COMMERCIAL IN CONFIDENCE	NUMBER: PL/0116/0011
APPLICATION FOR A VARIATION TO A PRODUCT LICENCE	PRODUCT NAME: HEMOFIL
PROPOSED CERTIFICATE/LICENCE HOLDER: Travenol Laboratories Ltd. Caxton Way THETFORD Norfolk, IP24 3SE	THERAPEUTIC CLASSIFICATION: BLOOD PRODUCT
	RECEIVED: May, 1983
	MEETING: September, 1983
MANUFACTURER OF DOSAGE FORM: Hyland Laboratories 3300 Hyland Avenue Costa Mesa California 92626, USA	COMMITTEE ON SAFETY OF MEDICINES
	SUB-COMMITTEE ON BIOLOGICALS
	CONSIDERATION BY OTHER COMMITTEES: -
LEGAL STATUS: POM	
SALE/SUPPLY: POM	ASSESSED BY: Dr. K. Fowler Dr. J. Purves

APPLICATION TO VARY AN EXISTING LICENCE

1. BACKGROUND

An application to vary the above Product Licence has been received from Travenol Laboratories Ltd.,

This application refers primarily to a change to the manufacturing process to incorporate a heat treatment step in the final stages of manufacture. In addition to this, a request has been made to delete dextrose from the formulation. In support of these changes, detials have been included of the work which has been performed on the product. This includes stability testing, product characterisation, animal safety studies and human safety and efficacy studies.

The company claims that the results from these tests demonstrate the equivalence of the heat treated product with that currently available.

2. DETAILS OF PROPOSED CHANGES TO THE LICENCE

Full details of the changes to the licence are highlighted at APPENDIX 1 and include,

2.1 inclusion of a heat treatment step (60°C for 72 hours) of the lyophilised product;

2.2 deletion of dextrose from the formulation, and

2.3 alteration of the period of time permitted for storage at ≯ 25°C.

3. SUPPORTIVE DATA FOR THE PROPOSED CHANGES

Three studies have been undertaken to support the proposed changes. These include,

3.1 A Characterisation Overview of the AHF - Treated Product;
(for full details see APPENDIX 2)

3.2 Stability Study
(for full details see APPENDIX 3)

3.3 In-vivo Studies
(for full details see APPENDIX 4)

. A CHARACTERISATION OVERVIEW OF THE AHF-TREATED PRODUCT (See APPENDIX 2)

The first of the studies cited at 3.1 relates to three topics, namely,

"4.1 To evaluate the biological activity, biochemical and immunological effect of heat treatment on antihemophilic factor (human) method IV (AHF) in the dried state in final container (sealed vials) when immersed and equilibrated in a water bath at 60°C for 72 hours.

4.2 To determine the effect on sterility, pyrogenicity, general safety, general product release parameters and product characterisation tests when antihemophilic factor (human) method IV (AHF) was heat treated as in study one.

4.3 The third study was conducted for the purpose of determining the effect of heating at 60°C on antihemophilic factor (human) method IV (AHF) for periods of time up to 28 days. It was projected that by extending the heating cycle, those effects, if any, seen after 3 days of heating would be magnified, in effect, by the longer heating cycle (28 days)."

Following the heat treatment mentioned in 4.1 Antihaemophilic Factor (Human) was examined for biological activity, biochemical and immunological properties in comparison with untreated control material. The range of tests utilised for this comparison were as follows,

1. One-stage factor VIII (AHF) assay for biological activity
2. Fibrinogen
3. Fibrin split products
4. Solubility
5. Globulins - IgG, IgA, IgM
6. Factor VIII coagulant antigen
7. Factor VIII related antigen
8. Gel electrophoresis
9. pH
10. Albumin
11. IgG by gel filtration

Results have been given in tabular form (see Table VI) of the Factor VIII coagulant activity, Factor VIII related antigen activity and Factor VIII coagulant antigen activity of the treated lots. It has been stated that these results are comparable to control lots but no data for control lots have been given.

Information on fibrinogen and fibrinogen split product concentrations of treated lots has been presented. Again no data have been given for control lots.

Statements have been made of how the heat treated material behaves relative to the unheated material with respect to solubility, globulins, gel electrophoresis, pH, albumin and gel filtration. Again no data are given of the results obtained with control material and more importantly, no evidence has been presented of the results obtained.

COMMENT : The data presented are inadequate and do not show, as the Company claims, that "heat treatment process results in little significant change in biological activity and no adverse effect on biological and immunological characteristics."

The second study involved the examination of both a heat treated batch and an unheated batch of Antihaemophilic Factor (Human). The criteria used for this was essentially that used in routine product release. It included tests on sterility, pyrogenicity, solubility, pH, potency, general safety as well as product characterisation tests for Factor VIII coagulant antigen, fibrinogen content, fibrin split product content and gamma globulin content.

The Company has claimed that the heat treatment (60°C for 72 hours) of the product in the dry state "was shown to have little adverse effect on the lots tested as all five lots passed product release criteria." This of course assumes that the release criteria for unheated product is adequate for heated product. Results for the five heat treated lots have been presented at Table IX, however, again no comparative results from an unheated control have been presented.

COMMENT : Information presented in support of the Company's claim might be considered inadequate in view of the paucity of comparative evidence from test and control batches.

The third study was set up to determine the effect of heating Antihaemophilic Factor (Human) in a water bath at 60°C but this time for periods up to 28 days.

The Company states that heating the product for this period of time resulted in a "linear progressive decline of Factor VIII biological coagulant activity." Heating the product for the shorter period of time "showed less than a 20% Factor VIII coagulant activity change in direct comparison to its control." It is interesting to note that no information on this loss of activity is evident from the previous results because of incomplete data presented by the Company. However, in mitigation reference is made in this section to the loss of activity of product subjected to the studies cited in 4.1 and 4.2.

