 countries and by IHPC. Data on 3-chloro-1,2-propanediol in soy sauces and related products for an additional country were available from the literature.

The average concentration of 3-chloro-1,2-propanediol present in soy sauce and soy sauce-related products was much higher (8 mg/kg; range, 0.01 to 44.1 mg/kg) than in any other food or food ingredient (< 0.3 mg/kg). Data from Japan show that soy sauce produced by traditional fermentation contains insignificant average amounts of 3-chloro-1,2-propanediol (0.003 mg/kg) compared with soy sauce made with acid-HVP (1.8 mg/kg).

## 5. ESTIMATED DIETARY INTAKE

### 5.1 National assessments of intake from diet

National dietary intake data for 3-chloro-1,2-propanediol were provided for 10 countries (Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden, UK, Thailand). These national intakes were calculated by linking data on individual consumption and body weight from national food consumption surveys with mean occurrence data obtained from food contamination surveys.

Consistent with their national food consumption data, all countries except Germany calculated estimates for the adult population and children. Mean occurrence data used in the calculation are those reported in section 4 of this monograph, in which all values classified as being less than the LOQ were assumed to contain 3-chloro-1,2-propanediol at a concentration equal to LOQ/2, except for Thailand where no information was available concerning the treatment of values less than the LOQ. Intake estimates were calculated on a per kilogram of body weight basis. Estimates reported by each country were described according to the

following two variables of intake distribution for mean and high-percentile dietary exposures: average estimates (average food consumption combined with mean occurrence data for food consumed); and the 95th percentile of the intake distribution (P95).

Table 2 summarizes estimates of national dietary intakes of 3-chloro-1,2-propanediol from various food sources, including soy sauce and soy sauce-based products.

*(a) Australia*

Australia submitted the results of an assessment of dietary exposure to chloropropanols (FSANZ, 2003). Intake calculations were made in a deterministic way using the dietary modelling computer program (DIAMOND), multiply mean concentration of chloropropanols in a wide range of foods, including soy and soy sauces, consumed by each individual reported in the 1995 National Nutrition Survey based on a 24 h-recall methodology. To provide a more refined estimate of dietary exposure, concentrations reported to be less than the reporting limits (LOR = 78%) were assigned by a concentration equal to the LOR.

Dietary exposure assessment was conducted using food groupings based on the Codex system of classification for food additives. Foods are classified according to major food types as raw and processed foods, and analytical concentrations fit easily to these to these food groupings. Calculations are made by assigning values for 3-chloro-1,2-propanediol concentrations to food groups for food eaten “as is”, as well as as used as ingredients in mixed foods. Where a single food from a food group was analysed, the concentration of 3-chloro-1,2- propanediol reported was assigned to the whole group, assuming that like foods would contain 3-chloro-1,2-propanediol at similar concentrations. This is likely to result in an over-estimation of dietary exposures, but assumes a worst-case scenario.

The intake estimates for the whole population aged more than 2 years ranged from an average of 0.20 µg/kg bw per day to 0.65 µg/kg bw per day for consumer sat the 95th percentile. Children aged from 2 to 12 years had exposures ranging from an average of 0.17 µg/kg bw per day to 0.18 µg/kg bw per day for consumers at the 95th percentile. Children aged from 13 to 19 years had exposures ranging from an average of 0.19 µg/kg bw per day to 0.65 µg/kg bw per day for the consumer at the 95th percentile. Adults aged 20 years and greater had exposures ranging from an average of 0.21 µg/kg bw per day to 0.75 µg/kg bw per day for consumers at the 95th percentile.

Major contributors to total exposure were soy and oyster sauce, ranging from 86% for children to 94% for adults

Where estimated exposure was expressed as a percentage of the PMTDI set by the Committee in 2001 (2 µg/kg/bw per day), the estimated dietary exposure to 3-chloro-1,2-propanediol from a wide range of foods, including soy sauce and related products, was far less than the PMTDI for all groups reported, both for mean consumption (< 10%) and for consumers at the 95th percentile (< 40%). Intakes ranged from 8.5% to 9% for children aged 2–12 years, from 9.5% to 33%, for children aged 13–19 years, and from 10% to 38% for adults aged 20 years and greater.

***Table 1. Summary of the distribution-weighted concentration of 3-chloro-1,2-propanediol in soy sauce and soy sauce-based products, in other foods and in food ingredients from various countries, 2001 to 2006***

| Product | Country and date | LOQ (mg/kg) | *N* | *N* < LOQ | Mean[a] (mg/kg) | Maximum (mg/kg) |
|---|---|---|---|---|---|---|
| Soy sauce and soy sauce-based products | EC (Scoop task 3.2.9, 2004) | 0.006–5.000 | 2035 | 1321 | 9.16 | 1779 |
| | Hong Kong SAR, 2003 | 0.0075 | 84 | 71 | 0.01 | 0.2 |
| | USA, 2003 | 0.012 | 55 | 8 | 44.1 | 876 |
| | Australia, 2001–2002 | 0.01 | 39 | 17 | 15.4 | 148 |
| | Japan, 2005–2006 | 0.002 | 200 | 1 | 1.8 | 33 |
| | Thailand, 2004–2005 | 0.01 | 190 | NA | 0.09 | 5 |
| | IHPC, 2004 | 0.01–0.1 | 26 | 15 | 0.18 | 4 |
| | All data | 0.006–5.000 | 2629 | 1433 | 8.39 | 1779 |
| Dairy products (cheeses) | EU, 1997–2002 | 0.01–0.02 | 137 | 126 | 0.007 | 0.095 |
| | Australia, 2001–2002 | 0.005 | 12 | 12 | 0.0025 | 0.0025 |
| | All | 0.005–0.010 | 149 | 138 | 0.007 | 0.095 |
| Fat and oils, and fat emulsions | EU, 1997–2002 | 0.01 | 34 | 20 | 0.09 | 1.5 |
| | Australia, 2001–2002 | 0.005 | 4 | 4 | 0.0025 | 0.0025 |
| | All | 0.005–0.010 | 38 | 24 | 0.081 | 1.5 |
| Nuts and seeds, processed vegetables | EU, 1997–2002 | 0.01 | 37 | 14 | 0.061 | 0.69 |

***Table 1.*** *(contd)*

| Product | Country and date | LOQ (mg/kg) | *N* | *N* < LOQ | Mean[a] (mg/kg) | Maximum (mg/kg) |
|---|---|---|---|---|---|---|
| Cereals and cereal products (flours and starch, pasta and noodles and bakery wares) | EU, 1997–2002 | 0.01–0.02 | 540 | 314 | 0.024 | 0.945 |
| | Australia, 2001–2002 | 0.005 | 37 | 34 | 0.0028 | 0.007 |
| | All | 0.005–0.020 | 577 | 348 | 0.023 | 0.945 |
| Meat and meat products | EU, 1997–2003 | 0.01 | 152 | 97 | 0.041 | 0.41 |
| | Australia, 2001–2002 | 0.005 | 99 | 73 | 0.006 | 0.069 |
| | All | 0.005–0.010 | 251 | 170 | 0.027 | 0.41 |
| Fish products | EU, 1997–2002 | 0.01 | 60 | 45 | 0.013 | 0.191 |
| | Australia, 2001–2002 | 0.005 | 29 | 23 | 0.01 | 0.083 |
| | All | 0.005–0.010 | 89 | 68 | 0.012 | 0.191 |
| Salts, spices, soup sauces, salads, protein products | EU, 1997–2002 | 0.01–2.5 | 454 | 248 | 0.286 | 50.7 |
| Foodstuffs intended for particular nutritional uses | EU, 1997–2002 | 0.01–0.08 | 131 | 122 | 0.007 | 0.02 |
| | Australia, 2001–2002 | 0.005 | 6 | 6 | 0.0025 | 0.0025 |
| | All | 0.005–0.080 | 137 | 128 | 0.007 | 0.02 |
| Ready-to-eat savouries | EU, 1997–2002 | 0.006–0.020 | 23 | 15 | 0.01 | 0.041 |
| Composite food | EU, 1997–2002 | 0.01 | 24 | 19 | 0.013 | 0.113 |

***Table 1.*** *(contd)*

| Product | | Country and date | LOQ (mg/kg) | *N* | *N* < LOQ | Mean[a] (mg/kg) | Maximum (mg/kg) |
|---|---|---|---|---|---|---|---|
| Eggs | | Australia, 2001–2002 | 0.005 | 8 | 8 | 0.0025 | 0.0025 |
| Root and tubers (potato crisps) | | Australia, 2001–2002 | 0.005 | 3 | 3 | 0.0025 | 0.0025 |
| Beverages: | Coffee roasted | EU, 1997–2002 | 0.01 | 20 | 20 | 0.005 | 0.005 |
| | | Australia, 2001–2002 | 0.005 | 3 | 3 | 0.0025 | 0.0025 |
| | | All | 0.005–0.010 | 23 | 23 | 0.005 | 0.005 |
| | Cocoa paste and chocolate products | Australia, 2001–2003 | 0.005 | 3 | 1 | 0.014 | 0.03 |
| | | EU, 1997–2002 | 0.01 | 12 | 12 | 0.005 | 0.005 |
| | | All | 0.005–0.080 | 15 | 13 | 0.007 | 0.005 |
| | Beer and malts beverages | EU, 1997–2002 | 0.01–0.08 | 100 | 92 | 0.008 | 0.017 |
| | | All | 0.005–0.080 | 138 | 128 | 0.008 | 0.03 |
| Confectionery, sugar-based (chewing gum, candy, nougats…) | | EU, 1997–2002 | 0.01 | 27 | 24 | 0.007 | 0.023 |
| Food ingredients | Acid HVPs | IHPC, 2000–2002 | 0.01–0.1 | 81 | 7 | 0.103 | 2.5 |
| | | EU, 1997–2002 | 0.01–1.15 | 146 | 89 | 0.171 | 1.84 |
| | | Japan, 2005–2006 | 0.004 | 148 | 0 | 0.047 | 0.14 |
| | | All | 0.01–1.15 | 375 | 96 | 0.107 | 2.5 |
| | Meat extracts | EU, 1997–2002 | 0.01–0.02 | 16 | 11 | 0.064 | 0.55 |

***Table 1.*** *(contd)*

| Product | Country and date | LOQ (mg/kg) | *N* | *N* < LOQ | Mean[a] (mg/kg) | Maximum (mg/kg) |
|---|---|---|---|---|---|---|
| Malts | EU, 1997–2002 | 0.01 | 63 | 32 | 0.096 | 0.85 |
| Modified starches | EU, 1997–2002 | 0.01 | 9 | 7 | 0.059 | 0.49 |
| Seasonings | EU, 1997–2002 | 0.02 | 15 | 11 | 0.016 | 0.06 |
| Others | EU, 1997–2002 | 0.01 | 11 | 9 | 0.008 | 0.025 |
| | All | 0.01–1.15 | 489 | 262 | 0.099 | 2.5 |

EU: European Union; IHPC: International Hydrolyzed Protein Council; LOQ: limit of quantification; NA: not available; SAR: Special Administrative Region of China.

[a] Data below the LOD or LOQ have been assumed to be half of those limits (except for Thailand in soy sauce, where no information was available) and the mean is weighted according to the number of samples per country

*(b) European Union*

Member States provided their own national estimates of dietary intake of 3- chloro-1,2-propanediol in reports on Tasks for Scientific Cooperation on levels of 3-chloro-1,2-propanediol and related substances in foodstuffs (European Union, 2004). As adequate national data were available, participants used their own data to calculate exposure to 3-chloro-1,2-propanediol from soy sauce. However, as adequate national data for other foods were not available, participants used pooled occurrence data to calculate dietary exposure to 3-chloro-1,2-propanediol from all other foods. Eight participants from the European community provided intake data: Denmark, Finland, France, Germany, Ireland, the Netherlands, Sweden and UK. Estimated intakes were described only for 3-chloro-1,2-propanediol in the Scoop task.

The intake estimates for the adult population aged greater than 18 years ranged from an average of 0.16 µg/kg bw per day to 1.38 µg/kg bw per day for consumers at the 95th percentile. Depending on country, children aged 2–14 years had exposures ranging from an average of 0.15 µg/kg bw per day to 2.3 µg/kg bw per day for consumers at the 95th percentile.

Where estimated exposure was expressed as a percentage of the PMTDI, results ranged from 8% to 69% for the adult population, and from 7.5% to 115% for children.

The highest contribution for high-level consumers was from soy sauces and soy sauce-based products, ranging from 5% to 86% of the PMTDI for children and from 1% to 54% of the PMTDI for adults.

*(c) Thailand*

Thailand submitted the results of dietary intake assessments carried out in 2004–2005 (Ministry of Agriculture and Cooperatives of Thailand, 2006). Intake calculations were obtained in a deterministic way by combining mean concentrations of 3-chloro-1,2-propanediol in a wide range of foods, including seasonings, by consumption data collected from different regions of Thailand using a household-seasoning-product disappearance method and a food-frequency method for the consumption of other foods. 1945 individuals in 512 households were surveyed. Per-capita intake per day was derived from the quantity of food available or consumed by the householder, divided by the number of family members. Daily dietary intake was converted to µg/kg bw per day using Thai reference body weights of 57 kg for adults.

The intake estimates for adults aged more than 16 years ranged from an average of 0.02 µg/kg bw per day to 0.06 µg/kg bw per day for consumers at the 95th percentile.

Where estimated exposure was expressed as a percentage of the PMTDI, results for the adult population were less than 5% of the PMTDI set by the Committee in 2001 (2 µg/kg bw per day).

The major contributor to total exposure was seasonings, sauce (acid-HVP) at 74%.

*(d) UK*

The UK submitted dietary intake assessments carried out in 2005 (Food Standards Agency, 2005b). Intake calculations were obtained using mean concentrations of 3-chloro-1,2-propanediol found in a wide range of foods, including compilation of occurrence data from the European Union task report and additional data obtained from Germany, Spain and Finland from activities to monitor 3-chloro-1,2-propanediol. Occurrence data for the products in which HVP may be found and previously not reported in the European Union report as potential sources of exposure, such as casseroles, pizzas and snacks, were therefore identified and incorporated using a recipe database. Consumption data for all foods including the percentage of ingredients in compound foods were derived from the UK National Diet and Nutrition Surveys (NDNS) realized in 1992–1993 for children and from the UK NDNS realized in 2000–2001 for adults aged 19–64 years. Exposure was estimated for consumers only using the UK FSA intake computer program. It was clear in the report that many of these estimations were done with occurrence data that preceded the introduction of European limits for 3-chloro-1,2-propanediol in liquid condiments.

The intake estimates for adults ranged from 0.40 µg/kg bw per day in average to 1.30 µg/kg bw per day for consumers at the 95th percentile. Children aged from 1.5 to 4.5 years had exposures ranging from an average of 0.70 µg/kg bw per day to 2.30 µg/kg bw per day for consumers at the 95th percentile.

Where estimated exposure was expressed as a percentage of the PMTDI, results for adults ranged from 20% to 65% and from 35% to 115% for young children.

The greatest contribution for consumers with a high intake was made by instant noodles for adult s and dark soy sauce for young children.

*Consumption of soy sauce and soy sauce-based products by consumers only from various countries and estimated dietary exposure to 3-chloro-1,2-propanediol*

The percentage of consumers of soy sauce and soy sauce-based products was reported to range from 1.4% to 8% in the adult population and from 1% to 8.7% in children population. Consumption, according to country, ranged from 1 to 30 g/day for the adult population and from 0.6 to 16 g/day in children.

Combining the average levels of contamination for soy sauce produced by traditional fermentation or using acid-HVP (from the Japanese submission) with a figure for daily consumption of soy sauce of 30 g (per-capita consumption for Japan and 95th percentile of consumption from Australia) resulted in dietary exposures of 0.0015 and 0.90 µg/kg bw per day, respectively, assuming a body weight of 60 kg.

*Summary of national intake estimates*

National dietary intake data for 3-chloro-1,2-propanediol were provided for 10 countries (Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden, Thailand, UK). These national intakes were calculated by linking data on individual consumption and body weight from national food consumption surveys with mean occurrence data obtained from food contamination surveys.

***Table 2. Estimated dietary intake of 3-chloro-1,2-propanediol from various food sources, including soy sauce and soy sauce-based products***

| Country | Population group | Intake (µg/kg bw per day) | |
|---|---|---|---|
| | | Mean | P95[b] |
| Australia | All (≥ 2 years) | 0.20 | 0.65 |
| | 2–12 years | 0.17 | 0.18 |
| | 13–19 years | 0.19 | 0.65 |
| | ≥ 20 years | 0.21 | 0.75 |
| Denmark | Adult | 0.21 | - |
| Finland | Adult | 0.23 | - |
| France | ≥ 18 years | 0.22 | - |
| | 3–14 years | 0.30 | - |
| Germany[a] | Adult | 0.17 | 0.72 |
| | 4 years | 0.48 | 1.70 |
| | 14 years | 0.15 | 0.53 |
| Ireland | Adult | 0.36 | 0.68 |
| Netherlands | Adult | 0.30 | 1.38 |
| | Children | 0.34 | 1.69 |
| Sweden | Adult | 0.16 | 0.48 |
| Thailand | Whole population | 0.02 | 0.06 |
| United Kingdom[a] | ≥ 18 years | 0.40 | 1.3 |
| | 1.5–4.5 years | 0.70 | 2.3 |

[a] Consumers only; [b]95th percentile: only 5% of consumers had exposures above this level.

The Committee concluded that, based on national estimates from a wide range of foods including soy sauce and soy-sauce related products, an intake of 3-chloro-1,2-propanediol of 0.7 µg/kg bw per day could be taken to represent the average for the general population, and an intake of 2.3 µg/kg bw per day could be taken to represent consumers with a high intake. In these estimates for average to high intake, young children are included.

When the estimated exposures are expressed as a percentage of the current PMTDI, the results at the national level ranged from 1% to 35% for average exposure in the general population. For the consumers at the high percentile (95th), the estimated intakes ranged from 3% to 85% and up to 115% in young children. These estimates are based on concentrations of 3-chloro-1,2- propanediol derived before any intervention to reduce occurrence had been undertaken by government or industry.

### 5.2 International estimates of intake for GEMS/Food Consumption Cluster Diets

The new descriptions of the GEMS/Food Consumption Cluster Diets allow a better refinement of intake assessments for 3-chloro-1,2-propanediol, e.g. information is provided on soy sauce consumed as is and oil of soya bean for other uses, cheese processed, which makes the diets more relevant than those used for the previous evaluation by the Committee.

Data on distribution of contamination from aggregate data on food items taking into account the weighting of samples from submitted and published results have been described according to the 13 GEMS/Food Consumption Cluster Diets (WHO, 2006). Table 1 summarizes the distribution-weighted concentration of 3-chloro-1,2-propanediol in soy sauce and soy sauce-based products, in other foods and in food ingredients from various countries from 2001 to 2006 reported as the best available occurrence data to 3-chloro-1,2-propanediol.

In general, the food items analysed were well characterized and it was possible to match sources, contamination and consumption for the 13 revised GEMS/Food Consumption Cluster Diets. Each weighted mean concentration of 3-chloro-1,2-propanediol was taken into account when quantified values for 3-chloro-1,2-propanediol and 1,3-dichloropropanol were reported for a food category or subcategory (e.g cheeses, soy sauce). These concentrations were multiplied by the total mean consumption of food in the corresponding category or subcategory to derive mean total intakes of 3-chloro-1,2-propanediol from all food sources for each cluster diet. Table 3 summarizes estimates of international dietary intake of 3-chloro-1,2-propanediol from all food sources (in µg/kg bw per day).

The range for international mean intakes of 3-chloro-1,2-propanediol from all sources was estimated to be between 0.28 µg/kg bw per day and 3.41 µg/kg bw per day for the 13 GEMS/Food Consumption Cluster Diets, assuming a body weight of 60 kg.

Where estimated exposure was expressed as a percentage of the PMTDI as proposed by the rules of the CCFAC (Codex Alimentarius Commission, 2005b), results ranged from 14% to 170% for the average in the general population. For three diets—clusters K, L and M—exposure to 3-chloro-1,2-propanediol was greater than the PTMDI.

