参考 4

European Food Safety Authority

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STATEMENT OF EFSA

On the Evaluation of a new study related to the bioavailability of aluminium in food[1]

European Food Safety Authority[2, 3]

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ABSTRACT

The European Food Safety Authority (EFSA) was asked to evaluate a new study provided by industry that reports on the bioavailability of aluminium from several aluminium compounds in the rat. EFSA was asked whether the scientific data provided by the study could trigger the revision of the safety evaluation performed by EFSA in 2008, for the different aluminium based food additives investigated in this report (in particular SALP acidic, also known as sodium aluminium phosphate, acidic form or E 541). In the new study, the oral bioavailability of aluminium was determined as the ratio of the fraction of radioactivity left in the carcass seven days after oral administration of the ^{26}Al-labelled compound of interest over the fraction of radioactivity left in the carcass seven days after intravenous administration of ^{26}Al-labelled aluminium citrate using accelerator mass spectrometry (AMS). The results from the study show that the oral bioavailability of aluminium from twelve different aluminium-containing compounds, including the food additives aluminium sulphate, Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate, ranges from 0.02 to 0.21%, and therefore falls within the overall 10-fold range of previously reported oral bioavailability values for aluminium from aluminium containing compounds. In the case of the two sodium aluminium phosphates, SALP acidic and SALP basic (KASAL), and aluminium metal, the measurements were below the limit of detection by AMS. In conclusion, the new study does not provide any additional information on the bioavailability of aluminium from aluminium-containing compounds that could modify the conclusions reached in 2008 by the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials. Therefore, EFSA concludes that this study does not give reason to reconsider the previous safety evaluation of aluminium-based food additives authorised in the European Union performed by EFSA in 2008.

KEY WORDS

(aluminum, bioavailability, toxicokinetics, elimination, rat)

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to evaluate a new study provided by industry that reports on the bioavailability of aluminium from several aluminium-containing compounds in the rat. EFSA was asked whether the scientific data provided by the new study could trigger the revision of the safety evaluation performed by EFSA in 2008, for the different aluminium-based food additives scrutinised in this report (in particular SALP acidic, also known as sodium aluminium phosphate, acidic form or E 541).

Aluminium occurs naturally in the environment, and is the most abundant metallic element in the earth's crust. The naturally occurring stable isotope is ^{27}Al. The isotope ^{26}Al has a long half life but a low natural abundance and is used as a tracer in biological studies. Aluminium is only found in nature as Al^{3+}.

The absorption, distribution and elimination properties of aluminium and several aluminium compounds in humans and experimental animals have been reviewed extensively. The gastrointestinal absorption of aluminium from aluminium compounds is determined to a large extent by its ionic availability in the gut content, and this is mainly related to the prevailing pH, the presence of complexing ligands with which the metal may form absorbable aluminium species and the chemical form of the ingested aluminium compound. It is thought that acid digestion in the stomach would degrade most of the ingested aluminium compounds to yield "free" and soluble Al^{3+}, i.e. hydrated Al^{3+}, part of which may be complexed with mono-, di- and tricarboxylic acids such as citric acid. By passing from the stomach to the intestines the increase in pH results in successive deprotonations and the formation of complexes of aluminium with hydroxide and finally, the formation of insoluble aluminium hydroxide at neutral pH. Therefore, as the pH is neutralised in the duodenum the aluminium ion is gradually converted to aluminium hydroxide and the majority is then expected to precipitate in the intestine, with subsequent faecal excretion, leaving only a minor fraction available for absorption.

Available studies indicate that the oral bioavailability of aluminium in humans and experimental animals from drinking water is approximately 0.3%, whereas the bioavailability of aluminium from food and beverages generally is considered to be lower, about 0.1%. However, considering the available human and animal data, it is likely that the oral absorption of aluminium from food can vary at least 10-fold depending on the chemical forms present in the intestinal tract. The total body burden of aluminium in healthy human subjects has been reported to be approximately 30–50 mg/kg bw. About one-half of the total body aluminium is in the skeleton. Aluminium has also been found in human skin, lower gastrointestinal tract, lymph nodes, adrenals, parathyroid glands, and in most soft tissue organs. In rats accumulation of aluminium after oral exposure was higher in the spleen, liver, bone, and kidneys than in the brain, muscle, heart, or lung. It has also been reported that aluminium can reach the placenta and fetus and to some extent distribute to the milk of lactating mothers.

The main carrier of Al^{3+} in plasma is the iron binding protein transferrin. Studies have demonstrated that about 90% of the Al^{3+} in plasma is bound to transferrin and about 10% to citrate. Cellular uptake of aluminium in organs and tissues is relatively slow. Absorbed aluminium is eliminated primarily by the kidneys, presumably as the citrate, and excreted in the urine. Unabsorbed aluminium is excreted in the faeces. Excretion via the bile constitutes a secondary, but minor route. Multiple values ranging from hours to days and years have been reported for the elimination half life of aluminium in humans and animals, suggesting that there are multiple compartments for aluminium storage from which aluminium is eliminated.

