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Dietary Reference Values for chloride

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) has derived dietary reference values (DRVs) for chloride. There are no appropriate biomarkers of chloride status, no balance studies and no adequate evidence on the relationship between chloride intake and health outcomes that can be used to set DRVs for chloride. There is a close relationship between sodium and chloride balances in the body. Sodium chloride is the main source of both electrolytes in European diets and similar urinary excretion levels of sodium and chloride (on a molar basis) are typically observed in Western populations. Hence, the Panel considered that reference values for chloride can be set at values equimolar to the reference values for sodium for all population groups, and are as follows: 1.7 g/day for children aged 1–3 years, 2.0 g/day for children aged 4–6 years, 2.6 g/day for children aged 7–10 years, 3.1 g/day for children aged 11–17 years and 3.1 g/day for adults including pregnant and lactating women. Consistent with the reference values for sodium, these levels of chloride intake are considered to be safe and adequate for the general EU population, under the consideration that the main dietary source of chloride intake is sodium chloride. For infants aged 7–11 months, an Adequate Intake of 0.3 g/day is set.

Key words: Chloride, Dietary Reference Value

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Summary

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA Panel) was asked to deliver a Scientific Opinion on Dietary Reference Values (DRVs) for the European population, including chloride.

Chloride (Cl^-) is the predominant anion in intracellular fluid and one of the most important extracellular anions. It contributes to many body functions including the maintenance of osmotic and acid–base balance, muscular and nervous activity, and the movement of water and solutes between fluid compartments.

Dietary chloride deficiency is rare. Sodium chloride added during industrial food processing, discretionary use or food preservation is the major source of dietary chloride in Western diets. Other sources of chloride include inherently food-borne sources, and chloride-containing food additives, in which chloride may be associated with cations other than sodium.

In healthy people, chloride is efficiently absorbed in the gut. Following absorption, chloride anions are freely transported in the blood, where their concentration is maintained within a narrow range. Renal excretion of chloride is coupled to that of sodium and potassium. The overall regulation of chloride balance is linked to that of sodium through hormonal control by the renin–angiotensin–aldosterone system and cortisol. The close interrelationship between sodium and chloride physiology and intakes are reflected by high correlations between sodium and chloride urinary excretion. Studies which quantified 24-hour urinary excretion of sodium and chloride in subjects from Western populations indicate that, on a molar basis, both electrolytes are excreted in similar amounts.

As for sodium, the amount of chloride excreted in the urine of an individual varies widely within the day and between days. In a long-term controlled feeding trial, a daily variation in chloride excretion with a seven-day rhythm was observed, which indicates that the day-to-day variation in chloride excretion is partly independent of chloride intake.

Because of its tight homeostatic regulation, serum chloride concentration is not a sensitive marker of chloride intake or status. Values outside the reference range are typically related to disorders affecting water and electrolyte balances. Overall, there are no appropriate biomarkers for chloride status that can be used for setting DRVs for chloride.

A few studies have measured chloride intake and losses and related chloride 'balance' in various experimental settings. These studies have important limitations. No balance studies can be used to set DRVs for chloride.

There is evidence that chloride can contribute to the effect of sodium chloride on blood pressure. Data from studies on hypertensive rats, and some clinical observations, suggest that the full expression of sodium-chloride-dependent elevation in blood pressure relies on the concomitant presence of both sodium and chloride. An independent effect of chloride on cardiovascular risk has also been explored in observational studies using serum/plasma chloride concentration. However, serum/plasma chloride concentration cannot be used as a marker of chloride intake. No studies are available which investigate the association between chloride intake or urinary excretion and cardiovascular disease-related health outcomes.

There are no data that can be used to determine Average Requirements and Population Reference Intakes for chloride. Hence, the Panel considered that reference values for chloride can be set at the value equimolar to the reference values for sodium for all population groups, and are as follows: 1.7 g/day for children aged 1–3 years, 2.0 g/day for children aged 4–6 years, 2.6 g/day for children aged 7–10 years, 3.1 g/day for children aged 11–17 years and 3.1 g/day for adults including pregnant and lactating women. Consistent with the reference values for sodium, these levels of chloride intake are considered to be safe and adequate for the general EU population, under the consideration that the main dietary source of chloride intake is sodium chloride. For infants aged 7–11 months, an Adequate Intake of 0.3 g/day is set.

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Background as provided by the European Commission

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community.[1] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the (SCF, 1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context the EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

Terms of reference as provided by the European Commission

In accordance with Article 29(1)(a) and Article 31 of Regulation No 178/2002,[2] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, trans fatty acids;
- Protein;
- Dietary fibre.

Following on from the first part of the task, the EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a

[1] Scientific Committee for Food, 1993. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st series. Food – Science and Technique, European Commission, Luxembourg, 248 pp.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, the EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

Data and methodology

The present Opinion addresses the Dietary Reference Values (DRVs) for chloride. The assessment is conducted in accordance with the NDA Panel's Scientific Opinion on principles for deriving and applying DRVs (EFSA NDA Panel, 2010).

The Opinion is structured as follows:

- **Sections 1–4** include relevant background information on chloride; this encompasses a brief introduction (Section 1), information on chemistry, function, physiology, metabolism, interaction with other nutrients and biomarkers for intake and status (Section 2), information on dietary sources and intake data (Section 3) and an overview of DRVs and recommendations from other bodies (Section 4).
- **Section 5** covers the assessment of the evidence on the criteria (endpoints) on which to base DRVs.
- **Section 6** provides the integration of the available evidence and derivation of DRVs.

In order to inform Sections 1 to 4 of the Scientific Opinion, a literature search covering chloride physiology and metabolism in healthy adults, biomarkers for intake, and genotypes affecting chloride metabolism was commissioned from the University of Hertfordshire (Lewis et al., 2014).

In order to complement the information gathered in a previous Opinion on the concentration of chloride in breast milk (SCF, 2003) (Section 2.3.3.4), a comprehensive review of the literature published since January 2000 on healthy women living in Europe, North America and Australia was conducted by LASER Analytica (LASER Analytica, 2014).

An ad hoc questionnaire developed by the members of the working group on DRVs for minerals was disseminated to EFSA's focal points and the members of the EFSA Food Consumption Network in order to collect information on the levels of urinary chloride excretion in European populations (Section 3.2).

To identify relevant health outcomes upon which DRVs for chloride could be based (Section 5.5), a comprehensive search of the literature published between 1990 and September 2012 was commissioned from Pallas health research and consultancy (Eeuwijk et al., 2013).