Soy sauce and oil of soya beans are the main contributors to intake of 3-chloro-1,2-propanediol for each diet, representing between 10% and 161% of the PMTDI. Intakes were estimated to be between 0.20 µg/kg bw per day and 3.22 µg/kg bw per day. Wheat-based products contribute more than 5% of the PMTDI in three diets (B, C and D). Others foods mainly contribute less than 5% of the PTMDI in all of the cluster diets.

***Table 3. Summary of estimates of international dietary intake of 3-chloro-1,2-propanediol from all food sources (in µg/kg bw day) for the 13 GEMS/Food Consumption Cluster Diets***

| Cluster Diet | Food category | | | | | | | | | | | | | | Total from all food sources | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat | | Soya [b] | | Nuts and oilseeds | | Meat[c] | | Animal oils and fats | | Beer [d] | | Other foodse | | | |
| | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake | % PTMDI |
| A | 0.033 | 1.7 | 0.220 | 11 | 0.032 | 1.6 | 0.009 | 0.4 | 0.001 | 0.1 | 0.018 | 0.9 | 0.01 | 0.8 | 0.33 | 16 |
| B | 0.150 | 7.5 | 0.913 | 46 | 0.070 | 3.5 | 0.049 | 2.4 | 0.015 | 0.8 | 0.011 | 0.6 | 0.01 | 0.4 | 1.22 | 61 |
| C | 0.161 | 8.0 | 0.849 | 42 | 0.041 | 2.1 | 0.011 | 0.6 | 0.001 | 0.1 | 0.001 | 0.0 | 0.01 | 0.6 | 1.08 | 54 |
| D | 0.147 | 7.4 | 0.559 | 28 | 0.035 | 1.8 | 0.023 | 1.1 | 0.009 | 0.5 | 0.009 | 0.4 | 0.01 | 0.5 | 0.79 | 39 |
| E | 0.089 | 4.5 | 0.911 | 46 | 0.069 | 3.4 | 0.038 | 1.9 | 0.021 | 1.1 | 0.032 | 1.6 | 0.02 | 0.7 | 1.18 | 59 |
| F | 0.082 | 4.1 | 1.030 | 52 | 0.038 | 1.9 | 0.058 | 2.9 | 0.011 | 0.6 | 0.022 | 1.1 | 0.01 | 0.5 | 1.25 | 63 |
| G | 0.065 | 3.3 | 1.525 | 76 | 0.043 | 2.2 | 0.022 | 1.1 | 0.003 | 0.2 | 0.003 | 0.1 | 0.01 | 0.7 | 1.68 | 84 |
| H | 0.030 | 1.5 | 1.487 | 74 | 0.034 | 1.7 | 0.039 | 2.0 | 0.037 | 1.9 | 0.014 | 0.7 | 0.01 | 0.5 | 1.65 | 83 |
| I | 0.026 | 1.3 | 0.277 | 14 | 0.033 | 1.6 | 0.011 | 0.5 | 0.001 | 0.1 | 0.015 | 0.7 | 0.01 | 0.4 | 0.37 | 19 |
| J | 0.016 | 0.8 | 0.196 | 10 | 0.040 | 2.0 | 0.008 | 0.4 | 0.001 | 0.1 | 0.007 | 0.4 | 0.01 | 0.4 | 0.28 | 14 |
| K | 0.043 | 2.2 | 2.730 | 137 | 0.028 | 1.4 | 0.037 | 1.8 | 0.008 | 0.4 | 0.013 | 0.7 | 0.00 | 0.2 | 2.86 | 143 |

***Table 3.***Contd

| Cluster Diet | Food category | | | | | | | | | | | | | | Total from all food sources | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat | | Soya [b] | | Nuts and oilseeds | | Meat[c] | | Animal oils and fats | | Beer [d] | | Other foodse | | | |
| | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake[a] | % PTMDI | Intake | % PTMDI |
| L | 0.039 | 2.0 | 3.224 | 161 | 0.100 | 5.0 | 0.025 | 1.3 | 0.006 | 0.3 | 0.011 | 0.6 | 0.01 | 0.5 | 3.41 | 171 |
| M | 0.088 | 4.4 | 3.126 | 156 | 0.037 | 1.8 | 0.070 | 3.5 | 0.033 | 1.6 | 0.035 | 1.8 | 0.01 | 0.5 | 3.40 | 170 |

GEMS/Food: Global Environment Monitoring System-Food Contamination Monitoring and Assessment Programme; PTMDI: provisional maximum tolerable daily intake.

[a] Weighted average concentration as reported in Table 2 for the food category or subcategory considered and food consumption in the new 13 GEMS/Food Consumption Cluster Diets.

[b] Soy sauce and oil of soya beans.

[c] Meat from cattle, pigs and sheep.

[d] From barley, maize, millet and sorghum.

[e] Others foods from all the following food category or subcategory (sugar confectionery, cocoa beans, spices, fish marine and freshwater, and cheeses from raw milk and processed) contributed less than 1% of the PTMDI of 2 µg/kg bw per day.

## 6. COMMENTS

*Toxicological data*

At its present meeting, the Committee evaluated two new short-term studies on the reproductive effects of 3-chloro-1,2-propanediol in rats.

In the first study, effects on fertility and sperm parameters were examined in male rats given 3-chloro-1,2-propanediol by oral gavage at doses ranging from 0.01 to 5 mg/kg bw per day for 28 days. The NOEL for effects on fertility was 1 mg/kg bw per day, in accordance with the results of earlier studies. With respect to the findings on sperm count, the nature of the dose–response relationship was unusual and was not in conformity, quantitatively, with results from earlier studies. The Committee considered that the data on sperm motility did not show any effect of treatment; the proportion of motile sperm in all treated groups was within 10% of the control value. On the basis of these considerations, the Committee concluded that this study should not be used as the pivotal study for risk assessment.

In the second new study it was shown that administration of 3-chloro-1,2-propanediol at doses of up to 25 mg/kg bw per day by gavage to pregnant rats on days 11 to 18 of gestation did not affect testicular organogenesis in the fetuses.

In a new study of neurotoxicity, rats given repeated oral doses of 3-chloro-1,2- propanediol at doses of up to 30 mg/kg bw per day for 11 weeks did not show neuromotor deficits. Previous studies in rats and mice had indicated that high daily doses (mice, 25–100 mg/kg bw; rats, 50–100 mg/kg bw) given intraperitoneally were associated with dose-related lesions of the central nervous system.

*Occurrence*

Acid-HVPs are widely used in seasonings and as ingredients in processed savoury food products. They are used to flavour a variety of foods, including many processed and prepared foods, such as sauces, soups, snacks, gravy mixes, bouillon cubes. As a result of those uses, 3-chloro-1,2-propanediol had been identified in many foods and food ingredients, most notably in acid-HVP and soy sauces.

Recent studies have demonstrated that 3-chloro-1,2-propanediol may also be formed in other processed foods, particularly in meat products (salami and beef burgers), dairy products (processed cheese and cheese alternatives), a range of cereal products subjected to heat treatments such as baking, roasting or toasting (toasted biscuits, doughnuts, malt and malt extract), and some other foods.

Data on the occurrence of 3-chloro-1,2-propanediol in food were provided by 14 countries and by the International Hydrolyzed Protein Council. Data on 3-chloro-1,2-propanediol in soy sauces and related products for an additional country were available from the published literature.

The average concentration of 3-chloro-1,2-propanediol present in soy sauce and soy sauce-related products was much higher (8 mg/kg, with a range of 0.01 to 44.1 mg/kg) than that present in any other food or food ingredient (less than

0.3 mg/kg). Data from Japan showed that soy sauce produced by traditional fermentation contains insignificant average amounts of 3-chloro-1,2-propanediol (0.003 mg/kg) compared with soy sauce made with acid-HVP (1.8 mg/kg).

Fatty acid esters of monochloropropanols have recently been identified in a range of processed and unprocessed foods. To date, only a limited number of analyses have been reported, but the amount of esterified 3-chloro-1,2- propanediol in many of the samples is higher than the amount of free (non- esterified) monochloropropanol in the same samples. The significance of the presence of esterified 3-chloro-1,2-propanediol in food has yet to be determined.

*Dietary exposure assessment*

National dietary intake data for 3-chloro-1,2-propanediol were provided for 10 countries (Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden, Thailand, UK). The national intakes were calculated by linking data on individual consumption and body weight from national food consumption surveys with mean occurrence data obtained from food contamination surveys. The estimated average intakes from a wide range of foods, including soy sauce and soy sauce-related products, ranged from 0.02 to 0.7 µg/kg bw per day in the general population. For consumers at a high percentile (95th), including young children, the estimated intakes ranged from 0.06 to 2.3 µg/kg bw per day.

Combining the average contamination levels for soy sauce produced by traditional fermentation or with acid-HVP (from the Japanese submission) with a daily consumption figure of soy sauce of 30 g (per-capita consumption for Japan and 95th percentile of consumption from Australia) resulted in values for dietary exposures of 0.0015 and 0.90 µg/kg bw per day, respectively, assuming a body weight of 60 kg.

## *7. EVALUATION*

As no new pivotal toxicological studies had become available, the Committee retained the previously established PMTDI of 2 µg/kg bw for 3-chloro-1,2- propanediol.

The Committee concluded that, based on national estimates from a wide range of foods, including soy sauce and soy sauce-related products, an intake of 3-chloro-1,2-propanediol of 0.7 µg/kg bw per day could be taken to represent the average for the general population, and that an intake of 2.3 µg/kg bw per day could be taken to represent high consumers. In the intake estimates for average to high intake, young children are also included.

When the estimated exposures are expressed as a percentage of the current PMTDI, the results at the national level ranged from 1% to 35% for average exposure in the general population. For consumers at the high percentile (95th), the estimated intakes ranged from 3% to 85% and up to 115% in young children. The estimates are based on concentrations of 3-chloro-1,2-propanediol derived before any remedial action had been taken by government or industry.

Because the distribution of 3-chloro-1,2-propanediol concentrations in soy sauce contains a number of highly contaminated samples, regular consumption of a certain brand or specific type of product could result in intakes greater than the PMTDI by such consumers. The Committee noted that reduction in the concentration of 3-chloro-1,2-propanediol in soy sauce made with acid-HVP could substantially reduce the intake of this contaminant by certain consumers of this condiment.

*Recommendation*

The Committee noted that it has been reported that fatty acid esters of 3-chloro-1,2-propanediol are present in foods, but there were insufficient data to enable either their intake or toxicological significance to be evaluated. The Committee recommended that studies be undertaken to address this question.

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## *METHYLMERCURY (addendum)*


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## *1. EXPLANATION*

Methylmercury was evaluated by the Committee at its sixteenth, twenty-second, thirty-third, fifty-third and sixty-first meetings (Annex 1, references 30, 47, 83, 143 and 166). At its sixty-first meeting, the Committee established a new provisional tolerable weekly intake (PTWI) of 1.6 µg/kg bw, after considering information that had become available since its fifty-third meeting. This information included the results of studies performed in laboratory animals and humans, and epidemiological studies of the possible effects of prenatal exposure to methylmercury on child neurodevelopment. Neurodevelopment was considered to be the most sensitive health outcome and development in utero the most sensitive period of exposure. Calculation of the PTWI was based on an average benchmark-dose level or no-observed-effect level (BMDL or NOEL) of 14 mg/kg (14 µg/g) for mercury in maternal hair in the studies of neurodevelopmental effects in cohorts of children from the Faroe Islands and the Seychelles. The concentration of mercury in maternal hair was calculated to be equivalent to a maternal blood methylmercury concentration of 0.056 mg/l (56 µg/l), which was calculated to arise from a daily intake of methylmercury of 1.5 µg/kg bw. The PTWI was derived by dividing this intake by a total uncertainty factor of 6.4 to give a value of 1.6 µg/kg bw. The PTWI established in 2003 was considered to be sufficient to protect the developing embryo and fetus, the most sensitive subgroup of the population. The new value of 1.6 µg/kg bw was a revision of the previous PTWI of 3.3 µg/kg bw, and the latter value should be considered as withdrawn.

After the establishment of this new PTWI, based on maternal–fetal exposure, Codex Commission on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session in 2005 (Codex Alimentarius Commission, 2005) considered a discussion paper on guideline levels (GLs) for methylmercury in fish. CCFAC noted that the Joint Expert Committee on Food Additives (JECFA) usually sets a single health-based guidance value for the whole population, which is protective for the most sensitive part of the population; however, in the case of guidance values based on developmental end-points, this may be overly conservative for some parts of the population. CCFAC further commented that in specific cases JECFA might consider setting separate values for subgroups of the population. This request to clarify the

PTWI for methylmercury in this context was considered by the Committee at its present meeting, taking into account relevant earlier and recent studies. The following issues were addressed:

- Clarification of the relevance of the PTWI of 1.6 µg/kg bw for different subgroups of the population;
- Assessment of the scientific evidence on the relevance of direct exposure to methylmercury to neurodevelopment in infants and young children;
- The impact of current GLs for methylmercury in fish on exposure and risk.

Descriptions of additional papers relevant to prenatal risks, published since the last review in 2004, are included for completeness.

## 2. BIOLOGICAL DATA

### 2.1 Biochemical aspects

#### *2.1.1 Absorption, distribution and excretion*

Details regarding the absorption and distribution of methylmercury in various species of experimental animals have been previously provided (Annex 1, reference 144). Briefly, methylmercury is effectively absorbed from the gastrointestinal tract and readily crosses the placenta and blood–brain barrier. Concentrations in the fetal blood and brain are generally greater than the corresponding maternal concentrations at parturition.

Recent analyses/assessments have further indicated that methylmercury tends to concentrate in the fetus. The relationship between mercury in erythrocytes and plasma fatty acid composition in 63 pairs of Japanese mothers and fetuses (umbilical cord blood) at delivery was recently reported. The geometric mean concentration of mercury in fetal erythrocytes was 13.4 ng/g, which was significantly higher than that in maternal erythrocytes (8.41 ng/g). The average ratio of mercury in fetal/maternal erythrocytes was 1.6 (range, 1.1 to 2.2). This suggests individual differences in methylmercury concentration ratios between maternal and fetal circulations at delivery. A significant correlation was observed between maternal and fetal concentrations of n-3 polyunsaturated fatty acids (PUFA) ($r = 0.37$, $p < 0.01$) and between concentrations of mercury in fetal erythrocytes and PUFA in fetal plasma ($r = 0.35$, $p < 0.01$). These results confirm that methylmercury and PUFA, which originated from fish consumption, transferred from maternal to fetal circulation (Sakamoto et al., 2004a).

The transport of methylmercury from maternal blood into human milk is less efficient than the transport across the blood–brain and blood–placenta barriers, and methylmercury concentrations in human milk are known to be very low (Skerfving, 1988; Oskarsson et al., 1995, 1996). Exposure of rodent offspring to methylmercury via lactation is lower than exposure in utero, with milk : blood ratios of the order of 0.03–0.2 (Annex 1, reference 144). Observations of decreasing concentrations of inorganic mercury in maternal blood during breastfeeding suggest that mercury may be excreted in human milk (Vahter et al., 2000); however the relevance of this

observation to methylmercury excretion via human milk is uncertain because of the kinetic differences between the two forms of mercury.

### *2.2 Toxicological studies*

#### *2.2.1 Reproductive and developmental toxicity*

Since polychlorinated biphenyls (PCBs) and methylmercury both occur as contaminants in some marine species and because co-exposure to PCBs was an issue in the studies in humans in the Faroe Islands, further studies were carried out to investigate the interactions of PCBs and methylmercury in development (Roegge et al., 2003; Widholm et al., 2004).

In the first study, the effect of pre- and postnatal exposure to PCBs and/or methylmercury on motor function was examined. Groups of at least 15 female Long-Evans rats (aged 60 days) were assigned to each of four exposure groups: (1) PCBs only, dissolved in corn oil and fed on a wafer cookie at 6 mg/kg bw per day; (2) methylmercury only, given at 0.5 µg/ml in the drinking-water (giving an average exposure of 41.90 µg/kg bw per day before mating and 58.90 µg/kg bw per day during gestation); (3) PCBs + methylmercury at the same doses; and (4) a control group given wafer cookies containing corn oil and pure drinking-water). It should be noted that the study was conducted in three cohorts spaced 6 months apart, with animals being assigned to the treatment groups evenly across each cohort. Mating commenced 28 days after the start of dosing and treatment continued for 66 days during mating, pregnancy and lactation until the offspring were aged 16 days. The offspring were weaned at age 21 days. The dams showed no overt signs of toxicity and there was no effect of treatment on length of gestation, dam body or liver weight, litter size or percentage of live births. The offspring of the groups exposed to PCBs or PCBs + methylmercury showed signs of toxicity, including significantly lower body weights, increased liver- and brain : body weight ratios and lower thymus : body weight ratio at age 21 days. After weaning and up to age 63 days, body weights were significantly reduced by 5–11% in the group exposed to PCBs and by 11–15% in the group exposed to PCB + methylmercury. Body weight was unaffected in the group exposed to methylmercury at up to age 63 days, but after this time, average body weight in this group was 7% lower than that of the controls, while average body weight in groups exposed to PCBs or PCBs + methylmercury was reduced by 8% and 11% respectively. Starting at age 60 days, one male and one female from each litter were tested over the next 4 weeks on three motor tasks that involved cerebellar function. Function measure by ability to traverse a rotating rod showed significant impairment in offspring exposed to PCBs + methylmercury, while offspring exposed to PCBs only were also impaired but not significantly. In the vertical-rope climb task, females exposed to methylmercury only were slightly impaired compared with controls, the difference being statistically significant on the third day of the 3 days of testing. In the parallel-bars test, males exposed to methylmercury only made significantly fewer hindlimb slips than did controls. There were no other significant effects of treatment in these two tests (Roegge et al., 2003).

In a further publication, describing the same experiment, one male and one female from each litter in the four treatment groups were tested on a spatial

alternation task at age 110 days. This test was chosen because it assesses both memory and learning and has previously been shown to be sensitive to disruption by PCBs. Methylmercury alone caused no overt toxicity. On the spatial alternation task, animals exposed to PCBs and/or methylmercury showed impaired function relative to controls. Specifically, there were significant reductions in non-cued alternation training in the groups treated with methylmercury only or PCBs + methylmercury, and reductions in delayed spatial alternation in the groups treated with PCBs only or methylmercury only. The group treated with PCBs + methylmercury showed a reduction of similar magnitude in delayed spatial alternation but the difference was not statistically significant because of variability within the group. Exposure to PCBs did not potentiate the effects of exposure to methylmercury. The authors concluded that the delays were indicative of either an associative or attentional impairment, rather than an effect on memory (Widholm et al., 2004).

Following up on earlier studies in which deficits in vision, hearing and fine motor control were observed during middle age in monkeys exposed to methylmercury at 50 µg/kg bw per day from birth to age 7 years or exposed at 10, 25 or 50 µg/kg bw per day in utero and after birth for 4–4.5 years (Rice & Gilbert, 1982, 1990; Rice & Hayward, 1999; Rice, 1999), a further study was conducted by the same group to examine the effects of methylmercury when exposure was limited to the prenatal period. To prevent postnatal exposure, the offspring were separated from their mothers at birth and reared separately. The mothers showed no overt signs of toxicity during gestation. Offspring of monkeys exposed orally to methylmercury at doses of 0, 50, 70 or 90 µg/kg bw per day before and throughout pregnancy were tested at 11–14.5 years of age on a visual contrast sensitivity task. There were 9, 8, 2 and 2 offspring in the 0, 50, 70 and 90 µg/kg bw per day groups, respectively. Monkeys exposed prenatally to methylmercury showed a significant loss of contrast sensitivity, particularly for higher frequency visual images. The degree of impairment was not related to maternal or newborn methylmercury body burden or clearance and in almost half the exposed animals function was unimpaired (Burbacher et al., 2005).

### *2.2.2 Relevance of studies of developmental toxicity in rodents to risk assessment in humans*

Although there are a number of studies in rodents that have addressed the issue of prenatal vs postnatal exposure, the utility of these studies to address the question of vulnerability of humans at different life stages is limited. The period during which brain growth is most rapid (brain growth spurt) during development occurs postnatally during the first 3 weeks of life in rats, while it occurs prenatally during the third trimester of gestation in humans (Dobbing & Sands, 1979; Rice & Barone, 2000). It has therefore been suggested that rats exposed to methylmercury postnatally during lactation could be used as models—at least with respect to the brain growth spurt—for human prenatal exposure during the last trimester. For example, administration of methylmercury during the postnatal phase only in rats (Wakabayashi et al., 1995) has resulted in central nervous system lesions that are similar to those of human fetal Minamata disease (Takeuchi, 1982), while prenatal administration of methylmercury to rats does not produce the same spectrum of

damage to the central nervous system as in humans exposed prenatally (Kakita et al., 2000a; 2000b; 2001).

