The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

London, 23 July 1998
CPMP/BWP/477/97

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

NOTE FOR GUIDANCE ON PHARMACEUTICAL AND BIOLOGICAL ASPECTS OF COMBINED VACCINES

DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY (BWP)	June 1997
TRANSMISSION TO THE CPMP	June 1997
RELEASE FOR CONSULTATION	June 1997
DEADLINE FOR COMMENTS	January 1998
ADOPTION BY THE CPMP	July 1998
DATE FOR COMING INTO OPERATION	January 1999

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Switchboard: (+44-171) 418 8400 Fax: (+44-171) 418 8551
E_Mail: mail@emea.eudra.org http://www.eudra.org/emea.html
©EMEA 1998 Reproduction and/or distribution of this document is authorised for non commercial purposes only provided the EMEA is acknowledged

PHARMACEUTICAL AND BIOLOGICAL ASPECTS OF COMBINED VACCINES

Table of Contents

Page

1. INTRODUCTION 2
   - 1.1 General considerations 2
   - 1.2 Constraints to Combined Vaccines Research and Development 2
   - 1.3 Additional points to consider 4
2. SCOPE 5
3. DEFINITIONS 5
4. MAIN CONCERNS 6
5. PHARMACEUTICAL ASPECTS 6
   - 5.1 Manufacturing and control requirements 6
   - 5.2 Formulation 7
   - 5.3 Adjuvants 7
   - 5.4 Antimicrobial agents 8
     - 5.4.1 General principles 8
     - 5.4.2 Inactivating agents 8
     - 5.4.3 Antibiotics used in production 8
     - 5.4.4 Preservatives present in the finished product 9
   - 5.5 Other ingredients 9
   - 5.6 Stability 10
6. RELEASE TESTING / POTENCY TESTING 10
   - 6.1 General 10
   - 6.2 Potency 11
   - 6.3 Additional points to consider for reconstituted vaccines 12
7. CONSISTENCY TESTING OF COMBINED VACCINES 12
8. IMMUNOGENICITY / BIOLOGICAL ACTIVITY STUDIES 13

©EMEA 1998

1. INTRODUCTION

1.1 General considerations

The concept of combined vaccines is not novel, since vaccines comprising combinations of inactivated diphtheria, tetanus and pertussis whole cell components or live measles, mumps and rubella viruses have been available for at least two decades. Combined multivalent vaccines like pneumococcal and meningococcal vaccines have also been extensively used over many years. The positive experience gained with these combinations has stimulated vaccinologists and vaccine manufacturers to contribute to this progress with numerous new vaccine designs: Vaccines comprising combinations of diphtheria, tetanus and pertussis components with Haemophilus influenzae b polysaccharide-protein conjugates have been available for some years. Combinations of diphtheria, tetanus, whole cell and acellular pertussis and Haemophilus influenzae type b components with additional components such as inactivated poliomyelitis vaccine, hepatitis B vaccine, have recently been introduced, while combinations with meningococcal - and pneumococcal polysaccharide-protein conjugates are under development. Combination vaccines against enteric infections including cholera, typhoid, shigella, and rotavirus may become available in the longer term.

One single vaccine containing protective antigens against different diseases for which universal immunisation is recommended could simplify the implementation, increase the acceptance, and lower the cost of immunisation programmes for the following reasons:

The use of combined vaccines can increase the convenience of vaccine delivery and thus improve compliance in the population. Fewer inoculations will be needed to protect against more diseases, thus enhancing the acceptance of immunisation programmes by both the general public and the medical profession. These cumulative favourable factors will boost the effectiveness and success of immunisation programmes by increasing vaccine coverage, while at the same time creating cost savings in healthcare budgets. In addition, changes in the vaccine schedule can be anticipated with the licensure of new combined vaccines, creating challenges for revision of the immunisation schedule and harmonisation of posology within the European Union.

In the near term, conventional methods of mixing existing live vaccines or killed antigens as described above will be favoured.

In the mid- and long-term more sophisticated methods made possible by recent scientific and technological breakthroughs will be employed, e.g. for vector based vaccines and polynucleotide vaccines. Although these approaches are very promising, they are still at the stage of feasibility testing and will not be considered in this guideline.