Subsequently, additional information was gathered by the members of the working group on DRVs for minerals and EFSA staff. Studies were retrieved through searches in bibliographic databases and selected based on their relevance to the assessment.

The draft scientific opinion is now open for a second public consultation. The opinion will then be finalised considering the comments received, where appropriate. A public consultation on the draft opinion on DRVs for sodium runs in parallel (EFSA NDA Panel, 20YY).

Assessment

1. Introduction

In 1993, the Scientific Committee for Food (SCF) adopted an Opinion on the nutrient and energy intakes for the European Community. With respect to chloride, the SCF acknowledged the absence of definitive information and since daily chloride was principally derived from the intake of sodium chloride, the Committee decided that chloride requirements should match those for sodium (SCF, 1993). For sodium, an Acceptable Range of Intakes (0.575–3.5 g/day, corresponding to 0.025–0.15 mol/day) was set for adults. This is equivalent to 0.89–5.4 g chloride/day.

The Panel notes the difficulty of dissociating the physiological effects of chloride from those of sodium or potassium and recommends that this Opinion should be read in conjunction with the Panel's Opinions on the dietary reference values for sodium and potassium (EFSA NDA Panel, 2016, 20YY).

2. Definition/category

2.1. Chemistry

Chlorine is a halogen and has an atomic mass of 35.5 Da, with two stable isotopes ^{35}Cl and ^{37}Cl which account for approximately 75% and 25% of the element's natural abundance (Wieser and Coplen, 2011). Chloride (Cl^-) as a monoatomic free hydrated anion (i.e. an electrolyte) is the form in which the element, in association with the cations sodium, potassium, calcium and magnesium, is essential for physiological processes in life forms. Cl^- can also form covalent organic compounds, but these fulfil pharmacological and toxicological roles and are not relevant for the derivation of DRVs.

Sodium chloride (NaCl) is table salt. One gram of salt consists of 17 mmol sodium and chloride, providing 0.4 g sodium and 0.6 g chloride.

2.2. Function of chloride

2.2.1. Biochemical functions

Chloride and bicarbonate (HCO_3^-) are the two dominant anions in the extracellular fluid, whereas in the intracellular fluid, Cl^- is the predominant anion. This compartmentalisation of chloride and bicarbonate, and of sodium (Na^+) and potassium (K^+), is achieved by the regulated exchange of the ions across the lipid membranes.

The transport of chloride across biological membranes is mediated by chloride channels, which are ubiquitously expressed. Chloride channels are classified into voltage-gated chloride channels (the CIC family), the cystic fibrosis transmembrane conductance regulator (CFTR), the Ca^{2+}-activated chloride channels, the volume-regulated anion channels and the ligand-gated anion channels (Berend et al., 2012; Kondratskyi et al., 2014).

These channels maintain and modulate membrane electropolarity, and osmotic and acid–base balance between intracellular compartments and the cytoplasm, as well as between the cytoplasm and extracellular fluid (Berend et al., 2012). They enable the generation of electrical signals in muscle and in the peripheral and central nervous systems, the transport of solutes across membranes (Greenwood and Earnshaw, 1997; Frausto da Silva and Williams, 2001), as well as the secretion and resorption of fluid, particularly in the lung alveoli where the lung air/fluid interface is central to gas absorption (Hollenhorst et al., 2011). Chloride-dependent secretion of water in the lung and exocrine organs moistens mucus and provides its fluidity; this is regulated by the CFTR channel, a defect in which is responsible for cystic fibrosis, which is also known as mucoviscidosis (Johnson et al., 2006).

Chloride secretion by channels in the parietal (oxyntic) cells of the gastric mucosa is crucial for the secretion of HCl (Berend et al., 2012). In erythrocyte membranes, a Cl^-/HCO_3^- exchange channel facilitates the uptake of oxygen and release of carbon dioxide in the lung vascular system and the release of oxygen and uptake of carbon dioxide in peripheral tissues. In the lungs the exchange channel releases CO_2 which has been taken up as bicarbonate and enables the entry of chloride ions which, in turn, induce a conformational change in haemoglobin that increases its affinity for O_2. This phenomenon is known as the 'chloride shift' (Prange et al., 2001; Fischer et al., 2007).

Other functions of chloride include the production by neutrophils of hypochlorous acid (HClO), the cytotoxic effect of which is a component of the innate cellular immune inflammatory response (Nauseef, 2014). It has been proposed that chloride has roles in the cell cycle and apoptosis (Nilius and Droogmans, 2003; Kondratskyi et al., 2014).

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

Dietary chloride deficiency is rare (Meletis, 2003). Low intakes of chloride have been described in two breast-fed infants whose mothers' milk was deficient in chloride (concentration of 2 mmol/L (70 mg/L) and undetectable, respectively) (Asnes et al., 1982; Hill and Bowie, 1983). Insufficient intakes have also occurred in infants given chloride-deficient breast milk substitutes (Rodriguez-Soriano et al., 1983; Kaleita, 1986) and among children and adult patients provided with chloride-deficient liquid

nutritional products (Miyahara et al., 2009). In infants, hypochloraemia features included growth failure, lethargy, irritability, anorexia, gastrointestinal symptoms, and weakness in addition to hypokalaemic metabolic alkalosis and haematuria (Grossman et al., 1980). These features are consistent with those seen in infants and children with hypochloraemia induced by congenital chloride diarrhoea (OMIM 214700) secondary to a defect in the ileal and colonic Cl^-/HCO_3^- exchange carrier for chloride absorption.

Hypochloraemia, which is characterised by abnormally low blood chloride concentration (below the reference range, typically 97–107 mmol/L; see Section 2.3.2), may be induced by excessive gastrointestinal and renal losses, as well as by acquired or inherited metabolic disorders (Tang et al., 2010; Berend et al., 2012).

2.2.2.2. Excess

Chloride excess secondary to dietary intake is uncommon. Hyperchloraemia, which is defined as a serum chloride concentration above the reference range (97–107 mmol/L), is usually caused by loss of bicarbonate in the faeces due to severe diarrhoea (metabolic acidosis). Hyperchloraemia may occur with several other conditions associated with abnormal losses of water (skin, renal or extra-renal), extracellular fluid volume depletion or an increase in the tubular chloride reabsorption. It can also be the result of excessive administration of salts (e.g. NaCl, NH_4Cl, $CaCl_2$) or intake of certain medications (e.g. cortisone preparations, acetazolamide).