However, there are known toxicokinetic differences between rodents and humans with regard to methylmercury (Annex 1, reference 144) that preclude simple extrapolation. In addition, a more recent study indicated that the postnatal rat receiving methylmercury via maternal milk may not be a suitable model for exposure of humans in the third trimester of gestation because blood and brain concentrations of methylmercury decline rapidly during the suckling period (Sakamoto et al., 2002a). The same group has shown that if methylmercury is administered directly to suckling rats via a micropipette, rather than via maternal milk, then the postnatal rat may serve as a useful model for exposure in humans during the third trimester (Sakamoto et al., 2004b).

## *2.3* Observations in humans

### *2.3.1 Prenatal exposure and neurodevelopment*

Cognitive development was investigated at age 1 year in a prospective cohort study of 233 infants born to mothers with generally low levels of exposure to mercury during pregnancy, in Krakow between 2001 and 2003. Samples of maternal and umbilical cord blood were taken at delivery. Cognitive function in the infants was assessed by Bayley Scales of Infant Development (BSID-II), mental and motor scales. Fish consumption in grams per week, assessed by questionnaire during pregnancy, was related to both maternal blood and cord blood mercury concentrations. The method of preparation of the fish (smoked, fried, roasted or grilled) had no effect on maternal blood levels of mercury. The geometric mean for maternal whole blood total mercury concentrations at delivery was 0.55 µg/l (range, 0.10–3.40) and for cord blood was 0.88 µg/l (range, 0.10–5.00), with a significant correlation between maternal and cord blood values. These values are consistent with published values from other countries. One hundred and ninety-seven infants showed normal development on the Bayley mental and motor scales and 36 showed delayed motor or psychomotor performance, with a significant difference between the mean psychomotor development Index for the two groups. There were no significant differences between the two groups in the proportions of mothers breastfeeding for given time intervals (< 25, 26–38, 39–51 and > 52 weeks). For the group of infants with normal developmental scores, the geometric mean for maternal blood mercury was significantly lower than that of the group with delayed development (0.52 µg/l; 95% confidence interval [CI] 0.46–0.58; and 0.75 µg/l; 95% CI, 0.59–0.94, in normal and delayed groups respectively). Cord blood mercury concentrations were also lower in the normal group (0.85 µg/l; 95% CI, 0.78–0.93 and 1.05 µg/l; 95% CI, 0.87–1.27, in normal and delayed groups respectively), but the significance was borderline ($p$ = 0.070). The relative risk (RR) for delayed performance increased more than three-fold (RR = 3.58; 95% CI, 1.40–9.14) if the cord blood mercury concentration was greater than the median value of 0.80 µg/l. Similar analysis for mercury concentrations in maternal blood showed a significantly increased risk for delayed performance (RR = 2.82; 95% CI, 1.17– 6.79) if maternal blood mercury concentration was greater than the median value of 0.50 µg/l (Jedrychowski et al., 2005).

The association between maternal fish consumption during pregnancy, umbilical cord mercury concentrations, child fish consumption and development were assessed in 7421 British children born in Bristol in 1991–1992 (part of the Avon Longitudinal Study of Parents and Children (ALSPAC) study. At age 15 months, a parent-completed assessment, based on the MacArthur Communicative Development Inventory (MCDI), assessed the children's vocabulary, comprehension and social activity. When the child was aged 18 months, the parents administered an adaptation of the Denver Developmental Screening Test (DDST) which screens for developmental differences in language, social skills, fine and gross motor skills. Eighty-eight percent of the mothers ate fish during pregnancy, and of these, 80% ate fish at least once a week. Higher mean developmental assessment scores were associated with a modest but consistent increase in maternal fish intake during pregnancy, particularly for the MCDI comprehension score. Tests for trend were significant for all measures except social skills in the DDST. These associations were not altered by the child's fish intake, but the child's intake at age 6 and 12 months was independently associated with an increase in all of the neurodevelopment scores except the language score in the DDST. Breastfeeding did not confound these relationships. Other potentially confounding factors (first born, female sex, higher home-observation-for-measurement of the environment (HOME) scores, higher maternal education, mother abstaining from alcohol during pregnancy) did not modify the relationship between maternal fish intake and neurodevelopment. Maternal fish intake was associated with wet weight umbilical cord mercury concentrations, but overall, umbilical cord concentrations were low in this population (median 0.01 µg/g, with only 33 cord concentrations being ≥ 0.1 µg/g) and when umbilical cord mercury concentrations were divided into quartiles, there was no association between the quartiles and the developmental scores. These concentrations are considerably lower that that reported to be the median for umbilical cords in the Faroe Islands cohort (Dalgard et al., 1994), which was 0.3 µg/g dry weight, equivalent to around 0.04 µg/g when adjusted to a wet weight value, based on a water content of 85–90%. These results indicate that these low levels of prenatal exposure are not associated with developmental effects and that, when fish is not significantly contaminated with mercury, fish intakes during pregnancy and lactation may be beneficial for development (Daniels et al., 2004).

A further statistical analysis has been undertaken of previously reported neurodevelopmental test data obtained at age 5.5 years in the Seychelles Child Development Study (SCDS) (Davidson et al., 1998). Linear measurement error models were used to correct bias resulting from unknown errors of measurement in data in order to obtain unbiased slope estimates that better approximate the true relationship between exposure and outcome. Reanalysis using the measurement-error-model approach indicated that adjustment for measurement errors in prenatal exposure and other explanatory variables had no appreciable effect on the original results (Huang et al., 2003).

Another further statistical analysis of the data from the SCDS at age 9 years has also been published. In the original analysis of the data at age 9 years (Myers et al., 2003), which has been reviewed previously by this Committee (Annex 1, reference 167), conventional linear regression models were used to analyse the data concerning prenatal exposure to methylmercury via maternal fish consumption

and neurodevelopmental test scores. Such models assume a linear relationship between exposure and outcome. However, if the true relationship between exposure and outcome were nonlinear, other statistical models would be more appropriate. Accordingly, the data from the SCDS gathered at age 9 years has been re-analysed using semi-parametric additive modelling with different degrees of smoothing to explore whether nonlinear effects of prenatal exposure were present. From the original 21 end-points yielded by the neurodevelopmental tests on 643 children, they selected the six end-points with a two-tailed $p$ value of less than 0.2. A nonlinear effect was identified with the smoother model on adjusted results for only one end-point, the grooved pegboard dominant hand. It suggested no effect of exposure up to a maternal hair total mercury concentration of 12 μg/g, but a slight effect above 12 μg/g. However, the authors noted that there were fewer data points above a maternal hair concentration of 12 μg/g and the curves were estimated with less precision. In the new analysis, the overall effect for prenatal exposure to total mercury was also significant ($p = 0.04$), while the overall effect in the previous linear analysis (Myers et al., 2003) was not ($p = 0.08$). The significant associations found in the previous linear analysis (adverse effect on grooved pegboard non-dominant hand in males and a beneficial effect on the Connors Teacher Rating Scale) remained significant in this new analysis. The authors concluded that this reanalysis confirms the findings of their previous linear regression analysis, with little evidence for adverse effects from prenatal methylmercury exposure in the Seychelles cohort, but that it does reveal a possible adverse effect in the uppermost exposure range at greater than 12 μg/g for maternal hair mercury (Huang et al., 2005). The authors further note that this possibility is consistent with the World Health Organization (WHO) analysis of the data from the Iraq poisoning incident, which also suggested a threshold for adverse effects on the offspring in the range of 10–20 μg/g for maternal hair mercury as a biomarker for prenatal exposure (WHO, 1990).

In the SCDS main study, at age 19 months, enhanced Motor Developmental Index scores were associated with increasing exposure to methylmercury in groups of caregivers with higher intelligence quotient (IQ) at several levels of family income. A similar analysis of the evaluations at 66 months to determine whether the modifying influences of social and environmental factors were consistent with those previously observed was performed. Children in the cohort ($n = 711$) were evaluated for cognitive ability (McCarthy Scales of Children's Abilities), language development (Preschool Language Scale), drawing and copying (Bender Gestalt Test), scholastic achievement (the Woodcock-Johnson Test of Achievement), and behaviour (the Child Behavior Checklist). Interactions between prenatal exposure to methylmercury and caregiver intelligence, socioeconomic status, home environment, and sex were examined by multiple regression analysis. The median prenatal exposure to methylmercury as measured by concentrations in maternal hair was 5.9 μg/g, with a range of 0.5– 26.7 μg/g. Prenatal exposure interacted with sex, one or more social or environmental covariates for general cognitive ability, overall language ability, and pre-arithmetic achievement. The effects were not consistent across either endpoints or covariate categories. The authors determined that a consistent pattern of effect modification was not observed, suggesting that the any statistically significant results were due to chance (Davidson et al., 2004).

The Faroe Islands cohort underwent detailed neurobehavioral examination at age 14 years. Prenatal exposure to methylmercury was determined by analyses of cord blood, cord tissue, and maternal hair. Of the 1010 living cohort members, 878 were available for examination. Eighteen participants with neurological disorders were excluded. The neuropsychological test battery was designed based on the same criteria as applied at the examination at age 7 years. Multiple regression analysis was carried out and included adjustment for confounders. Indicators of prenatal methylmercury exposure were significantly associated with deficits in finger tapping speed, reaction time on a continued performance task, and cued naming. Postnatal exposure to methylmercury had no discernible effect. These findings are similar to those obtained at age 7 years, and the relative contribution of mercury exposure to the predictive power of the multiple regression models was also similar. An analysis of the test score difference between results at age 7 and 14 years suggested that mercury-associated deficits had not changed between the two examinations. Exposure to methylmercury was significantly associated with deficits in motor, attention, and verbal tests. According to the authors, the effects on brain function associated with prenatal exposure to methylmercury appear to be multi-focal and permanent (Debes et al., 2006).

The associations between maternal fish intake during pregnancy and maternal hair mercury at delivery with visual brain processing among 135 mother–infant pairs was assessed in a prospective study of pregnancy and child cohorts in the United States of America (USA). Infant cognition by the percent novelty preference on visual recognition memory testing at age 6 months was determined. Maternal fish consumption averaged 1.2 servings per week during the second trimester. Mean maternal hair total mercury was 0.5 μg/g, with 10% of samples exceeding 1.2 μg/g. Mean visual recognition memory score was 59.8 with a range of 10.9–92.5. Higher fish intake was associated with higher infant cognition, after adjusting for participant characteristics using linear regression. The association strengthened after adjustment for maternal hair mercury concentration. For each additional weekly fish serving, offspring visual recognition memory score was 4.0 points higher. An increase of 1 μg/g in hair mercury concentration was associated with a visual recognition memory decrement of 7.5 points. Visual recognition memory scores were highest among infants of women who consumed more than two weekly servings of fish but had hair mercury concentrations of less than or equal to 1.2 μg/g (Oken et al., 2005).

In a similar study, the impact of long-term exposure to PCBs and methylmercury on visual brain processing in Canadian Inuit children was assessed. Concentrations of total mercury in blood and PCB 153 in plasma were measured at birth and again at the time of testing in 102 chidren of preschool age. Relationships between contaminants and pattern-reversal visual evoked potentials were assessed by multivariate regression analyses, taking into account several potential confounding variables. The possible protective effects of selenium and n-3 PUFA against toxicity caused by methylmercury and PCBs were also investigated. Results indicate that exposure to methylmercury and PCB from consumption of fish and sea mammals were associated with alterations in responses to visual evoked potentials, especially delays in the latency of the N75 and of the P100 components. In contrast, the concomitant intake of n-3 PUFA was associated with a shorter latency of the

P100. No significant interactions between nutrients and contaminants were found. Significant associations were found with concentrations of neurotoxicants in blood samples collected at the time of testing at preschool age (Saint-Amour et al., 2006).

The effects of prenatal and postnatal long-term exposure to mercury, polychlorinated PCBs and lead on the neuromotor development of preschool children was assessed in a study of 110 preschool Canadian Inuit children. Blood mercury, PCBs and lead concentrations were measured in cord blood and at the time of testing. Gross motor functions were evaluated and a neurological examination was performed. Fine neuromotor performance was assessed using quantitative measures of postural hand tremor, reaction time, sway oscillations, as well as alternating and pointing movements. Potential covariates were documented including demographic and familial characteristics, other prenatal neurotoxicants (alcohol, tobacco) and nutrients (selenium, n-3 PUFA). Hierarchical multivariate regression analyses were performed, controlling for significant covariates. Gross motor development was not linked to prenatal exposures. However, significant associations were observed between blood lead concentration at testing time and changes in reaction time, sway oscillations, alternating arm movements and action tremor. Negative effects of PCBs on neuromotor development were not clearly observed, neither were the potential beneficial effects of n-3 PUFA and selenium. Increased action tremor amplitude was related to blood mercury concentrations at testing time, which corroborates an effect reported among adults (Després et al., 2005).

In order to assess the association between mercury exposure in children with autistic spectrum disorder, a cross-sectional cohort study was performed over a 5-month period in 2000 to compare the hair and blood mercury concentrations of children with this disorder ($n$ = 82; mean age, 7.2 years) and a control group of normal children ($n$ = 55; mean age, 7.8 years). There was no difference in the mean mercury concentrations. The mean blood mercury concentrations for the autistic and control groups were 19.5 and 17.7 nmol/l, respectively ($p$ = 0.15), and the mean hair mercury concentrations of the autistic and control groups were 2.26 and 2.07 ppm, respectively ($p$ = 0.79). These results indicate that there is no causal relationship between mercury as an environmental neurotoxin and autism (Ip et al., 2004a).

### *2.3.2 Neurodevelopment and postnatal exposure to methylmercury in human milk during infancy*

Details of the critical studies of neurodevelopment from the Seychelles and the Faroe Islands underlying the establishment of the PTWI of 1.6 µg/kg bw in 2004 have been described previously (Annex 1, reference 167). Since then, further studies have been published that are relevant to the question of the possible additional role of postnatal exposure to methylmercury via human milk in contributing to the neurobehavioural changes.

Earlier observations from the Faroe Islands cohort where the length of breastfeeding showed a positive correlation with mercury concentration in the hair of the infants at age 12 months, indicated that, in addition to prenatal exposure, exposure also took place through human milk (Grandjean et al., 1994). In spite of

this, it was noted that breastfed children reached their developmental milestones before formula-fed children (Grandjean et al., 1995), suggesting an overall beneficial effect of human milk, despite the contamination with mercury. When examined in a series of neurobehavioural tests, in one test (reflecting attention) worse performance at age 7 years was associated with increased hair mercury concentrations at age 12 months, irrespective of whether the children were breastfed or not, but paradoxically, performance on a test of language skills increased with increasing hair mercury concentrations at age 12 months (Grandjean et al., 1999). The picture is further complicated by the observation, derived from a meta-analysis of available studies, that breastfeeding in general is associated with improvements in neurological and cognitive development across all ages from 6 months to 15 years and carries greater benefits in children of low birth weight (Anderson et al., 1999).

The role of breastfeeding on neuropsychological performance at age 7 years was examined in the Faroe Islands cohort of 905 children who had relatively high prenatal exposures to mercury. They compared the influence of breastfeeding exclusively for 0–4 months with breastfeeding for more than 4 months as well as total months of breastfeeding until weaning (0–6 months or > 6 months). All except 7.7% of the children were breastfed and 61% were exclusively breastfed for 0–4 months. The breastfeeding period exceeded 6 months for 55% of the children; of these only 5% were exclusively breastfed for more than 6 months, the remainder received other food in addition to human milk. Children who were breastfed longer (both exclusively and in total) performed slightly better on most neuro-psychological tests before adjustment for confounders (e.g. sex of child, parents professionally trained, father employed at examination, child in day care, etc). This effect was reduced after adjustment for these confounders, but children breastfed exclusively for more than 4 months or for longer in total still performed slightly better on most tests, with significantly better scores on the Boston Naming Test and Wechsler Intelligence Scale for Children—Revised, Block Designs. After further adjustment for cord-blood mercury and child hair mercury, the results were unaffected, except for the Boston Naming Test where the difference became non-significant. Adjusting for exposure to PCBs did not affect the associations found. Children who were not breastfed at all generally performed less well on most tests than those that were breastfed. The authors however, acknowledge the difficulty of differentiating between prenatal and postnatal exposure to mercury, because very few children in the cohort were not breastfed and most children were exposed to mercury both prenatally and early postnatally. Thus, in this cohort with relatively high prenatal exposure and demonstrated adverse effects from that exposure, breastfeeding was not associated with any deficit in neuropsychological performance at age 7 years, although breastfeeding appeared to be not as beneficial as previously reported by other investigators in non-exposed populations (Jensen et al., 2005).

### *2.3.3 Neurodevelopment and exposure to methylmercury during childhood*

In the re-analysis of the Seychelles (SCDS) data by Huang et al. (2003), described earlier in the section on prenatal exposure, the influence of postnatal exposure on neurodevelopment at age 5.5 years was also re-evaluated. Application

of linear measurement error models did not alter the significance levels for several beneficial effects compared with previous linear regression analysis (Davidson et al., 1998).

Murata et al. (2004) have studied the influence of exposure to methylmercury on brainstem auditory evoked potentials (BAEP) in children from the Faroe Islands cohort. A total of 878 children (87%) from the original cohort were given a thorough paediatric examination at age 14 years, hair samples were taken and analysed for mercury and BAEPs were determined in 859 of the children. Their hair mercury concentrations were increased compared with the previous examination of these children at age 7 years, as published previously (Budtz-Jørgensen et al., 2004); at age 14 years, geometric mean hair-mercury concentrations were 0.96 (range, 0.02–9.7) μg/g compared with 0.60 (range, 0.04–7.5) μg/g at age 7 years. However, these were still less than one quarter of the concentration in maternal hair at the time of their birth, which was 4.22 (range, 0.2–39.1) μg/g. Audiometry results generally showed normal hearing, but in accordance with their findings on the BAEP test at age 7 years (Grandjean et al., 1997), latencies of BAEP peaks III and V and especially the I–III interpeak interval were delayed in association with prenatal exposure to methylmercury, as measured by cord blood values. The delay was about 0.012 ms when cord blood mercury concentration doubled. The regression coefficients for the delay in latencies were approximately halved at age 14 years compared with age 7 years, suggesting that there may be some degree of compensation. More recent exposure, as measured by hair concentrations at age 14 years, was associated with a prolongation of the III–V interpeak interval. This change was not associated with prenatal exposure to methylmercury and analysis for exposure to PCBs did not affect the results.

The authors note that all these effects are subtle and not nearly as marked as the changes in BAEP that occur in disease states such as multiple sclerosis, acoustic neuroma or diabetes mellitus. They also comment that previous calculations based on the most sensitive end-point from all their neuro-psychological evaluations on this cohort, using a benchmark response of 5%, indicate a benchmark dose (BMD) lower 95% confidence limit (BMDL derived from the BMD) of about 10 μg/g for maternal hair, but the postnatal BMDL for the prolonged III–V interpeak interval is approximately one half of that. The authors acknowledge that the statistical uncertainties are such that the difference may not necessarily reflect the relative toxic potentials of prenatal and postnatal exposures. They also acknowledge that the postnatal exposure estimates were limited and may not necessarily represent the magnitude of exposure at susceptible time windows. Moreover, biomarkers of prenatal and postnatal exposure were highly associated. However, since biomarkers of prenatal and postnatal exposure were associated with effects on different peaks and/or peak intervals, this indicates that the differential effects of pre- and postnatal exposure may be robust. The authors conclude that the significance of postnatal methylmercury exposure needs to be documented further in independent studies with more frequent exposure assessments, but that the results suggests vulnerability to neurotoxicity attributable to methyl mercury may extend into the teenage period.