1.2 Constraints to Combined Vaccines Research and Development

Contrary to initial optimistic expectations based on the experience with the above mentioned diphtheria, tetanus and pertussis vaccines or live measles, mumps and rubella viruses vaccines and multivalent products against pneumo- and meningococci, the development of combined vaccines is not entirely straight-forward but can raise many complications. Each combination must be developed and studied individually in terms of quality, stability, safety, clinical tolerability and efficacy/immunogenicity. Initially, this includes the pharmaceutical development to establish the correct formulation, the stability of and compatibility between the individual components in the combined vaccine, including preservatives, excipients and adjuvants. This is exemplified with the following examples.

©EMEA 1998

In combined vaccines, the presence of more than one component often causes an interaction, leading to either a diminished or an increased response to individual components, compared to when the specific component(s) is administered alone.

Such interactions are often immunological in nature, but problems may also be caused by chemical and physical interactions between the different components of the vaccine. For example, there may be competition for adsorption sites on the adjuvant. In the case of live virus vaccines, interference between different virus strains used in combination, or induced by concomitant exposure to extraneous infection, may suppress proliferation of the vaccine strains resulting in a sub-optimal response.

Immunological interference is a broad description of a multitude of interferences, the nature of which are often only poorly understood. One or more of the following phenomena may be involved:

- antigenic competition,
- epitope specific suppression,
- an adjuvant effect, exerted by specific component(s) of the combined vaccine,
- an adverse adjuvant interaction.

Antigenic competition describes the phenomenon, that an immune response to a particular antigen may be diminished in the presence of other antigens, compared to when the same antigen is given alone. Several mechanisms are probably involved, but one mechanism seems to be intracellular competition among small peptides (T-cell epitopes) for binding to MHC-structures and subsequent presentation on the cell surface for T-cells.

Epitope specific suppression is described as a phenomenon, where pre-immunisation with a protein leads to a diminished response to a hapten, when the same protein is subsequently used as carrier for the hapten. Model systems with toxoids as carriers have often been used to demonstrate epitope-specific suppression. Hapten density influences the suppression. The mechanism involved is not fully clarified; some data point to the appearance of clonal expansion (dominance) of B-cell clones with specificity for carrier-B-cell epitopes as the cause, while other experiments seem to indicate a suppressor T-cell mechanism where carrier-specific suppressor cells influences the development of hapten-specific T-suppressor cells. A third proposed mechanism involves a defect in differentiation of hapten-specific B-cells.

An adjuvant effect, exerted by specific component(s) of the combined vaccine. Adjuvant effects from components of a combined vaccine are known from the presence of e.g. components of whole cell pertussis vaccine, which are known to function as a non-specific stimulant. Such added adjuvanticity is most often caused by substances which themselves function as B-cell mitogens or they are potent activators of the complement system.

An adverse adjuvant interaction caused by adsorption of more than one component. When an adjuvant is used to augment the immune response to a combined vaccine, special problems may appear. In the case of mineral adjuvants, adjuvanticity is dependent on the vaccine component being firmly bound to the adjuvant. The effect of adjuvants like mineral salts is thus strongly dependent on the amount of adjuvant, the amount of un-occupied adjuvant sites, the buffer used for adsorption (anions like phosphate and citrate effectively diminishes adsorption of some (mainly acidic) proteins onto Al (OH)3 and salt concentration (ionic strength). Also, the adsorption procedure must be carefully controlled as the sequence of adsorption's may influence the fraction of non-adsorbed individual vaccine components.

©EMEA 1998

The following well-known examples are given:

A drawback of combined live vaccines could be reduced immune response from interference between the individual components. The oral polio vaccine needs to be administered three or sometimes more times to achieve full immunisation due to mutual interference on their 'take' between the three viruses in the combined attenuated vaccine. With combined measles and mumps vaccines, there is a one-way interference between the mumps and measles components which reduces the immune response to mumps virus.

