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**Datasheet for the decision
of 25 September 2007**

Case Number: T 0067/05 - 3.3.04

Application Number: 93910244.8

Publication Number: 0652771

IPC: A61K 39/08

Language of the proceedings: EN

Title of invention:
Tetanus vaccine production

Patentee:
Evans Vaccines Limited

Opponent:
Sanofi Pasteur Limited

Headword:
Tetanus vaccine/EVANS

Relevant legal provisions (EPC 1973):
EPC Art. 54(1)(2), 56, 83, 84, 123(2)(3)
EPC R. 26(2)(c), 57a, 64(a), 65(2)

Keyword:
"Admissibility of appeal (yes)"
"Main request - inventive step (no)"
"Admission of auxiliary request into the proceedings (yes)"
"Auxiliary request: added subject-matter and extension of
scope of protection (no); novelty, inventive step and
sufficiency of disclosure (yes)"

Decisions cited:
T 0939/92, T 0149/93, T 0097/98, T 1329/04, T 1599/06

Headnote:
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Case Number: T 0067/05 - 3.3.04

DECISION
of the Technical Board of Appeal 3.3.04
of 25 September 2007

Appellant: Sanofi Pasteur Limited
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 4 November 2004
rejecting the opposition filed against European
patent No. 0652771 pursuant to Article 102(2)
EPC.

Composition of the Board:

Chairman: R. Moufang
Members: G. Alt
R. Gramaglia

Summary of Facts and Submissions

- I. European patent 0 652 771 is based on European patent application 93910244.8 filed as international application PCT/GB93/01037 and has the title "Tetanus vaccine production". It was granted with nine claims.

Claims 1 and 5 as granted read:

"1. A process for the preparation of tetanus toxoid, which process comprises incubating purified tetanus toxin having a specific activity of 2000 Lf/mg PN (Limes flocculationis/mg protein nitrogen) or more and an Lf content of 250 Lf/ml or more with 0.2 to 1% (V/V) formaldehyde in the presence of 0.005 to 0.25M lysine for from 24 to 32 days at a pH of from 6.0 to 8.0 and a temperature of from 30 to 45°C.

5. A process according to any one of the preceding claims, wherein the purified toxin is preincubated with the formaldehyde in the absence of the lysine for from 20 to 40 minutes at from 35 to 40°C."

- II. An opposition was filed by Aventis Pasteur Limited. The opposition was based on Article 100(a) EPC on the ground of lack of inventive step (Article 56 EPC) and on Article 100(b) EPC.

- III. The opposition division decided to reject the opposition pursuant to Article 102(2) EPC. As regards inventive step, it considered document D2 and the formaldehyde-based detoxification process disclosed therein as the closest prior art and identified the objective technical problem as how to provide an

improved process for the preparation of tetanus toxoid suitable for vaccine formulation, which does not exhibit adverse toxic reversion reactions, starting from a purified toxin. The opposition division held that even if the skilled person, despite being aware of the differences, had expected the reversion problem known for diphtheria also to occur in the context of tetanus detoxification, he/she would not have been in a one way street situation in overcoming the problem, since there had been conflicting views as to stabilising additives in documents D2, D6 and D9. The addition of lysine as a solution to the problem for the reason that it had been chosen for the detoxification of diphtheria toxin could therefore only be considered obvious with hindsight knowledge from the teaching of the contested patent.

With regard to the requirements of Article 83 EPC the opposition division essentially found that the opponent had not discharged its burden of proof for demonstrating that an undue burden was put on the skilled person wanting to rework the invention.

In the decision it was moreover held that document D14 filed by the opponent a few days before the oral proceedings was not admitted because it was not considered prima facie relevant.

- IV. An appeal was lodged against this decision on 13 January 2005 "on behalf of the opponent, Aventis Pasteur Limited". With the statement of the grounds of appeal, documents D11 to D13 and documents D15 to D22 were filed, as well as a complete copy of document D14.

The patentee (respondent) replied to the statement of grounds of appeal and filed documents D25 and D26.

Further written submissions were filed by both parties.

- V. Oral proceedings were held on 25 September 2007.
- VI. At the oral proceedings the board informed the parties about a letter submitted on behalf of the appellant in appeal case T 1193/03 on 24 January 2006. The letter contained information about a change of the appellant's name into "Sanofi Pasteur Limited" and included supporting documents. The appellant requested that the same change of name be recorded in the present case and that the erroneous statement of the name in the notice of appeal be corrected in accordance with Rule 65(2) EPC.
- VII. As a reaction to the conclusions of the board at the oral proceedings with respect to the main request, the respondent did not maintain its previous auxiliary request filed by letter of 5 December 2005, but submitted two new auxiliary requests ("Auxiliary Request 2" and "Auxiliary Request 3").

Claim 1 of auxiliary request 2 read:

"1. A process for the preparation of tetanus toxoid, which process comprises incubating purified tetanus toxin having a specific activity of 2000 Lf/mg PN (Limes flocculationis/mg protein nitrogen) or more and an Lf content of 250 Lf/ml or more with 0.2 to 1% (V/V) formaldehyde in the presence of 0.005 to 0.25M lysine for from 24 to 32 days at a pH of from 6.0 to 8.0 and a

temperature of from 30 to 45°C, wherein the purified toxin is preincubated with the formaldehyde in the absence of the lysine for from 20 to 40 minutes at from 35 to 40°C."