### 2.3.4 Methylmercury exposure and cardiovascular function in the young

To ascertain whether heart function in childhood is affected by exposure to methylmercury, the Faroese birth cohort was examined at age 7 and 14 years. Blood pressure, heart rate variability (HRV) and brainstem auditory evoked potentials (BAEPs) (see above) were measured. Mercury concentrations were determined in cord blood as the measure of prenatal exposure and in the child's hair as a measure of recent exposure. HRV, which reflects the continuous changes in central control of cardiac autonomic balance, was partitioned into its individual components by spectral analysis and the results expressed in terms of high frequency (HF) and low frequency (LF) components. HF components reflect parasympathetic activity and LF components reflect sympathetic activities. A doubling of prenatal methylmercury exposure was associated with significant decreases of about 6.7% in LF, mainly at 7 years of age, and in HF, mainly at 14 years of age. The coefficient of variation of the electrocardiograph R-R interval was also significantly decreased with increasing prenatal exposure to methylmercury at age 7 years and at 14 years. No discernible effect of methyl-mercury on blood pressure was apparent at age 14 years. Decreased LF was associated with increased latency of the BAEP peak III, but adjustment for prenatal and postnatal exposure substantially attenuated this correlation. The authors concluded that prenatal exposure was associated with decreased sympathetic and parasympathetic modulation of the HRV at age 14 years. The authors hypothesised that both the delay in one of the BAEP latencies and the effects on HRV parameters may be caused by underlying neurotoxicity of methylmercury to brainstem nuclei. The authors speculate on the possible clinical significance of the observed changes, citing clinical studies in adults showing that decreased vagal (parasympathetic) tone is associated with an increased risk of sudden cardiac arrest and coronary artery disease (Grandjean et al., 2004a).

In a similar study to that described above, the subclinical effects of prenatal exposure to methylmercury from fish consumption on the cardiac autonomic function were assessed in 136 Japanese children aged 7 years. In the children, cord tissue methylmercury concentration (range, 0.017–0.367, median, 0.089 μg/g) was not significantly correlated with hair total mercury at 7 years of age (range, 0.43–6.32, median, 1.66 μg/g). The principal finding was that prenatal exposure to methylmercury, as measured by cord tissue methylmercury concentration, was associated with decreased vagal modulation of cardiac autonomic function, in agreement with the findings in the Faroe Islands cohort at age 14 years. These associations remained after correcting for possible confounders such as age and sex. Hair mercury concentration was not significantly correlated with any cardiac autonomic indicators. The authors concluded that these findings suggest that prenatal exposure to methylmercury at a median of estimated maternal hair mercury concentration at parturition of 2.24 μg/g, may be associated with reduced parasympathetic activity and/or sympathovagal shift (Murata et al., 2006). The Committee noted that this is about six times lower than the value used to derive the PTWI in 2003.

### *2.3.5 Methylmercury exposure and neurological effects in adults*

Monoamine oxidase (MAO) regulates biogenic amine concentration in the brain and peripheral tissue and is known to be a molecular target of mercury compounds in animal models. Blood platelet MAO-B activity may reflect MAO function in the central nervous tissue. The relationship between platelet MAO-B and mercury exposure (blood and hair) in fish-eating adults ($n$ = 127) living along the St Lawrence River in Canada was assessed. A significant negative association was observed between platelet MAO-B activity and blood mercury concentration, but not with hair mercury concentrations. Multiple linear regression analysis demonstrated that blood mercury and heavy smoking were associated with reduced platelet MAO activity in the total population. The reduction in MAO-B activity appeared to be associated with blood mercury concentrations of greater than 3.4 µg/l (75th percentile). These results suggest that MAO-B activity in blood platelets may be a useful biomarker to assess the biochemical effects of exposure to mercury (Stamler et al., 2006).

Two groups of 22 Italian adult male subjects who were frequent consumers of tuna and 22 controls were examined in a cross-sectional field study. The assessment included neurobehavioral tests of vigilance and psychomotor function and hand tremor measurements. Mercury in urine and serum prolactin were measured in all exposed subjects and controls, while measurements of the organic component of mercury in blood (O-Hg) were available for only 10 exposed and six controls. Mercury in urine was significant higher among exposed subjects (median, 6.5 µg/g of creatinine; range, 1.8–21.5) than controls (median, 1.5 µg/g of creatinine; range, 0.5–5.3). The median blood concentrations of O-Hg were 41.5 µg/l among the tuna-fish eaters and 2.6 µg/l in the control group. Both mercury in urine and O-Hg were significantly correlated with the quantity of fish consumed per week. The neurobehavioral performance of subjects who consumed tuna fish regularly was significantly worse on colour word reaction time, digit symbol reaction time and finger tapping speed. After accounting for education level and other covariates, the multiple stepwise regression analysis indicated that O-Hg concentration was most significantly associated with individual performance on these tests, accounting for about 65% of the variance in test scores (Carta et al., 2003).

An annual follow-up involving multiple health examinations in about 1500 persons of age 40 years or more took place near Minamata City each summer from 1984 to 2004. Case–control studies were designed to estimate the role of risk factors for various health issues using geographical differences. The results of the study are summarized as follows. There were no significant differences in the prevalence of diseases associated with Minamata disease. Subjective complaints, which were related not only to neurological but also to general complaints, were consistently much more common in the polluted area than in the control area. Five percent of the inhabitants who were not certified as patients with Minimata disease had a high predicting index of Minimata disease and therefore could have been affected by methylmercury poisoning. No significant differences with respect to activities of daily living by residential area were observed. It is important to take into consideration mental stress not only from the physical effects but also from the secondary social damage experienced in these areas in making a differential diagnosis of Minimata disease (Futatsuka et al., 2005).

The thresholds of touch and two-point discrimination of residents near the Shiranui Sea and patients with Minamata disease were examined using the quantifiable instruments. Patients with Minimata disease could perceive the stimulation of touch although their touch thresholds were significantly increased in comparison with those of the control group. Their touch thresholds increased at the proximal extremities and the trunks as well as at the distal extremities. The evenly distributed increases at both distal and proximal parts indicated that the persistent somatosensory disturbances were not caused by the injuries to their peripheral nerves. The thresholds of two-point discrimination, which are associated with the function of the somatosensory cortex, increased at both forefingers and the lip in both groups. Apraxia limb kinetics, astereognosis and disorder of active sensation, which are all associated with damage to the somatosensory cortex, were detected in the patients with Minimata disease. The authors concluded that the persisting somatosensory disorders after discontinuation of exposure to methylmercury were induced by diffuse damage to the somatosensory cortex (Ninomiya et al., 2005).

A psychophysical study of tactile sensation to evaluate the somatosensory abilities was conducted in subjects living in a methylmercury-polluted area around Minamata City, Japan. Control subjects and methylmercury-exposed subjects with and without numbness were included in the study groups. A history of exposure to methylmercury was taken and a neurological examination was performed. Aluminum-oxide abrasive papers were used as stimuli in a psychophysical sensory examination of fine-surface-texture discrimination. Difference thresholds from 3 μm were calculated by the two-alternative, forced-choice technique. Difference thresholds in control subjects were also calculated. The difference threshold was 6.3 μm in exposed subjects with sensory symptoms, 4.9 μm in exposed subjects without sensory symptoms, and 2.7 μm in control subjects. Acuity of fine-surface-texture discrimination was disturbed not only in subjects with clinical complaints of hand numbness, but also in subjects without hand numbness who lived in the district where methylmercury exposure occurred. Sensory testing using a psychophysical test of fine-surface-texture discrimination in this population suggests that the number of individuals affected by methylmercury exposure in the polluted area was greater than previously reported (Takaika et al., 2004).

Neurological symptoms and signs of Minimata disease were assessed in another Japanese study by assessing neurological signs and symptoms temporally using multiple logistic regression analysis. The severity of predictive index in the study population declined over a 25 year period. Only a few patients showed aggravation of neurological findings, which were caused by complications such as spinocerebellar degeneration. Patients with chronic Minimata disease who were older than age 45 years had several concomitant diseases, so that their clinical pictures were complicated. It was difficult to statistically differentiate chronic Minimata disease based on sensory disturbance alone (Uchino et al., 2005).

A cross-sectional analysis was used to determine the effect of mercury concentrations on neurobehaviour in 474 subjects in the Baltimore Memory Study, a longitudinal study of cognitive decline in people aged 50 to 70 years. Total mercury in whole blood samples was analysed and multiple linear regression was used to examine its associations with neurobehavioral test scores. Twenty scores from 12 neurobehavioral tests were considered. The median blood mercury concentration

was 2.1 µg/l. After adjustment for covariates, increasing blood mercury was associated with worse performance on Rey complex figure delayed recall, a test of visual memory. However, increasing blood mercury concentrations were associated with better performance on finger tapping, a test of manual dexterity. Overall, the authors concluded that this study did not provide strong evidence that blood mercury concentrations are associated with worse neurobehavioral performance in this population of older urban adults (Weil et al., 2005).

### *2.3.6 Methylmercury exposure and cardiovascular effects in adults*

The association between mercury in blood and 24-h blood pressure in four groups of healthy subjects was assessed. The following describes the subjects in each group: group 1, Danes living in Denmark consuming European food; group 2, Greenlanders living in Denmark consuming European food; group 3, Greenlanders living in Greenland consuming European food; and group 4, Greenlanders living in Greenland consuming mainly traditional Greenlandic food. Mercury concentrations in blood were highest in Greenlanders and increased when they lived in Greenland and consumed traditional Greenlandic food (group 1: 2.2 µg/l (median), group 2: 4.8 µg/l, group 3: 10.8 µg/l, and group 4: 24.9 µg/l). The 24-h blood pressure was the same in all three groups of Greenlanders. However, 24-h diastolic blood pressure was significantly lower (71 vs 76 mm Hg) and 24-h pulse pressure was significantly higher (54 vs 50 mm Hg) among Greenlanders in comparison with Danes. Mercury in blood was significantly and positively correlated to pulse pressure (the difference between systolic and diastolic pressure). The differences in pulse pressure were undoubtedly due to the lower diastolic blood pressure noted in the Greenlanders (Pedersen et al., 2005).

The cross-sectional relationship between total blood mercury concentration and blood pressure was assessed in a representative sample of 1240 women, aged 16–49 years, from the National Health and Nutrition Examination Survey (NHANES) 1999–2000 in the USA. No overall association was found between blood mercury and blood pressure in multivariate models. Data by dietary fish intake was stratified resulting in 759 consumers of fish and 481 nonconsumers. Total blood mercury concentrations averaged 2.3 µg/l for the consumers and 0.8 µg/l for the nonconsumers. For each 1.3 µg/l (interquartile distance) increase in blood mercury, systolic blood pressure significantly increased by 1.83 mm Hg (95% CI, 0.36–3.30) among nonconsumers. A similar pattern was seen for diastolic blood pressure, although it was nonsignificant. No significant effects of blood mercury concentrations on blood pressure were seen among consumers of fish. While no adverse effect on blood pressure of mercury exposure at background levels was present when considering all study participants, an adverse association was present among young and middle-aged women who did not consume fish. The authors speculated that fish consumption may counter the effects of mercury on blood pressure regulation (Vupputuri et al., 2005).

Three Amazonian villages of the Tapajos Basin were studied in relation to fish mercury concentrations, mercury in hair (fish consumption) and erythrocytes, body mass index (body weight/height squared, $kg/m^2$), and blood pressure. The mean concentrations of mercury in fish were higher in predatory (578.6 ng/g) than in non-predatory species (52.8 ng/g). Overall, only 26% of concentrations of

mercury in fish were greater than 500 ng/g, and only 11% were greater than 1000 ng/g. There was no systematic trend in concentrations of mercury in fish from rivers with a history of gold-mining activities. The biomarker of fish consumption (hair mercury) was significantly associated with erythrocyte mercury ($r = 0.5181$; $p = 0.0001$). There was a trend of lower increase in blood pressure with age among consumers with a higher consumption. Summary clinical evaluation did not detect neurological complaints compatible with mercury intoxication (paraparesis, numbness, tremor, balancing failure), but endemic tropical diseases such as clinical history of malaria showed a high prevalence (55.4%). Fish is an abundant natural resource, important in the native diets, that has been historically consumed without perceived problems and can easily be traced through hair mercury. The authors concluded that exposure to methylmercury in freshwater fish is a less important health issue than endemic infectious diseases such as malaria and lack of basic medical services (Dorea et al., 2005b).

The incidence of myocardial infarction and death from all causes was reported during 24 years of follow-up of a prospective cohort study of 1462 women in Gothenberg, Sweden. There were a total of 87 cases of myocardial infarction, of which 39 died and 253 all-cause deaths. At baseline of the prospective study (1968–1969), sera from participants were collected and stored for future research. The sera samples were analysed for mercury concentrations in 1992–1993, and the correlation between mercury concentrations and disease or death was examined. An inverse correlation, though not statistically significant, was detected for myocardial infarction ($p = 0.10$ adjusted for age and $> 0.20$ when adjusted for age and education) and also for death ($p = 0.09$). A statistically significant inverse correlation between serum mercury and death was found when the correlation was adjusted for both age and education. No correlation coefficients were provided in the paper (Ahlqwist et al., 1999).

A case–control study nested within a larger ongoing prospective programme on cardiovascular disease and diabetes prevention was conducted in northern Sweden. Participants in the prospective programme had responded to health surveys and donated blood samples in 1994 that were stored for future research. From these participants, persons with first myocardial infarction with sufficient blood sample and without cancer ($n = 78$) were selected as cases, and persons matched by age, sex, date of health survey (± 1 year) and geographical region to the cases were selected as controls ($n = 156$). The average time to onset of first myocardial infarction for the cases after responding to the health survey was 18 months. The case–control study examined the association between first myocardial infarction and concentration of mercury in erythrocytes, as well as gluthathione peroxidase in erythrocytes (Ery-GSH-Px) and plasma concentrations of n-3 PUFA eicosapentaenoic and docosahexaenoic acids (P-PUFA), which the researchers considered to be biomarkers of fish intake. Fish consumption assessed by questionnaire was also evaluated. The results of the study showed a marginally significant inverse association between myocardial infarction and erythrocyte mercury in the univariate analysis. When the range of values for erythrocyte mercury (0.5 to 67 ng Hg/g erythrocytes) was divided into tertiles (< 3, 3–6, and > 6 ng Hg/g erythrocytes), the odds ratio for the highest vs lowest tertile was 0.43 (95% CI, 0.19–0.95). Erythrocyte mercury was found to be significantly correlated with reported weekly fish consumption. P-PUFA also showed an inverse association with myocardial

infarction and was significantly correlated with reported weekly fish consumption. No association between myocardial infarction and Ery-GSH-Px was detected. When erythrocyte mercury and P-PUFA and their interaction were analysed in a multivariate model that adjusted for smoking and body mass index, there was a significantly lower risk of myocardial infarction for the group that had both high erythrocyte mercury and high P-PUFA (OR = 0.16, 95% CI, 0.04–0.65) (Hallgren et al., 2001).

The correlation of hair mercury concentration with the risk of acute coronary events and cardiovascular and all-cause mortality was assessed in a study of men from eastern Finland. The population-based prospective Kuopio Ischaemic Heart Disease Risk Factor Study cohort of 1871 Finnish men aged 42 to 60 years and free of previous coronary heart disease or stroke at baseline was used. During an average follow-up time of 13.9 years, 282 acute coronary events and 132 cases of cardiovascular disease, 91 cases of coronary heart disease, and 525 all-cause deaths occurred. Men with hair mercury content within the highest third of the range (i.e. > 2.03 µg/g) had an adjusted 1.60-fold (95% CI, 1.24–2.06) risk of acute coronary event, 1.68-fold (95% CI, 1.15–2.44) risk of cardiovascular disease, 1.56-fold (95% CI, 0.99–2.46) risk of coronary heart disease, and 1.38-fold (95% CI, 1.15–1.66) risk of any death compared with men in the lower two thirds. High mercury content in hair also attenuated the inverse correlations of docosahexaenoic acid plus docosapentaenoic acid concentration with these cardiovascular risk factors (Virtanen et al., 2005).

The five epidemiological studies of mercury concentrations in adults in relation to cardiovascular disease or death are shown in Table 1. Three of these studies are described in this monograph (Virtanen et al., 2005; Hallgren et al., 2001; Ahlqwist et al., 1999) and two (Guallar et al., 2002; Yoshizawa et al., 2002) in the previous monograph on methylmercury prepared by the Committee at its sixty-first meeting (Annex 1, reference 166). One study (Salonen et al., 1995) in the previous monograph is not shown in the table because it concerns the same cohort of men in Eastern Finland as that described by Virtanen et al. (2005), with longer follow-up.

As the table demonstrates, the studies are not directly comparable owing to different biomarkers for ascertaining total mercury concentrations, i.e. hair, erythrocytes, toenails or serum. Two of the five studies (Virtanen et al., 2005; Guallar et al., 2002) found an increased risk of acute coronary event or myocardial infarction with higher mercury concentrations; one study (Hallgren et al., 2001) found a decreased risk of myocardial infarction with higher concentrations of mercury (the authors consider mercury concentrations to be a biomarker for fish consumption in this study); and the other two studies (Yoshizawa et al., 2002; Ahlqwist et al., 1999) did not show a statistically significant association between myocardial infarction and mercury concentrations. The reason for the differences in findings across studies and population groups is not clear but suggests that the evidence for the association between cardiovascular disease and methylmercury exposure is preliminary.

### *2.3.7 Dose–response assessments*

Exposure misclassification always constitutes an issue in dose–response relationships concerning epidemiology studies in humans. In additional work, the Faroese research team (Grandjean et al., 2005) considered the issue of imprecision

in analyses of mercury in cord blood and maternal hair. Laboratory imprecision for both chemical analyses was less than 5% coefficient of variation. Factor analysis and structural equation analysis were applied to assess the full extent of the imprecision. Calculated total imprecision exceeded the known laboratory variation: the coefficient of variation was 28–30% for the cord blood mercury concentration and 52–55% for the maternal hair mercury concentration. These findings illustrate that measurement error may be underestimated if judged solely on the basis of the ability of the laboratory to reproduce the data with check analysis. Adjustment by sensitivity analysis is meaningful only if realistic measurement errors are applied (Grandjean et al., 2004b; Budtz-Jørgensen et al., 2004).

### *2.3.8 Risk–benefit analyses*

When exposure recommendations are developed for foods that potentially have both harmful and beneficial qualities, a balance must be struck between the associated risks and benefits to optimize public health. Although quantitative methods are commonly used to evaluate health risks, such methods have not been generally applied to evaluating the health benefits associated with environmental exposures. A quantitative method for risk–benefit analysis has been used by several groups of investigators that allows for consideration of diverse health end-points (e.g. neurodevelopment, cardiovascular) that differ in their impact (i.e. duration and severity), using dose–response modelling by estimating the number of quality-adjusted life years saved.

To demonstrate the usefulness of this method, the risks and benefits of fish consumption were evaluated using a single health risk and health benefit end-point. The benefits of eating fish were defined as the decrease in mortality caused by myocardial infarction, and risk was defined as the increase in neurodevelopmental delay (i.e. delay in talking) resulting from prenatal exposure to methylmercury. Using the proposed framework, the net health impact of eating fish was estimated either in a whole population or in a population of women of childbearing age and their children. It was demonstrated that across a range of concentrations of methylmercury in fish (0–1 mg/kg) and a range of fish consumption levels (0–25 g/day), neurodevelopmental effects in the general population, including the embryo and fetus in women of childbearing age, would have to be weighed 6 times more and 250 times less than the cardiovascular benefits, when identifying optimal levels of fish consumption. These methods have the potential to evaluate the merits of other public health and risk management programmes that involve trade-offs between risks and benefits (Ponce et al., 2000).