Because of insufficient data, EFSA did not set a Tolerable Upper Intake Level (UL) for chloride (EFSA, 2005a, 2005b), but noted that current levels of intake among European populations exceeded amounts required for normal function and that increased intake of chloride, as sodium chloride, has been associated with a greater likelihood of elevated blood pressure, which can lead to cardiovascular and renal disease.

2.3. Physiology and metabolism

2.3.1. Intestinal absorption and secretion

Gastrointestinal secretions are rich in chloride, with gastric secretions being the predominant source. Chloride may also be actively secreted in the lumen, which is an important determinant of intestinal fluid secretion throughout the gastrointestinal tract (Murek et al., 2010).

Enterocyte absorption and secretion of chloride are regulated by endocrine, paracrine, autocrine, neuronal and immunological agents as part of the overall regulation of intestinal function (Kato and Romero, 2011; Malakooti et al., 2011).

In healthy people, chloride is efficiently absorbed in the gut and concentrations in faeces are low (10–15 mmol/L) (Kiela and Ghishan, 2016) (Section 2.3.3.2). Chloride is absorbed and transferred by the intestinal mucosa throughout the small and large intestine; the mechanisms involved vary with intestinal site (Strain and Cashman, 2009; Chang and Leung, 2014). Proximally, chloride is taken up actively by specific exchange mechanisms (e.g. Cl^-/HCO_3^-, Cl^-/OH^-) or passively by following electrochemical or concentration gradients. Net intestinal absorption of chloride occurs in the distal small intestine and proximal colon, where sodium and electrolyte salvage is achieved by electroneutral absorption of chloride ions coupled to the absorption of sodium. This is facilitated by two carrier proteins, the Na^+/H^+ and Cl^-/HCO_3^- exchangers (Sundaram et al., 1991; Gropper et al., 2013). In the ileum, colon and rectum, chloride is also absorbed by an HCO_3^--dependent pathway, probably involving a luminal membrane Cl^-/HCO_3^- exchanger not coupled to an Na^+/H^+ exchanger, as well as by voltage-dependent diffusion (Chang and Leung, 2014).

When the intestinal mucosa is stimulated by agents that increase intracellular second messengers, electroneutral sodium chloride absorption is inhibited and secretion of sodium chloride and potassium chloride is activated, facilitated by transport proteins in the intestinal mucosa (the most important being the CFTR channel) and basolateral membranes (Na^+-Cl^--K^+ cotransporter, K^+ channels, Na^+-K^+-ATPase) (Kato and Romero, 2011).

2.3.2. Transport in blood and distribution to tissues

Following absorption, chloride anions are freely transported in the blood (Gropper et al., 2009b). In healthy adults, serum chloride concentrations are approximately 97 to 107 mmol/L. Reference ranges of values vary slightly among different laboratories due to the variation in measurement techniques (Morimatsu et al., 2003).

The total body content of chloride has been estimated to be 85–115 g in an adult, corresponding to about 0.15% of total body weight (Pike and Brown, 1984; Yunos et al., 2010; Berend et al., 2012). Most systemic chloride (88% of total body content) is in the extracellular fluid. The concentration of chloride in the interstitial fluid is approximately 115 mmol/L (Bailey et al., 2014). Within cells, chloride is present at lower concentrations depending on the resting membrane potential of each cell type (Berend et al., 2012; Bailey et al., 2014). The variation in the resting membrane potential of cells drives the differences in the intracellular concentration of chloride (approximately 70 mmol/L in red blood cells and 3 mmol/L in muscle tissue) (Yunos et al., 2010; Berend et al., 2012).

Studies, primarily focusing on sodium, provide evidence that sodium chloride retention does not inevitably lead to extracellular fluid volume retention and that there are metabolically relevant electrolyte storage sites that are not controlled by the kidneys (Heer et al., 2000; Heer et al., 2009; McCallum et al., 2015; Titze, 2015; Birukov et al., 2016).

2.3.3. Elimination

Body chloride content is determined by the balance between dietary intake and renal excretion and closely follows that of sodium (Gropper et al., 2009b; Birukov et al., 2016).

2.3.3.1. Urine

The kidney has the capacity to filter large amounts of chloride, more than 99% of which is then reabsorbed (Greger, 2000). Most of the reabsorption of chloride occurs in the proximal tubule, by passive reabsorption, ion conductance or active coupled transport with other ions (Yunos et al., 2010). Under controlled conditions with constant chloride intake, the mean recovery rates of dietary chloride in 24-hour urine samples were 87–90%[3] (200 or 400 mmol chloride/day for 7-day periods) and 99–105% (100, 150 or 200 mmol chloride/day for periods > 29 days) (Luft et al., 1982a; Birukov et al., 2016).

Renal excretion of chloride is coupled to that of sodium and potassium (Brungel et al., 2001; Gropper et al., 2009a; Heer et al., 2009; Birukov et al., 2016). The overall regulation of chloride balance is linked to that of sodium through hormonal control by the renin–angiotensin–aldosterone system and cortisol. Studies in cohorts of four and six men lasting respectively 105 and 205 days consuming 4 g potassium per day, with periods of ingesting 6, 9 and 12 g sodium chloride per day, demonstrate an aldosterone- and cortisol-dependent weekly variation in daily sodium urinary excretion (Birukov et al., 2016; EFSA NDA Panel, 20YY). There is a similar periodicity for urinary loss of both chloride and potassium. Furthermore, the longer (i.e. over a month or more) rhythmic periodicity observed for sodium also occurs for chloride (Rakova et al., 2013; Birukov et al., 2016). The close interrelationships between sodium and chloride physiology and intakes are reflected by high correlations between sodium and chloride urinary excretions ($r \geq 0.86$ at various levels of intake) (Luft et al., 1982a, 1982b, 1985; Jeffery et al., 1987; Brungel et al., 2001; Birukov et al., 2016).

The Panel notes that the kidney is the main route of chloride excretion and that excretion of sodium and chloride in urine are closely related.

2.3.3.2. Faeces

Chloride excretion in faeces mainly consists of the ions lost after gastrointestinal secretion/absorption/recirculation (Gropper et al., 2009a).

Chloride losses in faeces are generally small (a few mmol/day) and relatively constant (Rose et al., 2015). The contribution of faecal excretion to overall losses can become significant when chloride

[3] Average recovery rate calculated from the mean 24-hour urinary chloride measured over the last 3 days of each regimen.

intakes are low, as observed in depletion studies (McCance, 1936; Dole et al., 1950), or in the rare condition of chloride malabsorption, such as congenital chloride diarrhoea (Section 2.2.2.1).