It is stated that no change in Factor VIII related antigen content was observed, in the two batches examined, after three days heating but a significant difference had occurred by the end of the 28 day test period. The Factor VIII coagulant antigen was found to be stable over the test period and fibronectin content remained within 90% of the initial values. Over the test period there was a decrease in the fibrinogen content with a consequential elevation of the fibrin split product content. Moisture content increased over the test period to $> 2.0\%$. Gel filtration studies over the 28 day period of heat treatment showed the native monomeric form is maintained for periods up to 21 days but that after 28 days aggregation of the gamma globulin was evident.

COMMENT : In the absence of evidence in the form of comparative data from heat treated product and unheated product, the Sub-Committee may consider the statements made inadequate.

ONCLUSION

The Company maintains that from these three studies it may be concluded that heat treated Hemofil remains essentially unaltered by heat treatment for periods of time up to three days.

As indicated in each of the comments above, there is a lack of evidence, in the form of comparative experimental data, from control and test batches that the heat treatment does not significantly effect the product.

5. STABILITY STUDY (see APPENDIX 3)

Full details of the stability data are given at the APPENDIX.

Essentially, the stability of the product has been evaluated using the following criteria, namely, Factor VIII potency, pH, physical appearance, moisture, solubility and heparin. Three formulations have been examined,

i. Antihaemophilic Factor (Human) containing 3% w/v dextrose (the currently marketed product Hemophil)

ii. Antihaemophilic Factor (Human) without dextrose

iii. Antihaemophilic Factor (Human) heat treated at 60°C for 72 hours.

The predicted (using the Arrhenius model) and observed rate of loss of activity for three lots of product - two with dextrose and one without dextrose - are summarised at Table I of APPENDIX 3. It is clear from these results that both the predicted and observed times for lots reaching 90% of initial potency are beyond the 24 months dating period presently being requested for AHF-treated.

Table II shows the assay results obtained using two batches of AHF which ha been heat treated (60°C for 72 hours) and two batches which have not been heat treated. Details of results obtained from another batch of non-heated material, for which a great deal of stability data is available, has also been presented.

Based on the above data and that presented at Tables III to VI, the Company has concluded that the potency of Antihaemophilic Factor (Human), heat treated, will remain above 90% of initial potency for at least two years when stored at 5°C. This shelf-life would include a period of storage at $\not>$ 25°C for one month. It would appear that the data do support this claim.

PHARMACEUTICAL COMMENT

The variation as requested by the Company has sought,

i. the deletion of dextrose from the formulation.

ii. the inclusion of an additional step which involves the heat treatment - 60°C for 72 hours - of the freeze-dried product, and,

iii. a reduction in the time period during which the finished product may be stored at room temperature.

It is noteworthy that,

iv. no positive information has been given on why the dextrose has been omitted and how this may ffect the finished product.

v. no reasons have been given for the inclusion of the heat-treatment step. This must raise questions about the justification of such a treatment in view of the fact that up to 20% of the activity of the product may be lost by virtue of its inclusion. This could lead to the product containing up to 20% of degradation products.

[NOTE : If it has been included to reduce the chance of transmission of viral infections, one would have expected to see some work done with the product spiked with known viruses.

Anecdotal information indicates this step has been included to minimise the chance of transmission of A.I.D.S. : but, this assumes A.I.D.S. is a viral mediated infection. There is no evidence to confirm this.]

vi. no reason has been given on why storage at room temperature should be reduced from ≯6 months to ≯1 month. In light of the stability data presented this might be considered surprising. However, there would appear to be no objection to this change.

Supportive data presented for this variation has already been considered and is addressed primarily to,

vii. Characterisation Overview of the AHF-Treated Product

viii. Stability Data, and,

ix. In-vivo Studies.

Although the Company's contention is that the "heat treatment process results in little significant change in biological activity and no adverse effect on biological and immunological characteristics", this claim is based on a series of statements without adequate supportive evidence in the form of suitable comparative studies and on an assumption that the release criteria for unheated product is satisfactory for heat-treated product. As a consequence the supportive data at APPENDIX 2 is considered inadequate.

Information presented on the stability of the heat-treated product would appear to justify the claimed shelf-life.

Comment on the 'In-vivo Studies' will be presented in the Medical Section of this report.

With reference to the proposed method of heat treatment, the Committee members may wish to consider the suitability of the type of method chosen by the Company, which involves heating the product ($60^{\circ}C/72$ hours) in the dry state. It has been established that micro-organisms can be protected from damage by heat, and other agents, by virtue of two factors, the dry state, and, the protective nature of protein envelopes. An example of such protection is seen with bacterial spores. It is also known that the efficacy of kill of sterilization procedures is less when a dry heat method is used as opposed to a wet method (eg $150^{\circ}C/1$ hour, dry heat against $121^{\circ}C/15$ minutes wet heat). In view of the possibility of viruses being afforded similar protection in this type of product, that is a lyophilized product which contains protein, should the Company not be asked to justify the method and the criteria of treatment chosen. Could the virus be susceptible to heat when on the surface of the product, but, protected when surrounded by protein?

The data above and that presented at APPENDICES 1 to 4 were presented by the Company in support of the request to vary the licence. However, subsequent to this application additional information has been received, totally independent of the application, which reflects fairly clearly the way in which Travenol would appear to be intending to promote this product, if the variation is granted (see APPENDICES 5 and 6). Has it been an inadvertent omission that an updated data sheet has not been included with the request to vary the licence?

In conclusion, the Sub-Committee may consider that insufficient information has been presented to permit the grant of this variation. Before it could be considered further, satisfactory additional information should be presented on

1. how the deletion of dextrose effects the product.

2. the justification for inclusion and choice of the heat treatment step.

3. the suitability of the current release criteria for the heat-treated product, in view of the possibility of the product containing up to 20% degradation products.

4. the characterisation of the heat-treated product, along with adequate supportive evidence.

5. satisfactory information on labels and data sheets that would have to be altered.

MEDICAL COMMENT

The company have applied to vary their product licence for a conventional FVIII concentrate, by the addition to the manufacturing process of a heat treatment step. No reason is given for this, but it may be assumed that they have a reason because the proposed treatment destroys about 20% of the coagulant activity yield. Two letters sent by the company to Regional Transfusion Directors and specialists in haeophilia, together with the reprint from Hospital Infection Control for May 1983, provide the answer. See APPENDICES 5,6 & 7.