As part of the effort to quantitatively integrate the benefits of fish consumption and the risks of exposure to methylmercury, the Harvard Center for Risk Analysis convened expert panels to quantify the net impact of changes in fish consumption by the US population of consumers. This included estimates of the effects on prenatal cognitive development, mortality from coronary heart disease, and stroke. Study weights were assigned to account for statistical precision, relevance of three end-point domains (general intelligence, verbal ability, and motor skills) to prediction of IQ, and age at evaluation. Eight randomized controlled trials comparing cognitive development in controls and in children who had received supplementation with n-3

***Table 1. Epidemiological studies on the association between cardiovascular disease or death with mercury concentrations in adults***

| Author/Country | Study design | Study participants | Ascertainment of mercury concentration | Disease or death | Results |
|---|---|---|---|---|---|
| Guallar et al. (2002)<br>Eight European countries and Israel | Case–control | Cases: 684 men<br>Controls: 724 men | Toenail Hg<br>(toenails collected after occurrence of MI, analysed in 1991–1992) | First acute MI | Range in toenail Hg concentrations: 0.14 to 0.57 μg/g (authors presented averages in control patients across study centres)<br>Adjusted OR for MI:<br>highest quintile of Hg compared with lowest quintile: 2.16 (95% CI, 1.09–4.29) |
| Yoshizawa et al. (2002)<br>USA | Case–control within prospective cohort study | Cases: 470 men<br>Controls: 464 men matched on age and smoking status | Toenail Hg<br>(toenails collected before the onset of CHD, analysed in 1987) | CHD | Toenail Hg concentration in controls:<br>range: 0.03–14.6 μg/g<br>mean (SD):<br>dentists: 0.91 (1.47) μg/g<br>others: 0.45 (0.40) μg/g<br>Adjusted OR for CHD:<br>highest quintile of Hg compared with lowest quintile in dentists: 0.97 (95% CI, 0.63–1.50)<br>Adjusted OR for CHD:<br>highest quintile of Hg compared with lowest quintile, excluding dentists: 1.27 (95% CI, 0.62 to 2.59) |
| Hallgren et al. (2001)<br>Sweden | Case–control within a prospective cohort study | Cases: 78 men and women | Hg in erythrocytes<br>(blood samples stored in 1985 for future | First MI | Range of erythrocyte Hg concentration: 0.6–67 ng/g erythrocytes<br>Adjusted OR for MI: |

| Author/Country | Study design | Study participants | Ascertainment of mercury concentration | Disease or death | Results |
|---|---|---|---|---|---|
| | | Controls: 156 men and women, matched for age, sex, date and geographic location | research purposes, analysed 1998) | | intermediate Hg (3–6 ng/g) compared with lowest Hg (< 3 ng/g): 0.9<br>highest Hg (> 6 ng/g) compared with lowest Hg (< 3 ng/g): 0.4 (95% CI, 0.19–0.95) |
| Ahlqwist et al. (1999) Sweden[a] | Prospective cohort study of women | 1462 women, enrolled in 1968–1969 | Serum Hg (blood samples collected in 1968–69, then 1980–81 for future research; mostly used earlier samples) | MI ($n$ = 87, 39 died); all-cause death ($n$ = 253) | An inverse, but not statistically significant correlation between serum Hg and MI was found<br>A statistically significant inverse correlation between serum Hg and death from all causes was found after adjusting for age and education |
| Virtanen et al. (2005) Eastern Finland | Prospective cohort study of men, 14 year follow-up | 1871 men who were free of CVD at baseline (1984–1989) | Hair Hg (hair collected before onset of disease or death, analysed in 1992–1993) | Acute CE ($n$ = 282); death from CVD ($n$ = 132), death from CHD ($n$ = 91), all-cause death ($n$ = 525) | Hair Hg concentration: mean: 1.9 µg/g; range: 0–15.7 µg/g<br>Adjusted RR for acute CE:<br>middle third of Hg compared with lowest third: 1.1<br>highest third of Hg compared with lowest third: 1.7*<br>Adjusted RR for CVD death:<br>middle third of Hg compared with lowest third: 0.7<br>highest third of Hg compared with lowest third: 1.3<br>Adjusted RR for CHD death: |

| Author/Country | Study design | Study participants | Ascertainment of mercury concentration | Disease or death | Results |
|---|---|---|---|---|---|
| | | | | | middle third of Hg compared with lowest third: 0.6<br>highest third of Hg compared with lowest third: 1.2<br>Adjusted RR for any death:<br>middle third of Hg compared with lowest third: 0.9<br>highest third of Hg compared with lowest third: 1.3*<br>*range of 95% CI above 1.0 |

[a]This study also examined the incidence of stroke ($n = 77$), diabetes ($n = 77$), and cancer ($n = 208$).

CE: coronary event; CHD: coronary heart disease; CI: confidence interval; CVD: cardiovascular disease; Hg: mercury; MI: myocardial infarction; OR: odds ratio; RR: relative risk.

PUFA (seven studies of formula supplementation and one study of maternal dietary supplementation) were aggregated. The results of this part of the analysis estimated that increasing maternal intake of docosahexaenoic acid by 100 mg/day increases child IQ by 0.13 points (Cohen et al., 2005b). The other major component of this assessment considered the impact of prenatal exposure to methylmercury on cognitive development. This analysis aggregated results from three major prospective epidemiology studies (from the Faroe Islands, Seychelles and New Zealand) to quantify the association between prenatal methylmercury exposure and cognitive development as measured by IQ. The outcome of this analysis demonstrated that prenatal exposure to methylmercury sufficient to increase the concentration of mercury in maternal hair at parturition by 1 μg/g decreased IQ by 0.7 points. This paper identified important sources of uncertainty influencing this estimate, and concluded that the plausible range of values for this loss is 0 to 1.5 IQ points (Cohen et al., 2005a).

The final integration of the quantitative consideration of risk and benefit showed that substituting fish with high methylmercury concentrations for fish containing less methylmercury in the diet of women of childbearing age would result in developmental benefits and few negative impacts. However, if women of childbearing age instead decrease overall fish consumption, there is a substantial reduction in benefits. If other adults reduce fish consumption, the net public health impact is negative. This analysis clearly shows that in considering the risk of any environmental contaminant in a food such as fish without a thorough and robust consideration of countervailing nutritional risks can result in unintended negative public health consequences (Cohen, et al., 2005c).

## 3. DIETARY INTAKE

### 3.1 Biomarkers of exposure

#### 3.1.1 Relationships between various biomarkers of exposure

The epidemiological studies have used various biomarkers of exposure, such as the concentrations in blood, hair or cord tissue, making immediate comparisons between studies difficult. Table 2 compares the mean ratios between various biomarkers, based on recent publications, to assist in exposure comparisons between studies. The data in the table are for methylmercury unless otherwise stated.

#### 3.1.2 Cord tissue as a biomarker of exposure

In the Faroe Islands study, the main biomarkers of exposure to methylmercury, i.e. the total mercury concentrations in cord blood and maternal hair obtained at parturition from 447 births, were compared. Umbilical cord tissue mercury was found to correlate closely with mercury in cord blood. Regression analyses showed that the cord dry-weight concentration of mercury was almost as good a predictor of methylmercury-associated neuropsychological deficits at age 7 years as was the concentration of mercury in cord blood. The authors noted that

mercury in cord blood is almost entirely in the methylated form and concluded that analysis of cord tissue can be used as a valid measure of prenatal exposure to methylmercury (Grandjean et al., 2005).

The relationship between total mercury and methylmercury concentrations among umbilical cord tissue and other tissues as biomarkers of fetal exposure to methylmercury were studied in a Japanese cohort. A total of 116 paired samples were collected in three Japanese districts. Total mercury was measured in hair and cord, while methylmercury was measured in cord tissue and blood and in maternal blood. More than 90% of the mercury in cord tissue, cord blood, and maternal blood was methylmercury. Total mercury and methylmercury in cord blood was about two times higher than in maternal blood. A strong correlation was found between total mercury and methylmercury in cord tissue and between cord tissue total mercury and methylmercury and cord blood mercury (Sakamoto, et al., 2006).

*Biomarkers of exposure via human milk*

A study in rats has shown that exposure during lactation is less than during gestation and that brain mercury concentrations decline during lactation. Ten female Wistar rats were given a diet containing methylmercury at a concentration of 5 mg/kg of diet (approximately equivalent to 0.32 mg/kg bw per day) before mating and during gestation and lactation. Offspring were provided with the same diet as their mothers after weaning. All treated offspring matured, with normal body-weight gain and without overt physical signs. They exhibited locomotor and behavioural deficits at age 5–6 weeks and histopathological changes in the brain postnatally that are typical of prenatal exposure to methylmercury in rats. At birth, blood and brain mercury concentrations in the offspring were 33 and 4.5 μg/g, respectively, concentrations that were approximately 1.5-fold those of their mothers. At weaning at age 30 days, blood and brain mercury concentrations in the offspring had declined to 12 and 1 μg/g, respectively (Sakamoto et al., 2002a).

In a subsequent study, the same group evaluated the time course of changes in brain total mercury concentrations after prenatal and postnatal exposure to methylmercury via the maternal diet. Female Wistar rats were given access ad libitum to a diet containing methylmercury at a concentration of 5 mg/kg of diet (approximately equivalent to 0.32 mg/kg bw per day), and when blood mercury concentrations had almost reached a plateau, after 8 weeks, they were mated. They continued on the same diet with access ad libitum during gestation and lactation until postnatal day 20. Maternal blood concentrations were lower during pregnancy than at mating, but blood mercury concentrations in the offspring were twice as high as in the mothers on the day of birth. Postpartum, maternal blood concentrations were higher on day 10 and 20 of lactation, comparable with the values observed at mating. During suckling, blood concentrations of mercury in the offspring declined rapidly and progressively. The offspring showed no overt signs of toxicity. Fetal brain mercury concentrations were around 4–4.5 μg/g on days 18, 20, and 22 of gestation and at birth, which was about 1.5–2 times higher than corresponding brain concentrations in the mothers. Postnatally, brain mercury concentrations in the offspring declined rapidly to about one tenth of that observed at birth; mercury concentrations in offspring liver also mirrored the pattern of the brain concentrations (Pan et al., 2004).

***Table 2. Relationship between common biomarkers of exposure to methylmercury***

| Sample | Concentration [a] | Comparison (corrected for different units) [b] | Mean ratio | Reference |
|---|---|---|---|---|
| Maternal blood at delivery [c] | 0.45 µg/l | — | | Björnberg et al. (2005) |
| Cord blood [c] | 0.99 µg/l | Maternal blood at delivery | 2.2 | Björnberg et al. (2005) |
| Infant blood at age 4 days | 1.10 µg/l | Maternal blood at delivery | 2.4 | Björnberg et al. (2005) |
| Milk at age 4 days (total mercury) [d] | 0.29 µg/l | Maternal blood at delivery | 0.64 | Björnberg et al. (2005) |
| Cord blood | 22.6 µgll | — | 5.4 | Budtz-Jorgensen et al. (2004) |
| Maternal hair (full length) (total Hg) | 4.22 µg/g | Cord blood | 187 [e] | Budtz-Jorgensen et al. (2004) |
| Blood at age 7 years (total Hg) | 1.93 µg/l | — | — | Budtz-Jorgensen et al. (2004) |
| Hair at age 7 years (total Hg) | 0.6 µg/g | Blood at age 7 years | 310 [f] | Budtz-Jorgensen et al. (2004) |
| Blood at age 14 years (total Hg) | 3.81 µg/l | — | — | Budtz-Jorgensen et al. (2004) |
| Hair at age 14 years (total Hg) | 0.96 µg/g | Blood at age 14 years | 252[g] | Budtz-Jorgensen et al. (2004) |
| Cord blood (total Hg) | 22.4 µg/l | — | — | Grandjean et al. (2005) |
| Maternal hair (full length) (total Hg) | 4.17 µg/g | Cord blood | 187 | Grandjean et al. (2005) |
| Cord tissue (wet weight) (total Hg) | 0.025 µg/g | Cord blood | 1.1 | Grandjean et al. (2005) |
| Cord tissue (dry weight) (total Hg) | 0.21 µg/g | Cord blood | 9.4 | Grandjean et al. (2005) |
| Maternal blood (total Hg) | 0.52 µg/l | — | — | Jedrychowski et al. (2005) |
| Cord blood (total Hg) | 0.85 µg/l | Maternal blood | 1.6 | Jedrychowski et al. (2005) |

Hg: mercury.

[a] Mean or median as reported.

[b] Assumes that 1 l of blood or milk = 1 kg.

[c] The corresponding concentrations of inorganic mercury were 0.09 µg/l in both maternal and neonatal infant blood

[d] The concentrations in milk correlated with maternal blood inorganic mercury concentrations; other data have shown a correlation between the concentrations in milk and the number of maternal amalgam fillings (Da Costa et al., 2005).

[e] The published mean ratio based on paired data was 370.

[f] The published mean ratio based on paired data was 370.

[g] The published mean ratio based on paired data was 264.

The same group has examined the contribution of mercury exposure via human milk to methylmercury concentrations in human infants, in order to evaluate the relative risks of fetal vs postnatal exposure, based on total mercury concentrations in erythrocytes. Seven pairs of maternal and infant blood samples were compared at parturition and 3 months after parturition. Six of the mothers consumed fish two or three times per week and the other mother once a week. Five infants were reared on human milk only and two were reared on human milk, supplemented with milk formula from age 4 weeks and 6 weeks. At parturition, erythrocyte mercury concentrations were higher in umbilical cord (geometric mean, 0.011 μg/g) than in the mothers (0.007 μg/g), with a strong correlation between the two. During the period of breastfeeding, all the infants showed declines in their erythrocyte mercury concentrations, reaching a geometric mean value of 0.006 μg/g at age 3 months. Mercury concentrations in human milk (geometric mean, 0.21 ng/g) were about 20% of those in maternal plasma; there were significant correlations between concentrations of mercury in maternal plasma and in milk, and between concentrations of mercury in maternal erythrocytes and in milk, as was also reported by Skerfving (1988) and Oskarsson et al. (1996). The study authors attributed the decline in infant erythrocyte mercury to the low amounts of mercury in human milk and the rapid growth rate of the infant. Noting the correlations between maternal blood concentrations and the concentrations of mercury in human milk, the authors concluded that there need be no concerns about breastfeeding if the concentrations of methylmercury to which the offspring is exposed are low enough during gestation to avoid adverse effects on the fetus (Sakamoto et al., 2002b).

A more recent study confirmed that exposure to methylmercury is higher in the fetus than in the breastfed infant. Twenty Swedish mothers with limited consumption of fish (especially of species potentially high in mercury) and few amalgam fillings were studied, together with their infants. Exposure to mercury was low. Methylmercury and inorganic mercury were measured in the blood of the mothers and their infants at delivery and when the infants were aged 4 days and 13 weeks. Total mercury was measured in human milk collected at 4 days, 6 weeks and 13 weeks after delivery. At delivery, infant blood methylmercury concentrations were highly correlated with maternal blood concentrations, although infant blood concentrations were more than twice as high as maternal ones. The concentrations of methylmercury in infant blood declined markedly during the 13 weeks after birth from a median of 1.1 μg/l at age 4 days to 0.38 μg/l at 13 weeks. Total mercury concentrations in human milk decreased significantly from postnatal day 4 (median, 0.29 μg/l) to postnatal week 6 (median, 0.14 μg/l), but did not change thereafter. Total mercury concentrations in human milk did not correlate with maternal blood methylmercury concentrations but did correlate significantly with methylmercury concentrations in infant blood at 13 weeks. Conversely, total mercury concentrations in human milk did correlate significantly with maternal blood inorganic mercury concentrations but did not correlate with inorganic mercury concentrations in infant blood at 13 weeks. From these results the study authors concluded that exposure to both forms of mercury is higher before birth than during the breastfeeding period. Also, that although inorganic mercury is more easily transported from maternal blood into human milk than methylmercury,

methylmercury contributes more than inorganic mercury to postnatal exposure, probably because it is more readily absorbed by the infant gastrointestinal tract (Björnberg et al., 2005).

The same authors (Björnberg et al., 2005) also discuss the potential of the breastfeeding infant to excrete methylmercury. They consider it to be limited because demethylating bacteria do not appear in the infant gut until after weaning (Rowland et al., 1983). Consequently, methylmercury would be reabsorbed via the enterohepatic circulation. They found the marked decline in infant blood methylmercury concentrations during breastfeeding surprising and they recommended that further studies were needed on total excretion of methylmercury in the infant. Although they did note that the decline could be partly related to the rapidly increasing body weight and decrease in erythrocyte volume fraction in the infant postnatally, they may not have considered other factors. These include the higher affinity of methylmercury for fetal haemoglobin (IPCS, 1990; Stern & Smith, 2003), the decline in concentrations of mercury in human milk during the breastfeeding period, and the possibility of uptake of methylmercury by various tissues, including the brain. Together all these factors could contribute significantly to the marked decline of methylmercury concentrations in infant blood (Björnberg et al., 2005).

A study of mercury concentrations in samples of colostrum from human milk from urban mothers and from mothers married to fishermen in Taiwan, did not find any significant difference between the two groups, despite higher fish consumption in the wives of fishermen. The geometric mean values (and ranges) for total mercury concentrations were 2.02 (0.24–9.35) µg/l in 56 urban mothers and 2.04 (0.26–8.62) µg/l in 12 fishermen's wives, values that are comparable to previously published measurements made in other countries. Some of the urban mothers consumed more shellfish and raw fish (sushi and sashimi) meals than the fishermen's wives. The authors estimated that breastfeeding contributed 96.3–99.6% of the total mercury exposure of infants, the remainder coming from air (inhalation) or water (dermal exposure). The concentrations of mercury in colostrum corresponded to mercury intakes of around 0.3 µg/kg bw per day for urban babies, assuming intakes of 1.04 µg/day and body weights of about 3.5 kg (Chien et al., 2006).

### *3.1.3 Blood methylmercury concentrations in childhood*

The concentrations of methylmercury in hair and whole blood have been followed in the children in the Faroe Islands cohort, for whom the prenatal exposure and neurodevelopmental findings have been described and discussed in detail elsewhere (Annex 1, reference *167* ). This study was designed to further elucidate the relationship between blood and hair biomarkers for methylmercury exposure, whole blood and hair mercury concentrations in the children at age 7 and 14 years. These children were exposed both prenatally and postnatally via maternal consumption and subsequently direct consumption of fish and whale. Although questionnaire information was obtained at both ages about the frequency of whale dinners, information on this aspect of the study was not reported. The biomarker data showed that postnatal exposure of the children was substantially lower than their prenatal exposure. The geometric means (and ranges) for whole blood mercury concentrations were 1.93 (0.3–12.6) and 3.81 (0.3–39.8) µg/l at age 7 and

14 years, respectively, compared with cord blood concentrations of 22.6 (0.9–351) µg/l. Similarly, hair mercury concentrations were 0.60 (0.04–7.5) µg/g and 0.96 (0.02–9.7) µg/g at age 7 and 14 years, respectively, compared with values for full-length maternal hair at the time of birth of 4.22 (0.2–39.1) µg/g (Budtz-Jørgensen et al., 2004).

Total blood mercury has been measured in children aged 1–5 years in the USA, as part of NHANES since 1999. The findings confirm that blood mercury concentrations in young children usually are below levels of concern. During 1999–2002, the geometric mean (and 95th percentile) of total blood mercury concentrations was 0.33 µg/l (2.21 µg/l). In almost all cases, inorganic mercury was not detectable in blood, indicating that total blood mercury mostly reflected exposure to organic mercury, especially methylmercury (Centers for Disease Control and Prevention, 2004).

### *3.1.4 Blood mercury concentrations in women of childbearing age*

The NHANES has measured blood mercury concentrations in women of childbearing age in the USA since 1999. The findings confirm that the blood mercury concentrations in this population usually are below levels of concern. During 1999–2002, the geometric mean (and 95th percentile) of total blood mercury concentrations for all women of childbearing age was 0.92 µg/l (6.04 µg/l). As for children, almost all inorganic blood mercury concentrations were undetectable, indicating that total blood mercury mostly reflected exposure to methylmercury (Centers for Disease Control and Prevention, 2004).

The NHANES for the years 1999–2002 was also used to obtain population estimates of blood mercury concentrations among women of childbearing age classified as belonging to the 'other' racial/ethnic group (Asian, Pacific Islander, native American, and multiracial). Blood mercury concentrations in this group ($n$ = 140) were compared with those among all other women participants ($n$ = 3497), classified as Mexican American, non-Hispanic black, non-Hispanic white, and 'other' Hispanic. An estimated 16.6% of the group designated as Asian, Pacific Islander, native American, and multiracial had blood mercury concentrations of greater than 5.8 µg/l. Among the remaining NHANES participants, 5.1% had blood mercury concentrations greater than 5.8 µg/l (Hightower et al., 2006).

### *3.1.5 Biomarkers of co-exposure to other environmental contaminants*

The studies from the Faroe Islands have not found a significant interaction between exposure to methylmercury and co-exposure to PCBs with respect to neurodevelopment. The extent of co-exposure has been documented in recent publications, showing that exposure to PCBs in the Faroese population are generally higher than those reported elsewhere in Europe and North America, and that there is also exposure to other persistent organic pollutants, such as polybrominated diphenyl ethers (PBDEs).

