EUROPEAN CHEMICALS AGENCY

Helsinki, 21 December 2017

Substance name: Aluminium chloride basic
EC number: 215-477-2
CAS number: 1327-41-9
Date of Latest submission(s) considered[1]: 11 March 2016
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[2] of Aluminium chloride basic (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Substance identity

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance **aluminium chloride basic** :

1.1 Maximum contents of relevant impurities Arsenic, Cadmium, Chromium, Mercury, Nickel, Lead, Antimony, Selenium and Beryllium for each registrant;

1.2 Contents of aluminium and chloride ions, Al/OH molar ratio (basicity grade) and pH for each composition for each registrant.

Human health endpoints

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the analogue substance **aluminium sulphate**:

1.3 Combined *in vivo* mammalian erythrocyte micronucleus test and *in vivo* mammalian comet assay with additional specific investigation on oxidative DNA damage on the following tissues: liver, kidney, glandular stomach and duodenum; test methods EU B.12./OECD 474 and OECD 489 in rats, oral route, using the analogue substance aluminium sulphate, including full-study report, as further specified in Appendix 1;

Exposure-related requests

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance **aluminium chloride basic** :

1.4 Refinement of exposure scenario with regard to the use of Riskofderm v2.1 model for workers dermal exposure estimates (Use of the 'hand' and 'whole-body' load

[1] This decision is based on the registration dossier(s) at the end of the 12 month evaluation period

[2] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

estimates instead of 'hands' only).

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the Chemical Safety Report by **28 June 2019**. The deadline takes into account the time that you, the Registrant(s), may need to agree on who is to perform any required tests.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3.

1. Who performs the testing

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all Registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

2. Appeal

You can appeal this decision to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on aluminium chloride basic and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (MSCA) to complete the evaluation of whether the substance constitutes a risk to human health.

0.0 ANALYSIS OF THE READ-ACROSS APPROACH

For the endpoint genotoxicity, a read-across has been proposed in the dossier of aluminium chloride basic (target substance) with aluminium sulphate (AS), aluminium chloride (AC), aluminium oxide, aluminium hydroxide and aluminium acetate. Very late in the process new data with dialuminium chloride pentahydroxide (CAS no. 12042-91-0) has also been included in your comment on proposals for amendments (PfA).

The read-across proposed by you is based on a category approach with the hypothesis that the registered (target) substance ACH and the source substances AC, AS, aluminium oxide, aluminium hydroxide, aluminium acetate and dialuminium chloride pentahydroxide have similar toxicological properties because they share a common metal ion aluminium and have expected similar bioavailability.

AS, AC andACH share structural similarity. The read-across is supported by similar physico-chemical properties and similar bioavailability.

With regard to the read-across with aluminium acetate and dialuminium chloride pentahydroxide, the read-across is supported by structural similarities and similar physico-chemical properties. However, ECHA does not have toxicokinetics data to fully validate the read-across.

With regard to the read-across with aluminium hydroxide and aluminium oxide, differences in physico-chemical properties, potential lower systemic toxicity and potential lower bioavailability does not support the read-across as these substances may underestimate potential hazard for AC, AS and ACH.

The detailed assessment of read-across in respect to all source substances, with justification, is elaborated below.

In short, results from the existing genotoxicity studies on AS and AC are considered appropriate for ACH and are used for the evaluation of this endpoint.

Substance identity and structural similarity

There is no issue identified with regard to substance identity as the target and source substances are all mono-constituent substances.

The proposed target and source substances are all inorganic salts of aluminium. The target substance ACH shares the ion aluminium +3 with other source substances ACH, AS, aluminium acetate and dialuminium chloride pentahydroxide. They only differ by their counter anions sulphate, chloride, acetate, hydroxychloride. ACH shares with aluminium

hydroxide and aluminium oxide the aluminium part but aluminium hydroxide and oxide are the most stable aluminium compounds and are in forms of particulates.

Physico-chemical data relevant for the toxicological endpoints

Differences in solubility were observed between the aluminium compounds. Indeed, AC, ACH, AS, dialuminium chloride pentahydroxide and aluminium acetate are soluble in water whereas aluminium hydroxide and aluminium oxide are insoluble in water. Although bioavailability appears to be generally correlated to solubility, insufficient data are available to directly extrapolate from solubility in water to bioavailability.

Moreover, aluminium oxide and hydroxide are amphoteric substances that can react both with acid and base whereas aluminium chloride is a strong lewis acid. Therefore, potential differences in reactivity are expected.

Toxicokinetics data

The study by Priest (2010, reviewed by EFSA in 2011) was conducted to compare the bioavailabilities of different ^{26}Al labelled aluminium compounds in groups of six female Sprague-Dawley rats. For the soluble aluminium compounds, it ranged from 0.05% to 0.2% (aluminium chloride, 0.054%; aluminium sulphate, 0.21%). Uptakes of the other insoluble compounds were lower (aluminium hydroxide, 0.025%; aluminium oxide, 0.018%). Uptake of aluminium metal could not be fully quantified because it was below the limit of detection (LOD). Under these experimental conditions, the most bioavailable aluminium compound was AS with an oral absorption rate of 0.21%.

The results of Priest (2010) show that the compounds administered as suspensions (hydroxide, oxide) were less bioavailable than the soluble compounds (AC and AS).

No toxicokinetic data are available with aluminium acetate and dialuminium chloride pentahydroxide.

In conclusion, AC, ACH and AS are expected to exhibit similar toxicokinetic behaviour and similar biological targets as soluble aluminium salts. As aluminium oxide and hydroxide were less bioavailable, toxicokinetics does not support the read-across with these salts.