Another example for bacterial vaccines is described in the following: There are indications from recent studies on diphtheria, tetanus, pertussis and Haemophilus influenzae type b vaccine combinations that these may not behave in control tests exactly as expected from the performance of the diphtheria, tetanus, pertussis and Haemophilus influenzae type b components administered separately. Thus there is evidence that the presence of diphtheria, tetanus and pertussis components enhances the antibody response to the Haemophilus influenzae type b component. This most likely arises from the adjuvant effect of the B. pertussis cells but a more complex antigen mimicking effect may also contribute. Another effect has been the enhancement of the tetanus antitoxin response when the Haemophilus influenzae type b component consists of a PRP-tetanus toxoid conjugate. This probably arises because the tetanus toxoid content of the conjugate is comparable with the quantity present in the diphtheria, tetanus and pertussis component. This raises the issue of possible excessive doses of tetanus toxoid if several conjugates containing this carrier were to be included in a combined vaccine. In such a situation the tetanus component of the diphtheria, tetanus and pertussis vaccine may not only be redundant, but possibly undesirable.

There is some evidence that vaccine combinations may prove more reactogenic than the individual components given separately. Examples have already been brought to the attention of the licensing authorities. Therefore, increased reactogenicity needs to be considered. The possibility of increasing endotoxin content, particularly where components derived from Gram negative bacteria are used, also needs to be taken into account. This would be a limiting effect, for example, in combinations of diphtheria, tetanus, pertussis and killed typhoid vaccine.

Another key issue for combined vaccines is the clinical evaluation which includes the determination of local and systemic tolerability and immunogenicity in well-controlled studies comparing the combination with its licensed vaccines containing the same components. The influence of the various combinations on the type of immune response must also be taken into consideration.

1.3 Additional points to consider

There is a strong demand for harmonised regulations and a common approach to combined vaccines in the European Union. The rules governing pharmaceutical products in the European Union are naturally applicable to combined vaccines to ensure their quality, safety and efficacy. This guideline is aimed at a product-specific harmonisation of the requirements for the registration of combined vaccines in the European Union and at giving an assurance that the research and development work carried out on combined vaccines will be considered as valid by all the Member States and will eventually lead to the marketing authorisation.

For pre-clinical and clinical studies the Note for Guidance on Pre-clinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95) be and the Note for Guidance on the Clinical Evaluation of New Vaccines (CPMP/EWP/436/97) should be consulted.

©EMEA 1998

2. SCOPE

This guideline gives advice to manufacturers and regulatory authorities to ensure that new combined vaccines and combined vaccines that are new to a particular manufacturer fulfil the highest possible standard of the quality, safety and efficacy. It will enable the competent authorities to arrive at their decisions by reference to uniform criteria for combined vaccines and will therefore avoid differences in evaluation.

This guideline does not address requirements for individual specific combined vaccines; such guidance will be provided through European Pharmacopoeia monographs (legally binding), WHO recommendations (the prevailing standard of scientific knowledge relevant to that type of product) and the Marketing Authorisations. For instance, the Ph. Eur. General Monograph on "Vaccines for Human Use" (1998:0153) already contains a statement on combined vaccines: "For a combined vaccine where there is no monograph to cover a particular combination, the vaccine complies with the monograph for each individual component, with necessary modifications approved by the competent authority".

In the sense of this guideline simultaneously (at separate injection sites) administered vaccines are not considered as combined vaccines.

3. DEFINITIONS

Combined vaccines are products intended

i) for protection against a single infectious disease complex caused by different strains or serotypes of organisms; or

ii) protection against multiple infectious diseases; or

iii) combinations of (i) and (ii).

In technical terms the following products are considered as combined vaccines and their use should be specifically addressed in the Summary of Product Characteristics:

i) the mixture of two separate vaccines in one vial or prior to administration;

ii) the use of by-pass or dual chamber syringes, containing a different vaccine in each chamber;

iii) vaccines for which the components are combined into one formulation at the final bulk stage.

Several rationals for combining vaccines are possible:

- Widely used current combinations of viral or bacterial antigens in a single product.

  Examples are:

  Measles, Mumps and Rubella Virus Vaccine Live;
  Diphtheria and Tetanus Toxoids and Whole Cell Pertussis Vaccine (Adsorbed).