2.3.3.3. Dermal losses

Chloride concentrations in sweat are typically around 20–40 mmol/L in healthy adults (Mishra et al., 2008; Taylor and Machado-Moreira, 2013). Chloride concentration in sweat is influenced by sweat rate (Dill et al., 1966; Taylor and Machado-Moreira, 2013), degree of heat acclimation (Fukumoto et al., 1988; Periard et al., 2015) and age (Mishra et al., 2008).

Assuming a sweat volume of 0.5 L/day (Shirreffs and Maughan, 2005) and a chloride concentration of 30 mmol/L, under conditions of moderate temperature and exercise levels, chloride losses via sweat can be estimated to be about 15 mmol/day (0.5 g/day).

2.3.3.4. Breast milk

Chloride concentration in breast milk decreases rapidly during the first days post-partum. This is followed by a more gradual decline in chloride concentration of mature milk (Atkinson et al., 1995).

The concentration of electrolytes, including chloride, in human milk is lower than in plasma. It is determined by an electrical potential gradient in the mammary epithelial cells regulated through membrane transport pathways (Wack et al., 1997; Truchet and Honvo-Houeto, 2017). Chloride concentration in breast milk is not influenced by nutritional factors (Lonnerdal, 1986; Atkinson et al., 1995). Diurnal variations in breast milk chloride concentration have been reported and are similar to the diurnal pattern of breast milk sodium concentration (Keenan et al., 1982; Keenan et al., 1983). Factors which have been associated with increased chloride concentration in breast milk include premature birth (Gross et al., 1980) or pathological processes such as mastitis (Ramadan et al., 1972).

Appendix A reports data on chloride concentration in breast milk from studies which involved mothers of term infants in Western populations. Mean chloride concentrations are between 339 and 586 mg/L from six studies which analysed mature breast milk (Atkinson et al., 1980; Gross et al., 1980; Picciano et al., 1981; Lemons et al., 1982; Neville et al., 1991; Wack et al., 1997) and 387 mg/L from one study which reported on mixed samples (collected between 1 and 8 weeks post-partum) (Bauer and Gerss, 2011). The Panel notes that in some studies chloride concentrations in breast milk vary widely across subjects.

Based on available data, the Panel considers an approximate midpoint of chloride concentration in mature breast milk of women from Western countries to be 400 mg (11.3 mmol)/L. Based on a mean milk transfer of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009) during the first six months of lactation in exclusively breastfeeding women, the Panel estimates a loss of chloride of 320 mg (9 mmol)/day.

2.3.4. Interactions with other nutrients

The interaction of chloride with other nutrients and metabolites, predominantly involves sodium and potassium, and bicarbonate. It is fundamental for their effective physiological function, which depends on their existence as free ions in aqueous media and on the ability of selective and specific ion channels across lipid membranes to distribute the ions such that their individual physicochemical properties can control membrane polarisation, the transport of solutes and water across membranes (for example in intestinal absorption and exocrine function), and the generation of electrical signals in muscle, and in peripheral and central nervous systems (Berend et al., 2012; Imbrici et al., 2015) (Section 2.2.1). It is noteworthy that some roles of chloride are independent of sodium and the other counter ions. Chloride is rate-limiting for the transport of sodium and chloride in the thin ascending loop of Henle, because of the differences in the affinities of sodium and chloride for the cotransporters, and the availability of chloride having a determinant effect on the release of renin (Kotchen et al., 1987).

Data from studies on hypertensive rats, and some clinical observations, suggest that the full-expression of sodium-chloride-dependent elevation in blood pressure relies on the concomitant presence of both sodium and chloride: sodium chloride causes a greater elevation of mean blood

pressure, in both normotensive and hypertensive subjects, than does sodium combined with other anions (e.g. citrate, phosphate, bicarbonate) (Kurtz et al., 1987; Shore et al., 1988; Luft et al., 1990; Kotchen and Kotchen, 1997; McCallum et al., 2015). As yet, mechanisms by which chloride may have a direct effect on blood pressure, independent of sodium, have not been established (McCallum et al., 2015).

The Panel notes that there is evidence that chloride can contribute to the effect of sodium chloride on blood pressure.

2.4. Biomarkers

2.4.1. Biomarkers of intake

Chloride is efficiently absorbed (Section 2.3.1) and most ingested chloride has been observed to be excreted in urine across a wide range of chloride intakes (Luft et al., 1982a; Birukov et al., 2016) (Section 2.3.3). As for sodium, the amount of chloride excreted in the urine of an individual varies widely during the day (e.g. lower concentration in nocturnal vs diurnal samples) (Wang et al., 2013) and between days (Wang et al., 2013; Birukov et al., 2016; Terry et al., 2016).

The validity of using 24-hour chloride urinary excretion as a biomarker of chloride intake was assessed in a long-term well-controlled feeding trial in which 10 healthy young men received constant amounts of sodium chloride (Birukov et al., 2016) (Section 2.3.3). A daily variation in chloride excretion with a seven-day (infradian) rhythm was observed, which indicates that the day-to-day variation in chloride excretion is partly independent of chloride intake. Through the use of Bland–Altman plots, Birukov et al. (2016) concluded that single 24-hour urine collection misclassified chloride intake half of the time. Accuracy improved as the number of collections increased and reached 72% when three 24-hour urine samples were used to predict intake.

In a feasibility study by the US National Health and Nutrition Examination Survey (NHANES), 282 subjects collected one 24-hour urine sample, and 108 of them collected a second 24-hour sample after 3–10 days. Although urinary excretions of chloride differed between collections at the individual level, mean daily excretions of the study groups did not differ significantly between the first and second 24-hour urine collections, overall, by sex or by race (Terry et al., 2016).

The Panel notes the similar characteristics of urine chloride and urine sodium as biomarkers of intake (EFSA NDA Panel, 20YY). The Panel considers that a single 24-hour excretion of chloride may be a valid marker for groups' average intake of chloride. The Panel notes that a single 24-hour urine collection does not reliably reflect an individual's usual intake, primarily due to the day-to-day variability in intake and excretion.