This application concentrates on the coagulant properties remaining in the product after heat treatment and skates over the toxic potential of degradation products associated with the loss of 20% of the coagulant activity.

The brief summaries of results obtained from animal and human studies are inadequate for the purposes of assessing this Variation. It will be seen that the reports from in vivo animal studies are conclusions rather than results; there are no data. Although the reports of studies in humans are more forthcoming with regard to data, they are still seriously lacking in experimental and clinical detail.

Numbers are small in all the in vivo studies, both animal and human, so even if a full, detailed description of the work had been provided, it might still have been thought insufficient to support this Variation.

MEDICAL RECOMMENDATION

That this Variation be refused on the grounds that the evidence of safety and efficacy was inadequate.

REMARK

The Committee may feel that on the evidence of the two letters sent by Travenol to Transfusion and Haemophilia specialists, that the company are already promoting heat treated Hemofil as being less likely to transmit viral infection, with particular reference to hepatitis B, non-A non-B hepatitis and possibly AIDS.

JMc/15

APPENDIX 1

Present	Proposed
PRODUCT LICENCE REVIEW FILE FOR HEMOFIL (Antihaemophilic Factor (Human) Method Four RA 191 - SUPPLEMENT Sent February 28, 1978	
PART 1A - PRODUCT PARTICULARS	PART 1A - PRODUCT PARTICULARS
1. Name of product HEMOFIL Antihaemophilic Factor (Human) Method Four	1. Name of product HEMOFIL-T Antihaemophilic Factor (Human) Method Four, Dried Heat Treated
PART II - PHARMACEUTICAL DATA ON THE DOSAGE FORM	PART II - PHARMACEUTICAL DATA ON THE DOSAGE FORM
1. FINISHED PRODUCT	1. FINISHED PRODUCT
1.2.2 Other constituents used in the manufacture	1.2.2 Other constituents used in the manufacture
Heparin Sodium USP Dextrose Anhydrous USP Sodium Chloride USP Glycine USP Sodium Citrate Dihydrate USP Polyethylene Glycol 4000 USP Acetic Acid USP)used for pH Sodium Hydroxide USP)adjustment	Heparin Sodium USP Sodium Chloride USP or Ph Eur Glycine USP or BP Sodium Citrate Dihydrate USP or Ph Eur Polyethylene Glycol USP or Belgian P Acetic Acid USP or BP)used for pH Sodium Hydroxide USP or BP)adjustment
2. MANUFACTURE OF DOSAGE FORM	2. MANUFACTURE OF DOSAGE FORM
2.1 Antihaemophilic Factor (Human)	2.1 Antihaemophilic Factor (Human)
(Page 3 of Review File Supplement)	(Page 3 of Review File Supplement)
The AHF-rich precipitate is then re-dissolved in citrated saline/ dextrose solution (0.02M sodium citrate, 0.12M sodium chloride) to a volume intended to place the AHF activity per ml in the range required by the product specification. The resulting solution is clarified by centrifugation, the activity is adjusted as necessary with the citrated saline/dextrose solution and the pH is adjusted to app. 6.84. The solution is further clarified by filtration prior to sterilisation and filling.	The AHF-rich precipitate is then re-dissolved in citrated saline solution (0.02M sodium citrate, 0.12M sodium chloride) to a volume intended to place the AHF activity per ml in the range required by the product specification. The resulting solution is clarified by centrifugation, the activity is adjusted as necessary with the citrated saline solution and the pH is adjusted to app. 6.84. The solution is further clarified by filtration prior to sterilisation and filling.

Present	Proposed
The final solution is sterilised by filtration, collected into a single, sterile container and thoroughly mixed. Under sterile conditions, the solution is aseptically filled, in one continuous operation, into sterile vials which are then frozen and lyophilised (see flow-chart below).	The final solution is sterilised by filtration, collected into a single, sterilse container and thoroughly mixed. Under sterile conditions, the solution is aseptically filled, in one continuous operation, into sterile vials which are then frozen and lyophilised (see flow-chart below).
When dried, the vials are capped, under vacuum, and placed in refrigerated storage (2° - $8^{\circ}C$) while awaiting completion of the tests as required by the product specification.	After lyophilisation the stoppers are sealed under vacuum, and the vials are removed from the freeze-dryer and capped.
Upon satisfactory completion of the tests, the vials are shipped to the facility in Lessines, Belgium where they are labelled and packed into 'kits' ready for sale.	At the schedule time, the vials are placed in a large stainless steel water bath and heated to $60^{\circ}C \pm 1^{\circ}C$. They are held at that temperature for 72 hours. Following this heating step, samples of the batch are taken for final container testing. Upon completion of final testing, the vials are inspected visually for the presence of container-closure defects of foreign matter. Vials exhibiting such defects are removed from the lot. The Antihaemophilic Factor (Human), Method Four, Treated is then labelled and packaged.
3. QUALITY CONTROL	3. QUALITY CONTROL
3.1.2 Other Constituents	Delete Dextrose Anhydrous USP
Dextrose Anhydrous USP	Delete Test
3.3.1 Test and Limits Applied	
Anhydrous Dextrose Content	
5. STABILITY	5. STABILITY
(Pages 14 and 15 of Review File Supplement)	
5.8 Storage conditions included on the labelling are:	5.8 Storage conditions included on the labelling are:
STORE BETWEEN 2° AND 8° (35° AND $45^{\circ}F$)	STORE BETWEEN 2° AND 8° (35° AND $45^{\circ}F$)
Avoid freezing which might damage diluent bottle.	Avoid freezing, which might damage diluent bottle.
May be stored at room temperature (not to exceed $25^{\circ}C$ or $77^{\circ}F$) for time periods up to 6 months within dating period.	May be stored at room temperature (not to exceed $25^{\circ}C$ or $77^{\circ}F$) for time periods up to 1 months within dating period.

Characterisation Overview of the AHF-Treated Product

In vitro and in vivo biological activity, biochemical and immunological studies have shown antihemophilic factor (human) method IV, treated (AHF treated) to be essentially equivalent to the present product, antihemophilic factor (human) method IV (AHF). The following pages review the biological activity and biochemical and immunological characteristics as well as general safety testing of this proposed new product, antihemophilic factor (human) method IV, treated (AHF treated).