The request contained seven dependent claims.

VIII. The appellant requested that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the appeal be dismissed or, in the alternative, that the decision under appeal be set aside and the patent be maintained in amended form on the basis of auxiliary requests 2 or 3.

IX. At the end of the oral proceedings the board announced the decision.

X. The following documents are referred to hereinafter:

D2: Rappuoli, R., "New and improved vaccines against diphtheria and tetanus" (chapter 17); in: Woodrow, G.C. and Levine, M.M. (eds), "New generation vaccines", 1990, pages 251-268

D6: GB-A-969772

D9: US-A-4996299

D10: Pappenheimer, Jr., A.M., "Diphtheria"; in: Germanier, R. (ed), "Bacterial vaccines", 1984, pages 1 to 36

D14: World Health Organisation, WHO Technical Report Series 800, 1990, pages 87-179, "Requirements for diphtheria, tetanus, pertussis and combined vaccines"

D22: Linggood, F.V. et al., British Journal of Experimental Pathology, 1962, vol. XLIV, No. 2, pages 177-188

D23: Affidavit of Prof. C. Montecucco dated 21 March 2005

D25: Relyveld, E.H. and Ben-Efraim, S., Methods in Enzymology, vol. 93, 1983, pages 24-60, "Preparation of vaccines by the action of glutaraldehyde on toxins, bacteria, viruses, allergens, and cells"

XI. The appellant's arguments, as far as they are relevant for the present decision, may be summarised as follows:

Main request

Inventive step

Detoxification of pre-purified tetanus toxin was known, for example, from document D2 and also its detoxification with formaldehyde, being qualified in document D2 as the "conventional" detoxification treatment.

The problem underlying the patent was to provide a method for generating stable tetanus toxoid vaccine preparations.

This problem was not solved by all process variants included in claim 1. Firstly, depending on the degree of purity of the starting material, for some of the variants the problem did not arise. Secondly, due to the broad parameter ranges by which process conditions were characterised, it was inherently unlikely that all embodiments resulted in stable tetanus toxoid preparations. Therefore, in view of decision T 939/92, since the problem as formulated was not solved by substantially all embodiments, it could not be taken into account for the assessment of inventive step.

Moreover, the patent did not make it plausible that the problem was solved, because the only example did not fall under the claim. Following decision T 1329/04 this was a reason for denying inventive step.

It was known from document D14 that tetanus vaccines made from pre-purified toxin and toxoided with formaldehyde were unstable.

Lysine had successfully been used to prevent reversion of numerous other toxoids generated from purified toxins, for example diphtheria and pertussis, see documents D2, D6, D9, D10, D14 and D22. Therefore it was obvious to use this compound also for the stabilisation of tetanus toxoid preparations.

The remaining features of claim 1 were routinely used by the skilled person in formaldehyde-based detoxification methods, see documents D2 and D22.

Auxiliary request 2

Admission

In the written submissions the respondent had stated that it could not identify auxiliary requests other than the one then submitted. It would therefore come as a surprise and be consequently unfair to admit a new auxiliary request which is different from the one on file.

Inventive step

Pre-incubation with glutaraldehyde as a detoxifying agent for toxoiding tetanus toxin in combination with the later addition of lysine was disclosed in Table V of document D25. Therefore, the new feature in claim 1 belonged to the common general knowledge of the skilled person and would therefore be routinely applied by him/her. Consequently, the subject-matter of claim 1 of this request did not involve an inventive step either.

Sufficiency of disclosure

The example in the patent was not performed according to the conditions specified in the claim, because the Lf content of the starting material was not 250 Lf/ml as required by the claimed process but 200 Lf/ml. Therefore, the disclosure of the invention in the patent was insufficient.

XII. The respondent's arguments, as far as they are relevant for the present decision, may be summarised as follows:

Main request

Inventive step

The formaldehyde-based method disclosed in document D2 was the closest prior art.

The problem underlying the patent was the provision of an alternative or improved method for producing tetanus toxoid suitable for inclusion in a tetanus vaccine.

The burden was with the appellant to show that the claimed subject-matter was not a solution to the problem.

The problem of reversion of tetanus vaccines made from pre-purified toxin and toxoided with formaldehyde was neither derivable from document D14 nor from document D16. In particular, the statement in document D14 in paragraph A.3.3.4 on page 115 that care must be taken to avoid reversion was a standard safety warning of the World Health Organisation (WHO) and would not have been taken seriously by the skilled person without support from scientific evidence.

Due to structural differences between the different toxins, a skilled person would not have used lysine for stabilising tetanus toxoid, even though it had been used for this purpose for diphtheria or pertussis toxoids.

Even if the skilled person had identified lysine as a possible stabilising agent, he/she would not have used

it, because there was no reasonable expectation of success in the context of tetanus toxin detoxification.

Auxiliary request 2

Admission

The request did not raise formal or new substantive issues. It should therefore be allowed into the proceedings.

Rule 57a EPC

The request was filed as an answer to the board's conclusion that the subject-matter of the main request lacked an inventive step.