The specific aim of the study under consideration was to provide experimental data on the oral bioavailability of a number of aluminium-containing compounds for which there were limited or no data on the toxicokinetic properties. The experimental approach adopted by the authors of the study was to prepare ^{26}Al-labelled compounds with sufficiently high levels of ^{26}Al relative to the stable ^{27}Al isotope to enable the detection of the radiolabel by accelerator mass spectrometry (AMS) in the

carcass of the dosed animals. Bioavailability was determined as the ratio of the fraction of radioactivity left in the carcass seven days after oral administration of the ^{26}Al-labelled compound of interest over the fraction of radioactivity left in the carcass seven days after intravenous administration of ^{26}Al-labelled aluminium citrate. Oral administration was as solutions in the case of the citrate, nitrate, sulphate and chloride salts of aluminium. In contrast, aluminium hydroxide, aluminium oxide, the two sodium aluminium phosphates, SALP acidic and SALP basic (KASAL), and sodium aluminium silicate were insoluble, and were administered as suspensions in carboxymethylcellulose. In the case of Allura RedAC aluminium lake (FD&C red 40 aluminium lake), powdered pot electrolyte and aluminium metal, the particles were too large for administration by gastric feeding tube; instead, they were mixed with honey for administration to the back of the rat tongue.

The results of the analysis of the control (untreated) animals presented in the study under consideration showed that the mean background ^{26}Al:^{27}Al ratio was 5 x 10^{-13}. Seven days after ^{26}Al citrate injection (iv), the ratio was approximately 500 times higher. This represented only 8.6% of the injected dose. The ^{26}Al:^{27}Al ratios in the oral dosing study were much lower, being only 1.5- to 15-fold higher than the mean background ^{26}Al:^{27}Al ratio obtained from control (untreated) animals. For the soluble aluminium citrate, chloride, nitrate and sulphate salts, the fraction absorbed ranged from 0.045 to 0.21% of the dose. In the case of the following aluminium compounds administered as suspension, aluminium hydroxide, aluminium oxide, Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate, the percentage of the aluminium dose absorbed ranged from 0.018 to 0.12%. However, the measured ^{26}Al:^{27}Al ratios for the two sodium aluminium phosphates, SALP acidic and SALP basic (KASAL), and aluminium metal were below the limit of detection by AMS.

EFSA notes that the measurements of the remaining quantity were made on day 7 following the administration. The authors argued that this extended time span ensures that all ingested aluminium had been cleared from the gastrointestinal tract and the phase of rapid excretion of aluminium in urine following its uptake into blood (short-term clearance) had been complete. However, the limitation of their approach is that less than 10% of the bioavailable dose remains in the experimental animals after administration of the compounds. Also, the authors of the study assumed that the single time point ^{26}Al:^{27}Al ratio measurements accurately reflect the toxicokinetics of aluminium.

In the case of aluminium metal and the two sodium aluminium phosphate forms, SALP acidic and SALP basic (KASAL), the AMS measurements were below the experimental limit of detection. The authors of the study acknowledged that for SALP and SALP basic (KASAL), this was due to the low level of ^{26}Al that was incorporated into the test product relative to the ^{27}Al levels. While this makes it impossible to derive bioavailability data, from the limits of detection provided by the authors, the bioavailability of aluminium metal and SALP basic (KASAL) can be estimated to be <0.015% and <0.024% for SALP acidic. However, in the case of aluminium metal, the conclusion that its bioavailability is <0.015% is only valid if assuming that the size of the aluminium metal particles had no impact on its absorption.

The oral bioavailability of aluminium in humans and experimental animals from drinking water is approximately 0.3%, whereas the bioavailability of aluminium from food and beverages generally is considered to be lower, about 0.1%. The results presented in the study discussed in this Statement not only confirm these findings but also extend them to several aluminium-containing food additives authorised in the EU that had not previously been assessed for their bioavailability. The bioavailability of aluminium from SALP, acidic, which could not be determined due to the technical reasons outlined above, has recently been studied in the rat using SALP acidic incorporated in a biscuit and SALP basic (KASAL) in cheese. The latter study found the bioavailability aluminium from biscuit and cheese to be around 0.1% and 0.1-0.3%, respectively.