- New combinations of viral and/or bacterial antigens in a single product.

  Examples are:

  Diphtheria and Tetanus Toxoids and Whole Cell Pertussis Vaccine
  Adsorbed + Hepatitis B Surface Antigen;
  Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine

©EMEA 1998

(Adsorbed) + Hepatitis B Surface Antigen;
Diphtheria and Tetanus Toxoids and Whole cell /Acellular Pertussis
Vaccine (Adsorbed) and Haemophilus Type b Conjugate Vaccine + Inactivated Polio Vaccine;
Hepatitis A and B Vaccine.

- Vaccine combinations designed to prevent a single disease complex.

  Examples are:

  Multivalent Meningococcal Conjugate Polysaccharide Vaccine, Groups A, C, Y and W135;
  Multivalent Pneumococcal Conjugate Polysaccharide Vaccine;
  Rotavirus Vaccine.

4. MAIN CONCERNS

The potential problems associated with combined vaccines are summarised as follows:

1. Enhanced reactogenicity through
   - a) excessive endotoxin load
   - b) excessive toxoid load
   - c) additive or synergistic effects
   - d) displacement of antigen from adjuvant
   - e) stimulation of excessive or inappropriate immune responses
2. Sub-optimal immunogenicity through
   - a) antigenic competition
   - b) epitope suppression
   - c) excessive antigen : adjuvant ratio.
3. Excessive batch-to-batch variation resulting from complex interactions of multiple components.
4. Sub-optimal stability, e.g. from conjugation of combined vaccines.

5. PHARMACEUTICAL ASPECTS

5.1 Manufacturing and control requirements

The basic requirements for manufacture and control of combined vaccines are essentially the same as for other biological products and are given in relevant guidelines for assuring the quality of biological products, e.g. "Good Manufacturing Practices for Biological Products" in: *WHO Expert Committee on Biological Standardisation. Forty-second report. Geneva, World Health Organisation*, *1992*, Annex 1 (WHO.Technical Report Series No. 822). For some combined vaccines there are already requirements for the individual components available, as for example diphtheria, tetanus and pertussis vaccines or live measles, mumps and rubella viruses vaccines, and influenza- or poliomyelitis-vaccines. The methods of preparation vary according to the type of vaccine, as described in individual European Pharmacopoiea

©EMEA 1998

monographs, WHO requirements and manufacturers production and control records. These methods are designed to guarantee the quality, safety and efficacy of each vaccine component, with greatest emphasis on maintaining the appropriate antigenic properties and to ensure freedom from contamination with extraneous agents. Vaccines are as far as possible free from ingredients known to cause toxic or allergic reactions in man: Ph. Eur. General Monograph on "Vaccines for Human Use" (1998:0153).

5.2 Formulation

The quality of all components of the final preparation forming the combined vaccine shall comply with the specifications of the relevant monographs of the European Pharmacopoeia, where existing, and the final vaccine preparation shall comply with the requirements of the relevant general and specific monographs. However, additional considerations are necessary for the formulation of combined vaccines and attention is drawn to the chapter MAIN CONCERNS above. These products are complex mixtures of antigens, preservatives, adjuvants and stabilisers and in addition may contain contaminants derived from fermentation, cell culture and other production processes.

The physical and chemical compatibility of these components requires carefully drafted formulation studies preceding any clinical trials. Such physical, chemical and biochemical studies go far beyond those required for the individual components. For example, for live viral vaccines possible interferences between viruses should be studied and adjustment of virus titers might become necessary. Possible deleterious effects of vaccine components on each of the active constituents of both viral and bacterial products should be controlled. Also of concern is the possibility that there might be interference with the immunological response to one or more antigens; relevant studies in animals may help to solve this problem.

With an increasing number of components to be incorporated into a combination the problem of the volume to be administered arises. In this context, studies to reduce the antigen amount in a given vaccine or to concentrate it will have to be performed. Reduction of the dose of antigens through the use of novel immunologic adjuvants may facilitate the formulation of combined vaccines, which will incorporate multiple antigens.