Three separate studies were conducted in the laboratories of Quality Control in Glendale, California and of Coagulation Research in Costa Mesa, California. References to these studies are as follows:

1. To evaluate the biological activity, biochemical and immunological effect of heat treatment on antihemophilic factor (human) method IV (AHF) in the dried state in final container sealed vials when immersed and equilibrated in a water bath at 60°C for 72 hours.

2. To determine the effect on sterility, pyrogenicity, general safety, general product release parameters and product characterisation tests when antihemophilic factor (human) method IV (AHF) was heat treated as in study one.

3. The final third study was conducted for the purpose of determining the effect of heating at 60°C on antihemophilic factor (human) method IV (AHF) for periods of time up to 28 days. It was projected that by extending the heating cycle, those effects, if any, seen after 3 days of heating would be magnified, in effect, by the longer heating cycle (28 days).

Discussions on individual studies listed previously are as follows:

1. In the first study, seven lots of antihemophilic factor (human) method IV (AHF) were heated in the dried state in final container sealed vials. The vials were immersed in a water bath at 60°C for 2 hours for thermal equilibration following which the heat treatment was continued for 3 days. Thermal monitoring of the dried cake in the vials and the water in the surrounding bath was conducted to establish and maintain thermal

equivalency during the heat cycle. This material following heat treatment will subsequently be referred to as antihemophilic factor (human) method IV, treated (AHF treated).

Following termination of the heat cycle, the antihemophilic factor (human) method IV, treated (AHF treated) was characterised for biological activity, biochemical and immunological properties in direct comparison to the same nonheated control material (lots) known as antihemophilic factor (human) method IV (AHF).

The following tests were employed in this evaluation:

1. One-stage factor VIII (AHF) assay for biological activity
2. Fibrinogen
3. Fibrin split products
4. Solubility
5. Globulins - IgG, IgA, IgM
6. Factor VIII coagulant antigen
7. Factor VIII related antigen
8. Gel electrophoresis
9. pH
10. Albumin
11. IgG by gel filtration

Tables VI, VII and VIII display data relative to these determinations on the seven (7) treated lots. The results of the control lots are not shown, however, a review of their comparisons to treated material is given below:

a. Factor VIII coagulant (FVIII:C) activity, Factor VIII related antigen (FVIIIR:Ag) activity and FVIII coagulant antigen (FVIIIC:Ag) activity in the treated lots are comparable to the control lots. The differences are within the acceptable, allowable analytical variance of the assay systems.

b. Fibrinogen concentration in the treated lots is also comparable to the control lots. Also, there was no change in fibrinogen split products.

c. The pH of heat treated material remained constant.

d. Solubility times of heated material were still within the product specification of 10 minutes.

e. Gel electrophoresis migration patterns of the test article were similar to the control.

f. The albumin content of treated material changed less than 20 percent when compared to unheated controls.

g. Immunoglobulin (IgG, IgA, IgM) content showed slight variations in concentration when comparing treated test article to the control. Such variations are within the allowable variance of the assay system.

The molecular structure of gamma globulin present in AHF was also examined via gel filtration. No difference in structure could be demonstrated in test versus control article.

Thus, the study shows that heat treatment process results in little significant change in biological activity and no adverse effect on biological and immunological characteristics.

TABLE VI

F-VIII CHARACTERISTICS IN HEMOFIL® T

SAMPLES	F-VIII:C (u/ml)	FVIIIC:Ag (u/ml)	F-VIIIR:Ag/ (u/ml)	F-VIIIC:Ag/ F-VIII:C	F-VIIIR:Ag/ F-VIII:C
Lot No 1	33	53	96	1.6	2.9
Lot No 2	35	29	87	0.8	2.5
Lot No 3	26	22	104	0.8	4.0
Lot No 4	27	44	95	1.6	3.5
Lot No 5	28	44	79	1.6	2.8
Lot No 6	34	38	180	1.1	5.3
Lot No 7	26	36	106	1.4	4.1
AVERAGE	30	38	107	1.3	3.6

TABLE VII

CO-FRACTIONATED PROTEINS IN HEMOFIL®T

SAMPLES	TOTAL PROTEIN gm/dl	FIBRINOGEN mg/dl	FIBRINOGEN % of T.P.	FSP ug/ml	FIBRONECTIN mg/ml	FIBRONECTIN % of T.P.
Lot No.1	2.0	493	25	80-160	-	-
Lot No. 2	2.0	382	19	80-160	12.2	61
Lot No. 3	1.6	407	25	80-160	9.3	58
Lot No. 4	2.1	477	23	80-160	-	-
Lot No. 5	2.0	490	24	80-160	11.0	55
Lot No. 6	2.3	595	26	80-160	17.1	74
Lot No. 7	2.0	408	20	80-160	12.1	60
AVERAGE	2.0	464	23	80-160	12.3	62

TABLE VIII

IMMUNOGLOBULINS AND ALBUMIN LEVELS IN HEMOFIL® T

	Radial Immunodiffusion (Mg/dl)				
SAMPLE	IgG mg/dl	IgA mg/dl	IgM mg/dl	ALBUMIN mg/dl	IgG AGGREGATION
Lot No 1	120	7.2	30	<8.7	NC
Lot No 2	110	9.5	36	<8.7	NC
Lot No 3	105	3.6	24	<8.7	NC
Lot No 4	100	8.3	34	<8.7	NC
Lot No 5	45	7.4	30	<8.7	NC
Lot No 6	129	8.3	34	<8.7	NC
Lot No 7	129	7.2	34	<8.7	NC
AVERAGE	105	7.4	32	<8.7	NC

NC - No change of IgG molecular size.

2. In the second study, five lots of antihemophilic factor (human) method IV (AHF) were heat treated as in study one at 60°C for 72 hours. This heat treated material, antihemophilic factor (human) method IV, treated (AHF treated), was then tested in direct comparison to the non-heat treated material, antihemophilic factor (human) method IV (AHF), which served as its control.

