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Was amorphous aluminium hydroxyphosphate sulfate adequately evaluated before authorisation in Europe?

Sesilje B Petersen ,[1] Christian Gluud[2]

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[1]Department of Occupational and Environmental Medicine, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, Copenhagen, Denmark
[2]The Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Correspondence to:
Dr Sesilje B Petersen,
Dept. of Occupational and Environmental Medicine, Bispebjerg Hospital, Kobenhavn, Denmark; sesilje.elise.bondo.petersen@regionh.dk

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ABSTRACT

The Merck Sharp & Dohme Corp aluminium adjuvant 'amorphous aluminium hydroxyphosphate sulfate' (AAHS), primarily used in the Gardasil vaccines against human papilloma virus, has been criticised for lack of evidence for its safety. Documentation from Danish authorities and answers from the European Medicines Agency (EMA) suggest that AAHS may not have been sufficiently evaluated. Documentation from the Danish Medicines Agency shows discrepancies in the trial documents of two prelicensure clinical trials with Gardasil in 2002 and 2003. For both trials, the Agency seems to have authorised potassium aluminium sulfate as the adjuvant and not AAHS. In addition, the participants in the trial launched in 2002 were informed that the comparator was saline, even though the comparator was AAHS in an expedient consisting of L-histidine, polysorbate-80, sodium borate and sodium chloride. According to the EMA, AAHS was first introduced in Europe in 2004 as the adjuvant in Procomvax, a vaccine against the hepatitis B virus and Haemophilus influenza type b. The EMA reports that AAHS was introduced without any prelicensure safety evaluation. The adjuvant is described by the company to be both physically and functionally distinct from all other previously used aluminium adjuvants. There is a need for rigorous evaluation of benefits and harms of the adjuvant AAHS.

Introduction

Aluminium is considered an effective adjuvant in vaccines, but its safety may not have been sufficiently in focus.[1] Aluminium is a known neurotoxin and inflammagen,[2] and interferes with several biomolecules and biochemical pathways, for example, disturbs calcium metabolism, increases oxidative stress, binds to phosphate groups of nucleoside diphosphates and triphosphates such as ATP, and competes with iron and magnesium.[3 4] Several research groups have raised concerns about the health effects of using aluminium in vaccines.[1 5–10] However, both the US Food and Drug Administration Center for Biologics Evaluation and Research and the US Agency for Toxic Substances and Disease Registry concluded that traditional aluminium adjuvants are safe.[11 12]

Aluminium adjuvants have been associated with a number of adverse effects, including injection site pain and tenderness, persistent lumps, granulomas, contact dermatitis and postimmunisation headache,[13] but also more severe adverse events such as macrophagic myofasciitis[14] and the autoimmune/inflammatory syndrome induced by adjuvants.[15] Animal models have demonstrated the toxicity of aluminium adjuvants[16] and their translocation away from the injection site.[17 18]

Criticisms have been raised of the prelicensure randomised clinical trials, that forms the body of evidence for the approval of Gardasil, a Merck Sharp & Dohme Corp manufactured human papilloma virus (HPV) vaccine made of recombinant HPV types 6, 11, 16 and 18 L1 virus-like particles.[19–23] One criticism is the use of amorphous aluminium hydroxyphosphate sulfate (AAHS) as a comparator in the prelicensure trials.[20 23] However, the European Medicines Agency (EMA) and the WHO conclude high vaccine safety and efficacy.[24 25] AAHS produced by Merck has a short history prior to the use in the Gardasil vaccine. A recent study by Doshi *et al* found that participants in Gardasil trials were not adequately informed that the placebo was AAHS.[23] As AAHS is both physically and functionally distinct from all previously used aluminium adjuvants,[26] it is crucial to know the body of evidence regarding safety that constitutes the basis of approval of randomised clinical trials using AAHS by medicines agencies. Here we describe some discrepancies in the documents that constitute the foundation for authorisation of two Gardasil randomised clinical trials in Denmark.