Overall, the study under consideration concludes that the oral bioavailability of twelve different aluminium-containing compounds, including the food additives aluminium sulphate, Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate, ranges from 0.02 to

0.21%, and thus falls within the overall 10-fold range of previously reported bioavailability values. Therefore, the study does not provide any additional information on the bioavailability of aluminium from aluminium-containing compounds that could modify the conclusions reached in 2008 by the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials. Therefore, EFSA concludes that this study does not give reason to reconsider the previous safety evaluation of aluminium-based food additives authorised in the European Union performed by EFSA in 2008.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

European Parliament and Council Directives 95/2/EC on food additives other than colours and sweeteners and 94/36/EC on colours for use in foodstuffs (as amended) allow a number of aluminium-containing additives to be used in some foodstuffs. Notably aluminium sulphates (E 520-523) are permitted to be used in egg white and candied, crystallised glace fruit and vegetables; acidic sodium aluminium phosphate (E 541) is permitted in scones and sponge wares; aluminium silicates (E 553-559) are permitted in a limited range of food categories and starch aluminium octenyl succinate (E 1452) is permitted in food supplements; aluminium metal (E 173) is authorised for the external coating of sugar confectionary and for the decoration of cakes and pastries. Moreover, the European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs (as amended) also permits the use of aluminium lakes of the permitted colours.

Food additives are reported to be the greatest contributors to intake of aluminium from food, but other sources also contribute to the overall intake, e.g. aluminium naturally present in plant products, migration from food contact materials and aluminium-based medicines.

The European Food Safety Authority (EFSA) evaluated the safety of aluminium from dietary intake in 2008. In view of the cumulative nature of aluminium in the organism after dietary exposure, the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) considered it more appropriate to establish a tolerable weekly intake (TWI) for aluminium rather than a tolerable daily intake (TDI). Based on the combined evidence from the available studies, the Panel established a TWI of 1 mg aluminium/kg bw/week. However, the Panel also noted that the estimated daily dietary exposure to aluminium in the general population, assessed in several European countries, varied from 0.2 to 1.5 mg/kg bw/week at the mean and was up to 2.3 mg/kg bw/week in highly exposed consumers. The TWI of 1 mg/kg bw/week is therefore likely to be exceeded in a significant part of the European population.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission is currently working on the revision of the conditions of use and use levels of all aluminium based food additives in order to ensure that the TWI is not exceeded anymore. Industry has recently issued a new study[4] related to the bioavailability of several aluminium compounds in the rat. The Commission asks EFSA to evaluate whether the scientific data provided by this study could trigger the revision of the safety evaluation performed by EFSA in 2008, for the different aluminium based food additives scrutinised in this report (in particular E 541, sodium aluminium phosphate, acidic form - SALP).

[4] Report: The bioavailability of ingested Al-26 labelled aluminium and aluminium compounds in the rat. General Nuclear Product GNP-121100-REPT-001.

ASSESSMENT

1. Introduction

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to evaluate a new study[5] provided by industry that reports on the bioavailability of aluminium from several aluminium compounds in the rat. EFSA was asked whether the scientific data provided by the new study could trigger the revision of the safety evaluation performed by EFSA in 2008, for the different aluminium based food additives scrutinised in this report (in particular SALP acidic, also known as sodium aluminium phosphate, acidic form or E 541).

The EFSA 2008 assessment established a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw, identical to the TWI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2007.

The text following in sections 2, 3 and 4 is based on the EFSA 2008 opinion. New data published since that opinion have also been included.

2. Chemistry of Aluminium

Aluminium is a silvery, white metal. It is ductile and malleable, non-magnetic and non-combustible (Krewski et al., 2007). It is the thirteenth element in the periodic system, with atomic number 13 and a relative atomic mass of 26.98. Its melting point is 660°C and its boiling point is 2467 °C. The density is 2.7 g/cm^3. The naturally occurring stable isotope is ^{27}Al. The isotope ^{26}Al has a long half life but a low natural abundance and is used as a tracer in biological studies (Jouhanneau et al., 1993; Priest, 2004). Aluminium is only found in nature as Al^{3+} (Martin, 1986, 1992).

In aqueous media, water molecules form relatively strong bonds with the Al^{3+} ion, and it has been recognised that in aqueous solution the ligands that form stable complexes with the Al^{3+} ion are fluoride ion and ligands coordinating by means of oxygen donor atoms. It is well known that the number of water molecules in this first sphere of coordination is six, and that these water molecules are regularly coordinated in an octahedral geometry, forming the species $[Al(H_2O)_6]^{3+}$, usually abbreviated as Al^{3+}. This species has a greater tendency to exchange protons than water molecules. In fact the $[Al(H_2O)_6]^{3+}$ ion behaves as a weak acid due to ion dipole forces between Al^{3+} and the oxygen atoms of the coordinated water molecules. It should be stressed that whatever ligands may be present in biological systems the equilibrium between aluminium and the hydroxide anion must be always considered (Martin, 1986, 1992). In acidic aqueous solutions with pH <5, the aluminium ion exists mainly as $[Al(H_2O)_6]^{3+}$. With increasing pH, in less acidic solutions, successive deprotonations of $[Al(H_2O)_6]^{3+}$ occur to yield $Al(OH)^{2+}$, $Al(OH)_2^+$ and soluble $Al(OH)_3$, with a corresponding decrease in the number of coordinating water molecules. Neutral solutions give an $Al(OH)_3$ precipitate that redissolves owing to the formation of the aluminate anion $Al(OH)_4^-$; a mixture of these species occurs in the pH range of 5-7, but at pH > 6.2 $Al(OH)_4^-$ is the predominant soluble aqueous species(Martin, 1986, 1992).