   The tests employed for this evaluation emphasised routine product release criteria, such as sterility, pyrogenicity, solubility, pH, potency, general safety, as well as product characterisation tests for factor VIII coagulant antigen, fibrinogen content, fibrin split product content, and gamma globulin content.

   Heat treatment of antihemophilic factor (human) method IV in final container dried state was shown to have little adverse effect on the lots tested as all five lots passed product release criteria (see Table IX) as well as product characterisation parameters. This testing was conducted in comparison to its own control lot, antihemophilic factor (human) method IV (AHF).

3. The third study employed heat and time parameters designed to facilitate evaluation of the magnitude in effect this heat process had on two lots of antihemophilic factor (human) method IV, treated (AHF treated). As in studies one and two, antihemophilic factor (human) method IV (AHF) was immersed in a water bath at 60°C but for periods of time up to 28 days.

   Continuance of heating for 28 days showed a linear progressive decline of factor VIII biological coagulant activity. When treated for 3 days, however, both lots showed less than a 20% factor VIII coagulant activity change in direct comparison to its control. This was likewise seen in studies one and two.

   While no change in factor VIII related antigen content was observed in two lots of antihemophilic factor (human) method IV, treated (AHF treated) following three days of heating, however, a significant difference had occurred in these two lots by the end of 28 days in direct comparison to their control, antihemophilic factor (human) method IV AHF lots.

   When examined, the factor VIII coagulant antigen was found to be a very stable component in the antihemophilic factor (human), treated (AHF treated) product when heated at 60°C for periods of time up to 28 days.

The fibronectin content of antihemophilic factor (human) method IV, treated (AHF treated) remained within 90% of the initial values following extension of heating time up to 28 days.

As expected, the extension of heating time resulted in a decrease in fibrinogen content of antihemophilic factor (human) method IV, treated (AHF treated) with a subsequent elevation in fibrin split product content by day 21 and a further increase in content demonstrated by day 28.

These two lots of antihemophilic factor (human method IV, treated (AHF treated) with an initial moisture content of less than 1% maintained a moisture content of less than 2% up to 14 days. Longer heating (28 days) elevated the moisture content above 2%.

These two lots of anithemophilic factor (human) method IV, treated (AHF treted) gamma globulin content, as measured by gel filtration, showed that the native monomeric form is maintained for periods of time up to 21 days when heated at 60°C in the dried state. However, aggregation of the gamma globulin was observed after 28 days of heating.

From the three studies reviewed here, it is concluded that the proposed product, antihemophilic factor (human) method IV, treated (AHF treated), remains essentially unaltered by heat treatment for periods of time up to 3 days. Based on the above testing parameters, the heating process has been found to be reproducible in effect as monitored by in vitro and in vivo (animal) model systems.

TABLE IX

Test Results on Representative Lots

RESULTS OF TESTS PERFORMED ON FINAL CONTAINER VIALS OF 5 LOTS
ANTIHEMOPHILIC FACTOR (HUMAN) MEHTOD FOUR, TREATED (AHF-TREATED)

TEST	2850T142HT	2830T286	2750T001	2750T002	2750T003
1. Fill Volume, ml	30	10	30	30	30
2. Total Protein, gm/vial	0.6	0.2	0.5	0.6	0.6
3. Moisture, %	0.6	1.9	0.9	1.0	0.9
4. Solubility	P	P	P	P	P
5. pH	6.8	6.8	6.9	6.9	6.8
6. Polyethylene Glycol, gm/100 ml	0.06	0.06	0.05	0.05	0.05
7. Glycine, M	0.27	0.26	0.23	0.25	0.23
8. Vacuum	P	P	P	P	P
9. Potency, U/ml	33.0	31.0	25.0	27.0	28.0
10. Hepatitis B Surface Antigen	P	P	P	P	P
11. Heparin, U/ml	0.5	0.2	0.4	0.5	0.7
12. Protein Identity	P	P	P	P	P
13. Sterility	P	P	P	P	P
14. Pyrogen	P	P	P	P	P
15. Specific Activity AHF U/g Protein	1624	1614	1553	1567	1496
16. General Safety	P	P	P	P	P

P = meets specification

Stability data

The active constituent in Antihemophilic Factor (Human), Method Four, Treated, (a lyophilised biological product reconstituted at the time of use) is Factor VIII, which is the most important item to be considered in reviewing the stability of this product. Other test parameters viewed in connection with the product include: pH, Physical Appearance, Moisture, Solubility, and Heparin. These items will be commented upon in a different context than the stability of the Factor VIII, the active ingredient.

In conjunction with this review, the following points are noteworthy for understanding the studies conducted and in following the conclusions drawn. The analytical method for determining Factor VIII potency is a (mechanised) clotting method with a precision of ± 10%. This is due to reagent, day, technician and/or instrument variation. In conjunction with a lack of tight precision, the difficulty of proving stability with the product is compounded by a lack of an absolute and stable standard which can be used with confidence over many discrete test intervals. However, the challenge in proving stability of the product is met, to an extent, by use of -25° C-stored samples of each lot of product on test. The -25°C-held samples are assayed in parallel with the test (stability) samples. Thus, assay variations as indicated above are reduced and differences in assay results between stability samples and samples held at -25°C would be more attributable to potency loss than other causes. It is expected that very little change in Factor VIII potency would occur on samples stored at -25°C, even after many years of storage. All test results on stability samples, then, are expressed as a percent of the -25°C-held sample, assayed in parallel. 1-2

The following terms will be used in this review:

ANTIHEMOPHILIC FACTOR (HUMAN), METHOD FOUR, DEXTROSE (AHF - DEXTROSE).

This is a currently marketing lyophilised product and contains 3% (w/v) dextrose.

ANTIHEMOPHILIC FACTOR (HUMAN), METHOD FOUR, (AHF)

This material is identical to the AHF-Dextrose, without the sugar.

ANTIHEMOPHILIC FACTOR (HUMAN), METHOD FOUR, TREATED (AHF - TREATED)

This material is AHF subjected to 60°C for 72 hours.