It is important to stress that we are not against safe vaccines in general. However, inadequacies in the regulation of vaccine adjuvants may fuel concerns—rightly or wrongly—in the highly polarised environment that surrounds vaccine sciences. It is therefore imperative to ensure transparent documentation and adequate informed consent in randomised clinical trials.

The Future II and Future K trials

Some of the prelicensure randomised clinical trials with the Gardasil vaccine were conducted in Europe. Data access to detailed trial information for the Danish part of the Future II trial (501-015) and the Future K trial (501-018) was obtained from the Danish Medicines Agency and the Danish National Committee on Health Research Ethics. Access was permitted to Merck protocols, recruitment brochures, informed consent forms and to selected sections of the quality, manufacture and control section of the Investigational Medicinal

Table 1 Adjuvants and excipients in the experimental vaccine and the comparator used in the Danish part of the Future II trial (501-015) according to information from the Danish Medicines Agency and the Danish National Committee on Health Research Ethics

Agency/committee	Composition of the experimental vaccine	Composition of the comparator
Danish Medicines Agency Danish National Committee on Health Research Ethics	225 mcg aluminium as potassium aluminium sulfate 9.56 mg sodium chloride 0.78 mg L-histidine 50 mcg polysorbate 80 35 mcg borax	225 mcg aluminium as potassium aluminium sulfate 9.56 mg sodium chloride 0.78 mg L-histidine 50 mcg polysorbate 80 35 mcg borax
Protocol	225 mcg aluminium as amorphous aluminium hydroxyphosphate sulfate (Merck aluminium adjuvant)	Merck standard aluminium diluent (225 µg alum) in normal saline, unique selling proposition (NaCl 0.9%) or Merck aluminium adjuvant placebo
Recruitment brochure	No information	Saline
Informed consent form	No information	Saline

Product Dossier (IMPD) regarding the Future II trial, and composition tables for experimental vaccine and comparator regarding the Future K trial.

In the Future II trial launched in 2002, 12 167 women aged 16–23 years participated at 90 trial centres in 13 countries.[27 28] In the Future K trial launched in 2003, 1781 children aged 9–15 years participated at 47 trial centres in 10 countries worldwide.[29] Both the Future II and the Future K trials have been described elsewhere.[27–29]

What do the trial documents show?

The IMPD written by Merck dated April 2002 relates to the manufacture of three container lots of the experimental vaccine and four lots of the comparator used in the Nordic parts of the Future II trial.[30] In the section of drug description and in the section of adjuvant preparation, the adjuvant is described as AAHS in a solution with the excipients L-histidine, polysorbate-80, sodium borate and sodium chloride. However, in the remaining parts of the IMPD, the type of aluminium is described as potassium aluminium sulfate and not AAHS.[30] The document describes the required quality test based on potassium aluminium sulfate, and in the composition table of the experimental vaccine and the comparator, the adjuvant is also listed as potassium aluminium sulfate (table 1; online supplementary figure S1).[30]

Regarding the Future K trial, the Danish Medicines Agency only released the composition table for the experimental vaccine and the comparator (online supplementary figure S2), which showed that the adjuvant was listed as potassium aluminium sulfate and not AAHS (table 2). In all documents, the adjuvant was defined as an inactive component.

Regarding the recruitment brochures and informed consent forms, the use of an adjuvanted comparator was not mentioned and the comparator was defined as saline (tables 1 and 2). The Future II recruitment brochure stated that the trial was not a safety trial as the vaccine was already tested for adverse events.[31] It further stated that no adverse events were found except for light redness and tenderness at the injection site.[31]

In the Merck protocols, the adjuvant was specified as 225 mcg aluminium as AAHS (Merck aluminium adjuvant) (tables 1 and 2), but in the section that describes the clinical material in the Future II protocol, the comparator was specified as Merck standard aluminium diluent (225 µg alum) in normal saline, unique selling proposition (NaCl 0.9%). In other sections of the protocol, the comparator is described as Merck aluminium adjuvant placebo (table 1). For the Future K trial, a non-aluminium containing comparator was mentioned in the Merck protocol; however, the composition of the vaccine and the comparator was not specified (table 2). For both trials, the excipients were not mentioned in the Merck protocols, recruitment brochures or the informed consent forms (tables 1 and 2).