Inventive step

It was taught on page 31 of document D25 that lysine inhibited the detoxification of tetanus toxin by glutaraldehyde. Therefore, the disclosure in document D25 rather gave the skilled person the suggestion to avoid lysine. Consequently, there was no reason for him/her to even consider pre-incubation with the detoxifying agent before addition of the allegedly stabilising agent.

Sufficiency of disclosure

An example was not required in order to fulfil the requirements of Article 83 EPC.

Reasons for the Decision

Admissibility of the appeal

1. The opposition was filed in the name of "Aventis Pasteur Limited". In view of a document submitted in a different case before it (see above section VI), the board became aware of the fact that this name was changed on 16 December 2004, the new name being "Sanofi Pasteur Limited". The opponent's notice of appeal was filed with a letter dated 13 January 2005 in the name of "Aventis Pasteur Limited", i.e. the former company name which, however, was no longer the correct name at the time of filing the appeal.

Hence, when the appeal was filed it did not comply with the provisions of Rule 64(a) EPC in combination with Rule 26(2)(c) EPC, because the name indicated in the notice of appeal was not the correct name of the appealing company.

- 1.1 Upon invitation by the present board at the oral proceedings, pursuant to Rule 65(2) EPC, the appellant has requested the correction of the name.

The correction of an appellant's name is allowable if it transpires from the facts that a mistake has been made and if the correction does not reflect a change of mind as to who the appellant should be (see for example decision T 97/98, OJ EPO 2002, 183).

- 1.2 Since it is obvious in the present case that the old name of the same legal person was erroneously used when

the appeal was filed, the board accepts the requested correction of the name.

Admission into the proceedings of documents D11 to D26

2. Documents D11 to D14 were filed shortly before the oral proceedings held during opposition proceedings. The opposition division, in exercising its discretion in accordance with Article 114(2) EPC, decided not to admit these documents. With regard to document D14 the opposition division criticised that it was not a complete document. With the statement of grounds of appeal, the appellant filed documents D11 to D13 again, together with a complete copy of document D14.
3. Article 114(2) EPC allows the board to decide on the admission of documents filed during appeal proceedings. Therefore, the board does not deem it necessary to consider whether or not the opposition division has correctly exercised its discretion under Article 114(2) EPC not to admit documents D11 to D14 to the opposition proceedings.
4. The board considers documents D11 to D14 relevant for finding a decision since their disclosure allows the board to get a more complete picture of the field of toxoids used as vaccines before the priority date of the patent. Documents D15 to D24, filed with the statement of grounds of appeal, and documents D25 and D26, filed with the respondent's response to that statement, are considered relevant for the same reason. Moreover, all the documents were filed at the earliest point in time during the appeal proceedings. None of the parties objected to their admission. Hence the

board has decided to admit documents D11 to D26 into the proceedings.

Main request

5. The present main request corresponds to the sole request before the opposition division. The only relevant issues with regard to it are that of inventive step and sufficiency of disclosure.

Inventive step

6. To assess inventive step, this board, in line with the normal practice of the boards of appeal of the European Patent Office, will apply the "problem and solution approach". This involves as a first step identifying the closest prior art.

The closest prior art

7. The closest prior art relates to subject-matter from which the claimed invention could most easily be made by the skilled person and thus provides the strongest basis for a challenge of obviousness. According to the case law this requirement is fulfilled by prior art disclosing subject-matter conceived for the same objective as the claimed invention (Case Law of the Boards of Appeal of the European Patent Office, 5th edition 2006, I.D.3.1).
8. The objective of the present invention is to generate tetanus toxoid preparations from pre-purified tetanus toxin that are stable during storage or, in other words, that do not revert to toxicity so that they can be used

as vaccines without any danger of intoxication (see for example paragraphs [0001], [0004] and [0005] of the patent).

9. Document D2 was considered by both parties as the closest prior art document. This document is chapter 17 of a book published in 1990 and discloses inter alia processes for the preparation of tetanus and diphtheria vaccines. In part VI of it (starting on page 259) entitled "Detoxification of purified diphtheria and tetanus toxins" it is stated:

"Several methods have been described for the purification of diphtheria and tetanus toxins: [...]. The partially purified or highly purified toxin preparations can then be detoxified by the conventional formaldehyde treatment."

On page 260 it is then stated:

"Early studies carried out with purified diphtheria toxin showed that the toxoids prepared from highly purified toxin became toxic after dilution and removal of excess formaldehyde [8,49-51]. Later on it was shown that if lysine, 0,0025 M, was included in the detoxification solution, the toxoids obtained were stable and immunogenic [8,50,52]."

And finally it is stated on the same page:

"An alternative way to formaldehyde detoxification has been developed by Relyveld using glutaraldehyde. Diphtheria and tetanus toxins can be completely and irreversibly inactivated by treatment for a short time

with 0.025 M or 0.0025 M glutaraldehyde, respectively. Lysine is still necessary for the irreversible detoxification of diphtheria, but not for tetanus, toxins."

