EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

16 March 2018
EMA/133566/2018
European Medicines Agency

Dear Mr Catalano,

Thank you for your query on vaccines constituents.

For most of the substances for which you requested information, there are no European mandatory limits for vaccines for human use. For any medicine or vaccine, all constituents and quantities used must be justified with appropriate clinical and safety data.

It is important to note that governments may sometimes use unauthorised vaccines to immunise military personnel after carrying out their own benefit-risk evaluation In addition, the vaccines they use may not be intended for routine mass immunisation e.g. smallpox, and therefore have a completely different risk benefit balance to vaccines intended to be used for mass immunisation in the general population. In addition, levels of ingredients (apart from the active substance) in these vaccines are often different to those used in standard vaccines. This is because acceptable limits are determined on product specific basis and taking into account the overall benefits and risks of a particular vaccine.

Below, we provide you with several important references that provide guidance on the use of excipients, residuals, adjuvants and other constituents in medicines, including vaccines. After this, we provide information about some of the substances from your list where specific guidance or information applies.

1.1. Excipients

Many of the substances you mention are listed in the European Pharmacopeia[1], a compendium which lists active substances and ingredients used to prepare pharmaceutical products in Europe and which is used as an official reference for the quality control of medicines in Europe. For these substances compliance with the corresponding/equivalent monograph is expected.

In addition the '*Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product*'[2] describes the information that an applicant needs to submit in relation to excipients in the context of applications for marketing authorisations. For novel excipients (excipients that are being used for the first time) for example, full details of manufacture, characterisation and controls with cross references to supporting safety data should be provided.

1 https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition
2 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003382.pdf

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1.2. Residuals

Regarding residuals (process-related impurities) in the final product, the manufacturing procedure will influence the nature, range and amount of potential residual in the final product and purification processes must be in place to remove them.[3]

In general, during the evaluation of a vaccine, the purification procedures of the manufacturing process to remove residuals resulting from the manufacturing process (e.g. unwanted variants, host cell proteins, nucleic acids, media components, viruses and reagents used in the modification of the protein) to acceptable levels is thoroughly evaluated. For further information please refer to the Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission (scope excludes vaccines, but principles of the guidance may apply depending on the specific nature of the product).[4]

For certain residuals, testing of either the drug substance or the drug product may not be necessary and may not need to be included in the specifications if it can be demonstrated that controls are in place that ensure the removal of the residual to acceptable levels.[5] Although, as stated above, specific uniform limits for most of the substances listed in your enquiry do not apply, there are requirements for product label warnings under specific circumstances for a number of substances.[6] Additionally, there is guidance for the description in the product information of a number of commonly used excipients and presence of residual substances left from the manufacturing-process that might generate a safety/ toxicity concern (e.g. antibiotics, free formaldehyde).[7]

Many ingredients specified in your request, such as amino acids, are products of media fermentation and no specific uniform limits for these are applicable.

You may wish to consult the Material Safety Data Sheets for thesesubstances to obtain information on their toxic levels. The European Food Safety Authority (EFSA) website,[8] may also provide useful information on toxic levels of these substances when ingested orally.

Please find below additional information about some of the constituents from the listwhere information on their inclusion in medicinal products is covered by European guidance/monographs. The information given is not exhaustive and the key point to note is that, other than where specified, there are no uniform limits for these substances in vaccines.

[3] Production And Quality Control Of Medicinal Products Derived By Recombinant DNA Technology
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003433.pdf
[4] Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission- scope excludes vaccines, but principles of the guidance may apply depending on the specific nature of the product.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/04/WC500205447.pdf
[5] ICH Q6B Specifications: test procedures and acceptance criteria for biotechnological/biological products- note that conventional vaccines are excluded from the scope of this document, but principles may apply according to the specific product concerned.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002824.pdf[6] Excipients in the label and package leaflet of medicinal products for human use
[6] Excipients in the label and package leaflet of medicinal products for human use
https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-2/c/guidelines_excipients_july_2013_rev_1.pdf and its Annex http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001646.jsp
[7] Guideline on quality aspects included in the product information for vaccines for human use. This link is the revised draft which is open for consultation
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2018/02/WC500242956.pdf
[8] http://www.efsa.europa.eu/en/publications/