Concentrations of hydroxylated polychlorinated biphenyls (OH-PCBs), PCBs and PBDEs in serum and milk samples from pregnant Faroese women and serum samples from their 7-year-old children were studied to determine the possible impact of the consumption of fatty fish and whale blubber on the body burdens of

environmental contaminants. High concentrations of OH-PCBs and PCBs were found in some of the serum samples, the respective ranges being 19–1800 ng/g lipid weight and 150–22 000 ng/g lipid weight. The relative congener distributions were similar to those observed elsewhere. 4-Hydroxy-2,2,3,4,5,5,6-heptachlorobiphenyl was the most abundant OH-PCB metabolite in all samples analysed, with four other OH-PCB congeners as the dominant metabolites. More than 25 additional OH-PCBs were detected. Maternal serum was dominated by 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), while 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153) prevailed in the children's serum 7 years later. PBDE was present in serum of both mothers and children up to 3 and 6 ng/g lipid weight, respectively. Concentrations of the 18 PCB congeners analysed in the children's serum averaged about 60% of that in their mothers, with median concentrations for both beign greater than 1 µg/g lipid weight and with similar PCB congener patterns. Serum concentrations of OH-PCBs from the mothers and their children showed ranges of 1.8–36 ng/g wet weight and 0.49–22 ng/g wet weight, respectively, with nearly all OH-PCB congener concentrations being lower in the children. In milk samples OH-PCBs were present in trace amounts only, at levels of approximately 1% of the PCB concentrations. PBDE concentrations showed a clear increase over time, and their concentrations in human milk are among the highest reported so far from Europe, with results of individual samples ranging from 4.7 to 13 ng/g lipid weight. The PBDE concentrations were similar in both mothers and their children. This study confirmed the presence of high concentrations of organohalogen substances in the Faroese population (Fangstrom et al., 2002, 2005a, 2005b).

In a follow-up study of the main birth cohort in the Faroe Islands study, data were collected by questionnaire on potential predictors of persistent organic pollutant concentrations, such as duration of breastfeeding and blubber consumption. Exposure to PCBs (37 congeners), to *p,p'*-dichlorodiphenyl trichloroethane (*p,p*'-DDT) and its primary degradate *p,p'*-dichlorodiphenyl dichloroethylene (*p,p*'-DDE) was assessed from in utero until age 14 years, using 316 umbilical cord samples, 124 serum samples collected from participants at approximately age 7 years, and 795 serum samples collected from participants at age 14 years. Measurements of more highly chlorinated PCB congeners made on individuals' serum samples collected at age 7 and 14 years were highly correlated. Concentrations at age 7 years were generally two to three times higher than at age 14 years. Umbilical cord tissue PCB concentrations were correlated with PCB concentrations in serum samples at age 7 and at age 14 years. Duration of breastfeeding and consumption of blubber were significant predictors of serum PCB concentrations at age 7 and 14 years. Multivariate analyses showed that breastfeeding duration was the primary contributor to serum concentrations of PCBs at age 7 years, and blubber consumption was the primary contributor at age 14 years. Thus although consumption of pilot whale blubber appears to be a significant source of methylmercury and of PCBs, human milk is only a significant source for PCBs (Barr et al., 2006).

### *3.1.6 Consumption of fish and whale and exposure to methylmercury*

After an official recommendation in the Faroe Islands that women should abstain from eating pilot whale because of mercury contamination, a survey was carried out to obtain information on dietary habits and hair samples for mercury

analysis. A letter was sent to all 1180 women aged 26–30 years who resided within the Faroes, and the women were contacted again 1 year later. A total of 415 women responded to the first letter; the second letter resulted in 145 repeat hair samples and 125 new responses. Questionnaire results showed that Faroese women, on average, consumed whale meat for dinner only once every second month, but the frequency and meal size depended on the availability of whale in the community. The geometric mean hair-mercury concentration at the first survey was higher in districts with available whale than in those without (3.03 vs 1.88 μg/g; $p = 0.001$). The concentration of mercury also depended on the frequency of whale meat dinners and on the consumption of dried whale meat. The 36 women who did not eat whale meat at all had a geometric mean hair-mercury concentration of 1.28 μg/g. At the time of the second survey, the geometric mean had decreased to 1.77 μg/g ($p < 0.001$), although whale was now available in all districts. In comparison with previously published data on hair-mercury concentrations in pregnant Faroese women, these results document substantially lower exposures as well as a further decrease temporally associated with the issue of stricter dietary advice (Weihe et al., 2005).

Fetal exposure to mercury was determined in 308 women in the Hawaii Islands by measuring concentrations of mercury in cord blood and analysing the association with fish consumption during pregnancy. Of the 308 women who were enrolled, 275 completed a dietary survey. The mean mercury concentration in cord blood was 4.82 μg/l. A significant relationship was noted between the amount of fish consumed during pregnancy and elevated mercury concentrations in cord blood (Sato et al., 2006).

The impact of fish consumption on the nutritional status of Indian children of eastern Amazonia has been evaluated. Weight-for-height Z score was measured, and hair mercury was determined in 203 children (age < 10 years) in three villages. There was significantly higher consumption of fish in Kayabi children (16.6 μg/g, as measured by hair mercury concentration) than in children of the villages of Missao-Cururu (4.8 μg/g) and Kaburua (2.9 μg/g). Mean weight-for-height Z scores of -0.27, -0.22, and 0.40 were noted for Kayabi, Missao-Cururu and Kaburua villages, respectively. There was no significant correlation between weight-for-height Z score and total hair mercury concentration (Dorea et al., 2005a).

The relationship between dietary habits and exposure to mercury was studied in a cohort of Chinese children. The hair and blood mercury concentrations of children aged more than 3 years were studied. Sociodemographic data, dietary habits of the past 6 months, and other risk factors for environmental mercury exposure were collected from the 137 Chinese children (mean age, 7.2 years) recruited. The mean hair mercury concentration was 2.2 μg/g and the mean blood mercury concentration was 17.6 nmol/l (3.8 μg/l). There was a strong correlation ($r = 0.88$) between hair and blood mercury concentrations. Frequency of fish consumption correlated with hair ($r = 0.51$) and blood ($r = 0.54$) mercury concentrations. For those children who consumed fish more than three times per week, hair and blood mercury concentrations were twice as high as in those who consumed fish one to three times per week, and three-fold higher than in those who never consumed fish. Both blood and hair (i.e. tissue) mercury concentrations of children in Hong Kong were elevated and correlated with the frequency of fish consumption (Ip et al., 2004b).

In the 1999–2000 NHANES, 1250 children aged 1 to 5 years and 2314 women aged 16 to 49 years were selected to participate in the survey. The data gathered are based on analysis of cross-sectional data for the non-institutionalized, US household population and are considered to be nationally representative. The survey consisted of interviews conducted in participants' homes and standardized health examinations conducted in mobile examination centres. Household interviews, physical examinations, and blood-mercury concentration assessments were performed on 705 children (response rate, 56%) and 1709 women (response rate, 74%). Blood mercury concentrations were approximately three times higher in women than in children. The geometric mean concentration of total blood mercury was 0.34 µg/l in children and 1.02 µg/l in women. Geometric mean mercury concentrations were almost four times higher among women who had eaten three or more servings of fish in the past 30 days compared with women who ate no fish in that period (1.94 µg/l vs 0.51 µg/l) (Schober, 2003).

Exposure to mercury was also assessed in 838 US children, aged 1–5 years, and 1726 women, aged 16–49 years, using hair mercury analysis during the 1999–2000 NHANES. The association of hair mercury concentrations with sociodemographic characteristics and fish consumption was reported. Geometric mean hair mercury was 0.12 µg/g in children, and 0.20 µg/g in women. Among frequent fish consumers, geometric mean hair mercury concentrations were three times higher for women (0.38 vs 0.11 µg/g) and twice as high for children (0.16 vs 0.08 µg/g) compared with non-consumers. Hair mercury concentrations were associated with age and frequency of fish consumption (McDowell et al., 2004).

#### *3.1.7 Consumption of other foods and exposure to methymercury*

The use of fish meal as a source of protein for poultry and swine may lead to exposure to methylmercury in addition to that which arises from the consumption of fish. A study in Sweden measured concentrations of inorganic mercury and methylmercury in blood in 9 men and 18 women, aged 20–58 years, who stated that they had consumed no fish for a period of 2 years or more. Participants answered a food-frequency questionnaire and reported number of dental amalgam fillings. Methylmercury concentrations in blood were very low. A significant association between the number of dental amalgam fillings and the inorganic mercury concentration in blood was found. Total hair mercury concentration was significantly associated with methylmercury in blood, but not with the inorganic mercury (Lindberg et al., 2004).

### *3.2 The effect of GLs for methylmercury on exposure and risk*

CCFAC at its Thirty-seventh Session (Codex Alimentarius Commission, 2005) asked the Committee to consider the impact on exposure and risk of the current GLs for methylmercury in fish, set at 1 mg/kg for predatory fish, and 0.5 mg/kg for non-predatory fish.[1]

[1] See http://www.codexalimentarius.net/download/standards/21/CXG_007e.pdf

### 3.2.1 The nature of GLs

Previous meetings of the Committee have considered the dietary impact of limits for other contaminants on exposure. For instance, differing GLs for aflatoxin $M_1$ and ochratoxin A were evaluated by the Committee at its fifty-sixth meeting in 2001 (Annex 1, reference 152). In each of these examples, the effect of setting limits was found to be minimal, only affecting exposure at the extreme tail of the intake distributions. It has been noted at previous meetings of the Committee that using GLs to influence exposure is, in general, not effective until a large fraction of the affected commodity has been withdrawn from the marketplace.

A GL is risk management tool set to protect a consumer of an affected food from exposure to a toxic dose of a substance. For substances with acutely toxic effects, GLs that are enforced can limit exposure. However, when the toxic effect of a substance results from long-term exposure, GLs will have a much smaller protective effect as the consumer will be exposed to the substance at the mean level of its distribution in the affected food[2]. The shape of the distribution of the levels of the substance in affected food will determine the extent of impact of a GL on exposure. The further the GL is set from the average of the distribution, the smaller the impact that GL will have on exposure.

In responding to the CCFAC request concerning the dietary impact of GLs for methylmercury, the Committee noted that no alternatives to the current GLs are under consideration. The situation is complex as many fish species are consumed as food and the terms predatory and non-predatory species have not been clearly defined. These terms are primarily used to designate fish that are higher on the food chain from those lower on the chain. In practice, non-predatory species are those typically containing low average concentrations of methylmercury and predatory species are those with higher average concentrations. This is owing to the bioaccumulation of methylmercury, especially in larger, older fish. Another complicating factor is the presumed non-random nature of consumer choice among fish species, thus fish eaters will experience some weighted average of mercury concentrations across species. Because of the nature of mercury accumulation in different species, GLs will have differing impacts on individual species, and thus on overall exposure based on the individual's choices.

Default assumptions were required for this analysis of the dietary impact of GLs. The Committee chose to compare the current situation with a scenario where no GLs are in effect or are enforced.

### 3.2.2 Data submitted to the Committee

The Committee received dossiers containing total mercury and/or methylmercury levels in finfish and/or shellfish from France, Japan, and the United Kingdom (UK), and obtained additional analytical data from the USA and from the published literature. Each of the dossiers contained analyses on individual samples. Additionally, two papers were reviewed from the USA and France, which considered

[2] This assumes that the portions consumed over a lifetime are of approximately the same magnitude, as would be expected for a primary food in the diet.

***Table 3. Data on total mercury in finfish, from the UK***

*Mean values:*

| Samples | Mean (mg/kg) | No. of samples |
|---|---|---|
| All samples | 0.25 | 282 |
| < 1.0 mg/kg | 0.14 | 263 |
| < 0.5 mg/kg | 0.09 | 240 |

*Values within species*

| Species | No. of samples | Mean (mg/kg) | | % Reduction |
|---|---|---|---|---|
| | | All samples | Violative samples removed | |
| *> 1.0 mg/kg* | | | | |
| Shark | 5 of 5 | 1.52 | 0.00 | 100 |
| Tuna | 1 of 74 | 0.25 | 0.23 | 8 |
| Swordfish | 11 of 17 | 1.36 | 0.43 | 68 |
| Marlin | 2 of 4 | 1.09 | 0.51 | 53 |
| *> 0.5 mg/kg* | | | | |
| Shark | 5 of 5 | 1.52 | 0.00 | 100 |
| Tuna | 11 of 74 | 0.25 | 0.17 | 32 |
| Swordfish | 13 of 17 | 1.36 | 0.22 | 84 |
| Orange roughy | 6 of 6 | 0.60 | 0.00 | 100 |
| Halibut | 2 of 8 | 0.29 | 0.19 | 34 |
| Ice fish | 1 of 1 | 0.66 | 0.00 | 100 |
| Marlin | 3 of 4 | 1.09 | 0.41 | 62 |

United Kingdom (2006) Dossier on fish mercury concentrations submitted to the Committee. UK, United Kingdom

risk management options for controlling exposure to methylmercury (Carrington, 2004; Crépet, 2005).

*Data from the UK*

The dossier from the UK (Food Standards Agency) contained analytical results on total mercury from 336 samples, comprising 282 finfish and 54 shellfish, collected in 1999–2002. Because the maximum concentration found in shellfish was only 0.25 mg/kg, one half the GL for non-predatory fish, they will not be considered further. The only species found to contain total mercury at concentrations greater than 1.0 mg/kg were shark, swordfish, marlin, and tuna. Three additional species, orange roughy, halibut, and Atlantic icefish, were found to contain total mercury at concentrations greater than 0.5 mg/kg (Table 3).

***Table 4. Data on total mercury in finfish, from France***

*Mean values:*

| Samples | Mean (mg/kg) | No. of samples |
|---|---|---|
| All samples | 0.099 | 1254 |
| < 1.0 mg/kg | 0.089 | 1245 |
| < 0.5 mg/kg | 0.077 | 1219 |

*Values within species*

| Species | No. of samples | Mean (mg/kg) | | % Reduction |
|---|---|---|---|---|
| | | All samples | Violative samples removed | |
| *> 1.0 mg/kg* | | | | |
| Tuna | 2 of 64 | 0.32 | 0.28 | 12 |
| Shark | 1 of 16 | 0.43 | 0.31 | 28 |
| Barracuda | 5 of 14 | 0.85 | 0.59 | 31 |
| Ray | 1 of 23 | 0.16 | 0.09 | 44 |
| *> 0.5 mg/kg* | | | | |
| Tuna | 13 of 64 | 0.32 | 0.21 | 34 |
| Shark | 4 of 16 | 0.43 | 0.23 | 47 |
| Barracuda | 10 of 14 | 0.85 | 0.23 | 73 |
| Ray | 1 of 23 | 0.16 | 0.09 | 44 |
| Rainbow trout | 1 of 604 | 0.05 | 0.05 | 0 |
| Marine fish | 5 of 91 | 0.18 | 0.16 | 11 |
| Ling | 1 of 14 | 0.26 | 0.24 | 8 |

France (2006) Dossier on fish mercury concentrations submitted to the Committee.

*Data from France*

The dossier submitted by France contained analytical results on total mercury from 1661 samples; 1254 finfish and 407 shellfish, collected in 1998–2002. Because the maximum concentration found in shellfish was only 0.43 mg/kg, 80% of the GL for non-predatory fish, shellfish were not considered further. The only species found to contain mercury at concentrations greater than 1.0 mg/kg were shark, tuna, barracuda, and ray. Three additional species were found to contain mercury at concentrations greater than 0.5 mg/kg—rainbow trout, marine fish, and ling (Table 4).

*Data from the USA*

The information received from the USA contained analytical results on total mercury from 952 samples, comprising 903 finfish and 49 shellfish, collected in 2002–2005. The maximum concentration found in shellfish was 0.30 mg/kg, which

***Table 5. Data on total mercury in finfish, from the USA***

*Mean values:*

| Samples | Mean (mg/kg) | No. of samples |
|---|---|---|
| All samples | 0.27 | 903 |
| < 1.0 mg/kg | 0.25 | 885 |
| < 0.5 mg/kg | 0.19 | 780 |

*Values within species:*

| Species | No. of samples | Mean (mg/kg) | | % Reduction |
|---|---|---|---|---|
| | | All samples | Violative samples removed | |
| *> 1.0 mg/kg* | | | | |
| Swordfish | 10 of 14 | 1.33 | 0.72 | 46 |
| Tuna | 3 of 531 | 0.26 | 0.26 | 0 |
| Grouper | 2 of 30 | 0.45 | 0.40 | 11 |
| Snapper | 1 of 26 | 0.18 | 0.14 | 22 |
| Bass | 1 of 52 | 0.32 | 0.28 | 13 |
| Sable fish | 1 of 2 | 0.64 | 0.22 | 66 |
| *> 0.5 mg/kg* | | | | |
| Swordfish | 13 of 14 | 1.33 | 0.16 | 88 |
| Tuna | 54 of 531 | 0.26 | 0.22 | 15 |
| Grouper | 8 of 30 | 0.45 | 0.29 | 36 |
| Snapper | 1 of 26 | 0.18 | 0.14 | 22 |
| Bass | 9 of 52 | 0.32 | 0.21 | 34 |
| Sable fish | 1 of 2 | 0.64 | 0.22 | 66 |
| Orange roughy | 17 of 29 | 0.54 | 0.40 | 26 |
| Weakfish | 4 of 24 | 0.24 | 0.16 | 33 |
| Blackfish | 6 of 6 | 0.61 | 0.00 | 100 |
| Char | 2 of 26 | 0.08 | 0.04 | 50 |
| Bluefish | 6 of 30 | 0.35 | 0.31 | 14 |
| Halibut | 1 of 14 | 0.23 | 0.21 | 9 |
| Tilefish | 1 of 14 | 0.13 | 0.11 | 15 |

United States (2006) Private communication on fish mercury concentrations made to the Committee.

is 60% of the GL for non-predatory fish; these results were not considered further. The only species found to contain mercury at concentrations greater than 1.0 mg/kg were swordfish, tuna, sablefish, bass, and snapper. Seven additional species, orange roughy, bluefish, blackfish, weakfish, char, halibut, and tilefish, were found to contain mercury at concentrations greater than 0.5 mg/kg (Table 5).

*Data from Japan*

The information received from Japan contained aggregated analytical results on total mercury and methylmercury from 227 fish species (including marine mammals) and shellfish. There were 6707 samples (1146 from marine mammals) and 918 shellfish, collected in 2003–2005. The fish species found to contain total mercury at concentrations greater than 1.0 mg/kg were dolphin (marine mammal), whale (marine mammal), tuna, alfonsino (related to red snapper), swordfish, porpoise (marine mammal), bluefish, halibut, and grouper. Additionally, the following species were found to have samples containing total mercury at concentrations greater than 0.5 mg/kg: rockfish, bluefish, snapper, marlin, stargazer, shark, sablefish, thornyhead, saucord, flounder, yellowtail, fusiliner, seaperch, brotula, sea bream, mackerel, and John Dory. Because these data were aggregated, it is not possible to determine the number of samples or the percentage within a species that exceeded either of the two limits. However, the species that contained mercury at average concentrations of greater than 1.0 mg/kg were dolphin, whale, marlin, porpoise (all marine mammals), and grouper. The species containing mercury at average concentrations greater than 0.5 mg/kg were swordfish, snapper, alfonsino, and shark.

Methylmercury concentrations were also measured in many of the samples. Species found to contain methylmercury at concentrations greater than 1.0 mg/kg were dolphin, whale, tuna, alfonsino, and swordfish. Yellowtail, seaperch, grouper, shark, sablefish, thornyhead, flounder, snapper, marlin, porpoise, saucord, marlin, rockfish, bluefish, and stargazer were found to have samples containing methyl mercury at concentrations greater than 0.5 mg/kg. Again, because these were aggregated data, it was not possible to determine the number of samples or the percentage within each species above either of the two limits.

Individual sample raw data were submitted for a number of species known to contain higher levels of mercury. These were tuna, marlin, swordfish, alfonsino, sablefish shark, red snow crab, and the finely-striated buccinum, a gastropod. Of the 501 individual samples measured, 53 (11%) were found to contain total mercury at greater than 1.0 mg/kg, with 208 (40%) greater than 0.5 mg/kg. The average mercury content of this sub-sample was 0.54 mg/kg. Exclusion of the samples containing mercury at greater than 1.0 mg/kg reduces the average for the remaining samples to 0.42 mg/kg, while exclusion of all samples containing mercury at greater than 0.5 mg/kg lowers the average to 0.27 mg/kg.