3. Sources

Aluminium occurs naturally in the environment, and is the most abundant metallic element in the earth's crust where it is frequently found as alumino-silicates, hydroxides, phosphates, sulphates and cryolite (WHO, 1997). The production of aluminium metal requires the refining and smelting of aluminium oxide into metallic aluminium and oxygen. Other elements may subsequently be added to yield different alloys (Krewski et al., 2007). Alternatively, aluminium may form inorganic compounds and compounds with organic moieties especially with organic acids (e.g. lactic acid, stearic acid etc), that are produced for different purposes (Martin, 1986, 1992).

[5] Report: The bioavailability of ingested Al-26 labelled aluminium and aluminium compounds in the rat. General Nuclear Product GNP-121100-REPT-001.

4. Toxicokinetic data on aluminium compounds

The absorption, distribution and elimination properties of aluminium and several aluminium compounds in humans and experimental animals have been reviewed extensively (Martin, 1992; Krewski et al., 2007; ATSDR, 2008; EFSA, 2008). In its previous evaluation, the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) noted that most of the biochemical and toxicological studies did not measure the "normal" aluminium content of the basal diet fed to the animals, and therefore the stated dose in such studies is likely to be an underestimate of the total aluminium exposure. In contrast, the actual level of Al^{3+} in test solutions of aluminium compounds for toxicological studies could be dramatically lower than the nominal level if the procedure used for adjusting pH, filtering, and measuring the remaining aluminium in the preparations were not adequately controlled (EFSA, 2008).

The gastrointestinal absorption of aluminium from aluminium compounds is determined to a large extent by its ionic availability in the gut content, and this is mainly related to the prevailing pH, the presence of complexing ligands with which the metal may form absorbable or unabsorbable aluminium species and the chemical form of the ingested aluminium compound (ATSDR, 2008). It is thought that acid digestion in the stomach (pH~2) would degrade most of the ingested aluminium compounds to yield "free" and soluble Al^{+3}, i.e. hydrated Al^{3+}, $(Al(H_2O)_6)^{3+}$, part of which may be complexed with mono-, di- and tricarboxylic acids such as citric acid. By passing from the stomach to the intestines the increase in pH results in successive deprotonations and the formation of complexes of aluminium with hydroxide and finally, the formation of insoluble aluminium hydroxide at neutral pH. Therefore, as the pH is neutralised in the duodenum the aluminium ion is gradually converted to aluminium hydroxide and the majority is then expected to precipitate in the intestine, with subsequent faecal excretion, leaving only a minor fraction available for absorption.

Although the water solubility of aluminium compounds appears to be one of the major factors affecting their bioavailability, it is not possible to extrapolate from solubility in water to bioavailability. Additionally, due to available dietary ligands that may either increase (e.g. citrate, lactate, and other organic carboxylic acid complexing agents, fluoride), or decrease the absorption (such as phosphate, silicon, polyphenols) the bioavailability of any particular aluminium compound can be markedly different depending on the presence or absence of particular food and beverages in the intestines(Martin, 1992; Krewski et al., 2007; ATSDR, 2008; EFSA, 2008).

Available studies indicate that the oral bioavailability of aluminium in humans and experimental animals from drinking water is approximately 0.3%, whereas the bioavailability of aluminium from food and beverages generally is considered to be lower, about 0.1% (Priest, 2004; Krewski et al., 2007; ATSDR, 2008). However, considering the available human and animal data, it is likely that the oral absorption of aluminium from food can vary at least 10-fold depending on the chemical forms present in the intestinal tract. Except for aluminium sulphate and sodium aluminium phosphate (SALP), acidic, none of the aluminium compounds authorised as food additives in the EU have been studied for their oral bioavailability. The oral bioavailability of aluminium from SALP, acidic, in the rat, when incorporated in a biscuit was found to be about 0.1% (Krewski et al., 2007; Yokel and Florence, 2008). In the same study but using SALP, basic, incorporated in cheese, oral bioavailability of aluminium was 0.1-0.3% (Krewski et al., 2007; Yokel and Florence, 2008).