There are certain constituents mentioned in the European Pharmacopoeia, for which limits do apply for human vaccines (aluminium, phenol, calcium, free formaldehyde). See monograph *Vaccines for human use*.[9] Note also that specific limits for residual host cell DNA, host cell proteins (e.g, ovalbumin, or residual bovine serum albumin [BSA)) or other substances may be specified in vaccine-specific monographs of the Ph.Eur. Some examples are given below and these are not exhaustive. Also, note that these limits may not necessarily apply for combination vaccines, so specific combination-vaccine monographs of the Ph.Eur. also would need to be consulted. For any information regarding these, please contact the European Directorate for the quality of medicines and Healthcare (EDQM) which publishes the Ph.Eur.[10]

1.3. Host cell DNA and host cell proteins (HCPs)

There are no uniform mandatory European limits that apply to all medicinal products.[11] However, there are specific vaccine monographs in the European Pharmacopoeia which set limits for certain vaccines e.g. for inactivated, adsorbed hepatitis A vaccine(not combined with other vaccines), if a continuous cell line is used for virus propagation, the content of residual host-cell DNA should not be greater than 100 picograms in the equivalent of a single human dose. A similar limit is given for rotavirus vaccine (live, oral). For hepatitis B (rDNA) vaccine (not combined with other vaccines) for host-cell- and vector-derived DNA, if mammalian cells are used for production, not more than 10 picograms of DNA in the quantity of purified antigen equivalent to a single human dose of vaccine is permissible. A similar limit is also given for human papillomavirus vaccine (rDNA), smallpox vaccine (live), rabies vaccine prepared in cell culture and cell-propagated influenza vaccines.

Additionally, in order to mitigate potential adverse effects (e.g. immunogenicity), HCP content should always be reduced to the lowest possible level. HCP clearance during the purification process must be assessed and the HCP content determined using an HCP assay that has been evaluated and validated for a given product. You should also consult specific monographs of interest from the European Pharmacopoeia as they may specify limits. For example, for influenza vaccine (live, nasal) and other egg-propagated influenza vaccines and mumps-measles-rubella vaccines, a limit for ovalbumin is specified of not more than 1 micrograms per human dose. For yellow fever vaccine (live) a maximum 5 micrograms of ovalbumin per human dose is set.

1.4. Thiomersal

EMA evaluated the safety of the preservative thiomersal on a number of occasions. In its latest position paper[12] EMA's scientific committee, the CHMP concluded that epidemiologic studies show no association between the vaccination with thiomersal-containing vaccines and specific neurodevelopmental disorders. EMA re-emphasised that immunisation with vaccines containing thiomersal continues to offer outstanding benefits to the general population, including infants.

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[9] Vaccines for human use monograph (Ph.Eur. 07/2017 0153) Aluminium (2.5.13): maximum 1.25 mg of aluminium (Al) per single human dose where an aluminium adsorbent has been used in the vaccine, unless otherwise stated. Calcium (2.5.14): maximum 1.3 mg of calcium (Ca) per single human dose where a calcium adsorbent has been used in the vaccine, unless otherwise stated.
Free formaldehyde (2.4.18): maximum 0.2 g/L of free formaldehyde in the final product where formaldehyde has been used in the preparation of the vaccine, unless otherwise stated.
Phenol (2.5.15): maximum 2.5 g/L in the final product where phenol has been used in the preparation of the vaccine, unless otherwise stated.

[10] https://www.edqm.eu/en/EDQM-contact-685.html

[11] DNA and host cell protein impurities, routine testing versus validation studies
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003322.pdf

[12] Thiomersal: implementation of the warning statement relating to sensitisation
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003905.pdf

EMA noted that, during some manufacturing processes, the use of organic mercury compounds is necessary and in such cases, very small levels might be present in the final product. Nevertheless, EMA re-iterated that, in line with the global goal of reducing exposure to mercury, the development of vaccines without thiomersal or with the lowest possible levels of thiomersal and other mercury containing preservatives should continue to be promoted. When the use of thiomersal as a preservative is necessary therefore, the levels are approved on a product-specific basis. For residual levels (i.e. below 40 nanograms of thiomersal) in the finished product, the CHMP considered that there is no scientific evidence suggesting that such levels could trigger hypersensitivity reactions.

According to the European Pharmacopoeia *Vaccines for human use*[9] monograph, preservatives are normally only acceptable for use in multi-dose vaccines (whereas most vaccines approved for use in Europe are single-dose). It should be noted that sometimes, ingredients such as thiomersal and 2-phenoxyethanol which may be used as preservatives are often only present as a residual in the manufacturing process (e.g. where they have been used as an inactivating agent for a bacterium/virus in preparation of the active substance).