You commented that the study by Priest (2010), demonstrates that the mean fractional absorptions of aluminium chloride and aluminium hydroxide only differed by roughly a factor of 2. Moreover, you pointed out that according to the Poirier (2010) toxicokinetic study, there is no difference in organ hydroxide/sulfate/chloride aluminium concentrations for kidneys and practically also no differences for bone suggesting no differences in bioavailability between these aluminium compounds. The only outstanding results were observed with aluminium citrate. You also highlighted that the relatively high value obtained for AS surprised the authors but they accepted the value because it was similar to the mean result of a study using just two human volunteers. According to you the real value for AS would be more similar to those of AC or aluminium nitrate.

ECHA agrees that results of the study of Priest (2010) should be considered with care as some weaknesses in the study have been identified. The limitation pointed out in the opinion of EFSA (2011) on this study was that the measurements were made 7 days following administration and less than 10% of the bioavailable dose remains in experimental animals at this time point. The following limitation was also noted by the authors "given the level of uncertainty in the mean (average SD~13% of mean) bioavailability the ranked values given above should be treated with caution". Nevertheless, this study shows that soluble aluminium salts were all more bioavailable than insoluble aluminium salts.

With regard to the Poirier (2010) study quoted by you in your comments, it is concluded by the author that the oral dose used in the study was insufficient to lead to any appreciable biodistribution for any salts "as dosing had little influence on systemic levels in aluminium for most tissues, the observed concentration were likely the results of previous exposure to aluminium from regular feed and water". The higher values obtained with citrate are expected as it is known that dietary ligands such as citrate increase the bioavailability of any particular aluminium compounds (ATSDR, 2008; EFSA, 2008). This study does not contradict Priest data: hydroxide had the lowest results in bone, the others being more or less similar. It is therefore very difficult to conclude, based on this study, on differences in aluminium bioavailability from the different salts.

Other studies investigating the differences in bioavailability of soluble aluminium salts show similarities among the tested compounds supporting the read-across between AC, ACH and AS. Slightly higher results were obtained with aluminium chloride or aluminium chloride basic than aluminium sulphate in some studies (Cunat, 2000; unpublished report, 2007) supporting your comment that similar bioavailability is expected between the soluble aluminium salts.

The reasonable worst case salts of aluminium needs to be determined with the available data even if judged of poor quality. Therefore, based on the toxicokinetic study of Priest (2010) and in absence of reliable results to contradict this study, it can be concluded that insoluble aluminium hydroxide and aluminium oxide cannot be considered as a worst case for soluble aluminium salts.

Comparison of human health data

Differences in the toxicity profile between soluble and insoluble compounds is supported by published developmental studies such as Colomina et al. (1992). In this study, general systemic toxicity and developmental toxicity was observed with aluminium lactate whereas neither maternal toxicity nor developmental effects were observed with aluminium hydroxide.

As stated in the review of Willhite et al. (2014) referring to the work of Mujika et al. (2011), the toxicity of soluble aluminium forms is expected to depend upon the delivered dose of Al^{3+} to target tissues. Trivalent aluminium is predicted to react with water, under the specific condition of the study, inducing formation of oxygen radicals that accounts for the oxidative damage that would lead to intrinsic apoptosis. In contrast, the toxicity of the

insoluble aluminium oxides and hydroxide will depend primarily on their behaviour as particulates.

You noted that the Colomina et al. (1992) study displayed severe insufficiencies. You also argue that all the effects (general toxicity and developmental toxicity) may have been due to lactic acid. You also pointed out in their comments that the formation of ROS by Al^{3+} in water described by Willhite et al. is purely hypothetical. You also add that aluminium is in principle a non-redox metal and is not expected to promote oxidative stress.

Although ECHA agrees that the study shows deficiencies, the animals were treated with aluminium hydroxide and aluminium lactate under the same protocol which has the same limitations. ECHA agrees that developmental effects observed in the Colomina *et al.* (1992) with aluminium lactate may be partly due to lactic acid and occurred at maternal toxic dose. Nevertheless, neither martenal toxicity nor any effects were observed with aluminium hydroxide and lactic acid in contrast to aluminium lactate showing potential differences in the systemic toxicity of the two compounds. Differences in toxicity between soluble and insoluble aluminium compounds were also suggested by other studies such as sub-chronic toxicity in dogs (FAO/WHO, 2007).

Consequently, ECHA concluded that the available toxicological database suggest differences in the toxicological profile between soluble aluminium compounds such as AC, ACH, AS and insoluble aluminium hydroxide and aluminium oxide. Such differences may be explained by lower bioavailability of insoluble test compounds.

Conclusion on read-across

In conclusion, based on the above considerations, it can be concluded that the results of the genotoxicity studies conducted with AC, AS are likely to predict the properties of ACH and a joint assessment of AC, ACH and AS is justified based on read-across. As AC is classified skin corrosive in category 1B and as aluminium sulphate anhydrous is not irritating to the skin, for animal welfare reasons, AS seems a more suitable substance to investigate the systemic effects of aluminium soluble salts than AC. Therefore, AS is considered the most appropriate test material for the genotoxicity testing of the three aluminium compounds under evaluation (AC, ACH and AS).

The read-across with dialuminium chloride pentahydroxide and aluminium acetate may be relevant for AC, ACH, AS due to similar solubility of the compounds. However, as no specific toxicokinetic is available on these compounds, no final conclusion can be made.

The read-across with insoluble aluminium hydroxide and aluminium oxide is not supported as potential differences in the absorption, bioavailability, reactivity and toxicity among soluble and insoluble salts have been identified.