With respect to safety particular attention must be paid to the possibility that adverse reactions might be either additive or enhanced. Where required, the test for reversion to toxicity and/or virulence should be performed on both the single vaccine component and the combined product.

5.3 Adjuvants

New combined vaccines, except for live virus vaccines, may be proposed with new adjuvant compositions. The reasons for introducing new adjuvants can be (i) an increased antigen purity, (ii) the lack of the adjuvant effect of other vaccine components, e.g. through omission of pertussis whole cells, and (iii) the need to target a specific type of immune response. This may well result in the introduction of new and more powerful adjuvants or optimisation of the one used currently. Manufacturers and researchers are looking at an extensive range of possible alternatives. Novel adjuvants, presently under preclinical and clinical testing with vaccines, include e.g. detoxified lipid A, emulsions, liposomes, microspheres, muramyl peptides, and saponins.

At present, according to the European Pharmacopoeia, the vaccines may be adsorbed on aluminium hydroxide, aluminium phosphate, calcium phosphate or other adsorbents prescribed

©EMEA 1998

in the monograph. "The adsorbents are prepared in special conditions which confer the appropriate physical form and adsorptive properties" (Ph. Eur.). However, it is well known that the different procedures used for adjuvanting antigens with aluminium can lead to variances in product stability or immunogenicity, e.g. through different adsorption capacity and desorption. Different forms of aluminium compounds can further add to the problem. In addition, different manufacturers make their antigens by various procedures. They in turn may individually require different adjuvanting conditions, even with the same aluminium compounds.

In addition, certain antigens may best be presented within a new combination with a completely new adjuvant. However this does not mean that the other antigens in that same combination will be equally adjuvanted by the same new adjuvant. Thus, there is the necessity to demonstrate compatibility between possible different adjuvants within a single combination product.

As a result, adjuvants in combined vaccines need considerable attention and manufacturers should provide assurance that they are adapted to each individual product, that they comply with the European Pharmacopoeia, that the combinations are safe and that they do not give rise to unacceptable reactogenicity. This includes: (i) demonstration of the compatibility of the adjuvant/adjuvants with all antigenic components present in a combined product; (ii) proof of an efficient adsorption of all antigenic components present in a combined product, where relevant; (iii) demonstration that no significant desorption takes place in the course of the shelf-life of the product, where relevant; and (iv) demonstration that the adjuvant is non-toxic.

5.4 Antimicrobial agents

5.4.1 General principles

In view of the desirability of excluding potentially toxic or allergenic substances from medicinal products whereever possible, and the emphasis on material selection, control, processing and GMP, as a means of achieving a sterile product, antimicrobial agents should only be used in the manufacture of a medicinal product when specifically justified, and then, must never be used as a substitute for any aspect of GMP.

5.4.2 Inactivating agents

In the case of inactivating agents used in the manufacture of bacterial vaccines

- traces appearing in the finished product should be minimised and controlled;

and

- the labelling and package leaflet texts should state the presence of any traces present.

5.4.3 Antibiotics used in production

Antibiotics should not normally be used during vaccine production to ensure bacterial sterility or to reduce bioburdens. In the case of any vaccine in which it is proposed to include antibiotics in production

©EMEA 1998

- traces appearing in the finished product should be minimised and controlled;

and

- the labelling and package leaflet texts should state the presence of any traces present.

5.4.4 Preservatives present in the finished product

Antimicrobial preservatives should not be included in the finished product unless their use is justified by quality and/or safety considerations. Their use is never acceptable in live vaccines, but may be justified in the case of inactivated vaccines which:

- are present in multi-dose containers and/or
- are presented as suspensions so that sterilisation/filtration is impossible.

In all cases where products are proposed to contain antimicrobial preservatives:

- a benefit risk analysis should be presented in the application dossier; any potential toxicity and/or potential allergenicity should be addressed in this presentation,
- the concentration of the antimicrobial preservatives should be controlled in the bulk and in the finished product specifications in accordance with European Pharmacopoiea limits,
- the efficacy of preservation should be tested according to the Ph.Eur. requirements for human vaccines,
- the maintenance of preservative concentration or efficacy throughout the period of the period of validity should be demonstrated,
- the name and concentration of the antimicrobial preservatives should be stated on the labelling.