1.5. Lactose, other bovine-derived materials such as polysorbate and human serum albumin (SA)

The safety of bovine-derived materials used in the manufacture of vaccines is described in the *Questions and answers on bovine spongiform encephalopathies (BSE) and vaccines*[13] and the parent guideline, *Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*.[14] Regarding lactose, the milk used for lactose production must be collected under the same conditions as milk for human consumption. This is to ensure that milk comes from healthy animals, controlled by veterinary welfare systems.

Regarding polysorbate, many vaccines now use vegetable-sourced polysorbate. However, bovine-derived polysorbate is used in a small number of vaccines. Bovine serum is obtained only from countries with a negligible or controlled BSE risk and from animals which are fit for human consumption (see EMA scientific guideline[13] for further requirements for bovine sera). Although EMA's scientific guideline does not specify a limit for any of these substances, there are sometimes limits for bovine serum albumin (BSA) specified for certain vaccines in the respective European Pharmacopoeia monograph e.g. vaccines containing inactivated polio antigens (50 ng per single human dose), hepatitis A vaccine (inactivated, adsorbed) depending on the product and the materials used for production, cell-propagated influenza vaccines, mumps vaccine, rabies vaccine prepared in cell culture, tick-borne encephalitis vaccine (inactivated). For varicella vaccine (live), a BSA maximum 0.5 µg per human dose is currently set. For shingles (herpes zoster) vaccine (live), a BSA limit of 0.65 µg per human dose is specified (see specific monographs for all vaccine types of interest).

According to the CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products,[15] use of substitutes for human plasma-derived albumin is encouraged and should be considered as a long-term approach and for many products. However, even when human-plasma albumin is used, there are product-specific limits for its inclusion.

[13] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2018/02/WC500242954.pdf

[14] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf

[15] http://www.ema.europa.eu/docs/en_GB/document_library/Position_statement/2011/06/WC500108071.pdf

1.6. Organic and inorganic impurities (listed as contaminants in your question)

For details about safe limits (permitted daily exposure) of organic impurities and inorganic impurities e.g., residual solvents and elemental impurities such as arsenic and other metals, see ICH Q3C[16], and Q3D[17]. Note that ICHQ3D (elemental impurities) excludes vaccines, however the principles described may apply.

1.7. Aluminium as adjuvant

For your information, please note that the use of aluminium hydroxide and aluminium phosphate in vaccines as adjuvants (used to enhance the immune response) has been well established for many years. These substances are defined in the European Pharmacopoeia.[9] In particular, for vaccines for human use the pharmacopeia specifies a maximum 1.25 mg of aluminium per single human dose when aluminium is used in this way, unless otherwise justified and approved for a specific product. Similar standards apply elsewhere in the world.

There are currently 17 vaccines for human use that have been approved through EMA (via the so-called centralised procedure), as well as many other vaccines authorised at national (Member State) level, that use aluminium hydroxide, aluminium phosphate or amorphous aluminium hydroxyphosphate sulphate as adjuvant.

The benefits of an adjuvant in a vaccine must be weighed against the risk of any adverse reaction inherent to it. The current attitude regarding risk-benefit of vaccination favours safety over efficacy when a vaccine is given to a healthy population. A final safety evaluation of the newly developed vaccine formulation can only be conducted on the basis of clinical trials.[18]

For further information on adjuvants please refer to the following webpage:

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000904.jsp&mid=WC0b01ac058002956b

1.8. Squalene

Squalene is a component of some adjuvants that are added to vaccines to enhance the immune response. Since 1997, an influenza vaccine (FLUAD, Chiron) which contains about 10 mg of squalene per dose, has been approved in health agencies in several European countries. Squalene is present in the form of an emulsion and is added to make the vaccine more immunogenic.[19]

1.9. Latex and silicon

Latex is not a specific constituent of medicinal products, but certain packaging components e.g. rubber stoppers, may be manufactured using latex, therefore label warnings[6] for potential allergic reactions are given. Silicon is not a specific constituent of medicinal products but is used to lubricate cartridges and syringes and prevent the medicinal product solution from interacting with the glass surface. The

[16] ICH Q3C (R6) Residual solvents
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/03/WC500104258.pdf

[17] ICH Q3D Elemental impurities
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/01/WC500180284.pdf

[18] Adjuvants in vaccines for human use
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003809.pdf

[19] http://www.who.int/vaccine_safety/committee/topics/adjuvants/squalene/questions_and_answers/en/

siliconisation process is controlled and potential leachables/extractables studied in individual product stability studies.

We hope you find this information useful. To help us understand how we have dealt with your enquiry, please would you take a short survey on our service through the following link:

https://ec.europa.eu/eusurvey/runner/AskEMA

Best regards,

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