You argue that the studies presented by ECHA to reject the read-across approach between soluble and insoluble aluminium compounds have major inconsistencies and that data indicate that the bioavailability of the compounds are rather similar. Thus you do not agree that a read-across approach should be rejected between soluble and insoluble aluminium

compounds. ECHA considers that the available data suggest differences between soluble and insoluble aluminium compounds and that aluminium hydroxide and oxide cannot be considered as a worst case with regard to soluble aluminium compounds.

1.1. MAXIMUM CONTENTS OF RELEVANT IMPURITIES SUCH AS ARSENIC, CADMIUM, CHROMIUM, MERCURY, NICKEL, LEAD, ANTIMONY, SELENIUM AND BERYLLIUM FOR EACH COMPOSITION

According to the "Guidance for identification and naming of substance under REACH and CLP", impurities present in a concentration > 1% should be specified and impurities that are relevant for classification and/or PBT assessment shall always be specified irrespective of the concentration. Presence of Arsenic, Cadmium, Chromium, Mercury, Nickel, Lead, Antimony, Selenium and Beryllium is expected in the registered substance. All these impurities are of toxicological concern and assigned to harmonised classification or self-classification. According to their level in the registered substance, these impurities can be relevant for classification and risk assessment.

Consequently, each registrant is required to specify a maximum concentration for each impurity.

It should be noted that the European standard EN 883:2004 and EN 881:2004 describe the characteristics and specifies the requirements of aluminium chloride basic, in particular the maximum content of Arsenic, Cadmium, Chromium, Mercury, Nickel, Lead, Antimony and Selenium. Three grades of purity are described as type 1, 2 or 3. Each registrant has the possibility to attest that the maximum concentration of each impurity complies with specifications (type 1, 2 or 3) of the European standard EN 883:2004 and EN 881:2004.

Consideration of Registrants' comments

You agreed to provide the maximum concentration of each impurity in compliance with the specifications (type 1, 2 or 3) of the appropriate EN standards. You commented that Beryllium is not listed in the EU standards regulating drinking water and that Beryllium is not expected in the registered substance.

ECHA considers that according to literature data and certificate of analysis of some aluminium salts reporting the presence of Beryllium at a concentration range from 0.03 to 0.1 mg/kg, it is justified to expect the presence of Beryllium in the registered substance: therefore each registrant is required to specify a maximum concentration for Beryllium as well.

1.2. CONTENTS OF ALUMINIUM AND CHLORIDE IONS, AL/OH MOLAR RATIO (BASICITY GRADE) AND PH FOR EACH COMPOSITION FOR EACH REGISTRANT

Aluminium chloride basic is an aluminium salt having an empirical formula Al(OH)xCl(3-x), where the basicity (representing the ratio between OH and Al) varies from low (>0.03 e.g. x = 0.1) to high (>0.7, e.g. x = 2.3). The substance is manufactured and supplied only as aqueous solution in which various aluminium hydroxyl complex ions (monomer, dimers and also higher polymeric forms) might be present depending on the aluminium concentration, the degree of hydrolysis with respect to the basicity grade and the pH.

However the complexes cannot be quantified or identified specifically or separated according you.

According to the "Guidance for identification and naming of substance under REACH and CLP", the composition shall normally be described up to 100%.

For this substance supplied as aqueous solution, water shall be regarded as a solvent which cannot be removed without composition change. Each constituent shall be reported as it is present in the aqueous solution and required a complete chemical specification, including structural information and content.

ECHA recognises that regarding the complex behavior of aluminium chloride basic in aqueous solution, it is not possible to give an accurate description of the composition and a complete chemical specification of each constituent. However, further information on composition shall be included in section 1.2. to characterise the registered substance and to verify the identity of the registered substance. Each registrant is requested to provide:

- The aluminium ion content and the chloride ion content in the registered substance as supplied (i.e. aqueous solution) expressed as grams per kilogram of solution.

The content of aluminium and chloride ions is necessary to use the read-across between AC, ACH and AS for risk assessment to cover the substance as placed on the market.
It should be underlined that aluminium ion content is a critical value as it is used to calculate the content of Aluminium chloride basic, as a purity criteria according to the EN 883:2004 and to calculate the relevant impurities contents, the limits given in the EN 883:2004 being expressed in mg/kg of aluminium.

- The Al/OH molar ratio, as registration may cover three grades of basicity;

- The pH of the aqueous solution, because it provides an estimation of predominant aluminium species present in solution.

1.3 COMBINED *IN VIVO* MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST AND *IN VIVO* MAMMALIAN COMET ASSAY WITH ADDITIONAL SPECIFIC INVESTIGATION ON OXIDATIVE DNA DAMAGE ON THE FOLLOWING TISSUES: LIVER, KIDNEY, GLANDULAR STOMACH AND DUODENUM; TEST METHODS EU B.12./OECD 474 AND OECD 489 IN RATS, ORAL ROUTE, USING THE ANALOGUE SUBSTANCE ALUMINIUM SULPHATE, INCLUDING FULL STUDY REPORT

Based on envisaged read-across (structural similarity validated but pending on substance composition clarification), genotoxicity of the three aluminium salts currently under substance evaluation ACH, AS and AC has been evaluated jointly. The reasoning for the combined assessment is explained above (see point 0.0).

<u>The Concerns Identified</u>

The available data are not sufficient to clarify the identified concern on potential genotoxicity of AC, ACH and AS *in viv*o that needs to be clarified with further information.