- 9.1 Thus, document D2 discloses that tetanus vaccines can be prepared by detoxifying purified tetanus toxin with either formaldehyde or glutaraldehyde. As regards stability, it is explicitly stated that the glutaraldehyde-based process results in stable preparations. No explicit comment is given anywhere in document D2 about the stability or instability of tetanus vaccine preparations obtained from purified tetanus toxins after detoxification with formaldehyde. However, given the disclosure in document D2 of the instability of diphtheria vaccines made from purified diphtheria toxin with formaldehyde, the board considers the disclosure of document D2 to implicitly convey that there is a risk of instability of tetanus toxoids generated from purified tetanus toxins by formaldehyde detoxification.
- 9.2 Therefore, since the objective of the patent is the provision of a **stable** tetanus vaccine (see point 8 above), the board concludes that the glutaraldehyde-based detoxification process disclosed in document D2 is closest to the claimed invention.
10. The problem arising for the skilled person in view of the closest prior art is the provision of an alternative process for the preparation of stable tetanus toxoids from purified tetanus toxin.

11. According to the appellant there are two reasons why claim 1 must be regarded as involving subject-matter which does not solve this problem and the problem cannot therefore be taken as a basis for the assessment of inventive step.

11.1 The appellant submits that it is known, for example from document D2 (page 256; see also paragraph 12.1 below), that impurities present in the medium of the toxin preparation add to a stabilising effect during detoxification with formaldehyde. Therefore, the stability of tetanus toxoid preparations generated by the formaldehyde-based tetanus toxin detoxification method (as for example disclosed in document D2 on page 256) depended on the purity of the tetanus toxin used as a starting product. Given that the specific activity of the starting product according to claim 1 may be as low as 2000 Lf/mg PN (corresponding to a purity of about 70%, see paragraph [0009] of the patent), it necessarily follows that the claim encompasses process variants which would result in stable preparations even without the addition of lysine. The above formulated problem does not apply to those variants.

However, the board has no evidence at its disposal that tetanus toxoid preparations made from tetanus toxin purified to the lower purity levels encompassed by the claim would indeed be stable without the addition of lysine. For that reason alone, the board is not convinced by the appellant's argument.

11.2 Moreover, the appellant referred to decision T 939/92 (OJ EPO 1996, 309) where the board held that a problem

can only be considered as solved and therefore be taken into account for assessing inventive step if it is solved by substantially all the subject-matter of a claim. The appellant submits that, in the present case, due to the definition of process parameters by broad ranges of values, it is inherently unlikely that all possible process variations falling under the terms of claim 1 result in stable tetanus toxoid preparations. For example, if tetanus toxin is incubated at pH 6.0 and a temperature of 45°C for several weeks, then a large volume of precipitate is formed which would hamper further processing of the preparation (see appellant's submission of 14 March 2005).

11.3 The board considers that a claim to a process defining different process parameters by broad ranges of values can formally encompass process variants which may not achieve the desired result if the extreme values of ranges are combined, as in the appellant's example. However, in the board's view, the skilled person would be aware that such combinations of process conditions might not generate the desired result and would not apply them. Therefore, an argument to the effect that a claim directed to a process encompasses variants not solving the problem, where that argument relies solely on rather unrealistic combinations of process conditions, does not convince the board.

12. The appellant also referred to decision T 1329/04 of 28 June 2005 in which the board held in point 12 of the reasons that the definition of an invention as being a contribution to the art, i.e. as solving a technical problem and not merely putting one forward, required that it is at least made plausible by the disclosure in

the application that its teaching indeed solves the problem it purports to solve. The appellant argues that the patent does not make it plausible that the problem is solved because one of the conditions of the only example disclosing a process generating stable detoxified tetanus toxin preparations lies outside claim 1, namely the toxin concentration of the starting product (paragraph [0029]). It is 200 Lf/ml according to the example, whereas it is 250 Lf/ml or more according to claim 1.

- 12.1 The detoxification of tetanus toxin relies on a reaction of the free amine group of a lysine residue in the toxin molecule with the aldehyde group of formaldehyde, leading to either intra- or intermolecular cross-linking or to a linkage with peptidic impurities in the reaction medium. The final result of this reaction is the formation of a methylene bridge. If a sufficient amount of these bridges have formed, the toxin is no longer toxic and the toxoiding is irreversible, i.e. the preparation is stable (document D2, page 256). Given this underlying chemistry of the detoxification process, the board considers that in fact the effectiveness of the reaction is not dependent on the concentration of tetanus toxin alone but on the relation of concentrations between tetanus toxin, aldehyde and lysine. The example disclosed in paragraph [0029] illustrates these relations. Therefore, in the board's view, although the initial toxin concentration of the example is not within the claimed range, the patent makes it plausible that the problem is solved.

Obviousness

13. The process according to claim 1 and the process identified as the closest prior art (see point 9.2 above) differ in the following features:
- The starting product, purified tetanus toxin, has a specific activity of 2000 Lf/mg PN or more and an Lf content of 250 Lf/ml or more; in document D2 the concentration of the starting product is not disclosed.
 - The starting product is detoxified with formaldehyde in a concentration of 0.2 to 1% (V/V); according to the process of the closest prior art, detoxification is made with glutaraldehyde at a concentration of 0.0025 M.
 - According to claim 1 detoxification takes place in the presence of lysine at a concentration of 0.005 to 0.25 M; lysine is absent in the glutaraldehyde-based detoxification of tetanus toxin according to document D2.
 - The reaction takes place from 24 to 32 days, at a pH from 6.0 to 8.0 and at a temperature of 30 to 45°C. As to the reaction conditions, it is only mentioned in document D2 that the inactivation treatment is carried out for "a short time".
14. For the assessment of whether or not the solution proposed in claim 1 was obvious, it has thus to be examined whether the skilled person starting from the closest prior art process - the detoxification of

purified tetanus toxin by glutaraldehyde - was led in an obvious manner by the prior art on file to solve the problem of providing an alternative process for the preparation of stable tetanus toxoids from purified tetanus toxin by modifying the features of the closest prior art process such as to arrive at the combination of features according to the claimed process.