After absorption, aluminium distributes unequally to all tissues in humans and accumulates in some. The total body burden of aluminium in healthy human subjects has been reported to be approximately 30–50 mg/kg bw. Normal levels of aluminium in serum are approximately 1–3µg/L (Krewski et al., 2007). For example, the mean serum aluminium level in 44 non-exposed persons who did not use antacids was found to be 0.06 µM (1.6 µg/L) (Valkonen and Aitio, 1997). However, values that were ten-fold higher were reported in haemodialysis patients (Chen et al., 2010). About one-half of the total body aluminium is in the skeleton. Reported normal levels in human bone tissue range from 5 to 10 mg/kg. Aluminium has also been found in human skin, lower gastrointestinal tract, lymph nodes, adrenals, parathyroid glands, and in most soft tissue organs. In rats accumulation of aluminium after

oral exposure was higher in the spleen, liver, bone, and kidneys than in the brain, muscle, heart, or lung. It has also been reported that aluminium can reach the placenta and fetus and to some extent distribute to the milk of lactating mothers. Aluminium levels have been found to increase with age in a number of tissues and organs (bone, muscle, lung, liver, and kidney) of experimental animals. Moreover, aluminium has been shown to rapidly enter the brain extracellular fluid and the cerebrospinal fluid, with smaller concentrations in these than in the blood (Martin, 1992; Krewski et al., 2007; ATSDR, 2008; EFSA, 2008).

The main carrier of Al^{3+} in plasma is the iron binding protein transferrin. Studies have demonstrated that about 90% of the Al^{3+} in plasma is bound to transferrin and about 10% to citrate (Martin, 1986; Hemadi et al., 2003; Chen et al., 2010). Cellular uptake of aluminium in organs and tissues is relatively slow and was believed to occur from the aluminium bound to transferrin by transferrin-receptor mediated endocytosis. However, recent evidence has shown that the Al-transferrin complex does not bind to the transferrin-receptor (Hemadi et al., 2003; Sakajiri et al., 2010), and consequently, alternative pathways of cellular uptake of aluminium must exist.

The distribution of aluminium may be modulated by several factors. Although citrate and fluoride have been shown to reduce tissue accumulation of aluminium and increase its renal excretion in experimental animals, this only occurs when the aluminium concentration exceeds the transferrin metal binding capacity. In humans this is not expected to occur frequently (EFSA, 2008). The iron status is negatively correlated with aluminium accumulation in tissues and animal experiments have shown that calcium and magnesium deficiency may contribute to accumulation of aluminium in the brain and bone.

Following ingestion, absorbed aluminium from the blood is eliminated primarily by the kidneys, presumably as the citrate, and excreted in the urine (Krewski et al., 2007; ATSDR, 2008). Unabsorbed aluminium is excreted in the faeces. Excretion via the bile constitutes a secondary, but minor route. Mean urine aluminium level in 44 non-exposed persons who did not use antacids was 0.33 µM (8.9 µg/L) (Valkonen and Aitio, 1997). Median urine aluminium concentration was 3.3 µg/L in 67 office workers who had not been exposed to aluminium (Liao et al., 2004).

Multiple values have been reported for the elimination half life of aluminium in humans and animals, suggesting that there is more than one compartment of aluminium storage from which aluminium is eliminated. Within the first day after receiving a single intravenous injection of ^{26}Al citrate, approximately 59% of the dose was excreted in the urine of six subjects. At the end of 5 days, it was estimated that 27% of the dose was retained in the body. However, when ^{26}Al levels were monitored for more than 3 or 10 years in a single subject that had received an intravenous injection of ^{26}Al citrate, half-lives of approximately 7 years and 50 years were estimated (Talbot et al., 1995; Priest et al., 1996; Priest, 2004).

Initial half-lives of 2-5 hours were reported in rats, mice, rabbits and dogs after intravenous injection of soluble aluminium salts, such as aluminium chloride, aluminium nitrate and aluminium citrate (Krewski et al., 2007; ATSDR, 2008). When the sampling time was prolonged the half-life of aluminium in rabbits was estimated to be 113, 74, 44, 42, 4.2 and 2.3 days in spleen, liver, lung, serum, kidney cortex, and kidney medulla, respectively. A second half-life in the kidney greatly exceeded 100 days. In rats, the whole organism elimination half-life was estimated to be 8 to 24 days in serum, kidney, muscle, liver, tibia and spleen. Aluminium persists for a very long time in the rat brain following intravenous injection of very small doses of ^{26}Al for which a half-life of 150 days has been reported. However, this estimate is not expected to have a high degree of accuracy as brain samples were not obtained for at least 3 half-lives. Based on calculations for offspring of rats that were given ^{26}Al injections daily from day 1 to 20 postpartum and thereafter examined on days 40, 80, 160, 320 or 730 postpartum, elimination half-lives of approximately 13 and 1635 days in the brain were suggested. Half-lives of 7 and 520 days were suggested for parietal bone. For liver and kidneys half-lives were suggested to be 5 and 430 days and 5 and 400 days, respectively. In blood the values were 16 and 980 days. Based on the above findings, a physiologically based biokinetic (PBBK) model for

aluminium has been developed to describe the absorption, distribution, and excretion of aluminium (Kislinger et al., 1997; Nolte et al., 2001; Steinhausen et al., 2004). However, there is little published information on allometric scaling of aluminium elimination rates that can be used to extrapolate these results from the rat to the human.