In selecting a preservative system the applicant should consider:

- the effectiveness against potential microbial contaminants,
- possible interaction with the formulation or container (for example, thiomersal may be ineffective in the presence of sera, and can bind to -SH groups and polymeric material; for toxoid vaccines phenol might impair the antigenicity),
- possible effects on testing in biological systems.

If replacement of preservatives is considered on the basis of side effects or for other reasons, a risk/benefit evaluation should be made, taking into consideration that such a change implies a new formulation with on a case by case basis the need for additional studies for sterility, potency, stability and their clinical implications.

5.5 Other ingredients

The use of different buffers, salts or varying concentrations of them, changes in pH, and other chemical factors may affect the safety, purity and potency of the final combined vaccine. These factors should be considered during the formulation development of the product.

©EMEA 1998

5.6 Stability

The studies should be based on the requirements of the ICH "Guideline for the stability testing of new active substances and medicinal products" in connection with its annex for biological and biotechnological products. This guideline on "Quality of biotechnological products: Stability testing of biotechnological/biological products" requires that primary data to support a requested storage period for either drug substance (bulk material) or drug product (final container product) should be based on long-term, real-time, real-condition stability studies. Accelerated stability testing data may be used as supporting data but cannot be used to assign the expiry date. In the case of combined vaccines there are additional points to be considered: stability data are requested for each individual component before combination, after combination into the bulk product, and for the combined vaccine as finished product; stability data on at least three batches of each of these three manufacturing steps of the combined vaccine should be provided for the requested dating period. For details cross-reference is made to the ICH stability testing documents mentioned above. The period of validity for a given batch begins on the day on which the assay is started. Where there is no assay for a given vaccine, some other date should be specified in the marketing authorisation, for example, the date of an approved stability-indicating test, the date of freeze-drying or the date of filling. The maximum length of the storage period should always be based upon the expiry date of the least stable component.

6. RELEASE TESTING / POTENCY TESTING

6.1 General

The aim of the release testing of a given vaccine batch is to show that this batch is consistent with and equivalent to the successive batches produced by a given manufacturer and to the batches that have been shown safe and efficacious in clinical trials in man. Among all tests included in the specifications for batch release most have to be carried out on the product in its final container; i.e. volume, pH, sterility, endotoxins, preservatives / adjuvants / excipient contents, qualitative and quantitative identification of the adsorbed antigens, freedom from abnormal/specific toxicity, toxicity reversal and potency or antigen content.

However, on a case by case basis and provided that adequate data have been presented some of the tests may be performed on the final bulk product, e.g. (i) the quantification of preservatives and excipients, (ii) the abnormal toxicity test, (iii) the *in vivo* potency testing, (iv) the pyrogenicity test for vaccine components where it is required, even if combined with other vaccine components for which pyrogenicity testing is not foreseen in the European Pharmacopoiea monograph, (v) toxicity reversal testing, (vi) physico-chemical analysis for purity and integrity in cases where it is not required for the final product but for the bulk component. In such cases the additional manufacturing process should be demonstrated to have no effect on the release specifications of the finished product.

The tests for sterility, freedom from abnormal/specific toxicity and identity should be performed according to the European Pharmacopoiea monographs or WHO requirements on both the products single components or on the combined product depending on the presentation (see also 6.3). Similarly, each vaccine component should meet the purity characteristics according to the European Pharmacopoiea monographs or WHO requirements at the appropriate stages of manufacture.