Thus, the first question to be considered is whether or not it was obvious to use formaldehyde instead of glutaraldehyde as a detoxification agent.

Formaldehyde as a detoxifying agent

15. The formaldehyde-based treatment as an effective method to detoxify tetanus and diphtheria toxins was discovered in the 1920s inter alia by Ramon (document D2, Introduction). The use of glutaraldehyde for the detoxification of toxins for the preparation of vaccines only started in the early 1970s (document D25, the paragraph bridging pages 24 and 25). However, at the priority date of the patent, the use of formaldehyde was still more widespread. It is stated in the introduction of document D2, published in 1990, i.e. approximately two years before the priority date of the patent:

"Diphtheria and tetanus vaccines are presently still prepared using the method described by Ramon."

This is confirmed by a statement in document D14, also published in 1990, on page 111:

"The most common method of preparing toxoids from toxins is by means of formaldehyde."

Thus, the board concludes that, at the priority date of the patent, formaldehyde was the means of first choice for toxoiding bacterial toxins for vaccine preparation.

16. It is an issue, however, whether or not the skilled person would have considered tetanus vaccine preparations made from purified tetanus toxins with formaldehyde to be stable upon storage. The respondent maintains that the skilled person would not be aware of an instability of such preparations and that, therefore, the addition of lysine as a stabilising agent could not be obvious. In particular, in the absence of any supporting scientific evidence on the real existence of such a danger for reversion, the skilled person would not have taken seriously the advice in document D14 on page 115 that "particular care must be taken to avoid a reversion to toxin" if detoxification starts from purified tetanus toxin, but would have regarded it as a standard warning passage which the WHO included in relation to all toxin-derived vaccines, as could be seen from the equivalent passage in relation to diphtheria toxin.

- 16.1 However, document D14 is a publication of the WHO describing "Requirements for diphtheria, tetanus pertussis and combined vaccines". It is not imaginable for the board that the skilled person would ignore an advice, even if it is made in the absence of scientific support, given by a renowned organisation such as the WHO in the context of such a serious issue as the preparation of vaccines made from toxins. This is

especially so since tetanus toxin is the second most poisonous substance known (Affidavit of Prof. Montecucco dated 21 March 2005 = document D23, point 11). Moreover, as already noted above in point 9.1, the risk of instability in tetanus vaccine preparations made from purified tetanus toxins with formaldehyde transpires also from the disclosure of document D2.

Lysine as a stabilising agent

17. The next question arising with regard to the evaluation of the obviousness of the claimed process is whether or not the skilled person would have considered lysine as a means of stabilisation.

17.1 Documents D2, D6, D9, D10, D14 and D22 relate to the subject of the stability of diphtheria and pertussis vaccine preparations generated by formaldehyde treatment. Thus, none of the above-mentioned documents is explicitly concerned with the stabilisation of tetanus toxoids. Therefore, the respondent maintains that, given size and sequence differences between tetanus toxin and the other bacterial toxins, the skilled person would not have considered applying the teaching of any of these documents.

17.2 The board disagrees. The reaction mechanism underlying the process of detoxification with formaldehyde is explained on page 256 of document D2. It involves a cross-linkage between the ϵ -amino groups of lysine residues in the toxin, resulting in a) internal cross-linking within one toxin molecule, b) cross-linking of different toxin molecules or c) cross-linking between

toxin molecules and peptide molecules present in the medium. This basic reaction is common to all formaldehyde-based detoxification processes, which is also accepted by the respondent (page 8 of the submission dated 5 December 2005). Therefore, in the board's view, the skilled person aiming at providing an alternative process for the preparation of stable tetanus toxoids would not see size and sequence differences as a reason deterring him/her from considering prior art dealing with the detoxification of toxins other than tetanus toxin.

- 17.3 Document D2 discloses on page 260 that lysine has been successfully used to stabilise diphtheria toxoids prepared from purified toxin (see point 9.1 above). Also, document D10 on page 18 and document D14 on page 98 disclose that lysine is added during detoxification as a means of preventing reversion of diphtheria toxoids. In document D22 the effects of various amino acids during the detoxification of purified diphtheria toxin with formaldehyde on a number of properties of the toxoids produced, inter alia their stability, are compared. When discussing the results the authors come to the conclusion that "[o]f all the amino acids tested, lysine is the only one which gives a product having all the required desiderata of high antigenicity and stability, freedom from reversal and good adsorption on to aluminium phosphate." (page 187, paragraph 4 of the Discussion). In the worked examples of document D6 purified diphtheria toxin is toxoided in the presence of formaldehyde and: lysine (example 1), lysine and alanine (examples 2 and 3) and ethylene-diamine (examples 4 and 5). No explicit stability measurements are reported. It is mentioned however on

page 3 that the lysine-treated toxoid of example 1 has been incorporated in a diphtheria vaccine. Finally, document D9 discloses a method for preparing stable pertussis toxoids by a treatment of the toxin with formaldehyde in the presence of lysine or glycine in combination with N-acetyltryptophane, where the combination with lysine had the best effect (column 4).