5. Evaluation of a new study on the bioavailability of ingested Al-26 labelled aluminium and aluminium compounds in the rat

5.1. Overview

The specific aim of the study was to provide experimental data on the oral bioavailability of a number of aluminium-containing compounds for which there were limited or no data on toxicokinetic properties (Table 1).

Table 1: Aluminium-containing compounds used in the study.

Compound name	E number	Administered form	^{27}Al dose per animal (mg)	^{26}Al dose per animal (ng)
Aluminium citrate	*	Solution	0 (inj.); 50 (oral)	0.19 (inj.); 1.47 (oral)
Aluminium chloride	*	Solution	50	1.24
Aluminium nitrate	*	Solution	50	1.77
Aluminium sulphate	E 520	Solution	50	2.44
Aluminium hydroxide	*	Suspension[5]	17	12.2
Aluminium oxide	*	Suspension[5]	23	17.9
Aluminium metal	E 173	Suspension[6]	6.9	1.4
Powdered pot electrolyte	*	Suspension[6]	26	2.40
FD&C red 40 aluminium lake[1]	E 129	Suspension[6]	414[7]	0.96
Sodium aluminium phosphate, acidic[2]	E 541	Suspension[5]	10	0.46
Sodium aluminium phosphate, basic[3]	*	Suspension[5]	10	0.31
Sodium aluminium silicate[4]	E 554	Suspension[5]	27	0.60

* Not authorised in the EU as food additive
[1] Synonym: Allura Red AC aluminium lake
[2] Synonym: SALP acidic ($NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$)
[3] Synonyms: SALP basic; KASAL (approx. $Na_8Al_2(OH)_2(PO_4)_4$ + 30% NaH_2PO_4)
[4] Synonym: Sodium aluminosilicate
[5] Administered as a suspension in carboxymethylcellulose
[6] Administered mixed with honey for administration to the back of the rat tongue.
[7] Total mass of product (dye lake)

The experimental approach adopted by the authors of the study was to prepare ^{26}Al-labelled compounds with sufficiently high levels of ^{26}Al relative to the stable ^{27}Al isotope to enable the detection of the radiolabel by accelerator mass spectrometry (AMS) in the carcass of the dosed animals. The intent was to administer either approximately 1.47 ng ^{26}Al for soluble compounds or >10 ng for the insoluble compounds. In the case of iv injected aluminium citrate, the dose of ^{26}Al was 0.19 ng. Bioavailability was determined as the ratio of the fraction of radioactivity left in the carcass seven days after oral administration of the ^{26}Al-labelled compound of interest over the fraction of radioactivity left in the carcass seven days after intravenous administration of ^{26}Al-labelled aluminium citrate.

5.2. Experimental design

All the test materials were prepared from aluminium stock solutions containing ^{26}Al as a tracer. Administration was as ultra-filtered solutions in the case of the citrate, nitrate, sulphate and chloride salts of aluminium. Where the test materials were insoluble, these were administered as suspensions in carboxymethylcellulose (Table 1). In the case of Allura Red AC aluminium lake (FD&C red 40 aluminium lake), powdered pot electrolyte and aluminium metal, the particles were too large (~1 mm) for administration by gastric feeding tube; instead, they were mixed with honey for administration to the back of the rat tongue. The oral dose of ^{26}Al per animal ranged from 0.3 to 17.9 ng depending on the compound (Table 1). The doses of ^{26}Al and total ^{27}Al administered per compound were confirmed by AMS.

The study used female Sprague-Dawley rats (10-12 week old). In the first part of the study 12 rats received an iv injection into the saphenous vein of ^{26}Al citrate (1.49 ng per animal). After seven days the animals were sacrificed, and following removal of pelt, gastrointestinal tract, paws, tail and head (to remove potential sources of external contamination), the ^{26}Al remaining in the carcass was determined as follows. After ashing of the carcass, each sample aliquot was spiked with 10.003 mg ^{27}Al tracer and the aluminium present in the sample was converted to aluminium oxide before analysis and determination of the ^{26}Al:^{27}Al ratio by AMS. Samples from a further six control (untreated) animals were generated to produce background ^{26}Al:^{27}Al ratios. In the second part of the study, the oral bioavailability of the aluminium-containing compounds listed in Table 1 was studied in rats (6 animals/group) using oral administration by gastric feeding tube or, in the case of large particles, by coating with honey and administration to the back of the rat tongue (Table 1). The doses are reported in Table 1, and the procedure was as described above.