The dossier from Japan contained data on the consumption of fish and fish species known to be high in mercury (Table 6). The data were compiled in 2001 and 2002. Overall mean consumption of fish was found to be approximately 80 g/person per day, with consumption at the 90th percentile being approximately 170 g/person per day. It is important to note that, with the exception of tuna, both canned and fresh (approximately 40% eaters), no species is consumed by more than 5% of the population surveyed, with all except bream species being consumed by less than 1% of the survey population. Intakes and percentages of eaters were not significantly different for the population subgroups, women aged 19–45 years, and all women aged more than 20 years.

***Table 6. Data on total mercury in fish and marine mammals, from Japan***

| Species of fish or marine mammal consumed | All people who ate fish or marine mammals | | | | |
|---|---|---|---|---|---|
| | Average (g/day) | 50th percentile (g/day) | 90th percentile (g/day) | 95th percentile (g/day) | No. of people |
| Average for all fish | 82.7 | 72.0 | 168.0 | 208.1 | 18839 |
| Blue marlin and striped marlin | 69.8 | 70.0 | 100.0 | 138.5 | 135 |
| Alfonsino | 79.2 | 71.0 | 133.3 | 160.0 | 144 |
| Shark species | 76.3 | 64.0 | 125.0 | 125.0 | 6 |
| Bream species | 56.2 | 43.7 | 105.0 | 140.0 | 708 |
| Tuna species | 26.3 | 18.8 | 65.3 | 100.0 | 6043 |
| Big-eye | 88.0 | 76.0 | 125.0 | 250.0 | 38 |
| Marine mammals | 94.1 | 82.0 | 150.0 | 250.0 | 17 |
| Canned tuna | 21.6 | 17.5 | 50.0 | 60.0 | 1461 |

Japan (2006) Dossier on fish mercury concentrations submitted to the Committee.

### *3.2.3 The impact of risk management options on exposure*

The Committee considered two recent publications that dealt directly with the present analysis. A paper from the USA evaluated exposure scenarios based on curtailing or removing exposure to mercury from fish species known to contain mercury at typically high levels (Carrington, 2004). This paper used food intake data from the USA with mercury analyses of domestic (USA) and imported seafood. The scenarios evaluated exposure based on limits on seafood consumption of 6, 12 or 18 ounces per week, replacing fish containing mercury at high concentrations with fish containing mercury at intermediate or low concentrations, and considered exposure when no limits on consumption were necessary, but when fish containing mercury at high concentrations were excluded from the diet. These scenarios bracket the advice given to seafood consumers in the USA concerned with reducing exposure to mercury[3]. Fifty-one types of finfish and nine shellfish were included. Of these, 11 finfish had maximum mercury concentrations greater than 1.0 mg/kg. Gulf tilefish was the only type to have a mean concentration greater than 1.0 mg/kg. Additionally, shark, swordfish, king mackerel, grouper, and orange roughy had mean mercury concentrations greater than 0.5 mg/kg. Water loss on preparation and market share were also taken into consideration in the exposure modelling.

[3] The advice issued in the USA concerning mercury recommends that pregnant women (or those planning on becoming pregnant) avoid consuming shark, swordfish, king mackerel, and tilefish, and include up to 12 ounces per week of a variety of other fish in their diets.

The paper concludes: "In general, reducing overall fish consumption appears to have more impact on the overall population distributions than reducing or eliminating levels of high-level mercury species only." With respect to the current analysis, reducing or eliminating high-level mercury species is more drastic than simply removing fish of those species in which the GL is exceeded, which simply reduces the mean of the remaining distribution of mercury levels in those species.

A second paper, published in 2005, considers the question in the current analysis, the effect of enforced GLs on exposure, and the effect of excluding or limiting predatory fish intake, as in the analysis by Carrington et al., discussed above (Crépet, 2005). In this publication, exposure to methylmercury was determined using French data on food intake and analyses of fish and fishery products available in the French marketplace. Mean mercury contamination levels from 89 individual food items (from a total of 2818 individual analyses) containing fish were used and combined with appropriate 7-day average food intakes for the 89 items. Correction factors (0.84 for finfish, 0.36 for shellfish and 0.43 for molluscs) were applied to convert total mercury analyses to methylmercury levels in each food. Actual body weights from surveyed consumers in the food intake survey were used.

In one scenario, all predatory fish containing mercury at concentrations greater than 1.0 mg/kg and all non-predatory fish (includes all shellfish and molluscs) with mercury concentrations greater than 0.5 mg/kg were excluded and the mean mercury concentration of the food type was recalculated. In this scenario, 2.3% of predatory fish samples, tuna, shark, swordfish, rays, and marlin, would be excluded. In a second scenario, all fish containing mercury at concentrations greater than 0.5 mg/kg were excluded. In this scenario, 8.8% of predatory fish samples would be excluded. In the first scenario, only 27 fish food types had their mean mercury concentration lowered by the exclusion, most by less than 15%. The largest reduction, of 42%, was for fried, simmered, or oven-baked skate. The second, more drastic scenario resulted in the further lowering of the mean methylmercury concentration of 18 fish food types, but did not lower the mean of any food type not affected by the conditions of the first scenario.

Methylmercury exposures in each scenario were calculated for children aged 3–6 years and 7–10 years, and for women of childbearing age, as were the probabilities of exceeding the PTWI of 1.6 µg/kg bw (Annex 1, reference 166). In the first scenario, there were no significant differences between the probabilities of exceeding the PTWI from compliance with the GLs or baseline (no GLs). In the second scenario, for children aged 3–6 or 7–10 years there were also no significant differences in the probabilities of exceeding the PTWI, but the difference for women of childbearing age did reach significance (4.4% for no GLs vs 0.6 for a uniform GL of 0.5 mg/kg). The authors note that the impact of reducing exposure to predatory fish would be greater for women of childbearing age because products derived from predatory fish make up a larger proportion of their diets than in the case of children, and is a larger vector of exposure for those that would exceed the PTWI (23% for children vs 70% for women).

## 4. COMMENTS

### 4.1 Vulnerability of the embryo and fetus

The Committee noted that the new toxicokinetic, toxicological and epidemiological studies available since its previous evaluation in 2003 further confirmed the embryo and fetus as the most vulnerable stage of life with respect to the adverse effects of methylmercury. The new data do not suggest the need for revision of the previously established PTWI of 1.6 µg/kg bw, with respect to maternal intakes and this life stage.

### 4.2 Vulnerability of the infant and child

In reviewing the available studies relevant to risk assessment for infants and young children exposed after birth via human milk and via the diet in general, the Committee noted that few studies have attempted to separate the potential effects of postnatal exposure to methylmercury from the known neurodevelopmental effects of prenatal exposure.

There is clear evidence from the concentrations of mercury in human milk and in the blood of infants that, compared with exposure in utero, postnatal exposure to methylmercury is considerably lower in infants who are breastfed and, similarly, postnatal exposure is lower in those that are formula-fed. The Faroe Islands study reported earlier developmental milestones in breastfed compared with formula-fed infants and lack of any independent association between breastfeeding and neurological deficits at age 7 years. The study authors suggested that breastfeeding is beneficial even in a population with a relatively high prenatal exposure to methylmercury because of maternal consumption of fish and whale. This suggestion is compatible with other extensive data showing that breastfeeding per se offers benefits for cognitive development.

It is clear from the earlier major poisoning incidents in Japan and Iraq that methylmercury did cause neurotoxicity when exposure of children was limited to the postnatal period. However, the incidents do not give much insight into the question of whether children may be more vulnerable than adults to exposure at low levels, since in most cases there was prenatal as well as postnatal exposure to methylmercury and the exposures were very high. Similarly, while monkeys exposed to methylmercury from birth to early adulthood (age 7 years), but not exposed in utero, showed deficits in fine motor control (clumsiness) beginning in middle age and restrictions in visual fields during old age, the exposure levels in those studies, at 50 µg/kg bw per day, were high relative to dietary exposures in humans. Data from the Faroe Islands have suggested a subtle but measurable effect of postnatal exposure on latency in a single interpeak interval in brainstem auditory evoked potentials measured at age 14 years. The health significance of this observation, if any, remains unclear.

Knowledge of human brain development raises the theoretical possibility of continuing vulnerability to neurodevelopmental effects from postnatal exposure to methylmercury, but there is no clear evidence on this. For example, the influence of exposure to methylmercury on synaptogenesis, which continues well into

adolescence in humans, is not known. However, in rats given methylmercury as a single, high, oral dose at 8 mg/kg bw administered by gavage during the late fetal period, synaptogenesis had been shown to be affected. Similarly, both neuronal myelination and remodelling of the cortex of the brain occur postnatally in humans and have a protracted time course, continuing through adolescence until about age 17 years, but again there was no evidence as to whether exposures to methylmercury at low levels might affect these potentially vulnerable processes.

In summary, there are insufficient data from the studies previously reviewed by the Committee and the more recent studies reviewed at the present meeting to draw conclusions regarding the vulnerability of infants and children to methylmercury. It is clear that they are not more vulnerable than the embryo and fetus, but the information available to date does not enable any firm conclusions to be drawn on whether infants and children, including adolescents, are more, or less, vulnerable than adults.

### *4.3 Vulnerability of adults*

For adults, the previously established PTWI of 3.3 µg/kg bw, which was revised in 2003, was regarded by the Committee in 1988 at its thirty-third meeting (Annex 1, reference 83) as adequate to take account of neurotoxicity, excluding developmental neurotoxicity; the Committee at its present meeting considered that this remained the case. Concerning other health aspects, the Committee gave further consideration to previous and more recent studies on methylmercury exposure and cardiovascular findings and concluded that the weight of evidence at the current time did not indicate an increased risk of adverse cardiovascular events. The Committee also noted that fish consumption in general is associated with cardiovascular benefits.

### *4.4 Impact of current GLs for methylmercury in fish on exposure and risk*

The Committee evaluated the impact of current Codex GLs for methylmercury in fish (predatory fish, 1.0 mg/kg; non-predatory fish, 0.5 mg/kg) on exposure and risk. Submissions were received from France, Japan, and the UK, and additional information on the distribution of mercury and methylmercury in various fish species was obtained from the USA. Additionally, two recent publications concerning risk management options for the control of exposure to methylmercury were considered by the Committee.

Previous Committees have noted that excluding foodstuffs containing a contaminant at a concentration that is at the high end of a log-normal distribution of concentrations is not an effective method for reducing overall exposure to that contaminant in the general population. Large proportions of foodstuffs must be excluded from the market before the average exposure to the contaminant is significantly reduced. The data from France, Japan, the UK and the USA reviewed at this meeting support this conclusion for methylmercury in fish. In each of those countries, the total market—and hence the total distribution of methylmercury in seafood—is dominated by species that do not contain a high concentration of mercury. If it were the case that seafood consumers randomly chose from the total

market over their lifetime, their mean level of exposure to methylmercury in seafood would not be substantially reduced by excluding fish containing methyl-mercury at concentrations greater than the GLs of 1.0 mg/kg for predatory fish and 0.5 mg/kg for non-predatory fish, and the numbers of individuals exposed to methylmercury at intakes greater than the PTWI would not be lowered significantly.

For individual consumers whose preferred choice of fish comprises species that are known to accumulate methylmercury at higher concentrations, exclusion from their diets of all fish found to exceed the GLs may significantly limit their total exposure to methylmercury. The information submitted by France, the UK and the USA showed that excluding fish samples found to contain methylmercury at concentrations greater than the current Codex GLs would reduce the mean concentration of methylmercury in those species by 30–100% in fish available on the market. This would, however, be at the cost of removing the majority of samples of those species from the market. The French analysis suggests that the impact of those exclusions on an individual's intake of methylmercury may not be great, as the percentage of women exceeding the PTWI for methylmercury would only be reduced significantly if all fish containing methylmercury at concentrations greater than 0.5 mg/kg (one half of the GL for predatory species) were removed from their diets, while the percentage of children aged 3–10 years with exposures greater than the PTWI would still not be significantly reduced.

In other populations (e.g. Japan, where the mean consumption of seafood in the population is higher than that in France, the UK or the USA), exclusion from the population diet of all fish exceeding the Codex GLs may have a greater impact on the percentage of individuals with exposures greater than the PTWI. It was not possible from the data submitted by Japan to determine the percentage of samples in the Japanese market that exceeded the current Codex GLs for each marine species, and therefore, not possible to estimate the extent or significance of any reduction in exposure to methylmercury resulting from the removal of such fish from the market. The species containing the highest concentrations of methylmercury are not consumed by large percentages of the Japanese population, suggesting that GLs would not be very effective in reducing the overall number of vulnerable individuals in the population who would have exposures greater than the PTWI.

## *5. EVALUATION*

At its present meeting, the Committee made it clear that the previous PTWI of 3.3 µg/kg bw had, in fact, been withdrawn in 2003. The Committee confirmed the existing PTWI of 1.6 µg/kg bw, set in 2003, based on the most sensitive toxicological end-point (developmental neurotoxicity) in the most susceptible species (humans). However, the Committee noted that life stages other than the embryo and fetus might be less sensitive to the adverse effects of methylmercury.

In the case of adults, the Committee considered that intakes of up to about two times higher than the existing PTWI of 1.6 µg/kg bw would not pose any risk of neurotoxicity in adults, although in the case of women of childbearing age, it should be borne in mind that intake should not exceed the PTWI, in order to protect the embryo and fetus.

Concerning infants and children aged up to about 17 years, the data did not allow firm conclusions to be drawn regarding their sensitivity compared with that of adults. While it was clear that they are not more sensitive than the embryo or fetus, they might be more sensitive than adults because significant development of the brain continues in infancy and childhood. Therefore, the Committee could not identify a level of intake higher than the existing PTWI that would not pose a risk of developmental neurotoxicity for infants and children.

The Committee had previously noted that fish makes an important contribution to nutrition, especially in certain regional and ethnic diets. The present Committee recommended that the known benefits of fish consumption need to be taken into consideration in any advice aimed at different subpopulations. Risk managers might wish to consider whether specific advice should be given concerning children and adults, after weighing the potential risks and benefits. The Committee was unable to offer any further advice in this regard since it is not within its remit to examine the beneficial aspects of fish consumption. The Committee also noted that the relative benefits of fish consumption will vary from situation to situation, depending on, for instance, the species of fish consumed and the relative nutritional importance of fish in the diet.

The Committee concluded that the setting of GLs for methylmercury in fish may not be an effective way of reducing exposure for the general population. The Committee noted that advice targeted at population subgroups that might be at risk from methylmercury exposure could provide an effective method for lowering the number of individuals with exposures greater than the PTWI.

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# ANNEXES

## *ANNEX 1*

## *REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES*

1. **General principles governing the use of food additives** (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. **Procedures for the testing of intentional food additives to establish their safety for use** (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. **Specifications for identity and purity of food additives (Antimicrobial preservatives and Antioxidants)** (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as **Specifications for identity and Purity of food additives, Vol. I. Antimicrobial preservatives and Antioxidants**, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. **Specifications for identity and purity of food additives (Food colours)** (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. **Evaluation of the carcinogenic hazards of food additives** (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. **Evaluation of the toxicity of a number of antimicrobials and antioxidants** (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. **Specifications for the identity and purity of food additives and their toxicological evaluation: Emulsifiers, stabilizers, bleaching and maturing agents** (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. **Specifications for the identity and purity of food additives and their toxicological evaluation: Food colours and some antimicrobials and antioxidants** (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. **Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants.** FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. **Specifications for identity and purity and toxicological evaluation of food colours.** FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. **Specifications for the identity and purity of food additives and their toxicological evaluation: Some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases** (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. **Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.** FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. **Specifications for the identity and purity of food additives and their toxicological evaluation: Some emulsifiers and stabilizers and certain other substances** (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. **Specifications for the identity and purity of food additives and their toxicological evaluation: Some flavouring substances and non nutritive sweetening agents** (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. **Toxicological evaluation of some flavouring substances and non nutritive sweetening agents**. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. **Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents**. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. **Specifications for the identity and purity of food additives and their toxicological evaluation: Some antibiotics** (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. **Specifications for the identity and purity of some antibiotics**. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. **Specifications for the identity and purity of food additives and their toxicological evaluation: Some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances** (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. **Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances**. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. **Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives**. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. **Evaluation of food additives: Specifications for the identity and purity of food additives and their toxicological evaluation: Some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents**. (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. **Toxicological evaluation of some extraction solvents and certain other substances**. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. **Specifications for the identity and purity of some extraction solvents and certain other substances**. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. **A review of the technological efficacy of some antimicrobial agents**. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. **Evaluation of food additives: Some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants** (Fifteenth report of the Joint FAO/

WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. **Toxicological evaluation of some enzymes, modified starches, and certain other substances**. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. **Specifications for the identity and purity of some enzymes and certain other substances**. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
29. **A review of the technological efficacy of some antioxidants and synergists**. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
30. **Evaluation of certain food additives and the contaminants mercury, lead, and cadmium** (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. **Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate**. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. **Toxicological evaluation of certain food additives with a review of general principles and of specifications** (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. **Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents**. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. **Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers**. FAO Food and Nutrition Paper, No. 4, 1978.
35. **Evaluation of certain food additives** (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
36. **Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives**. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. **Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives**. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. **Evaluation of certain food additives: Some food colours, thickening agents, smoke condensates, and certain other substances**. (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
39. **Toxicological evaluation of some food colours, thickening agents, and certain other substances**. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. **Specifications for the identity and purity of certain food additives**. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. **Evaluation of certain food additives** (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 10, 1976.

43. **Specifications for the identity and purity of some food additives**. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. **Evaluation of certain food additives** (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. **Summary of toxicological data of certain food additives**. WHO Food Additives Series, No. 12, 1977.
46. **Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others**. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. **Evaluation of certain food additives and contaminants** (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. **Summary of toxicological data of certain food additives and contaminants.** WHO Food Additives Series, No. 13, 1978.
49. **Specifications for the identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 7, 1978.
50. **Evaluation of certain food additives** (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 14, 1980.
52. **Specifications for identity and purity of food colours, flavouring agents, and other food additives**. FAO Food and Nutrition Paper, No. 12, 1979.
53. **Evaluation of certain food additives** (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 15, 1980.
55. **Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).** FAO Food and Nutrition Paper, No. 17, 1980.
56. **Evaluation of certain food additives** (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 16, 1981.
58. **Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives**). FAO Food and Nutrition Paper, No. 19, 1981.
59. **Evaluation of certain food additives and contaminants** (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 17, 1982.
61. **Specifications for the identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 25, 1982.
62. **Evaluation of certain food additives and contaminants** (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. **Toxicological evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 18, 1983.
64. **Specifications for the identity and purity of certain food additives.** FAO Food and Nutrition Paper, No. 28, 1983.

65. **Guide to specifications, general notices, general methods, identification tests, test solutions, and other reference materials.** FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. **Evaluation of certain food additives and contaminants** (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 19, 1984.
68. **Specifications for the identity and purity of food colours**. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. **Specifications for the identity and purity of food additives**. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. **Evaluation of certain food additives and contaminants** (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. **Specifications for the identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 34, 1986.
72. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. **Evaluation of certain food additives and contaminants** (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. **Specifications for the identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 37, 1986.
76. **Principles for the safety assessment of food additives and contaminants in food**. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.
77. **Evaluation of certain food additives and contaminants** (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987 and corrigendum.
78. **Toxicological evaluation of certain food additives**. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. **Specifications for the identity and purity of certain food additives.** FAO Food and Nutrition Paper, No. 38, 1988.
80. **Evaluation of certain veterinary drug residues in food** (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41, 1988.
83. **Evaluation of certain food additives and contaminants** (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. **Evaluation of certain veterinary drug residues in food** (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.

86. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 25, 1990.
87. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. **Evaluation of certain food additives and contaminants** (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 26, 1990.
90. **Specifications for identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 49, 1990.
91. **Evaluation of certain veterinary drug residues in food** (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 27, 1991.
93. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. **Evaluation of certain food additives and contaminants** (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 28, 1991.
96. **Compendium of food additive specifications** (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agricultural Organization of the United Nations, 1992 (2 volumes).
97. **Evaluation of certain veterinary drug residues in food** (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. **Toxicological evaluation of certain veterinary residues in food**. WHO Food Additives Series, No. 29, 1991.
99. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. **Guide to specifications—general notices, general analytical techniques, identification tests, test solutions, and other reference materials**. FAO Food and Nutrition Paper, No. 5, Ref. 2, 1991.
101. **Evaluation of certain food additives and naturally occurring toxicants** (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.
102. **Toxicological evaluation of certain food additives and naturally occurring toxicants**. WHO Food Additive Series, No. 30, 1993.
103. **Compendium of food additive specifications: Addendum 1**. FAO Food and Nutrition Paper, No. 52, 1992.
104. **Evaluation of certain veterinary drug residues in food** (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 31, 1993.
106. **Residues of some veterinary drugs in animals and food**. FAO Food and Nutrition Paper, No. 41/5, 1993.

107. **Evaluation of certain food additives and contaminants** (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 32, 1993.
109. **Compendium of food additive specifications: Addendum 2**. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. **Evaluation of certain veterinary drug residues in food** (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 33, 1994.
112. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. **Evaluation of certain veterinary drug residues in food** (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 34, 1995.
115. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/7, 1995.
116. **Evaluation of certain food additives and contaminants** (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 35, 1996.
118. **Compendium of food additive specifications: Addendum 3**. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. **Evaluation of certain veterinary drug residues in food** (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. **Toxicological evaluation of certain veterinary drug residues in food**. WHO Food Additives Series, No. 36, 1996.
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## *ANNEX 2*

## *ABBREVIATIONS USED IN THE MONOGRAPHS*

| | |
|---|---|
| 3MS | Modified Mini-Mental State Examination |
| ADH | alcohol dehydrogenase |
| ADI | acceptable daily intake |
| ALAT | alanine aminotransferase |
| ALDH | aldehyde dehydrogenase |
| ALS | amyotrophic lateral sclerosis |
| AOAC | Association of Official Analytical Chemists |
| ASAT | aspartate aminotransferase |
| ATP | adenosine triphosphate |
| AUC | area under the curve |
| BAEP | brainstem auditory evoked potentials |
| BMD | benchmark-dose |
| BMDL | benchmark-dose level |
| BSID | Bayley Scales of Infant Development |
| bw | body weight |
| cAMP | adenosine 3',5'-cyclic monophosphate |
| CCFAC | Codex Commission on Food Additives and Contaminants |
| cDDP | cisplatin |
| $C_{max}$ | maximum plasma concentration |
| CI | confidence interval |
| coA | coenzyme A |
| CNS | central nervous system |
| CT | computer tomography |
| CYP | cytochrome P450 |
| DDST | Denver Developmental Screening Test |
| DEN | diethylnitrosamine |
| DES | dialysis encephalopathy syndrome |
| DMBA | 7, 12-dimethylbenz[*a*]anthracene |
| DMH | dimethylhydrazine |
| DNA | deoxyribonucleic acid |
| ECG | electrocardiogram |
| ELISA | enzyme-linked immunosorbent assay |
| F | female |
| FAO | Food and Agriculture Organization of the United Nations |
| GC/MS | gas chromatography-mass spectrometry |
| GEMS | Global Environmental Monitoring System |
| GEMS/Food | Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme |
| GL | guideline levels |
| GLP | good laboratory practice |
| GSFA | General Standard for Food Additives |
| GSH | glutathione |

| | |
|---|---|
| GST | glutathione *S*-transferase |
| HDL | high-density lipoprotein |
| HF | high frequency |
| $^1$H NMR | proton nuclear magnetic resonance spectroscopy |
| HPLC | high-performance liquid chromatography |
| HRV | heart rate variability |
| HS GC/MS | gas chromatographic separation and mass spectrometric with an automated headspace procedure |
| HVP | hydrolysed vegetable protein |
| ICP-MS | inductively coupled plasma-mass spectrometry |
| IDL | intermediate-density lipoprotein |
| Ig | immunoglobulin |
| IPCS | International Programme on Chemical Safety |
| IQ | intelligence quotient |
| $k_m$ | affinity constant |
| $LD_{50}$ | median lethal dose |
| LDL | low-density lipoprotein |
| LF | low frequency |
| LOAEL | lowest-observed-adverse-effect level |
| LOEL | lowest-observed-effect level |
| LSC | liquid scintillation counting |
| M | male |
| MAO | monoamine oxidase |
| MCDI | MacArthur Communicative Development Inventory |
| MMSE | Mini-Mental State Examination |
| mRNA | messenger RNA |
| MS | mass spectrometry |
| MTT | 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide |
| $NAD^+$ | nicotinamide adenine dinucleotide |
| NADH | nicotinamide adenine dinucleotide, reduced |
| NADPH | nicotinamide adenine dinucleotide phosphate, reduced |
| NCE | normochromatic erythrocytes |
| NHANES | National Health and Nutrition Examination Survey |
| NINCDS | United States National Institute of Neurological and Communicable Disorders and Stroke and Alzheimer Disease and Related Disorders |
| NOEL | no-observed-effect level |
| O-Hg | organic component of mercury in blood |
| OH-PCB | hydroxylated polychlorinated biphenyl |
| PARP | poly-ADP-ribose polymerase |
| PCB | polychlorinated biphenyl |
| PBDE | polybrominated diphenyl ether |
| PDAT | presenile dementia of the Alzheimer type |
| PMTDI | provisional maximum tolerable daily intake |

| | |
|---|---|
| PUFA | polyunsaturated fatty acids |
| QA | quality assurance |
| RT-PCR | reverse transcription-polymerase chain reaction |
| S9 | 9000 × *g* microsomal fraction of rat liver |
| SALP | sodium aluminium phosphate |
| SCDS | Seychelles Child Development Study |
| SCE | sister chromatid exchange |
| SEC | size exclusion chromatography |
| SMR | standardized mortality ratio |
| SPMSQ | Short Portable Mental Status Questionnaire |
| $t_{1/2}$ | half-life |
| TOS | total organic solids |
| TPA | 12-*O*-tetradecanoylphorbol-13-acetate |
| TPN | long-term parenteral nutrition |
| TPPO | triphenyl phosphine oxide |
| UDPGT | UDP-glucuronosyltransferase |
| UK | United Kingdom |
| USA | United States of America |
| VEP | visual evoked potentials |
| VLDL | very-low-density lipoprotein |
| Vs | versus |
| WHO | World Health Organization |
| w/w | weight for weight |

## *ANNEX 3*

## *SIXTY-SEVENTH MEETING OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES*

Rome, 20–29 June 2006

### Members

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Dr J.C. Larsen, Senior Consultant, Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Vice-Chairman*)

Dr A. Mattia, Division Director, Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, MD USA.

Mrs I. Meyland, Senior Scientific Adviser, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Chairman*)

Dr M.V. Rao, Director, Central Laboratories Unit, United Arab Emirates University, Al Ain, United Arab Emirates

Dr J. Schlatter, Head of Food Toxicology Section, Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health, Zurich, Switzerland

Dr P. Verger, Director of INRA Unit 1204—Food risk analysis methodologies, National Institute for Agricultural Research, Paris, France

Professor R. Walker, Emeritus Professor of Food Science, Ash, Aldershot, Hampshire, England

Mrs H. Wallin, Director of the Steering Unit, National Food Safety Authority (Evira), Helsinki, Finland

Dr B. Whitehouse, Consultant, Bowdon, Cheshire, England

### Secretariat

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Dr D. Benford, Principal Toxicologist, Food Standards Agency, London, England *(WHO Temporary Adviser)*

Ms R. Charrondiere, Nutrition Officer, Nutrition Planning, Assessment and Evaluation Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization, Rome, Italy *(FAO Staff Member)*

Dr M.L. Costarrica, Senior Officer, Food Quality and Standards Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization, Rome, Italy *(FAO Staff Member)*

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## *ANNEX 4*

## *TOXICOLOGICAL RECOMMENDATIONS AND INFORMATION ON SPECIFICATIONS*

### Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

| Food additive | Specifications[a] | Acceptable daily intake (ADI) and other toxicological recommendations |
|---|---|---|
| Annatto extracts | R | ADI for bixin: 0–12 mg/kg bw<br>Applicable to the following annatto extracts, provided they comply with the respective specifications:<br>—solvent-extracted bixin (≥ 85% bixin, ≤ 2.5% norbixin)<br>—aqueous processed bixin (≥ 25% bixin, ≤ 7% norbixin)<br>Does not apply to oil-processed bixin (≥10 % bixin)<br>Group ADI for norbixin and its sodium and potassium salts: 0–0.6 (expressed as norbixin)<br>Applicable to the following annatto extracts, provided they comply with the respective specifications:<br>—solvent-extracted norbixin (≥ 85% norbixin)<br>—alkali-processed norbixin, acid-precipitated (≥ 35% norbixin) and not acid precipitated (≥ 15% norbixin)<br>In re-evaluating the studies of toxicity with solvent-extracted bixin (92% bixin) and solvent-extracted norbixin (91.6% norbixin) and in light of the additional compositional data, the Committee considered that ADIs could be allocated to these pigments, on the basis of studies conducted on the extracts.<br>The Committee established an ADI for bixin of 0–12 mg/kg bw based on the NOEL of 1311 mg/kg bw per day from a 90-day study in male rats fed an extract containing 92% bixin, corrected for pigment content and applying a safety factor of 100<br>The Committee established a group ADI for norbixin and its sodium and potassium salts of 0–0.6 mg/kg bw (expressed as norbixin) on the basis of the NOEL of 69 mg/kg bw per day from a 90-day study in male rats fed an extract containing 91.6% norbixin, corrected for pigment content and applying a safety factor of 100. |

| Food additive | Specifications[a] | Acceptable daily intake (ADI) and other toxicological recommendations |
|---|---|---|
| | | On the basis of compositional data and toxicological data on aqueous processed bixin and alkali-processed norbixin (acid precipitated), the Committee concluded that the use of these annatto extracts as sources of bixin or norbixin would not raise safety concerns, provided that they complied with the relevant specifications. Accordingly, the ADIs given above could be applied to bixin and norbixin derived from these annatto extracts.<br><br>The Committee noted that the pigment in alkali-processed norbixin (not acid-precipitated) consists of sodium or potassium salts of norbixin and that compositional data on this extract, complying with the specifications, did not raise safety concerns. Consequently, the Committee concluded that the group ADI for norbixin and its sodium and potassium salts is applicable to norbixin salts from this source.<br><br>As no NOEL could be identified for oil-processed bixin and no compositional data were available, the Committee decided that the above evaluation could not be applied to this extract.<br><br>Assuming all annatto-derived pigment were bixin, the estimated intake would amount to approximately 0.2% of the ADI (0–12 mg/kg bw). Assuming all annatto derived pigment were norbixin, the estimated intake would amount to approximately 4% of the ADI (0–0.6 mg/kg bw).<br><br>Specifications were established for all extracts covered by the established ADIs, and tentative specifications were established for oil-processed bixin. |
| Lycopene (synthetic) | N | The Committee established an ADI of 0–0.5 mg/kg bw for synthetic lycopene based on the highest dose of 50 mg/kg bw per day tested in the 104-week study in rats (at which no adverse effects relevant to humans were induced) and a safety factor of 100. This ADI was made into a group ADI to include lycopene from *Blakeslea trispora*, which was also under consideration at the present meeting and was considered to be |

| Food additive | Specifications[a] | Acceptable daily intake (ADI) and other toxicological recommendations |
|---|---|---|
| | | toxicologically equivalent to chemically synthesized lycopene.<br><br>The estimate of high exposure (> 95th percentile) of 30 mg/person per day, equivalent to 0.5 mg/kg bw per day, which includes background exposure plus additional exposure from food additive uses, is compatible with the ADI. |
| Lycopene from *Blakeslea trispora* | N | Lycopene from *Blakeslea trispora* is considered to be toxicologically equivalent to chemically synthesized lycopene, for which an ADI of 0–0.5 mg/kg bw was established. This was given further credence by the negative results obtained for lycopene from *B. trispora* in two tests for genotoxicity, and the absence of adverse effects in a short-term toxicity study considered at the present meeting. The ADI for synthetic lycopene was therefore made into a group ADI of 0–0.5 mg/kg bw to include lycopene from *B. trispora*.<br><br>The exposure estimate is the same as for synthetic lycopene. |
| Natamycin<br><br>(also known as pimaricin)<br><br>(exposure assessment) | N | The data as a whole, including estimations based on GEMS/Food Consumption Cluster Diets and calculations for consumers with a high intake and children, confirm the results of the assessment made by the Committee at its fifty-seventh meeting and show that the current ADI of 0–0.3 mg/kg bw is unlikely to be exceeded. |
| Propyl paraben<br><br>(also known as propyl para-hydroxybenzoate) | W | In view of the adverse effects in male rats, propyl paraben (propyl *p*-hydroxybenzoate) should be excluded from the group ADI for the parabens used in food. This conclusion was reached on the grounds that the group ADI was originally set on a NOEL of 1000 mg/kg bw per day for a different toxicological end-point—growth depression—taken from the range of studies then available for the methyl, ethyl and propyl parabens. Propyl paraben has shown adverse effects in tissues of reproductive organs in male rats at dietary doses of down to 10 mg/kg bw per day, which is within the range of the group ADI (0–10 mg/kg bw), with no NOEL yet identified. |

| Food additive | Specifications[a] | Acceptable daily intake (ADI) and other toxicological recommendations |
|---|---|---|
| | | The specifications for propyl paraben were withdrawn.<br>The group ADI of 0–10 mg/kg bw for the sum of methyl and ethyl esters of *p*-hydroxybenzoic acid was maintained. |

GEMS: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; NOEL: no-observed-effect level.

[a]N: new specifications prepared; R: existing specifications revised; W: specifications withdrawn.

## Food additives considered for specifications only

| Food additive | Specifications[a] |
|---|---|
| Acetylated oxidized starch | R |
| Annatto extracts (oil-processed bixin) | R, T |
| Butyl *p*-hydroxybenzoate (butyl paraben) | W |
| Carob bean gum | R, T |
| Carob bean gum (clarified) | N, T |
| Ethylene oxide | W |
| Guar gum | R, T |
| Guar gum (clarified) | N, T |
| DL-Malic acid and its calcium and sodium salts | R |
| Maltitol | R |
| Titanium dioxide | R |
| Zeaxanthin (synthetic) | R |

[a]N: new specifications prepared; R: existing specifications revised; T: tentative specifications; W: specifications withdrawn.

## Contaminants evaluated toxicologically

| Contaminant | Tolerable intake and other toxicological recommendations |
|---|---|
| Aluminium (from all sources including food additives) | PTWI : 1 mg/kg bw expressed as Al<br>The previously established ADIs and PTWI for aluminium compounds were withdrawn.<br>The Committee concluded that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than those used in establishing the previous PTWI and the PTWI was therefore revised.<br>The available studies have many limitations and are not adequate for defining dose–response relationships. The Committee therefore based its evaluation on the combined evidence from several studies. The relevance of studies involving administration of aluminium compounds by gavage was unclear because the toxicokinetics after gavage were expected to differ from toxicokinetics after dietary administration, and these gavage studies generally did not report total aluminium exposure including basal levels in the feed. The studies conducted with dietary administration of aluminium compounds were considered most appropriate for the evaluation. The lowest LOELs for aluminium compounds in a range of different dietary studies in mice, rats and dogs were in the range of 50–75 mg/kg bw per day, expressed as Al.<br>The Committee applied an uncertainty factor of 100 to the lower end of this range of LOELs (50 mg/kg bw per day expressed as Al) to allow for inter- and intraspecies differences. There are deficiencies in the database, notably the absence of NOELs in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. These deficiencies are counterbalanced by the probable lower bioavailability of the less soluble aluminium compounds present in food. Overall, it was considered appropriate to apply an additional uncertainty factor of three. The Committee confirmed that the resulting health-based guidance value should be expressed as a PTWI, because of the potential for bioaccumulation.<br>The Committee noted that the PTWI is likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing additives. The Committee also noted that dietary exposure to Al is expected to be very high for infants fed on soya-based formula. |

| Contaminant | Tolerable intake and other toxicological recommendations |
|---|---|
| 3-Chloro-1,2-propanediol | As no new pivotal toxicological studies had become available the Committee retained the previously established PMTDI of 2 µg/kg bw for 3-chloro-1,2-propanediol.<br><br>Estimated exposures at the national level considered a wide range of foods, including soy sauce and soy-sauce related products, ranged from 1% to 35% of the PMTDI for average exposure in the general population. For the consumers at the high percentile (95th), the estimated intakes ranged from 3% to 85% and up to 115% of the PMTDI in young children. These estimates are based on concentrations of 3-chloro-1,2-propanediol derived before any remedial action had been taken by government or industry.<br><br>The Committee noted that reduction in the concentration of 3-chloro-1,2-propanediol in soy sauce and related products made with acid-HVP could substantially reduce the intake of this contaminant by certain consumers of this condiment. |
| 1,3-Dichloro-2-propanol | The Committee concluded that the critical effect of 1,3-dichloro-2-propanol is carcinogenicity. The substance yielded negative results in two new studies on genotoxicity in vivo, but limitations in these studies and positive findings in tests for genotoxicity in vitro as well as lack of knowledge on the modes of action operative at the various tumour locations led the Committee to the conclusion that a genotoxic mode of action could not be excluded. Accordingly, the cancer dose–response data were analysed by dose–response modelling to calculate $BMD_{10}$ and $BMDL_{10}$ values.<br><br>The Committee concluded that a representative mean intake for the general population of 1,3-dichloro-2-propanol of 0.051 µg/kg bw per day and an estimated high-level intake (young children included) of 0.136 µg/kg bw per day could be used in the evaluation. Comparison of these mean and high-levels intakes with the lowest $BMDL_{10}$ of 3.3 mg/kg bw per day, which was the $BMDL_{10}$ for incidence data on tumour-bearing animals for all treatment-affected locations, indicates margins of exposure of approximately 65 000 and 24 000, respectively. Based on these margins of exposure, the Committee concluded that the estimated intakes of 1,3-dichloro-2-propanol were of low concern for human health.<br><br>The available evidence suggests that 1,3-dichloro-2-propanol occurs at lower concentrations than 3-chloro-1,2-propanediol in soy sauce and related products, and also in acid-HVP food ingredients. However, in meat products the concentrations of 1,3-dichloro-2-propanol are generally higher than the concentrations of 3-chloro-1,2-propanediol. |
| Methylmercury | The Committee made it clear that the previous PTWI of 3.3 µg/kg bw had, in fact, been withdrawn in 2003. The Committee confirmed the existing PTWI of 1.6 µg/kg bw, set in 2003, based on the most sensitive toxicological |

| Contaminant | Tolerable intake and other toxicological recommendations |
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| | end-point (developmental neurotoxicity) in the most susceptible species (humans). However, the Committee noted that life-stages other than the embryo and fetus may be less sensitive to the adverse effects of methylmercury.<br><br>In the case of adults, the Committee considered that intakes of up to about two times higher than the existing PTWI of 1.6 µg/kg bw would not pose any risk of neurotoxicity in adults, although in the case of women of childbearing age, it should be borne in mind that intake should not exceed the PTWI, in order to protect the embryo and fetus.<br><br>Concerning infants and children aged up to about 17 years, the data do not allow firm conclusions to be drawn regarding their sensitivity compared to that of adults. While it is clear that they are not more sensitive than the embryo or fetus, they may be more sensitive than adults because significant development of the brain continues in infancy and childhood. Therefore, the Committee could not identify a level of intake higher than the existing PTWI that would not pose a risk of developmental neurotoxicity for infants and children.<br><br>The Committee has previously noted that fish makes an important contribution to nutrition, especially in certain regional and ethnic diets. The present Committee recommends that the known benefits of fish consumption need to be taken into consideration in any advice aimed at different subpopulations. Risk managers may wish to consider whether specific advice should be given concerning children and adults, after weighing the potential risks and benefits.<br><br>The Committee concluded that the setting of guideline levels for methylmercury in fish may not be an effective way of reducing exposure for the general population. The Committee noted that advice targeted at population subgroups that may be at risk from methyl mercury exposure may provide an effective method for lowering the number of individuals with exposures greater than the PTWI. |

ADI: acceptable daily intake; Al: elemental aluminium; LOEL: lowest-observed-effect level; NOEL: no-observed-effect level; PMTDI: provisional maximum tolerable daily intake.