A battery of negative standard *in vitro* test (Ames, thymidine kinase locus of L5178Y mouse lymphoma cells assay, micronucleus assay) with AC, ACH or AS has been

submitted. Positive published *in vitro* results have also been observed with AC, ACH and AS. The discrepencies between the published results and standart tests are not known but may be due to differences in protocol or test material (e.g. purity not reported). Some of these positive studies suggest potential aneugenicity of the test material and also potential induction of oxidative damage. Indeed, positive results on *in vitro* comet assays has been published and may be explained by the potential of aluminium compounds to induce oxidative stress. This is supported by numerous *in vitro* or *in vivo* studies in several species showing induction of oxidative stress by AC or AS. Therefore, a concern on potential mutagenicity of AC, ACH and AS mainly *via* oxidative stress mode of action *in vivo* is raised. Moreover, *in vitro* data suggest that aneugenicity may be induced by aluminium compounds.

In vivo, the genotoxicity potential of ACH, AC and AS has been assessed in many genotoxicity assays available in the registration dossiers and in publicly available literature. Few had reliability index of 1 or 2 and give contradictory results. All the *in vivo* genotoxicity assays performed with AC, ACH or AS have reported positive effects. Nevertheless, these studies had deficiencies due to lack of reporting or deviations from OECD test guidelines A negative result has been obtained with aluminium hydroxide (unpublished report, 2010) in an OECD, GLP micronucleus study but was not considered relevant for AC, ACH and AS as no proof of exposure was available in the study and as the substance may be of lower bioavailability than AC, ACH and AS. *An in vivo* micronucleus and a comet assays have been published with aluminium oxide (Balasubramanyam, 2009). In this study, aluminium oxide as nanoforms were positive and increase level of aluminium was observed in tissues whereas aluminium oxide as microsized was negative but not significantly found in the tissues. Moreover, a negative result has been observed in an *in vivo* micronucleus assay with dialuminium chloride pentahydroxide (unpublished, 1999). This result is not in line with the consistent positive results available with soluble aluminium compounds. Nevertheless, as jusitified in section 0.0 of the decision, as aluminium chemistry and absorption is complex and as no toxicokinetics data are available, this study is not sufficient to exclude concerns raised by the positive *in vivo* assays observed with soluble aluminium compounds AC, ACH and AS.

In summary, the positive studies available with the three salts under evaluation have all deficiencies in reporting or deviations (e.g. single dose testing, no positive control data) that impaired to classify directly AC, ACH and AS for mutagenicity. Nevertheless, overall the findings observed in this studies cannot be dismissed by the observed limitations and raised a concern on potential genotoxicity of AC, ACH and AS *in viv*o that need to be clarified with further information.

Further information on genotoxicity, specifically chromosomal effects (aneugenicity/clastogenicity) and DNA damage *via* oxidative stress of soluble aluminium salts such as AC, is thus required *in vivo*. Moreover, available literature data suggest that aluminium is capable of reaching the germ cells. Therefore, it is justified that more information is required in order to determine the appropriate classification of aluminium salts for mutagenicity in somatic and germ cells. This request could also impact risk assessment by identifying a threshold-based mechanism (e.g. aneugenicity) of action to be considered for risk assessment and control of risk to humans.

Why new information is needed

The genotoxicity of various aluminium compounds has been reviewed in detail on a number of occasions (EFSA, 2008; ATSDR, 2008; FAO/WHO 2007). A large number of studies

investigating the genotoxicity of AC and aluminium compounds have been published; however many of the available studies had shortcomings. New reliable studies on the genotoxicity of registered aluminium chloride and analogue substances aluminium hydroxide, aluminium chloride basic and aluminium sulphate, using the standardized OECD guidelines, became available in 2010.

The EFSA scientific opinion from 2008 concluded that "Al compounds were non-mutagenic in bacterial and mammalian cell systems, but some produced DNA damage and effects on chromosome integrity and segregation *in vitro.* Clastogenic effects were also observed *in vivo* when Al sulfate was administered at high doses by gavage or by the intraperitoneal route. Several indirect mechanisms have been proposed to explain the variety of genotoxic effects elicited by aluminium salts in experimental systems. Cross-linking of DNA with chromosomal proteins, interaction with microtubule assembly and mitotic spindle functioning, induction of oxidative damage, damage of lysosomal membranes with liberation of DNAase, have been suggested to explain the induction of structural chromosomal aberrations, sister chromatid exchanges, chromosome loss and formation of oxidized bases in experimental systems. The Panel noted that these indirect mechanisms of genotoxicity, occurring at relatively high levels of exposure, are unlikely to be of relevance for humans exposed to aluminium via the diet". It should therefore be noted that EFSA did not conclude on the hazard but on the risk associated with the use of aluminium in diet.

Chromosomal aberration and micronuclei have been observed *in vitro* with aluminium chloride or other soluble aluminium salts in studies with some shortcomings (EFSA, 2008; Patel e*t al.*, 2009). Standardized *in vitro* tests for genotoxicity endpoints with AC, ACH or AS were negative (Ames, thymidine kinase locus of L5178Y mouse lymphoma cells assay, micronucleus assay). These discrependies may be explained by differences in methods and other parameters such as the purity of the test material. Increased DNA damage have been observed consistently in three *in vitro* alkaline comet assays (Pereira et al., 2013 ; Lankoff et al., 2006; Lima et al., 2007). Although these studies had limitations, they consistently show that AC, ACH or AS may induce DNA damages. Some *in vitro* studies have shown that AC, ACH or AS may induce oxidative damage. This mechanism of genotoxicity may explained the positive results observed in the *in vitro* comet assays.