Thus, documents D2, D10 and D14 disclose lysine as the sole stabilising agent. Documents D6, D9 and D22 disclose lysine as a stabiliser among others or in combination with others, but lysine is either explicitly (D22) or implicitly (D6 and D9) favoured.

17.4 Hence, in view of these disclosures, the board concludes that, at the priority date of the patent, lysine was one of the known stabilising agents, if not the preferred agent, in formaldehyde-based detoxification processes of diphtheria and pertussis toxoids. Therefore, in the board's view, the skilled person would also have envisaged it for stabilising tetanus toxoid preparations made from purified tetanus toxin.

18. The respondent submitted that even if the skilled person had identified lysine as a possible candidate for stabilisation of such tetanus toxoids, it was not obvious to use it because there was a lack of reasonable expectation of success in the context of tetanus toxin detoxification.

18.1 However, in the board's view, consideration of the expectation of success is not appropriate in the present case for assessing obviousness. This approach

has been developed in the field of genetic engineering to take account of the fact that in this field one may easily conceive of inventions to be made by genetic engineering, yet realising them may cause problems in view of difficulties known or experienced when starting the project. Thus, the evaluation of the "reasonable expectation of success" involves an analysis of the prior art with the aim of determining the degree of confidence conveyed by it to the skilled person that an envisaged result, which has never before been achieved, will be obtained.

According to the case law, a skilled person who, in view of the teaching in the prior art, has already clearly envisaged a group of compounds or a compound and then determines by routine tests whether such compound or compounds have the desired effect, is in a try and see situation (see e.g. decision T 1599/06 of 13 September 2007, point 20.2 of the reasons).

- 18.2 In the present case lysine had already been identified in the prior art to prevent diphtheria and pertussis vaccine preparations from reversion (see point 17.3 above). For the determination of whether it has this same activity in the context of the detoxification of tetanus toxin, it is enough to perform well-known tests. It is true that the outcome of such tests is not clearly predictable. However, this would not have deterred the skilled person from performing them in the light of the activity reported in the prior art (in this context see decision T 149/93 of 23 March 1995, point 5.2 of the reasons).

19. In summary, the board concludes that the skilled person would have chosen lysine as stabilising agent in an obvious way when aiming at detoxifying purified tetanus toxin.

Other features of claim 1

Specific activity of toxin

20. In document D2, in the part relating to detoxification of purified diphtheria and tetanus toxins it is stated (page 259): "Several methods have been described for the purification of diphtheria and tetanus toxins: [...]. With these methods, diphtheria and tetanus toxins can be easily obtained at a purity ranging from 85% to 95%. The partially purified or highly purified toxin preparations can then be detoxified by the conventional formaldehyde treatment."

According to paragraph [0009] of the patent a specific activity of 2000 Lf/mg is equivalent to a purity of about 70%. The starting material according to the process of claim 1 has a specific activity of 2000 Lf/mg or more.

Toxin concentration

21. Document D22 discloses detoxification of purified tetanus toxin with formaldehyde at a starting concentration of 500 Lf/ml (page 177, last paragraph). According to claim 1 of the patent an initial concentration of 250 Lf/ml or more of purified tetanus toxin is used.

Formaldehyde and lysine concentration, pH, temperature, duration

22. Document D2 teaches the detoxification of the purified toxin preparation by the conventional formaldehyde treatment (see quotation in point 20 above). A "conventional formaldehyde treatment" is disclosed in document D2 in part V entitled "Preparation of conventional diphtheria and tetanus vaccines". The detoxification reaction is carried out by adding formaldehyde to a final concentration of 0,5%, at a pH of 7.6, at 37°C for four weeks. According to claim 1, purified tetanus toxin is incubated with formaldehyde at a concentration of 0.2 to 1%, at a pH of 6.0 to 8.0, at 30 to 45°C, for 24 to 32 days.

Furthermore, document D2 discloses on page 260 a concentration of lysine of 0,025 M in a detoxification process of purified diphtheria toxin. According to claim 1, the concentration is between 0.005 and 0.25 M.

23. The board thus concludes that the further process conditions recited in claim 1, i.e. specific activity and concentration of toxin, formaldehyde and lysine concentration, pH, temperature and duration of the reaction, encompass conditions which would be ordinarily applied by the skilled person.
24. Hence, all features of claim 1 encompass obvious modifications for a skilled person in view of the problem to be solved. Therefore, the subject-matter of claim 1 of the patent as granted does not involve an inventive step.

Auxiliary request 2

Admission of the request

25. This request was filed at the oral proceedings after the board had announced its decision that the main request lacks an inventive step (see above section VII). Article 10b(3) of the Rules of Procedure of the Boards of Appeal (RPBA) stipulates that amendments sought to be made after oral proceedings have been arranged may not be admitted if they raise issues which the board or the other party or parties cannot reasonably be expected to deal with without adjournment. The subject-matter of present claim 1 corresponds to that of claim 5 of the main request. Therefore, it was at stake when considering the patentability of the main request. Moreover, the amendment does not raise formal issues (see below). Consequently, the board considered that the new request could be dealt with properly by the appellant and the board at the oral proceedings. Therefore, in accordance with Article 10b(1) RPBA, the board has decided to admit the request.