Stability data are computer-generated predictions based on the Arrhenius model:[3]

$$\log K = \log A - \frac{Ea}{2.303\ R}\frac{1}{T}$$; where k is the specific reaction rate;

A, the frequency factor; Ea, the energy of activation; R, the gas constant; T, the absolute temperature.

In all calculations, the order of reaction (decomposition) of Factor VIII is assumed (and appears) to be zero, or pseudo zero. Whether this is true or not, zero order calculations for rate losses at each temperature are a "worst case" situation, and thus predictions based on these rate losses would be the most conservative.

Table I summarises the predicted and observed rate losses for three lots of product. Note that the predicted times to a 10% loss, or the times for the lots to reach 90% of initial potency (T90) are well beyond the 24 months dating presently being requested for AHF-Treated. The "agreement" between the predicted T90 and that actually observed on product stored at 5°C (and the 95% confidence limits about the observed T90 on product actually stored at 5°C) is eventually anticipated to be better. The explanation for this is that these lots of product are "only" 60 months old. From the decay rates on product actually stored at 5°C only about a 10% loss is anticipated at 60 months. Because of assay variation, 5°C stored sample of sufficient age to show greater than an actual 10% loss are necessary to obtain a firmer estimate of the decay rate at that temperature, but are not yet available. Each of these lots does, however, exhibit, with 95% confidence, a shelf-life (T90) in excess of 24 months.

Table II details assay data observed at various temperatures on:

1. The lot of AHF for which many years' data are available.
2. Two lots of AHF-Treated.
3. Two control (unheated) lots of AHF.

Based on these comparative data, it is concluded that the potency of AHF-Treated will remain above 90% of initial potency for at least two years when stored at 5°C. This conclusion is based on the following:

1. The rate losses (see Table III) on product subjected to 60 and 47.5°C are nearly identical for the lot of AHF for which many years' stability data are available and which serves as a model for the two AHF-Treated lots and their controls. The rate losses of these lots stored at 35.5 and 25°C are of the same magnitude. Variations from the model in rate losses observed at these temperatures are eventually anticipated to be reduced, demonstrating even better agreement, when the AHF-Treated and control lots are held at these temperatures for longer times and additional data points are obtained.

2. The predicted shelf-life for AHF-Treated and control lots (see Table III) indicates that the product will remain above 90 ± 1% of potency for the requested shelf life of 23 months at 5°C plus one month at room temperature (not to exceed 25°C). This data was obtained on the 30 ml vial size.

   Table IV shows equivalent data obtained on 2 lots of the 10 ml vial size.

3. With respect to other test parameters - pH, Moisture, Physical Appearance, Solubility, Heparin, Safety, Pyrogen, Sterility and Haemolysins - Tables V and VI detail the tests and results. From the data obtained at elevated storage temperature the product can be expected to remain stable for at least 23 months stored at 5°C, plus one month at Room Temperature.

[1]Kirkwood, TBL, Predicting and Stability of Biological Standards and Products, Biometrics 33, 736-742, 1977.

[2]Barrowcliffe, TW and Kirkwood, TBL, An International Collaborative Assay of Factor VIII Clotting Activity, Thrombos Haematos 40, 260-271, 1978.

[3]Martin, AN, Physical Pharmacy, pp 478-506, Lea & Febiger, 1960.

TABLE I

Comparison of Predicted & Actual Rate Losses & Time to 90% of Activity (T90) on 3 Lots of AHF

	AHF	AHF, Dextrose	
Set Down:	1308	1616	1617
Lot #:	0591X073A1	0591C046A	0591C045A
5°C: Rate Loss (%/Mo)			
Predicted	0.124[1]	0.160[2]	0.243[2]
Observed	0.185	0.123	0.150
Higher - 95% Conf. Limit	+ 0.105	+ 0.234	+ 0.114
5°C: T90 (Months)			
Predicted	81	62	41
Observed[3]	54	81	67
Lower - 95% Conf. Limit	34	28	38

[1] Based on 47.5, 35.5, 25 and 14.5°C rate data

[2] Based on 35.5, 25 and 14.5°C rate data

[3] Based on data derived on samples stored for up to 60 months

TABLE I

TABLE II

AHF and AHF, Treated Stability

Factor VIII (u/ml)*

Temp.	Age (Months)	Set Down: 1308 Lot: 0591X073A1	%	One Parent Lot of Product: 2256 2750T001 (Treated)	%	2257 800401AH11	%	One Parent Lot of Product: 2270 2750T002 (Treated)	%	2271 810113AH11	%
60/-25	1 day	**32.6/32.7	100	-		-		-		-	
	1 day	30.7/31.6	97	-		-		-		-	
	2 days	**33.2/32.7	102	-		-		-		-	
	2 days	33.6/31.6	106	-		-		-		-	
	4 days	-		24.5/24.2	101	26.0/27.4	95	26.0/27.7	94	24.9/31.1	8
	37 days	-		**22.8/24.2	94	20.0/27.4	73	19.6/27.7	71	21.3/31.8	6
	37 days	-		18.3/25.9	71	21.7/28.3	77	-		-	
	60 days	15.2/34.5	44	-		-		-		-	
47.5/-25	0.3	27.4/30.2	91	-		-		-		-	
	0.5	27.0/30.2	89	-		-		-		-	
	0.7	26.1/30.2	86	-		-		-		-	
	3	26.3/32.8	80	21.1/25.9	81	22.1/28.3	78	22.1/29.5	75	20.0/27.8	7
	5	22.0/33.0	67	-		-		-		-	
	5	24.2/31.6	76	-		-		-		-	
	6	-		17.7/29.7	60	16.5/28.5	58	19.5/29.5	66	16.4/27.8	5
	11	-		-		-		12.0/28.4	42	13.3/27.2	4
	12	-		11.0/23.6	47	11.2/25.7	44	-		-	
35.5/-25	1	28.5/30.2	94	-		-		-		-	
	5	28.0/33.0	85	-		-		-		-	
	5	29.1/31.6	92	-		-		-		-	
	6	-		21.6/29.7	73	24.0/28.5	84	23.7/29.5	80	21.6/27.8	7
	8	26.7/31.1	86	-		-		-		-	
	11	24.8/30.8	80	-		-		-		-	
	12	-		18.6/23.6	79	16.4/25.7	64	18.1/28.4	64	19.2/27.2	7
	14	22.0/31.0	71	-		-		-		-	
25/-25	7			21.6/24.0	90	23.0/25.6	90	24.7/29.3	84	26.2/28.7	9
	8	30.5/31.1	98	-		-		-		-	
	12	-		19.5/23.6	83	21.3/25.7	83	24.1/28.4	85	21.8/26.1	83
	14	26.0/31.0	84								
	17	26.9/30.5	88								
	24	**32.6/32.7	100								
14.5/-25	24	-									
	30	26.1/31.7	82								
	49	**33.4/32.7	102								
	49	33.0/37.6	88								
	67	21.1/30.4	69								
5/-25	Initial	28/-		25/-		32.2/-		27.4/-		32.9/-	
	1	31.1/30.2	103	24.2/-		27.4/-		-		-	
	6	-		26.8/29.7	90	27.7/28.5	97	27.0/29.5	92	27.7/27.8	10
	7			24.6/24.0	102	23.5/25.6	92	26.9/29.3	92	28.3/28.7	9
	11	29.9/30.8	97	-		-		-		-	
	12	-		23.0/23.6	98	24.2/25.7	94	27.0/28.4	95	25.0/26.1	9
	14	31.0/31.0	100								
	24	-									
	30	29.1/31.7	92								
	50	**32.2/32.7	99								
	51	32.4/37.6	86								
	60	30.0/34.5	87								
	77	27.3/30.4	90								