2.4.2. Biomarkers of status

Serum chloride concentration is tightly regulated by homeostatic mechanisms due to its role in maintaining serum osmolarity, fluid balance, membrane electroneutrality and polarisation (Section 2.3.3.). Thus, serum chloride concentration is not a sensitive marker for chloride status. Reference serum chloride concentrations are in the range of 97 to 107 mmol/L (Section 2.3.2.). Values outside the reference range (i.e. hypo- and hyperchloraemia) are typically related to disorders affecting water and electrolyte balances, and are seldom due to inappropriate chloride intake (Section 2.2.2).

The Panel notes that there is no biomarker of chloride status that can be used for setting DRVs for chloride.

2.5. Effects of genotype

Mutations affecting genes of all classes of chloride channels and ion exchange transporters have been identified (OMIM database[4]). These affect plasma membrane chloride channels (i.e. chloride channelopathies) or chloride transporters (mostly Cl^-/H^+ exchangers), mainly located in intracellular compartments (e.g. endosomes, lysosomes, synaptic vesicles). Mutations of the CFTR channels (OMIM 602421) are responsible for variants of cystic fibrosis.

[4] Online Mendelian Inheritance in Man, available at: https://www.omim.org/

Overall, inherited disease genotypes produce a range of phenotypic conditions and diverse diseases nearly all of which are unresponsive to chloride intake (a possible exception is congenital chloride diarrhoea (OMIM 214700)) (Puljak and Kilic, 2006; Planells-Cases and Jentsch, 2009).

The Panel considers that, as yet, no genotype has been identified that requires consideration in the estimation of DRVs for chloride in the general population.

3. Dietary sources and intake data

3.1. Dietary sources

All unprocessed foods contain chloride, albeit at low levels. The chloride content of unprocessed meat and fish may be up to 4 mg/g, whereas fruit and vegetables contain generally less than 1 mg/g (Scherz and Senser, 2000; UK Food Standards Agency, 2002; Anses, 2016). Chloride content can be substantially higher than sodium in fruit and vegetables, while sodium is found in somewhat higher or equimolar concentrations compared with chloride in animal tissues. Analyses of 14 experimental one-day diets free from added sodium chloride were found to contain between ca 20 and 60 mmol (900–2,700 mg) chloride (energy content ranged between 1,900 and 2,300 kcal) (Hulet, 1955), which indicates the 'natural' content of chloride in the diet. The sodium content of these diets was between 10 and 35 mmol (230–805 mg).

The chloride content of drinking water is affected by anthropogenic sources (e.g. use of inorganic fertilisers or treatment with chlorine or chloride for disinfection purposes). Concentrations of chloride in tap water are typically below 50 mg/L (WHO, 2003). The Panel notes that the water chloride content is low as compared with dietary sources and the contribution of drinking water to overall chloride intake is expected to be small.

Chloride may be added to food as sodium chloride ('table salt') or as mixtures of sodium chloride and potassium chloride. Other chloride-containing food additives include chloride in conjunction with calcium, chromium (III), magnesium, manganese and zinc, as well as thiamin hydrochloride and pyridoxine hydrochloride, which may be added to both food[5] and food supplements,[6] and thiamine monophosphate chloride and thiamine pyrophosphate chloride, which can be added to food supplements only.[7] The chloride content of infant and follow-on formula is regulated.[7]

A study of processed foods in the Netherlands reported average chloride content of between 3 mg/g in cakes and pastries and more than 10 mg/g in chips/nuts, sauces, processed meat and cheese (Capuano et al., 2013). The molar concentrations of chloride and sodium were similar in about half of the examined commodities, while they differed significantly in the other half. The largest differences were found for the group of cakes/pastries and processed meat, which was partly explained by the use of sodium-containing food additives in these products (e.g. sodium bicarbonate in pastries, sodium nitrate in processed meat). In the other food groups, differences were ±10%.

The Panel is not aware of any assessment of the relative contribution of sodium chloride vs chloride-containing food additives vs inherently food-borne sources of chloride to total chloride intake. In view of the low content of chloride in unprocessed (unsalted) foods relative to the levels of consumption of sodium chloride in Western countries, sodium chloride (from processed food and discretionary use) is considered to be the principal source of dietary chloride in Western diets. In studies which involved individuals consuming their habitual diet, the levels of excretion of sodium and chloride, in mmol/day, were found to be similar (Sanchez-Castillo et al., 1987b; Kübler, 1995; Wang et al., 2013; Curcio et al., 2016; Terry et al., 2016) (Appendix B.1). Differences (in mmol) in urinary excretions of sodium and chloride become more prominent when a no- or low-salt diet is consumed (Dole et al., 1950; Dole et al., 1951; Oliver et al., 1975). In a study of 26 Yanomamo Indians, average daily urinary excretion was 1.0 mmol sodium vs 13.7 mmol (about 0.5 g) chloride (Oliver et al., 1975).

[5] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26.

[6] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[7] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p.1.

The Panel notes that sodium chloride added during industrial food processing, discretionary use or food preservation is the major source of dietary chloride in Western diets. Other sources of chloride include inherently food-borne sources, and chloride-containing food additives, in which chloride may be associated with cations other than sodium.

3.2. Dietary intake

There is a paucity of publications providing estimates of daily chloride intake based on food consumption data, primarily reflecting limitations in capturing the intake of chloride sources (e.g. sodium chloride added at the table or in cooking) and the restricted knowledge of the chloride content of foodstuffs.

Since 24-hour urine excretion of chloride may be a valid marker of a population's average intake, the Panel launched a call to collect available data on urinary chloride levels in Europe. Replies were received from 20 out of 32 countries. Only one country (Austria) provided data, which were chloride concentrations in single spot urine samples (Elmadfa, 2012) and thus do not reflect daily chloride intake (Appendix B.2).

Studies which quantified 24-hour urinary excretion of sodium and chloride in subjects from Western populations are tabulated in Appendix B.1. These data indicate that, on a molar basis, both electrolytes are excreted in similar amounts.

The Panel notes that, in Western diets, sodium chloride is the major source of chloride intake which is reflected in the similar levels of urinary excretion of sodium and chloride, on a molar basis.

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The German-speaking countries (D-A-CH, 2016), the US Institute of Medicine (IOM, 2005) and the UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) derived an adequate intake for chloride in adults from the values of sodium, on an equimolar basis. For pregnant and lactating women, the same values as for other adults were adopted by these bodies.

The SCF (1993) did not set DRVs for chloride but stated that chloride intake should match the Acceptable Range of Intakes for sodium.