©EMEA 1998

6.2 Potency

Amongst the release tests, special attention has to be paid to the potency test, since potential interferences between various antigens of combined vaccines could lead to different and unstable results. Potency assays on biological medicinal products rely heavily on the use of reference preparations with an established potency. The “traditional” approach in biological standardisation of potency testing follows three principles:

- **The establishment of a WHO International Standard for global use.** These primary standards are - because of shortage - not for routine direct use in assaying batches of vaccines but for calibration of secondary reference preparations or in-house reference preparations.
- **The establishment of secondary regional or national reference preparations,** e.g. the European Pharmacopoiea Biological Reference Preparations (BRP). The European Pharmacopoiea Biological Reference Preparations are regional reference preparations for use by European manufacturers and Official Medicines Control Laboratories (OMCL’s). These reference preparations are always calibrated against the relevant WHO International Standard and are available in amounts suitable for their direct use as working references. They may also be used to calibrate national or in-house working reference preparations.
- **The calibration of in-house working reference preparations.**

  In general, this approach contributes significantly to the equivalence of the biological potency of vaccines all over the world and it should be used whenever relevant and possible.

  In situations where the traditional approach cannot be followed, an alternative solution must be developed:
- **The WHO International Standard or the regional or national reference preparations do not exist.** In this case, the appropriate mechanisms should be initiated to develop the necessary materials, e.g. the European Department for the Quality of Medicines should consider developing a regional reference preparation for use by European manufacturers and Official Medicines Control Laboratories.
- **Due to differences in composition between the WHO International Standard or the regional or national reference preparations and the combined vaccine to be assayed, it may be impossible to perform a valid comparison.** In this case, reference material should be developed which suitably resembles the composition of the vaccines to be assayed. In some cases, this would be a batch representative of a particular manufacturing process or even a batch representative of a particular product (“in-house” or “homologous reference”).

'In-house' or 'homologous reference' materials should preferably have been shown satisfactory in clinical trials in man carried out by the manufacturer or should have been shown equivalent to a vaccine lot previously tested in clinical trials and shown satisfactory in man. The appropriate mechanisms should be initiated to give official status to these process specific or product specific reference materials. This can be done through assessing, in collaborative studies organised by the European Department for the Quality of Medicines, the suitability and behaviour of such references for manufacturers and Official Medicines Control Laboratories. The specifications stating the acceptable limits of the tests, the conditions for its validity and

©EMEA 1998

the criteria should be determined and validated by the individual manufacturers in close collaboration with the authorities.

It is felt that for the newly developed vaccines (e.g. Haemophilus influenzae b, acellular pertussis) and especially for the newly developed combined vaccines the latter case will occur more frequently than in the past. For example, nearly all of the existing International Standards/references are monovalent preparations which, by definition, differ in composition from combined vaccines. Although the universal use of a monovalent Standard/ reference preparation has many practical advantages, the suitability of this approach should be carefully evaluated on a case by case basis for the new combined vaccines.

6.3 Additional points to consider for reconstituted vaccines

When a combined vaccine is composed of two separate preparations which shall be reconstituted with each other at the time of administration, full approved release testing operations carried out on each of the two preparations separately shall be deemed as sufficient for routine release testing purposes.

Repeating the tests on the reconstituted product, particularly those involving animals such as potency testing, is not required, provided that during development, duly validated studies demonstrating compatibility of the two components following reconstitution have been shown satisfactory by the manufacturer with due consideration of batch consistency, batch size and frequency of production.

These studies will show that components and the final reconstituted vaccine have sufficient comparability of purity and immunogenicity to meet the release specifications.

Any further requirements should be analysed on a case by case basis.

7. CONSISTENCY TESTING OF COMBINED VACCINES

Problems related to antigen-antigen compatibility, formulation with the proper adjuvants and preservatives and the optimising conditions that would maintain and ensure safety, purity and efficacy lead to the requirement of a proper demonstration and definition of consistency testing. Consistency of manufacture should be demonstrated after the establishment of the final manufacturing procedures by testing a logical defined subset of batches selected from a very large number of possible combinations.

The consistency of combined vaccines should be evaluated by first of all demonstrating the consistency of their single individual valency. At least three consecutive production runs (bulk antigens) would be required for each valency of the combined vaccine to show consistency of valency production. For a pentavalent vaccine this amounts to: A1, A2, A3, B1, B2, B3, C1, C2, C3, Y1, Y2, Y3, Z1, Z2, Z3.