Results reported as : value at indicated time and temperature/value at -25C

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**: Suspect assay

TABLE III

Factor VIII Decay Rates, AHF and AHF, Treated, (%/Mo)

Temp. °C.	Set Down: Lot:	1308 05S1X073A1	One Parent Lot of Product 2256* 2750T001 ≡	2257 800401AH11	One Parent Lot of Product 2270* 2750T002 ≡	2271 810113AH11
60		27.8	24.4	21.1	24.6	27.7
47.5		6.0	5.0	5.3	5.7	5.6
35.5		2.0	2.3	2.9	3.1	2.7
25 Actual		0.81	1.44	1.42	1.51	1.5
Predicted		0.92[1]	1.38[2]	1.47[2]	1.54[2]	1.35[2]
14.5		0.41				
5 Predicted		0.149[1]	.39[2]	.38[2]	.40[2]	.32[2]
2 - 8 Actual		0.17[3]	.32[4]	.63[4]	.73[4]	.29[4]
Potency remaining after 23 months @ 5°C plus 1 month @ 25°C (%)		95.7	89.7	89.8	89.3	91.3

TABLE III

*AHF, Treated
[1]Based on 47.5, 35.5, 25 and 14.5°C rate losses
[2]Based on 47.5, 35.5, 25°C rate losses
[3]Based on observed losses after 77 months
[4]Based on observed losses after 12 months

Potency (%) remaining after 23 months @ 5°C plus 1 month @ 25°C =

[23 X (predicted 5°C rate loss) + 1 X (predicted 25°C rate loss)] -100%.

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TABLE IV

FACTOR VIII DECAY RATES: AHF, TREATED, (%/MO)

Temp.°C.	Set Down: 2293 Lot: 2788T002AT	2319 2792V002AT
60	34.5	40.0
47.5	9.3	12.6
35.5	3.1	3.4
25 Actual	1.30	
Predicted	1.26[1]	1.01[3]
14.5		
5 Predicted	.17[1]	.08[3]
2 - 8 Actual	.30[2]	-1.9[4]
Potency remaining after 23 Mo @ 5°C plus 1 month @ 25°C (%)	94.8	97.2

[1]Based on 47.5, 35.5, 25°C rate losses

[2]Based on observed losses after 6 months

[3]Based on 47.5 and 35.5°C rate losses

[4]Based on observed losses after 3 months

Potency (%) remaining after 23 months @ 5°C plus 1 month @ 25°C = [23 X (predicted 5°C rate loss) + 1 X (predicted 25°C rate loss)] -100%

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TABLE V.

Stability Parameters

Temp. °C.	Time (Mo)	Lot*	pH 1	pH 2	pH 3	pH 4	Moisture (%) 1	Moisture (%) 2	Moisture (%) 3	Moisture (%) 4	Physical (Dry) 1	Physical (Dry) 2	Physical (Dry) 3	Physical (Dry) 4	Solubility (Min) 1	Solubility (Min) 2	Solubility (Min) 3	Solubility (Min) 4	Heparin (u/ml) 1	Heparin (u/ml) 2	Heparin (u/ml) 3	Heparin (u/ml) 4
60	1.2		6.9	6.9	6.8	6.8	1.3	1.1	1.3	1.0	Sat	Sat	Sat	Sat	7	5	5	6	-	-	-	-
47.5	3		6.9	6.9	6.8	6.8	1.5	0.8	1.5	1.2	Sat	Sat	Sat	Sat	5	5	3	9	-	-	.48	.37
	6		6.9	6.9	6.8	6.8	1.9	1.0	1.4	1.1	Sat	Sat	Sat	Sat	3	5	4	8	.66	.36	.48	.44
	11		-	-	6.8	6.8	-	-	2.5	1.2	-	-	Sat	Sat	-	-	7	10	-	-	-	-
	12		6.8	6.8	-	-	1.7	1.2	-	-	Sat	Sat	-	-	5	>10	-	-	-	-	-	-
35.5	6		6.9	6.9	6.8	6.8	1.4	1.2	1.4	1.5	Sat	Sat	Sat	Sat	3	2	5	11	.68	.37	.48	.30
	12		6.9	6.9	6.8	6.8	1.7	1.4	1.7	1.9	Sat	Sat	Sat	Sat	3	3	3	4	-	-	-	-
25	7		7.0	6.9	6.9	6.9	1.4	1.5	1.9	1.3	Sat	Sat	Sat	Sat	5	5	6	4	.38	.39	.43	.46
	12		6.9	6.9	6.8	6.8	0.7	-	0.9	1.1	Sat	Sat	Sat	Sat	4	5	3	3	-	-	-	-
5	Initial		6.9	6.9	6.9	6.8	0.9	0.8	1.0	0.8	Sat	Sat	Sat	Sat	4	3	3	3	.40	.39	.48	.35
	1		6.9	6.9	-	-	1.0	0.5	-	-	Sat	Sat	-	-	4	4	-	-	-	-	-	-
	6		6.9	6.9	6.9	6.9	1.2	1.0	1.2	0.6	Sat	Sat	Sat	Sat	4	3	4	7	.69	.36	.50	.37
	7		6.9	6.9	6.9	6.9	1.2	1.0	1.6	1.0	Sat	Sat	Sat	Sat	4	3	4	3	.38	.39	.50	.45
	12		6.9	6.9	6.9	6.9	0.6	0.7	1.3	1.4	Sat	Sat	Sat	Sat	3	4	2	4	.47	.50	.40	-