Rule 57a EPC

26. The amendment has been made in order to overcome the objection of lack of inventive step against claim 1 of the main request, i.e. of claim 1 as granted. Hence, the requirements of Rule 57a EPC are fulfilled.

Articles 84 and 123(2)(3) EPC

27. Since Article 84 EPC is not a ground of opposition, and since Article 100(c) EPC has not been invoked as a

ground of opposition in the present case, the claims of the auxiliary request are to be examined for the requirements of Articles 123(2) and 84 EPC only with regard to amendments in relation to the granted claims. The evaluation of the requirements of Article 123(3) EPC is also made by comparison with the granted claims.

27.1 Claim 1 of the auxiliary request is a combination of claims 1 and 5 as granted. In other words, present claim 1 and claim 5 as granted relate to the same subject-matter. Claims 2 to 8 of the auxiliary request correspond to claims 2 to 4 and 6 to 9 as granted. Hence, none of the present claims is open to objections under Article 84 EPC.

27.2 The newly added feature in claim 1 "wherein the purified toxin is preincubated with the formaldehyde in the absence of the lysine for from 20 to 40 minutes at from 35 to 40°C" is taken verbatim from claim 5 as originally filed (which is worded identically to claim 5 as granted; see section I above). The requirements of Article 123(2) EPC are therefore fulfilled.

27.3 Claim 1 results from a combination of two granted claims, i.e. claims 1 and 5, which combination results in the limitation of the scope with regard to claim 1 as granted. The requirements of Article 123(3) EPC are therefore fulfilled.

Inventive step

Closest prior art and problem

28. The board considers that the closest prior art with respect to claim 1 of this request and the underlying problem are the same as those defined with respect to claim 1 of the patent as granted (see points 9.2 and 10 above), i.e. the detoxification of purified tetanus toxin with glutaraldehyde disclosed in document D2 represents the closest prior art and the problem is to be formulated as the provision of an alternative process for the preparation of a stable tetanus toxoid from purified tetanus toxin.
29. The patent makes it plausible that the above-formulated problem is solved for the reasons given in respect of the main request in point 12 above and because the process described in the example of paragraph [0029] of the patent encompasses a pre-incubation step: "Aliquots were toxoided as follows: 1. 0,25% (v/v) formaldehyde in the form of formalin was added to one aliquot and the mixture was preincubated for 30 minutes at 37°C. L-lysine monohydrochloride was subsequently added...".

Obviousness

30. It has already been found above in relation to claim 1 of the patent as granted that the skilled person would have carried out the toxoiding reaction with formaldehyde in the presence of lysine in order to obtain stable tetanus toxoid preparations and that he/she would also ordinarily have applied process conditions encompassed by the remaining features of claim 1.
31. As regards the new feature of **pre-incubation** of tetanus toxin with formaldehyde in the absence of lysine, the

appellant submits that Table V of document D25 disclosed such a process step and that therefore it was an obvious alternative to the known simultaneous presence of lysine and formaldehyde in a process for preparing stable tetanus toxoids.

32. Table V of document D25 reads as follows:

TABLE V
EFFECT OF LYSINE ON THE DETOXICATION PROCESS OF TETANUS TOXIN BY
GLUTARALDEHYDE^a

GA final concentration	Conditions of treatment ^b		Lf/ml ^c	Initial toxicity ^d	Stability
	Time of contact (min)	Lysine addition			
0.00263 M	10	No	200	Not toxic	ND ^e
	90	Yes (procedure A)	370	Toxic	ND
	90	Yes (procedure B)	405	Toxic	ND
0.00131 M	10	Yes (after GA) ^f	280	Not toxic	ND
	10	No	285	Not toxic	No reversal
	90	Yes (procedure A)	400	Toxic	ND
0.00066 M	90	Yes (procedure B)	425	Toxic	ND
	10	Yes (after GA) ^f	280	Not toxic	No reversal
	10	No	340	Toxic	ND
	30	No	320	Not toxic	Toxic
	60	No	320	Not toxic	No reversal
	90	No	320	Not toxic	No reversal
	30	Yes (after GA) ^f	360	Toxic	ND
0.00033 M	60	Yes (after GA) ^f	345	Not toxic	Toxic
	90	Yes (after GA) ^f	330	Not toxic	Toxic
	90	No	380	Toxic	ND

^a Purified tetanus toxin 500 Lf/ml (2500 Lf/mg N) was prepared at the Pasteur Institute; GA 25% stock solution was purchased from Merck-Schuchardt, Germany, and diluted in buffer Na₂HPO₄, 0.07 M.

^b All treatments were performed at 37°; the preparations were dialyzed after addition of lysine. Lysine (L-lysine dichlorohydrate, Prolabo, France) solution was made in buffer Na₂HPO₄, 0.07 M, adjusted by NaOH at pH 8.55, and diluted to a final concentration of 0.01 M. Procedure A: rapid subsequent addition of toxin, GA, and lysine; procedure B: rapid subsequent addition of toxin, lysine, and GA. In cases when 10 min of contact with GA was sufficient for detoxication, same results were obtained after contact for 90 min with GA; in the case of preparations that remained toxic after 90 min of contact with GA, the same results were found when time of contact with GA was reduced to 60, 30, or 10 min, not shown in the table.