EMA's approval of AAHS

Two queries (ASK-50308 and ASK-53619) about how AAHS was approved and introduced by EMA were sent to EMA on 15 January and 4 April 2019. The answers show that the EMA has no specific safety studies comparing aluminium alone with an inactive comparator. The EMA stated in the answer that no new clinical safety studies were needed comparing aluminium alone versus inactive comparator for vaccines containing aluminium adjuvants (see the online supplementary file). The EMA explained that there was no need to further investigate an adjuvant alone when there are no new safety issues reported after decades of use. The EMA stated further that if the adjuvant is novel, then there should usually be enough safety data from the preclinical

Table 2 Adjuvants and excipients in the experimental vaccine and the comparator used in the Danish part of the Future K trial (501-018) according to information from the Danish Medicines Agency and the Danish National Committee on Health Research Ethics

Agency/committee	Composition of the experimental vaccine	Composition of the comparator
Danish Medicines Agency Danish National Committee on Health Research Ethics	225 mcg potassium aluminium sulfate 9.56 mg sodium chloride 0.78 mg L-histidine 50 mcg polysorbate 80 35 mcg borax	9.56 mg sodium chloride 0.78 mg L-histidine 50 mcg polysorbate 80
Protocol	225 mcg aluminium as amorphous aluminium hydroxyphosphate sulfate (Merck aluminium)	No aluminium
Recruitment brochure	No information	Saline
Informed consent form	No information	Saline

studies to allow for it to be given with antigen(s) from the outset (see the online supplementary file). In the answers, EMA specified that the applicability to established adjuvants (eg, aluminium hydroxide and aluminium phosphate) will vary on a case-by-case basis. EMA explained that no further non-clinical studies on the AAHS adjuvant were required, as AAHS is used in other vaccines approved in Europe. The EMA refers in the answer to the Guideline on Adjuvants in Vaccines for Human Use[32] (see the online supplementary file).

According to the EMA, AAHS was introduced in Europe in 2004, when the name of the adjuvant in the vaccine Procomvax was modified from aluminium hydroxide to AAHS (see the online supplementary file). The change was requested by the company to align the nomenclature of the adjuvant in all relevant authorised Merck vaccines at that time. The EMA explained that the adjuvant AAHS is the same chemical compound as the one initially called aluminium hydroxide. The change in name reflects a change in nomenclature that occurred after the initial authorisation of Procomvax. The change was accepted by the Committee for Medicinal Products for Human Use (see the online supplementary file).

Discussion

It was not possible to identify the type of aluminium adjuvant used in the Future II and the Future K trials from the accessed documents. None of the documents listed the composition of the vaccine and the comparator according to the Gardasil insert. It seems plausible that it was AAHS they used in the trials, as AAHS is the vaccine adjuvant in both trial protocols (tables 1 and 2). Doshi *et al* also came to the same conclusion that the comparator was AAHS.[23]

In the section for the clinical material description in the Future II protocol, the placebo is described as 'Merck standard aluminium diluent (225 µg alum) in normal saline, unique selling proposition (NaCl 0.9%)', which does not correspond to the description of AAHS. In other sections of the protocol, the placebo is described as 'Merck aluminium adjuvant placebo', but as aluminium hydroxide was used in Merck vaccines in 2002, and as AAHS according to the EMA was unknown by the authorities before 2004, we can speculate that the Committee on Health Research Ethics may have interpreted the placebo as being aluminium hydroxide.