Table 1: Overview of Dietary Reference Values for chloride for adults

	D-A-CH (2016)	IOM (2005)	DH (1991)
Age (years)		19–50	≥ 19
AI (mg/day)	2,300 [(a)]	2,300 [(a)]	2,500 [(a)(b)]
Age (years)		51–70	
AI (mg/day)		2,000	
Age (years)		> 70	
AI (mg/day)		1,800	

AI: Adequate Intake; D-A-CH: Deutschland-Austria-Confoederatio Helvetica; DH: Department of Health (UK); IOM: US Institute of Medicine of the National Academy of Sciences.
(a): the value also applies to pregnant and lactating women.
(b): Reference Nutrient Intake.

4.2. Infants and children

As for adults, the US Institute of Medicine (IOM, 2005) and the UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) derived adequate intakes for chloride in infants and children from the values of sodium, on an equimolar basis.

The German-speaking countries (D-A-CH, 2016) estimated values for infants based on chloride intake from human milk, while for older children values for chloride were set at a level equimolar to reference values for sodium.

466 **Table 2:** Overview of Dietary Reference Values for chloride for children

	D-A-CH (2016)	IOM (2005)	DH (1991)
Age (months)	4–< 12	0–6	4–6
AI (mg/day)	450	180	426 (a)
Age (months)		7–12	7–9
AI (mg/day)		570	497 (a)
Age (months)			10–12
AI (mg/day)			533 (a)
Age (years)	1–3	1–3	1–3
AI (mg/day)	600	1,500	781 (a)
Age (years)	4–6	4–8	4–6
AI (mg/day)	750	1,900	1,065 (a)
Age (years)	7–9		7–10
AI (mg/day)	1,150		1,775 (a)
Age (years)	10–12	9–18	11–18
AI (mg/day)	1,700	2,300 (b)	2,485 (a)
Age (years)	13–14		
AI (mg/day)	2,150		
Age (years)	15–18		
AI (mg/day)	2,300		

467 AI: Adequate Intake; D-A-CH: Deutschland-Austria-Confoederatio Helvetica; DH: Department of Health (UK); IOM: US Institute
468 of Medicine of the National Academy of Sciences.
469 (a): Reference Nutrient Intake (expressed in mmol/day in the original report).
470 (b): the value also applies to pregnant and lactating adolescents aged 14–18 years.

471 5. Criteria (endpoints) on which to base Dietary Reference Values

472 5.1. Biomarkers as indicators of chloride requirement

473 As stated in Section 2.4, the Panel considers that there are no appropriate biomarkers of chloride
474 status that can be used to set DRVs for chloride.

475 5.2. Balance studies

476 A few studies have measured chloride intake and losses and related chloride 'balance' in various
477 experimental settings (McCance, 1936; Falconer and Lyall, 1937; Dole et al., 1950; Heer et al., 2009).
478 One of them was a balance study designed to determine the basal requirement of sodium chloride
479 (Falconer and Lyall, 1937; Lyall, 1939). However, this study involved only three participants and used
480 the terms 'sodium chloride' and 'chloride' interchangeably without any adjustment to quantities, which
481 render its interpretation difficult. In the other studies the assessment of chloride 'balance' was part of
482 the data collected to characterise the metabolic effects of sodium chloride deficiency (McCance,
483 1936), the rice-fruit Kempner diet for treating hypertension (Dole et al., 1950) or of increasing sodium
484 intake (Heer et al., 2009). Furthermore, these studies bear important limitations such as: (a) the very
485 small number of study participants (two in McCance (1936)); (b) the inclusion of study participants
486 with pre-existing medical conditions (Dole et al., 1950); and (c) the absence of adaptation periods and
487 lack of measurements of both faecal and dermal losses (Heer et al., 2009).

488 The Panel considers that there are no balance studies that can be used to set DRVs for chloride.

489 5.3. Indicators of chloride requirement in pregnancy and lactation

490 At all gestational ages, foetal chloride concentration is 5 mmol/L higher than in maternal blood.
491 Chloride transport mechanisms across the human placenta have been characterised, although the
492 physiological roles of the chloride transporters and channels remain unclear (Riquelme, 2009;
493 Sadovsky and Jansson, 2015).

494 Pregnancy is associated with physiological adaptive changes in electrolytes' metabolism (Gupta and
495 Nath, 1964; Cheung and Lafayette, 2013; EFSA NDA Panel, 20YY). As for sodium, the Panel assumes

that these processes provide the chloride necessary for covering the need related to the expansion of the maternal extracellular fluid volume and the compositional requirements of the fetus, placenta and the amniotic fluid, without requiring an increase in maternal dietary intake.

Chloride losses in human milk are relatively low (a few mmol/day). Chloride concentration in human milk, as for that of other electrolytes, is regulated by the secretion mechanisms in the mammary cells and is not influenced by dietary factors (Section 2.3.3.4). The Panel considers that there is no evidence that the chloride requirement of lactating women differs from the requirement of non-lactating women.

5.4. Indicators of chloride requirement in infants and children

Fomon (1993) proposed a factorial approach for the determination of the chloride requirement of infants. The whole body content of chloride for each of the first 12 months was calculated from the amount of extracellular water and its chloride content (ca. 4 g/kg), on the assumption that the chloride not present in the extracellular water is negligible. The daily increment in body chloride was calculated by dividing the difference of the whole body chloride content between the beginning and the end of a month by the number of days. The average daily increment between age zero and 4 months (29 mg/day) and between age 4 and 12 months (16 mg/day) was added to the inevitable chloride losses via urine (assumed to be zero) and skin to calculate the chloride physiological requirement (76 and 74 mg/day for 0–4 and 4–12 month-olds, respectively). Assuming an absorption of 95% for dietary chloride, the chloride dietary requirement would be 78 and 76 mg/day for 0–4- and 4–12-month-olds, respectively. Fomon (1993) proposed a daily recommended intake of 120 mg (3.5 mmol) of chloride for infants throughout the first year of life in consideration of both the uncertainty created by the limited data available and the need for assumptions to be made, and the necessity to provide for individual variability of requirements. The Panel notes that the amount of chloride provided by human milk during the first six months of life (i.e. 320 mg/day assuming a volume of 0.8 L/day and a chloride concentration of 400 mg/L, see Section 2.3.3.4) is higher than this calculated physiological requirement.