5.3. Results

The results of the analysis of the control (untreated) animals presented in the study under consideration showed that the mean background ^{26}Al:^{27}Al ratio was 5×10^{-13}. Seven days after ^{26}Al citrate injection (iv), the ratio was approximately 500 times higher. This represented only 8.6% of the injected dose. According to the authors of the study, the seven day span was to ensure that in the oral dosing study, all ingested aluminium had been cleared from the gastrointestinal tract and the phase of rapid excretion of aluminium in urine following its uptake into blood (short-term clearance) had been completed.

The ^{26}Al:^{27}Al ratios in the oral dosing study were much lower, being only 1.5- to 15-fold higher than the mean background ^{26}Al:^{27}Al ratio obtained from control (untreated) animals. Table 2 shows the absorbed fraction computed by the authors of the study from the doses administered and the ^{26}Al:^{27}Al ratios determined at day seven. For the soluble aluminium citrate, chloride, nitrate and sulphate salts, the fraction absorbed ranged from 0.045 to 0.21% of the dose. In the case of following aluminium compounds administered as suspension, aluminium hydroxide, aluminium oxide, Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate, the percentage of the dose absorbed ranged from 0.018 to 0.12%. However, the measured ^{26}Al:^{27}Al ratios for the two sodium aluminium phosphates, SALP acidic and SALP basic (KASAL), and aluminium metal were below the limit of detection by AMS.

Table 2: Mean fractional absorption of the aluminium-containing compounds as computed by the authors.

Compound name	Administered form	Fraction of dose absorbed (mean % ± SD)
Aluminium citrate	Solution	0.079 ± 0.006
Aluminium chloride	Solution	0.054 ± 0.015
Aluminium nitrate	Solution	0.045 ± 0.013
Aluminium sulphate	Solution	0.210 ± 0.079
Aluminium hydroxide	Suspension[5]	0.025 ± 0.041
Aluminium oxide	Suspension[5]	0.018 ± 0.038
Aluminium metal	Suspension[6]	<0.015[7]
Powdered pot electrolyte	Suspension[6]	0.042 ± 0.004
FD&C red 40 aluminium lake[1]	Suspension[6]	0.093 ± 0.020
Sodium aluminium phosphate, acidic[2]	Suspension[5]	<0.024[7]
Sodium aluminium phosphate,basic[3]	Suspension[5]	<0.015[7]
Sodium aluminium silicate[4]	Suspension[5]	0.120 ± 0.011

[1] Synonym: Allura Red AC aluminium Lake
[2] Synonym: SALP, acidic
[3] Synonyms: SALP, basic; KASAL
[4] Synonym: Sodium aluminosilicate
[5] Administered as a suspension in carboxymethylcellulose
[6] Administered mixed with honey for administration to the back of the rat tongue.
[7] Reported as 50% of the mean detection limit.

6. Discussion of the results of the study

The study uses the well accepted approach of comparing the quantity of ^{26}Al tracer remaining in the experimental animals at a given point in time post-oral administration with the quantity remaining at the same point in time post-iv administration. However, EFSA notes that the measurements of the remaining quantity are made on day 7 following the administration. The authors argue that this extended time span ensures that all ingested aluminium had been cleared from the gastrointestinal tract and the phase of rapid excretion of aluminium in urine following its uptake into blood (short-term clearance) had been completed. However, the limitation of their approach is that less than 10% of the bioavailable dose remains in the experimental animals after administration of the compounds. Also, the authors of the study assumed that the single time point ^{26}Al:^{27}Al ratio measurements accurately reflect the toxicokinetics of aluminium. In the case of aluminium metal and the two sodium aluminium phosphate forms, SALP acidic and SALP basic (KASAL), the AMS measurements were below the experimental limit of detection. The authors of the study acknowledged that with SALP acidic and SALP basic (KASAL), this was due to the low level of ^{26}Al that was incorporated into the test product relative to the ^{27}Al levels. Similarly, the large size of aluminium metal particles administered to the animals may have prevented sufficient solubilisation in the gut for any detectable bioavailability to be measured. It is also noted that the intra-experimental variability within groups was generally very high, and in the case of aluminium nitrate, aluminium hydroxide and aluminium oxide, the coefficients of variation were >1. This may be linked to the fact that less than 10% of the total absorbed ^{26}Al remained in the animals after seven days.