In vivo, genotoxicity studies performed with soluble AC, AS, ACH, report positive results. Induction of chromosomal aberration, sister chromatid exchange and/or micronuclei have been observed with AS after administration *via* oral or intraperitoneal route in rats and mice (Roy 1991, 1992; Dhir 1990, 1993). Nevertheless, in these studies there were several shortcomings such as the lack of positive control (Roy 1991), and intraperitoneal route of exposure (Dhir 1990, 1993; Roy, 1992) and/or lack of reporting (Roy 1991, 1992; Dhir 1990, 1993), only two dose tested (Roy 1992). Moreover for all of these studies no information on purity was available.

In addition, after oral route, AC was positive in an *in vivo* liver micronucleus assay performed in rats (Türkez *et al*., 2010). The exposure of liver was shown by increased liver toxicity (biochemistry and histopathological findings). However, in this study only one dose level was tested (34 mg/kg aluminium chloride hexahydrate), environmental conditions were not described, the oral mode of administration was not further specified, the purity of AC and the vehicle was not specified, severe hepatic damage may be a confounding factor in the study to interprete genotoxicity. the general , . Interestingly, in this published study, simultaneous treatment with propolis significantly modulated the toxic effects of AC which may be due to an antioxidative effect. The reversion of

genotoxicity effects *in vivo* after administration of antioxidative agents were also observed in the studies of Roy et al., 1992 and Dhir et al., 1990 (quoted above) in mice. Therefore, *in vivo* there is evidence of genotoxicity effects linked to the production of reactive oxygen species.

A member state, in a proposal for amendment, requested further evidence of this oxidative MOA using relevant markers. In response, although some evidences were already provided in the draft decision, additional evidence are given here for further support. Indeed, there is convincing evidence that aluminium compounds can induce oxidative stress both in *vitro* and *in vivo*. Evidence for example of lipid peroxidation as measured by relevant markers such as increased malondialdehyde production has been observed with AC or AS in several studies in brain tissues or blood such as for example in Turgut (2006), Abd-Elghaffar et al. (2007), Candan et al. (2008), Sood et al. (2015). The oxidative mode of action may explain the positive results observed in *in vitro* comet assays and support the request of an *in vivo* comet assay.

In mice, aluminium acetate induced after single or repeated intraperitoneal exposure chromosomal aberrations which were dose and time-dependent. Micronulei induction was only observed after repeated exposure and not after single exposure. In this study, toxicity to germ cells was evident from a significant and dose-dependent increase in the percentage of abnormal spermatozoa and a reduction in sperm count (D'souza *et al.*, 2014). This study had limitations such as the absence of statement on the purity of the tested substance. Thus, there is concern from *in vivo* data that aluminium compounds could be germ cells mutagens.

In contrast, a negative GLP, OECD guideline *in vivo* mammalian erythrocyte micronucleus test was available with aluminium hydroxide (unpublished report, 2010). At the limit dose of 2000 mg/kg bw per day, no proof of exposure of bone marrow was provided. Moreover, this test material is not suitable as it may be of lower bioavailability than AC, AS or ACH.

An *in vivo* mammalian bone marrow chromosome aberration and comet assays with aluminium oxide have been published in 2009 and was negative for the micro-sized and positive for the nano-sized materials (Balasubramanyam *et al*., 2009). Nevertheless, as justified in section 0.0, the read-across between the target substance AC and the source substance aluminium oxide is not considered appropriate. It may be highlighted that positive results in this study were obtained concomitantly with proof of exposure (nano-sized) whereas, negative results were observed with the micro-sized particles showing no statistically significant increase of aluminium in tissues.

Carcinogenicity studies in animals with aluminium salts are not informative due to severe shortcomings in the study designs. The International Agency for Research on Cancer (IARC) has concluded that "the available epidemiological studies provide limited evidence that certain exposures in the aluminium production industry are carcinogenic to humans, giving rise to cancer of the lung and bladder." However, the aluminium exposure was confounded by exposure to other agents and IARC did not implicate aluminium itself as a human carcinogen.

In conclusion, *in vitro* genotoxicity data are inconsistent and positive results have been reported *in vivo* studies with aluminium soluble salts. As these studies have shortcomings, no firm conclusion can be done and further testing is needed.

The clarification of the *in vivo* genotoxic potential and mode of action of ACH is requested for both risk assessment and classification and labelling.

The use of the FISH technique in the requested micronucleus assay will investigate the aneugen or clastogen potential of the substance (OECD TG 474). This may be of particular importance for risk assessment as there is generally no threshold for mutagenicity but a threshold exist for aneugens.

The use of specific enzymes in the requested comet assay additionally to the standard comet assay will assess oxidative DNA damage in various tissues relevant for toxicological evaluation.

In case of negative results for both the micronucleus and the comet assay (including comet assay with a specific enzyme), the concern will be clarified as this combined test will allow to solve the initial concerns (clastogenicity/aneugenicity and oxidative DNA damage).

In case of positive results in the micronucleus test and/or the comet assay, depending on the nature of the results, further risk management measures such as classification and labelling or further testing on mutagenicity will be considered.

You provided in your comments on PfA an additional GLP, OECD guideline unpublished study from 1999, (previous version of the test guideline) reporting negative *in vivo* micronucleus test with dialuminium pentahydroxide. At the limit dose of 2000 mg/kg bw no effects were observed. However, the interpretation of the negative result is limited by the absence of proof of exposure. The substance is very soluble and would be expected to have the same bioavailability as AC, ACH and AS. As no proof of exposure are available in the study (no adverse clinical signs, body weight effects or mortality), the negative results cannot clarify the concerns raised with AC, ACH and AS.