5.5. Chloride intake and health consequences

5.5.1. Risk of cardiovascular diseases

The Panel notes that there is evidence that chloride can contribute to the effect of sodium chloride on blood pressure (Section 2.4). An independent effect of chloride on cardiovascular risk has been explored in observational studies using serum/plasma chloride concentration (McCallum et al., 2013). The Panel notes that serum/plasma chloride concentration cannot be used as a marker for chloride intake (Section 2.4.2). The Panel notes that no studies are available which investigate the association between chloride intake or urinary excretion and cardiovascular-disease-related health outcomes.

The Panel considers that the available evidence on cardiovascular disease cannot be used to set DRVs for chloride.

5.5.2. Gastric cancer

A number of prospective cohort studies have assessed the association between sodium chloride intake and gastric cancer incidence and/or mortality (EFSA NDA Panel, 20YY, Annex A). The population-based studies available in the literature evaluated associations between sodium chloride or sodium intake and gastric cancer risk and there are no studies that evaluated the independent role of chloride from sodium chloride in the disease occurrence.

The Panel considers that the available evidence on gastric cancer incidence and/or mortality cannot be used to set DRVs for chloride.

6. Data on which to base Dietary Reference Values

The Panel considers that there is no data that can be used to derive ARs and PRIs for chloride.

The Panel noted the close relationship between sodium and chloride balances in the body (Sections 2.3.3.1 and 2.4.1). Sodium chloride is the main source of both electrolytes in European diets and

similar urinary excretion levels of sodium and chloride (on a molar basis) are typically observed in Western populations (Section 3). Hence, the Panel considers that reference values for chloride can be set at values equimolar to the reference values for sodium (EFSA NDA Panel, 20YY), for all age and life-stage groups (Table 3). Consistent with the reference values for sodium, the values proposed for chloride are considered to be safe and adequate intakes for the general EU population, under the consideration that the main dietary source of chloride intake is sodium chloride (Section 3). BOX-1 provides an explanation for the use of the terms 'safe' and 'adequate'.

Table 3: Summary of Dietary Reference Values for chloride

Age	Safe and adequate intake for chloride[a] (g/day)
7–11 months	0.3[b]
1–3 years	1.7
4–6 years	2.0
7–10 years	2.6
11–17 years	3.1
≥ 18 years[c]	3.1

(a) Derived by multiplying the reference values for sodium (EFSA NDA Panel, 20YY) by 35.5/23 and rounded to the nearest 0.1.
(b) Adequate Intake.
(c) Including pregnant and lactating women.

BOX 1 - Safe and adequate intake: explanation for the terms

Safe: Although the term 'safe intake' is not defined in the principles on deriving and applying DRVs (EFSA NDA Panel, 2010), the concept of a *safe* intake has been used in previous assessments when providing advice on a daily intake of a nutrient which does not give rise to concerns about adverse health effects, in case a tolerable upper intake level (UL) could not be established (SCF, 2000; EFSA NDA Panel, 2012b).

Adequate: An adequate intake (AI) is the value estimated when a population reference intake (PRI) cannot be established because an average requirement (AR) cannot be determined (EFSA NDA Panel, 2010). The AI is the level of intake that is assumed to be sufficient based on observations from groups of apparently healthy people. It involves more judgement than is used for determining an AR or PRI. The practical implication of an AI is similar to that of a PRI i.e. to describe the level of intake of a nutrient that is considered adequate for good health. The distinction in the terms relates primarily to the different strength of the scientific basis on which they rest.

The reference values for chloride are set at values equimolar to the reference values for sodium, under the consideration that the main dietary source of chloride intake is sodium chloride. The reference values for chloride are called 'safe' and 'adequate' consistent with the use made of these terms for sodium (EFSA NDA Panel, 20YY).

Conclusions

The Panel concludes that there is insufficient evidence to derive an AR and a PRI for chloride. The Panel proposes references values for chloride which are derived from the reference values for sodium on an equimolar basis, for all age and life-stage groups (Table 3). Consistent with the reference values for sodium, the values proposed for chloride are considered to be safe and adequate intakes for the general EU population, under the consideration that the main dietary source of chloride intake is sodium chloride.

Recommendations for research

There is a need for studies, using robust assessment methods for chloride intake and the outcome of interest, to investigate the effects on health of chloride intake, independent from that of sodium. This will become particularly relevant if a significant proportion of sodium chloride becomes substituted by other chloride salts in the diet.

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Appendix A – Concentrations of chloride in breast milk from mothers of term infants

Reference	Number of women (number of samples)	Country	Stage of lactation (time post-partum)	Cl concentration mmol/L Mean ± SD[a]	Cl concentration mg/L Mean ± SD[a]	Analytical method
Allen et al. (1991) Neville et al. (1991)	12	USA	3 days	25.4 ± 1.9 [b]	902 ± 67 [b]	Samples collected by manual expression from each breast. Chloride analysed by automated colorimetric procedure.
			7–9 days	20.5 ± 1.9 [b]	728 ± 67 [b]	
			14 days	20.5 ± 1.6 [b]	728 ± 57 [b]	
			30 days	16.5 ± 0.7 [b]	586 ± 25 [b]	
			90 days	13.9 ± 1.0 [b]	493 ± 36 [b]	
			180 days	11.6 ± 0.8 [b]	412 ± 28 [b]	
			360 days	13.0 ± 1.1 [b]	462 ± 39 [b]	
Atkinson et al. (1980)	10 (32)	Canada	3–5 days	23.0 ± 0.15 [c]	817 ± 5 [c	24-hour milk samples collected by manual or electric breast pump 4–6 times per day. Chloride analysed by ashing followed by ion-specific electrode measurement.
			7–10 days	18.1 ± 1.8 [c]	645 ± 64 [c]	
			30 days	12.0 ± 1.0 [b][c]	426 ± 36 [b][c]	
Bauer and Gerss (2011)	10	Germany	1–8 weeks	10.9 ± 1.01	387 ± 36 [c]	Samples obtained mechanically with an electric breast pump. Chloride analysed by absorption spectrometer and colorimetric assay.
Gross et al. (1980)	(10)	USA	3 days	26.9 ± 2.4 [c]	955 ± 85 [c]	Samples collected by manual or mechanical emptying of both breasts. Chloride analysed by amperometric titration.
	(13)		7 days	21.3 ± 2.7 [c]	756 ± 96 [c]	
	(13)		14 days	14.5 ± 1.5 [c]	515 ± 53 [c]	
	(12)		21 days	15.2 ± 1.9 [c]	540 ± 67 [c]	
	(11)		28 days	13.1 ± 2.3 [c]	465 ± 82 [c]	
Lemons et al. (1982)	(7)	USA	7 days	13.9 ± 1.57 [d]	493 ± 56 [d]	Complete 24-hour milk expressions obtained by electric pump. Chloride analysed by automated colorimetric procedure.
	(7)		14 days	12.11± 1.82 [d]	430 ± 65 [d]	
	(7)		21 days	10.53 ± 1.17 [d]	374 ± 42 [d]	
	(7)		28 days	10.46 ± 0.91 [d]	371 ± 32 [d]	
Picciano et al. (1981)	26 (234)	USA	1 month	12.0 ± 2.37	426 ± 84	Samples collected with a manual breast pump or by manual expression. Chloride analysed by ashing followed by ion-
			2 months	11.7 ± 2.09	415 ± 74	
			3 months	11.93 ± 2.57	424 ± 91	