^c Lf titers were determined before dialysis only for procedures A and B; the titers were determined after dialysis for the other preparations.

^d Toxicity tests were performed by injecting 0.5 ml of nondiluted GA-treated toxoid i.m. into the leg of Swiss mice (17-20 g weight); the animals were observed for 2 weeks for signs of local paralysis and death.

^e ND, not done.

^f Lysine added after contact of toxin with GA; time of contact with lysine was 15 min for initial toxicity tests and 15 or 60 min for stability tests; no difference in results was observed between contact for 15 min with lysine vs. 60 min contact.

32.1 Thus, in point *f* of the legend to Table V a process where lysine is added only after the toxin was

contacted with the detoxifying agent is indeed disclosed.

33. However, it has to be borne in mind that for the purpose of the interpretation of a document in the context of inventive step, in order to avoid hindsight considerations, the teaching being perceived by a skilled person in view of the problem that he/she wants to solve must be determined by taking into account the whole information content of that document.

- 33.1 An evaluation of document D25 from the perspective of the skilled person looking for a process for the preparation of stable tetanus toxoids reveals, in the board's view, the following:

Table V reports on the effect of lysine on the stability of tetanus toxoids in a detoxification process of tetanus toxin by glutaraldehyde. Inter alia, glutaraldehyde concentrations were varied, i.e. they were 0.00263 M, 0,00131 M, 0,00066 M and 0.00033 M (see column 1), as were the order of the additions and the number of compounds, i.e. procedure A consisted in the rapid subsequent addition of toxin, glutaraldehyde and lysine (see legend to the table, point *b*); procedure B consisted in the rapid subsequent addition of toxin, lysine and glutaraldehyde (see legend to the table point *b*); furthermore, lysine was added after contact of toxin with glutaraldehyde ("preincubation"; see legend to the table, point *f*); and no lysine was added (column 3 of the table "No").

In the last column of the table, data on the stability of the different preparations are given, as far as they have been determined.

The results are as follows: The stability of preparations made at a glutaraldehyde concentration of 0,00263 was not determined (see first four lines of Table V, last column). At a concentration of 0.00131 M glutaraldehyde, stable preparations were obtained when no lysine was added (see line 5 of Table V; last column) and after pre-incubation of the toxin with glutaraldehyde (see line 8 of Table V; last column). The only stable preparations at a concentration of 0,00066 M glutaraldehyde were obtained in the absence of lysine and at a contact time between toxin and detoxifying agent of 60 and 90 minutes, while of the two pre-incubated preparations tested for stability, both reverted to toxicity.

In summary, three of four processes which resulted in stable solutions were carried out in the absence of lysine. The fourth stable preparation was obtained under pre-incubation conditions. However, a stable solution was also obtained with the same amount of glutaraldehyde and with the same time of contact but without pre-incubation.

Therefore, in the board's view, altogether, the results of the table would rather indicate to a skilled person that lysine is not necessary for stabilisation and not that the point in time of the addition of lysine has an influence on stability.

Moreover, in the board's view, the remainder of the document does not contain any information which would change this view. The comment in the text on the results in Table V is as follows (page 31, first full paragraph): "The effect of lysine on detoxication of tetanus toxin by GA (note by the board: "GA" is used as an abbreviation for glutaraldehyde) has been evaluated.²⁶ This was done in view of results showing the stabilizing effect of lysine on diphtheria toxoid.¹⁴ As shown in Table V, **addition of lysine inhibited detoxication of tetanus toxin by GA.**" (emphasis added by the board).

- 33.2 In view of the foregoing, the board thus concludes that, although document D25 discloses a process including pre-incubation of the detoxifying agent with the toxin before the addition of lysine in Table V, this step would not have been regarded as a suitable process step by the skilled person wanting to prepare stable tetanus toxoid preparations.

Hence, document D25 is not considered as rendering obvious the feature in claim 1 "wherein the purified toxin is pre-incubated with the formaldehyde in the absence of lysine for from 20 to 40 minutes at from 35 to 40°C". Since document D25 was the only document cited in this respect by the respondent and since the board is not aware of any other document on file disclosing said feature, it is concluded that the subject-matter of claim 1 as well as the subject-matter of the dependent claims 2 to 8 involves an inventive step.

The requirements of Article 56 EPC are fulfilled.

Sufficiency of disclosure

34. The appellant's argument that the disclosure is insufficient since the relevant example does not describe a process falling under the claim because the initial toxin concentration is 200 Lf/ml and not 250 Lf/ml or more as required by claim 1 (see also point 12 above), does not convince the board. An example is not a necessary prerequisite for the acknowledgement of sufficiency of disclosure, if the invention can be carried out in view of information given in the patent or known to the skilled person from the prior art. In the board's view, present claim 1 itself describes the invention, i.e. a process for the preparation of tetanus toxoid, in such a precise manner that, in the absence of any further relevant evidence, the board considers the requirement of Article 83 EPC already as fulfilled on the basis of the disclosure in the claim alone.

Therefore, the requirements of Article 83 EPC are considered as fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent in amended form on the basis of claims 1 to 8 of auxiliary request 2, filed at the oral proceedings, and a description still to be adapted.

The Registrar:

The Chairman:

U. Bultmann

R. Moufang