The Danish Medicines Agency authorisation of potassium aluminium sulfate and not AAHS for both trials is noteworthy, as potassium aluminium sulfate (also referred as alum) is a different aluminium salt than AAHS, and it has no record of being used as an adjuvant in European human vaccines.[33] Even known aluminium adjuvants such as AlHydrogel (aluminium hydroxide) and AdjuPhos (aluminium hydroxyphosphate) react quite differently as adjuvants.[34] Therefore, equating potassium aluminium sulfate with AAHS is questionable. Indeed, the only published study on the adjuvant properties of AAHS, a Merck in-house study in mice, concluded that the formulation of aluminium adjuvants has significant implications for their biological activity.[26] The manufacturer of AAHS seems to have prevented independent studies of AAHS, though experts in the characterisation of aluminium adjuvants have speculated that the inclusion of sulfate moieties will increase the acidity at the injection site with likely concomitant increased toxicity.[34]

In both the Future II and Future K trials, the trial participants do not seem to have been informed about the use of AAHS, which is in line with the findings from Doshi *et al*.[23] In all documents, the comparator is described as an inactive component. This is also questionable as aluminium is potentially reactive both when administered alone and in a vaccine formulation.[35]

Gardasil is also composed of the excipients polysorbate-80, sodium borate and L-histidine in addition to AAHS. However, the excipients do not seem to have been described in the protocols, even though they were part of the vaccine and the comparator according to the IMPD (online supplementary figure S1). The excipients are added with the purpose of stabilising the virus-like particles, as they are unstable during long-term storage.[36] The missing information is of relevance, as the safety of the excipients has been questioned.[37–39]

The answers from the EMA on queries ASK-50308 and ASK-53619 suggest that Merck did not inform the EMA about AAHS (see the online supplementary file). Merck seems to have told EMA that AAHS was identical to aluminium hydroxide, and that they requested nomenclature changes for all approved vaccines to secure identical nomenclature. In 2002, neither the Danish Medicines Agency nor the EMA seems to know about the adjuvant AAHS. The manufacturer cannot claim that this was because of another use of nomenclature as the aluminium adjuvant was correctly declared for the vaccine in the Future II and Future K study protocols, except for the excipients.

Merck seems to have been aware of the chemical and functional differences between aluminium hydroxide and AAHS, as they have published an in-house study where they compare AAHS with more traditionally used adjuvants.[26] Therefore, the change in nomenclature suggests that Merck may have got AAHS authorised without following the guidelines for new adjuvants.

According to EMA's own guidelines, an adjuvant should be tested alone in minimum two species unless otherwise justified, as adjuvants themselves might be immunogenic.[32] Full tissue examination is recommended in the case of novel adjuvants with no prior non-clinical and clinical experience.[32] It can be argued that the AAHS should have been handled as a novel adjuvant by the agencies, as it is a new type of aluminium adjuvant with excipients that have not been used earlier in EMA authorised vaccines. As EMA seems to be unaware of any safety studies where AAHS is tested against an inert comparator (a real placebo), one can question the safety of AAHS containing vaccines. A recent PhD thesis from the Nordic Cochrane Centre concluded that it is unclear to what extent the benefits of the Gardasil vaccine outweigh its harms, as almost all of the Gardasil studies used AAHS as comparator.[40] In the light of our observations, the benefit–harm balance of the vaccine may need a re-evaluation.

The present cases regarding the Future II and Future K trials from Denmark emphasise that the clinical trial authorisation processes have not been able to secure proper vaccine and control descriptions and sufficient informed consent. Merck initiated trials with a design, where the placebo seems unknown both for the authorities, the investigators and the trial participants. Moreover, the trial design, using a novel adjuvant as the placebo, seems unapproved by the authorities. If this is the case, then the trials raise ethical concerns. Based on the present cases, EMA should re-evaluate the guidelines for clinical trial authorisations and close any possible holes in the regulations to secure future vaccine safety. Comparable actions of the national regulatory authorities and ethics committees are likely needed.