Reference	Number of women (number of samples)	Country	Stage of lactation (time post-partum)	Cl concentration mmol/L Mean ± SD[a]	Cl concentration mg/L Mean ± SD[a]	Analytical method
						specific electrode measurement.
Wack et al. (1997)	30 (140)	USA	0–60 days	12.93 ± 3.49	459 ± 124	Samples collected by hand expression or breast pump from a single breast. Chloride determined by a potentiometric method using a Buchler Digital Chloridometer.
			61–120 days	11.32 ± 2.73	402 ± 97	
			121–180 days	9.55 ± 4.54	339 ± 161	
			181–240 days	12.96 ± 6.54	460 ± 232	
			241–300 days	11.83 ± 3.75	420 ± 133	
			301–360 days	10.82 ± 5.55	384 ± 197	
			> 360 days	11.18 ± 3.52	397 ± 125	

Studies were identified by a comprehensive literature search for publications from January 2010 to January 2014 (LASER Analytica, 2014) and from a previous review by Atkinson et al. (1995). If studies did not report whether infants were born at term or not, it was presumed that infants were born at term.
SD: standard deviation; SE: standard error; SEM: standard error of mean.

(a) Unless specified otherwise.
(b) This information is taken from Atkinson et al. (1995).
(c) Mean ± SEM.
(d) Mean ± SE.

Appendix B – Urinary excretion of sodium and chloride in Western adult populations

B.1 Daily urinary excretion

Reference	Country	Population	Age (years)	N	Na (mmol/day)		Cl (mmol/day)		Method
					Mean ± SD[(a)]	97th perc.[(a)]	Mean ± SD[(a)]	97th perc.[(a)]	
Sanchez-Castillo et al. (1987a)	UK		20–60						Multiple 24-hour urinary collection. Completeness checked based on the creatinine content of the samples (incomplete if < 2 SD below the mean creatinine output for the individual) and excluded. Cl- measured by the ferric ammonium sulfate /mercuric thiocyanate technique and Na+ by autoanalyser.
		Men		33	187 ± 55	-	182 ± 54	-	
		Women		50	131 ± 35	-	127 ± 35	-	
Wang et al. (2013)	USA		18–39						Single 24-hour urinary collection. Completeness checked based on the length of collection, urine volume, and responses to eight questions asked upon return of the specimens. If the participant was unable or unwilling to redo an incomplete collection, the existing sample was excluded. Na+ and Cl- measured using ion-selective electrodes.
		Men (non-black)		97	154 ± 62	200[(b)]	148 ± 61	181[(b)]	
		Women (non-black)		114	131 ± 53	172[(b)]	124 ± 49	153[(b)]	
		Men (black)		89	153 ± 70	179[(b)]	142 ± 64	169[(b)]	
		Women (black)		107	138 ± 57	163[(b)]	131 ± 57	150[(b)]	
Kübler	Germany		18–88						Single 24-hour urinary

Reference	Country	Population	Age (years)	N	Na (mmol/day)		Cl (mmol/day)		Method
					Mean ± SD[a]	97th perc.[a]	Mean ± SD[a]	97th perc.[a]	
(1995)		Men (Q1)		167	143.0[c]	304.6	143.5[c]	266.9	collection. Collection with creatinine < 4 mmol/24 h or > 20 mmol/24 h excluded. Na+ measured using flame-photometry and Cl- measured by colorimetry.
		Men (Q2)		178	173.5[c]	344.3	167.9[c]	360.3	
		Men (Q3)		181	198.5[c]	393.0	191.7[c]	352.7	
		Men (Q4)		167	202.4[c]	339.8	191.1[c]	334.8	
		Women (Q1)		224	110.9[c]	288.5	104.0[c]	264.4	
		Women (Q2)		245	132.3[c]	-	127.5[c]	244.3	
		Women (Q3)		244	138.1[c]	-	125.5[c]	280.0	
		Women (Q4)		233	153.1[c]	-	152.8[c]	283.3	
Curcio et al. (2016)	Switzerland				Median		Median		Single 24-hour urinary collection. Participants with an estimated GFR < 60 mL/min/1.73 m^2 or a 24-h urine collection of < 600 mL excluded. Na+ and Cl- measured using ion-selective electrodes.
		Men	20–89	121[d]	159[c]	326	160[c]	289	
		Women	19–82	118	121[c]	217	124[c]	207	

GFR: glomerular filtration rate; Q: quartile of intake, estimated by seven-day consumption diaries; SD: standard deviation; SE: standard error.
(a) Unless specified otherwise.
(b) 75th percentile.
(c) Median.
(d) Number of samples available for chloride analysis: N = 119.

876 **B.2 Urinary concentration**

Reference	Country	Population	Age (years)	N	Na (mmol/L)	Cl (mmol/L)	Method
Elmadfa (2012)	Austria	Boys	7–14	392[(a)]	144.2	107.7	Single spot urine samples
		Girls	7–14		132.8	107.1	
		Men	18–64	419[(a)]	108.5	106.1	
		Women	18–64		82.7	106.0	
		Men	65–80	196[(a)]	104.7	108.1	
		Women	65–80		85.2	107.6	

877 (a) Boys and girls.
878

879 **Abbreviations**

AI	Adequate Intake
CFTR	cystic fibrosis transmembrane conductance regulator
COMA	Committee on Medical Aspects of Food Policy
D-A-CH	Deutschland–Austria–Confoederatio Helvetica
DRV	Dietary Reference Value
FAO	Food and Agriculture Organization
IOM	US Institute of Medicine of the National Academy of Sciences
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NHANES	US National Health and Nutrition Examination Survey
OMIM	Online Mendelian Inheritance in Man
SCF	Scientific Committee for Food
WHO	World Health Organization

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