If consecutive lots are not used, a scientific explanation should be provided. At least three lots of combined vaccines should then be produced to demonstrate consistency of the combination. This amounts to:

A1............Z1

A2............Z2

A3............Z3

©EMEA 1998

The addition of one new valency (Z) to existing batches of a well established combination represents a common situation. For example, existing batches of diphtheria and tetanus will be combined with new batches of acellular pertussis (aP). For this situation D1T1aP1, D1T1aP2 and D1T1aP3 should generally adequately show consistency on the level of final bulk. In general, for the addition of a new valency (Z) to any number of existing and well established valencies (A1, B1, C1, ...) consistency is to be shown according to:

A1 B1 C1 ... Z1

A1 B1 C1 ... Z2

A1 B1 C1 ... Z3

where it is considered that the consistency of the well established combined vaccine has been already proven by numerous production batches that have been used in man.

The addition of more than one new valency (Y, Z) to a well established combination may arise, for example, when Hepatitis A and Hepatitis B are combined with diptheria, tetanus and pertussis to form a pentavalent vaccine. In this situation the possible combination of batches may easily become very large (different production scales, expiry dates, etc.). In this case consistency should be shown by three consecutive final bulks as follows:

A1 B1 C1 ... Y1 Z1 ...

A1 B1 C1 ... Y2 Z2 ...

A1 B1 C1 ... Y3 Z3 ...

This procedure may be extended to any combination of existing and new valencies and does not depend on the presence of any existing and well established valency in the combined vaccine (provided that the consistency of each valency has been shown separately).

8. IMMUNOGENICITY / BIOLOGICAL ACTIVITY STUDIES

New combination vaccines should be studied for their appropriate immunogenicity parameters in animal model(s), if available. Regarding the selection of animals the choice is dependant on the individual assay to be performed. Ideally, all new combined vaccine components should induce antibody titers in the appropriate species; these should be representative of the antibody responses achieved in humans.

The immunogenicity of combined vaccines in an experimental host may not always parallel exactly the response in humans, but over many years it has been shown that preparations which are protective in animal model systems also protect humans. It is important to note that immunogenicity studies like all other pharmacological and toxicological studies should be performed before the initiation of studies of human clinical trials.

The response to each of the antigens in the vaccine should be assessed as well as the quality of the response. This evaluation should include a characterisation of antibody class, avidity, affinity, half-life, or function, e.g., examining the ability to neutralise the target agent or toxin.

With regard to the selection of dosage and immunogenicity test methods manufacturers should, whenever possible, refer to WHO recommendations and European Pharmacopoiea methods. However, it is in the detail that difficulties can arise with immunogenicity testing of combined vaccines. Each new combined vaccine needs to be considered on a case-by-case basis taking into account progress with the developments in control procedures and the introduction of new

©EMEA 1998

immunogenicity testing methods by either the European Pharmacopoiea monographs or the WHO requirements. Dose responses for different combined vaccine batches of the same product should show very similar results, indicating a consistency in the product and in the resulting immune response.

The characteristics of the immune response depend on the type of cells producing the response and the antigens stimulating the process. If the combined vaccine is recommended for booster injections, boostability of the immune response should also be studied. Most of the vaccine antigens are proteins and induce B lymphocytes to produce antibody aided by T helper cells, the immune response is potentially boostable, and IgG antibody predominates. In contrast, unconjugated polysaccharide antigens stimulate B cells without T-cell help, producing a nonboostable response of both IgG and IgM antibodies. The boostability of the immune response should be studied in appropriate animals taking also into due consideration the time interval recommended between prime and booster dose.

It is preferable to study the new combination in comparison to the individual antigens in animals to determine if any augmentation, diminution or alteration of response occurs. Interference between live vaccine strains also may be studied in animal immunogenicity studies.

Animal protection studies are recommended using an animal model, wherever one is available, for new vaccines or combination vaccines with a new antigen that has not been studied in humans before. Protection should be demonstrated upon challenge with toxin or a virulent strain(s) of each organism against which the vaccine is intended to protect. As already pointed out in chapter 6.2 "Potency", in an ideal biological assay the test preparation and a standard preparation should be compared. The study should be conducted with statistically and scientifically valid procedures for verifying the results and these should be described.

©EMEA 1998