While reviewing this additional study from 1999 described above, evaluating MSCA also identified a new positive *in vivo* micronucleus assay with aluminium chloride (Paz et al., 2017). In this study a statistically significant dose-related increase in micronuclei was observed in females at all dose tested and in males a statistically significant effect was observed at all dose tested but without clear dose-response. Systemic and local toxicity has been shown in the study by decreasd weights of stomachs and kidneys without concomitant histopathological findings. The study was performed according to the previous version of the test guideline but has some shortcomings. The main limitations are the absence of nformation on the purity of the test material and the lack of reporting of the results of the positive control for the *in vivo* test. Moreover, the levels of micronuclei observed in the *in vivo* study were surprisingly high. Because of these limitations, this study cannot allow to classify AC for germ cell mutagenicity and further testing is still needed to clarify the concern.

<u>Considerations on the test method and testing strategy</u>

Test material:
The analogue substance AS (CAS no. 10043-01-3 ; EC no. 233-135-0) is considered the most appropriate test material for testing soluble aluminium salts as AC is a corrosive substance.

The analogue substance AS would be used in order to clarify concern for AC, ACH and AS. This will allow to limit animal testing. The purity of the test material should be compliant with type 1 of EN878:2004 with technical AS containing the highest content of Aluminium, as impurities are expressed based on aluminium mass (g/kg). Type 1 of EN878:2004 corresponds to the highest pure grade of technical aluminium sulphate and will thus cover other aluminium compounds such as AC and ACH.

No differences in the impurity profile between the salts is expected as grade 1, 2 and 3 of EN878:2004, EN883:2004 and EN881:2004 for AS, ACH and AC, respectively, are exactly the same with regard to the allowed level of impurities.

In the draft decision, initially, the lowest grade (grade 3) was proposed, but the highest purity may be more appropriate as the results will be representative for the three substances. In case of positive results, further testing, foreseen in the initial draft decision, will then not be needed to exclude the potential effects of impurities.

The certificate of analysis including the content of impurity used in technical aluminium sulphate shall be provided.

Route of exposure
The oral route is considered to be the most appropriate route of exposure for AS. Indeed it is the most common route of administration for this test combination. Furthermore, AS has a low vapor pressure and thus the inhalation route is not considered relevant. Although dermal exposure may occur in workers, the oral route is the most appropriate to investigate genotoxicity potential. Furthermore, the oral route is the most realistic route with regard to consumer exposure.

Tissues to be examined
Toxicokinetics data suggest that following oral exposure, ACH, AC and AS salts are mainly distributed in liver, kidney, bones and spleens (ATSDR, 2008). Therefore, the bone marrow is considered an appropriate target organ for the micronucleus analysis and the liver and kidney should be considered for the Comet assay.

Moreover, in order to also investigate genotoxicity at the site-of-contact, glandular stomach and duodenum shall also be investigated in the comet assay. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Specific investigation
The *in vivo* micronucleus test covers the endpoints of structural and numerical chromosomal aberrations. Clarification of relevant mode of action for micronuclei induction need to be considered using fluorescent *in situ* hybridization (FISH) with probes for DNA sequences in the centromeric region, or a labeled antibody to kinetochore proteins to discriminate between clastogenic and aneugenic effects in the MN assay.

The *in vivo* comet assay is not considered adequate to detect aneugens. However, comet

assay is an indicator test measuring DNA damage in various relevant tissues. Some compounds with specific mechanisms of genotoxicity (such as oxidative damage) may be more difficult to detect in the standard comet assay without modifying the protocol (according to JACVAM 2014 validation trial). The test thus needs to be complemented to identify oxidative DNA damage by using exogenous DNA repair enzymes such as human 8-hydroxyguanine DNA-glycosylase (hOGG1)or the formamidopyrimidine DNA-glycosylase (FPG). These enzymes recognise and remove oxidatively damaged purines, for example, 8-oxo-7,8-dihydroguanine formamidopyrimidine moieties.

The standard alkaline comet assay shall be performed together with the following modifications. The modification of the protocol consists to add additional slides in the standard alkaline comet assay (OECD TG 489) which will be treated with enzyme (hOGG1 or Fpg) between the lysis and alkaline treatment. It is recommended to use the protocol based on the publication from Ersson et al. (2013) and Dusinska et al. (2000). In addition, further technical specification to help you to do the method succesfully is suggested:

- Dilution of the enzyme in dilution buffer (IU/mL), amount of the diluted enzyme applied to comet preparations, and incubation time of comet assay slides with enzyme at 37 °C should be determined for each cell type separately to guarantee maximum dynamic range between negative and positive control slides. Positive controls should be obtained for each tissue examined by treating the comet assay slides prepared by agarose embedding of the tissues isolated from negative control animals prior to lysis such as RO 19-8022 treatment followed by light induction (Collins et al., 2014).

- With regards to the number of slides, it is recommended to prepare 2 sets of slides for each tissue and for each test condition: one set submitted to the standard protocol (without enzyme), the other submitted to a modified protocol (with enzymes). The enzyme buffer (without enzyme) will serve as control.

Any deviations from this suggested protocol should be scientifically justified.

Dose levels
According to ECHA guidance Chapter R.7a v4, July 2015 and EFSA journal 2012; 10(11):2977, 3 administrations should be performed. The top dose is recommended to be the limit dose of 2000 mg/kg bw or the highest dose should produce signs of clinical toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality.

The aluminium content in food and water shall be determined in order to have accurate dose levels of aluminium consumption.

Study report
In consideration of the complexity of the case, the evaluating MSCA must have access to the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA.