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ORCID iD
Sesilje B Petersen http://orcid.org/0000-0001-5543-7603

References

1 **Djurisic S**, Jakobsen JC, Petersen SB, *et al*. Aluminium adjuvants used in vaccines. *Cochrane Database Syst Rev* 2018;**347**:CD013086.
2 **Exley C**. Human exposure to aluminium. *Environ Sci Process Impacts* 2013;**15**:1807–16.
3 **Exley C**, Siesjö P, Eriksson H. The immunobiology of aluminium adjuvants: how do they really work? *Trends Immunol* 2010;**31**:103–9.
4 **Kisnierienė V**, Lapeikaitė I. When chemistry meets biology: the case of aluminium – a review. *Chemija* 2015;**26**:148–58.
5 **Crépeaux G**, Eidi H, David M-O, *et al*. Non-Linear dose-response of aluminium hydroxide adjuvant particles: selective low dose neurotoxicity. *Toxicology* 2017;**375**:48–57.
6 **Shaw CA**, Li D, Tomljenovic L. Are there negative CNS impacts of aluminum adjuvants used in vaccines and immunotherapy? *Immunotherapy* 2014;**6**:1055–71.
7 **Gherardi RK**, Aouizerate J, Cadusseau J, *et al*. Aluminum adjuvants of vaccines injected into the muscle: normal fate, pathology and associated disease. *Morphologie* 2016;**100**:85–94.
8 **Bergfors E**, Björkelund C, Trollfors B. Nineteen cases of persistent pruritic nodules and contact allergy to aluminium after injection of commonly used aluminium-adsorbed vaccines. *Eur J Pediatr* 2005;**164**:691–7.
9 **Cox NH**, Moss C, Forsyth A. Cutaneous reactions to aluminium in vaccines: an avoidable problem. *Lancet* 1988;**2**:43.
10 **Djurisic S**, Jakobsen JC, Petersen SB, *et al*. Aluminium adjuvants used in vaccines versus placebo or no intervention. *Cochrane Database Syst Rev* 2017;**347**:CD012805.
11 **Mitkus RJ**, King DB, Hess MA, *et al*. Updated aluminum pharmacokinetics following infant exposures through diet and vaccination. *Vaccine* 2011;**29**:9538–43.
12 **Agency for Toxic Substances and Disease Registry**. Public health statement: aluminium Cas # 7429-90-5, department of health and human services, public health service: agency for toxic substances and disease registry (ATSDR). Available: https://www.atsdr.cdc.gov/toxprofiles/tp22.pdf2008
13 **Petrovsky N**. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug Saf* 2015;**38**:1059–74.
14 **Gherardi RK**, Coquet M, Cherin P, *et al*. Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle. *Brain* 2001;**124**:1821–31.
15 **Guimarães LE**, Baker B, Perricone C, *et al*. Vaccines, adjuvants and autoimmunity. *Pharmacol Res* 2015;**100**:190–209.
16 **Sheth SKS**, Li Y, Shaw CA. Is exposure to aluminium adjuvants associated with social impairments in mice? A pilot study. *J Inorg Biochem* 2018;**181**:96–103.
17 **Khan Z**, Combadière C, Authier F-J, *et al*. Slow CCL2-dependent translocation of biopersistent particles from muscle to brain. *BMC Med* 2013;**11**:99.
18 **Asín J**, Molín J, Pérez M, *et al*. Granulomas following subcutaneous injection with aluminum adjuvant-containing products in sheep. *Vet Pathol* 2019;**56**:418–28.
19 **Tomljenovic L**, Shaw CA. Too fast or not too fast: the FDA's approval of Merck's HPV vaccine Gardasil. *J Law Med Ethics* 2012;**40**:673–81.
20 **Exley C**. Aluminium-based adjuvants should not be used as placebos in clinical trials. *Vaccine* 2011;**29**:9289.
21 **Brinth L**, Theibel AC, Pors K, *et al*. Suspected side effects to the quadrivalent human papilloma vaccine. *Dan Med J* 2015;**62**:A5064.
22 **Chandler RE**. Safety concerns with HPV vaccines continue to linger: are current vaccine pharmacovigilance practices sufficient? *Drug Saf* 2017;**40**:1167–70.