The calculation of the bioavailability involved the direct comparison between iv administered aluminium citrate and orally administered aluminium compounds. Except for orally administered aluminium citrate, the other eleven aluminium-containing compounds were chemically distinct from

each other and from the aluminium citrate used in the iv experiments. The chloride, nitrate and sulphate salts were given as solutions, whereas the others were administered as suspensions involving a large range of different particle sizes. Indeed, it is generally thought that the acid within the stomach would degrade most of the ingested aluminium compounds to yield "free" and soluble Al^{3+}, i.e. hydrated Al^{3+}, $(Al(H_2O)_6)^{3+}$ (Krewski et al., 2007; ATSDR, 2008; EFSA, 2008). The subsequent increase in pH when passing from the stomach to the intestines will lead to the formation of insoluble aluminium hydroxide at neutral pH. It is therefore likely that different compounds used in the study are broken down to yield Al^{+3}, and therefore, their absorption kinetics from the gastrointestinal tract may be comparable, with a similar minor fraction available for absorption. However, this fraction may be smaller in the case of Allura Red AC aluminium lake (FD&C red 40 aluminium lake), powdered pot electrolyte and aluminium metal depending on the degree of solubilisation in the rat stomach, particularly in the latter case of the 1 mm aluminium metal particles used by the authors.

Once absorbed, Al^{3+} will complex in the plasma with citrate and transferrin (Martin, 1986; Hemadi et al., 2003; Chen et al., 2010), and Al^{3+} spiking experiments have demonstrated that the distribution ratio between citrate and transferrin (approximately 1 to 9) is maintained as the aluminium concentration increases (Chen et al., 2010). Therefore, even though the doses of the different aluminium-containing compounds administered were different, once absorbed and available in the blood as Al^{3+}, the individual tissue distribution pattern and elimination kinetics would be expected to be similar. Consequently, the reported bioavailability data for the different compounds are expected to reflect their toxicokinetic properties in the rat; however, this is assuming that the single measurements taken after less than 10% of the absorbed dose was left in the treated animals can be extrapolated to accurately reflect the toxicokinetics of aluminium.

Table 2 shows that the mean bioavailability of the soluble aluminium salts ranged between 0.05% and 0.21%. Among the soluble aluminium salts, aluminium sulphate had the highest bioavailability. The bioavailability of the insoluble aluminium hydroxide, aluminium oxide and powdered pot electrolyte was somewhat lower (range 0.02-0.04%). In contrast the bioavailability of the insoluble Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate were 0.09 and 0.12%, respectively. These findings confirm that it is not possible to predict bioavailability from the physico-chemical form of an aluminium-containing compound and that there is no major difference in bioavailability between soluble and insoluble aluminium compounds.

In the case of aluminium metal and the two sodium aluminium phosphate forms, SALP acidic and SALP basic (KASAL), the AMS measurements were below the experimental limit of detection. The authors of the study acknowledged that for SALP and SALP basic (KASAL), this was due to the low level of ^{26}Al that was incorporated into the test product relative to the ^{27}Al levels. While this makes it impossible to derive bioavailability data, from the limits of detection provided by the authors, the bioavailability of aluminium metal and SALP basic (KASAL) can be estimated to be <0.015% and <0.024% for SALP acidic. However, in the case of aluminium metal, the conclusion that its bioavailability is <0.015% is only valid if assuming that the size of the aluminium metal particles had no impact on its absorption.

The oral bioavailability of aluminium in humans and experimental animals from drinking water is approximately 0.3%, whereas the bioavailability of aluminium from food and beverages generally is considered to be lower, about 0.1% (Priest, 2004; Krewski et al., 2007; ATSDR, 2008; EFSA, 2008). The results presented in the study discussed in this Statement not only confirm these findings but also extend them to several aluminium-containing food additives authorised in the EU that had not previously been assessed for their bioavailability. The bioavailability of aluminium from SALP, acidic, which could not be determined due to the technical reasons outlined above, has recently been studied in the rat using SALP incorporated in a biscuit and in cheese (Yokel and Florence, 2008). The latter study found the bioavailability from biscuit in the rat to be about 0.1%.

7. Conclusions

The study under consideration concludes that the oral bioavailability of twelve different aluminium-containing compounds, including the food additives aluminium sulphate, Allura Red AC aluminium lake (FD&C red 40 aluminium lake) and sodium aluminium silicate, ranges from 0.02 to 0.21%, and thus falls within the overall 10-fold range of previously reported bioavailability values (EFSA, 2008). Therefore, the study does not provide any additional information on the bioavailability of aluminium from aluminium-containing compounds that could modify the conclusions reached in 2008 by the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials. Therefore, EFSA concludes that this study does not give reason to reconsider the previous safety evaluation of aluminium-based food additives authorised in the European Union performed by EFSA in 2008.

DOCUMENTATION PROVIDED TO EFSA

1. Report: The bioavailability of ingested Al-26 labelled aluminium and aluminium compounds in the rat. General Nuclear Product GNP-121100-REPT-001.

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ABBREVIATIONS

AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AMS	Accelerator Mass Spectrometry
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
EC	European Commission
EFSA	European Food Safety Authority
JECFA	Joint FAO/WHO Expert Committee on Food Additives
SALP	Sodium Aluminium Phosphate
SD	Standard deviation
TDI	Tolerable Daily Intake
TWI	Tolerable Weekly Intake