<u>Alternative approaches and proportionality of the request</u>

Clastogenic effects (induced structural or numerical chromosomal aberrations) have been

shown *in vivo* in micronucleus tests. Some *in vitro* comet assays have shown increase in DNA strand breaks which may have been caused by oxidative stress. The genotoxic oxidative MOA is further supported by *in vitro/in vivo* animal data showing induction of oxidative stress by aluminium compounds. Consequently, the combined OECD 474 and comet OECD 489 with specific investigations on oxidative DNA damage, structural and numerical chromosomal aberrations and DNA damage and more particularly also damage induced via oxidative stress, is considered a suitable and proportionate option.

The transgenic rodent assay OECD TG 488, which measures gene mutations and small deletion/insertions is not considered a suitable option to clarify the concern. Moreover, eMSCA notes that no *in vitro* tests would be suitable to clarify the concern.

In case of positive results with AS in the requested *in vivo* micronucleus test with its somatic tissues distant from the portal of entry, AS should be assumed to reach other distant tissues such as the germ cells also, and therefore be considered to be a germ cell mutagen, if no counter evidence can be provided. In that case you will be invited to consider the option of self-classification Muta. 1B in an update of the registration dossier.

The request for the comet assay/micronucleus test is suitable and necessary to obtain information that will allow to clarify whether there is potential for mutagenicity. More explicitly, there is no equally suitable alternative way available for obtaining this information. Micronucleus assay is not designed to assess oxidative stressed-mediated genotoxicity. Where the data, once obtained, confirms that there is potential of mutagenicity, authorities will consider either further testing or regulatory risk management such as classification as germ cell mutagen .

Consideration of Registrants' comments

You consider that there is no mutagenicity concern and that the study should not be requested.

You argue that *in vitro* and *in vivo* studies quoted by ECHA to demonstrate potential concerns of genetic toxicity were not reliable and could not be used to justify the requested study. Moreover, you pointed out that they have provided a state-of-the-art GLP- and Guideline-compliant *in vitro* genetic toxicity test battery which gave no indication of genetic toxicity whatsoever. You disagree with ECHA's statement that the *in vitro* genotoxicity database is inconsistent and does not reflect the data set presented in the dossier. You highlighted the limitations of the quoted studies in the draft decision and more particularly the Migliore et al. (1999) and Banasik et al. (2000) studies. You do not agree with the demand for an *in vivo* genotoxicity study. According to you, this demand does not comply with animal welfare considerations and challenges scientific value of *in vitro* tests, and thus contests the concept of staggered genotoxicity testing as manifested in REACH.

ECHA firstly notes that substance evaluation allows departing from the standard information regimes, to ensure that additational concerns identified with a substance can be clarified. Although negative results were obtained from the three standard *in vitro* OECD tests, positive results in *in vitro* studies are available from the literature. For studies similar to these standard OECD test guideline, inconsistency may be due to differences in purity, test system, cells, concentrations, treatment periods, quality of the study etc. For non-guideline studies such as *in vitro* alkaline comet assay, the three available *in vitro* alkaline

comet assays were consistently positive (Pereira et al., 2013; Lankoff et al., 2006, Lima et al., 2007). In the study of Pereira et al. (2013), increased single strand breaks and DSB *via* induction of phosphorylated histone γ-H2AX were observed in Zebrafish fibroblasts. The biological relevance of embryonic zebrafish is unclear for human risk assessment but the method used was very sensitive. Although the two other *in vitro* alkaline comet assays had weaknesses, positive results were observed in human lymphocytes (Lankoff et al., 2006; Lima et al., 2007). There is suggestion that oxidative stress may be a possible mechanism of aluminium genotoxicity that may induce breaks observed in the comet assay *in vitro*. As no *in vivo* alkaline comet assay is available it is not possible to exclude that positive results observed in three alkaline comet *in vitro* assays may be relevant *in vivo* and for human health risk assessment. These results raised concern on potential mutagenicity of aluminium soluble salts *in vivo* link to the production of reactive oxygen species.

ECHA agrees that some published studies do have weaknesses in their reporting and were not performed according to OECD guidelines. They have been considered to have values in particular to evaluate mechanism of genotoxicity (e.g. oxidative stress, DNA repair). Moreover, other techniques such as FISH, not routinely performed in standard guideline studies provide useful information.

ECHA considers that the *in vivo* micronucleus assay (High quality) performed with aluminium hydroxide was considered not relevant to exclude the concern identified on AS (see read-across justification). Moreover, in this study or in any other studies in the literature performed with aluminium hydroxide, no proof of bone marrow exposure has been provided. Therefore, the published positive results reported in micronucleus or chromosomal aberration assays *in vivo* in bone marrow or liver may be due to differences in quality and methods used compared to the OECD TG micronucleus study or due to truely positive outcomes resulting from a higher bioavailability of Al soluble salts. Therefore, the results raised concern on potential genotoxicity *in vivo* of aluminium soluble salts.

You commented that although no proof of exposure was observed in bone marrow with aluminium hydroxide, the Poirier (2010) study shows that aluminium hydroxide reaches the bones. ECHA would like to emphasize that in this study, increased level of aluminium in bone was only statistically significant with AS and AC but not with aluminium hydroxide. In addition, ECHA considers the results of this study with caution because as stated by the authors "*These results suggest that dosing had little influence on systemic levels of aluminum in most tissues, and the observed concentrations were likely the result of previous exposure to aluminum from regular feed and water".* Thus, higher doses would have been needed to make any conclusion.

You commented that the formation of reactive oxygen species hypothesized by the ECHA (referring to a review paper by Willhite et al., 2014) is a theoretical process. You questioned its relevance in a physiological environment (specifically the nucleus).