23 **Doshi P**, Bourgeois F, Hong K, *et al*. Adjuvant-containing control arms in pivotal quadrivalent human papillomavirus vaccine trials: restoration of previously unpublished methodology. *BMJ Evid Based Med* 2020. doi:10.1136/bmjebm-2019-111331. [Epub ahead of print: 17 Mar 2020].
24 **Pharmacovigilance Risk Assessment Committee, European Medicines Agency**. Assessment report. review under article 20 of regulation (EC) No. 726/2004. human papillomavirus (HPV) vaccines European medicines Agency, 2015. Available: https://www.ema.europa.eu/en/documents/referral/hpv-vaccines-article-20-procedure-assessment-report_en.pdf
25 **World Health Organization (WHO)**. Global Advisory Committee on vaccine safety statement on safety of HPV vaccines, 2015. Available: https://www.who.int/vaccine_safety/committee/GACVS_HPV_statement_17Dec2015.pdf [Accessed 5 Feb 2019].
26 **Caulfield MJ**, Shi L, Wang S, *et al*. Effect of alternative aluminum adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice. *Hum Vaccin* 2007;**3**:139–45.
27 **FUTURE II Study Group**. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007;**356**:1915–27.
28 **Kjaer SK**, Nygård M, Dillner J, *et al*. A 12-year follow-up on the long-term effectiveness of the quadrivalent human papillomavirus vaccine in 4 Nordic countries. *Clin Infect Dis* 2018;**66**:339–45.
29 **Reisinger KS**, Block SL, Lazcano-Ponce E, *et al*. Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. *Pediatr Infect Dis J* 2007;**26**:201–9.
30 **Merck Research Laboratories**. *Clinical study authorization - chemistry, manufacturing and controls*, 2002.
31 **Sharp M**, Corp D, **Danish Cancer Societry**. *Brochure for the future 2 trial in Denmark*, 2002.
32 **The European Medicines Agency, Evaluation of medicines for human use (EMEA/CHMP/VEG/134716/2004)**. Committee for medicinal products for human use (CHMP). guideline on adjuvants in vaccines for human use, 2005. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-adjuvants-vaccines-human-use-see-also-explanatory-note_en.pdf
33 **Gupta RK**. Aluminum compounds as vaccine adjuvants. *Adv Drug Deliv Rev* 1998;**32**:155–72.
34 **Shardlow E**, Mold M, Exley C. Unraveling the enigma: elucidating the relationship between the physicochemical properties of aluminium-based adjuvants and their immunological mechanisms of action. *Allergy Asthma Clin Immunol* 2018;**14**:80.
35 **Exley C**. The toxicity of aluminium in humans. *Morphologie* 2016;**100**:51–5.
36 **Volkin DB**, Shi L, Mach H. Human papillomavirus vaccine formulations. patent number: wo 00/45841, 2000. Available: https://patentimages.storage.googleapis.com/bd/de/a3/7da6c68cfbc8e3/US6251678.pdf
37 **Holland M**, Rosenberg KM, Iorio ET. *The HPV vaccine on trial: seeking justice for a generation betrayed*. Skyhorse Pub Co Inc, 2018.

38 **Little DT**, Ward HRG. Adolescent premature ovarian insufficiency following human papillomavirus vaccination: a case series seen in general practice. *J Investig Med High Impact Case Rep* 2014;2:2324709614556129.
39 **American College of Pediatricians**. New concerns about the human papillomavirus vaccine, 2016. Available: https://www.acpeds.org/the-college-speaks/position-statements/health-issues/new-concerns-about-the-human-papillomavirus-vaccine [Accessed 5 Feb 2019].
40 **Jørgensen L**. PhD Thesis - Benefits and harms of the human papillomavirus (HPV) vaccines. Graduate School of Health and Medical Sciences, University of Copenhagen, 2018. Available: https://drive.google.com/file/d/1B823OIQ3-5pBlEqRvTpD5HAcu80o9vVP/view