With regard to aluminium toxicity and oxidative stress, although ECHA acknowledges that the reported mechanism of toxicity of soluble aluminium described in the review of Willhite et al. (2014) is theoretical, this hypothesis is further supported by *in vitro* and *in vivo* data. There is evidence from the literature that aluminium soluble salts induce reactive species in humans *in vitro* (e.g. brain, lymphocytes) and in repeated-dose toxicity studies *in vivo* (e.g. brain, blood, liver) in rabbits, mice and rats (Abd-Elghaffar et al., 2007, Candan et al., 2008, Prakash et al., 2009, Kaur et al., 2015, Lakshmi et al., 2015; Singh et al., 2015, Rani et al., 2015, Sood et al., 2015, Waly et al., 2014,). Several studies report, after

administration of AC, alteration of the antioxidative system (superoxide dismutase, glutathione peroxidase, catalase), increase of reactive species (nitrite, nitrate, oxidized dichlorofluoresceine) and lipid peroxidation (increase in malondialdehyde, 4-hydroxyalkenals, thiobarbituric acid reactive substances). In these studies, this mechanism has been confirmed experimentally by co-administration of antioxidants, which abrogated or reduced the effects of aluminium compounds on oxidative stress. Although, literature data suggested that aluminium compounds are able to induce reactive species and lipid peroxidation, the extent to which it can occur in cell nucleus is unknown but supported by the positive results in *in vitro* alkaline comet assays.

You also argued that high-quality *in vitro* mutagenicity Ames tests specifically designed to detect oxidative damage gave no indication of such effects (TA102 or E.coli WP2).

The fact that the Ames test performed with aluminium chloride basic included *E.coli* strain WP2 *uvr*A was negative should be interpreted with caution. Indeed Ames test may not be sensitive enough for the testing of metals (cf. REACH Guidance on information requirements and chemical safety assessment, Chapter R.7a, Version October 2015). HERAG (Health risk assessment guidance for metals No 5 Mutagenicity) fact sheet, also stated that "b*acterial mutagenicity tests appear to have little utility for the testing of metals (test results are always negative)".*

Interaction of aluminium with microtubule and mitotic spindle functioning has been suggested in some *in vitro* studies (Banasik et al., 2005, Migliore et al., 1999). Therefore, the FISH technique required in the micronucleus assay is considered proportionate.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the analogue substance aluminium sulphate: Combined *in vivo* mammalian erythrocyte micronucleus test and *in vivo* mammalian comet assay with additional specific investigation on oxidative DNA damage on the following tissues: liver, kidney, glandular stomach and duodenum; test methods EU B.12./OECD 474 and OECD 489 in rats, oral route.

Note for consideration

As already indicated above, further testing on mutagenicity will be considered in case of positive results in the comet assay. You are therefore invited to consider integrating a Pig-a assay in the requested study, which would not require additional animals. The results of the Pig-a assay would provide further information on mutagenicity and reduce the need for further testing.

Recommandation on this test methods already exist, and ECHA recommends to follow published existing protocols (Dobrovolsky et al., 2010, Dertinger et al., 2011, Kimoto et al., 2016, Gollapudi et al., 2015).

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1.4 REFINEMENT OF EXPOSURE SCENARIO WITH REGARD TO THE USE OF RISKOFDERM V2.1 MODEL FOR WORKERS DERMAL EXPOSURE ESTIMATES (USE OF THE 'HAND' AND 'WHOLE-BODY' LOAD ESTIMATES INSTEAD OF 'HANDS' ONLY).

Workers may be exposed via inhalation and/or dermal routes. You have used two exposure estimation models in your exposure scenarios for workers:

- ECETOC TRA (Workers 3.0)

- Riskofderm 2.0

The use of Riskofderm model may underestimate occupational exposure via dermal route:

You used only the 'hands' load estimate in its assessment with the use of spray cans (superior to 1 meter in the model). However the 'whole-body' exposure load has also to be taken into account, even if RMMs are considered in the assessment (i.e. body protective equipment in case of spraying). Measurements gathered in the framework of Riskofderm project contained data for each parameter to fulfil in the model (Warren N.D. 2006), including a distance superior to 1 meter between the source and the worker. In such situation the 'whole body' of the worker may also be exposed. In case of RMM, a safety factor may be then applied to reduce the estimated 'whole body' and 'hands' load.

Therefore, ECHA concludes that you are required to refine exposure scenarios for ACH with regard to the use of *Riskofderm v2.1* for workers dermal exposure estimates: use of the 'hand' and 'whole-body' load estimates instead of 'hands' only.

Consideration of Registrants' comments

You do not agree that the exposure scenario generated through the Riskofderm model underestimates occupational exposure via dermal route; however, you are ready to provide the refinements requested for spraying Contributing Scenarios, where Riskofderm 2.0 has been used (PROCs7 and PROCs11).

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected CMR (mutagenicity, reproductive toxicity), wide dispersive use, high Risk Characterisation Rations, aggregated tonnage, Aluminium chloride basic, CAS No 1327-41-9 (EC No 215-477-2) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The Competent Authority of France (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding neurotoxicity.

The evaluating MSCA considered that further information was required to clarify the following concerns: mutagenicity and developmental neurotoxicity. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 11 March 2016.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation.

ECHA notified you of the draft decision and invited you to provide comments.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took into account the comments from you, which were sent within the commenting period, and they are reflected in Appendix 1. Information requirement 1.3 from the draft decision sent to you on 26 April 2016 has been removed ("Certificate of analysis with the level of impurities of technical aluminium citrate used in the one-year developmental and chronic neurotoxicity study in Rats").

Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member Staes and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposals for amendment to the draft decision and modified the draft decision. They are reflected in the reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendments.

Your comments on the proposed amendments were taken into account by the Member State Committee.

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-56 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:

   Further advice can be found at http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.