	S/D #	Lot #			
1 -	2256	2750T001	-	AHF, Treated	One Parent Lot
2 -	2257	800401AH11	-	AHF	One Parent Lot
3 -	2270	2750T002	-	AHF, Treated	One Parent Lot
4 -	2271	810113AH11	-	AHF	One Parent Lot

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TABLE V

TABLE VI

Stability Parameters

Temp. °C.	Time (Mo)	Lot*	Safety 1	Safety 2	Safety 3	Safety 4	Pyrogen 1	Pyrogen 2	Pyrogen 3	Pyrogen 4	Sterility 1	Sterility 2	Sterility 3	Sterility 4	Hemolysins 1	Hemolysins 2	Hemolysins 3	Hemolysins 4
25	12		Sat	Sat	Sat	Sat	Sat	Sat	Sat	-	-	-	-	-	Neg	Neg	Neg	-
5	Initial		Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	-	Neg	Neg	Neg
	12		Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	Sat	-	Sat	-	Neg	Neg	Neg	Neg

* S/D # Lot #

1 - 2256 2750T001 - AHF, Treated)
2 - 2257 800401AH11 - AHF) One Parent Lot

3 - 2270 2750T002 - AHF, Treated)
4 - 2271 810113AH11 - AHF) One Parent Lot

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IN VIVO STUDIES

Animal Safety Studies - Toxicology and Pharmacology

Toxicology and pharmacological studies in three animal species have shown Antihemophilic Factor (Human), Method Four, Hemofil® T to be equivalent to the present Hyland Therapeutic product (Antihemophilic Factor (Human), Method Four, Hemofil®). The latter product has been licenced in the U.K. since 1973, is used widely throughout the world and has been found to be safe and efficacious in clinical use. The following reviews the animal experiments used to evaluate the Hemofil T.

Four separate studies were conducted in the laboratories of the Department of Safety Assessment, Travenol Laboratories, Morton Grove, Illinois, U.S.A. Reference to these studies are as follows:

1. A seven-day assessment of the comparative safety in rats of intravenously administered Antihemophilic Factor Heat-Treated, Hemofil T.

2. Effects of intravenous Antihemophilic Factor-Treated on the circulation and respiration of the anaesthetised dog.

3. Acute intravenous LD_{50} determination of Antihemophilic Factor-Treated following administration to rats and mice.

4. Effects of intravenous Antihemophilic Factor-Treated at various rates of administration on the systemic hemodynamics and respiration in the anaesthetised dog.

Discussion of Individual Studies

1. Comparative Safety in Rats

Antihemophilic Factor (Human), Method Four, Hemofil® T was given intravenously each day for seven consecutive days to groups of eight rats at doses of 20, 40 and 60 AHF units per kilogram. Comparisons were made with a reference agent, Antihemophilic Factor (Human), Method Four, Hemofil® at 60 units/kg and with a control substance, sodium chloride, 0.9 percent, in an equivalent volume.

Toxicity was assessed on the basis of overt signs of toxic behaviour, body weight gain, haematology, blood and urine chemistry profiles, opthamological examination, and gross and microscopic pathology.

Antihemophilic Factor (Human), Method Four, Hemofil® T rats were not significantly different from rats given the reference and control materials in any of the observed parameters. (All histophathology examinations were conducted by consultant pathologists from Northwestern University.)

2. Effect on the Circulation and Respiration of the Anaesthetised Dog

Five dogs, anaesthetised with pentobarbitone, received Antihemophilic Factor (Human), Method Four, Hemofil® T intravenously at a constant rate of 0.12 ml/kg/minute (or: 3.12 u/Kg/Min) until 8 ml/kg (or: 208 u/Kg) had been administered. This dose was chosen as twice the maximum human dose recommended, and the dose was given at three times the rate suggested for clinical use. Five other dogs were given Antihemophilic Factor (Human), Method Four, Hemofil® and five received normal serum albumin (5%).

Arterial blood pressures and electrocardiograms were continuously recorded. Also, measurements were recorded at 1, 2, 4 and 8 ml/kg of each of the following: cardiac output, heart rate, body temperature, respiratory rate and volume, arterial blood pH, pCO_2, base excess, HCO_3, and CO_2 content.

Antihemophilic Factor (Human), Method Four, Hemofil® T had no effect on heart rate, arterial blood pressure or cardiac output, indicating no adverse effects on the myocardium or arterial vasculature. Dose-related increases in respiration were seen, but were not significantly different from those produced by the reference article.

3. Acute LD_{50} Determination in Rats and Mice

LD_{50} studies were done in mice and rats, comparing Antihemophilic Factor (Human), Method Four, Hemofil® T to Anithemophilic Factor (Human), Method Four, Hemofil® as a reference material and to saline as a control article. The test and reference materials were made from the same pooled human plasma.

The maximum dose administered of both the test and reference materials was 40 ml/kg, with administration rates of 2 ml/minute in rats and 1 ml/minute in mice. It was not considered reasonable to go to higher doses.

Because there was less than 50 percent lethality among rats and mice at the 40 ml/kg dosage levels of either test or reference materials, the LD_{50} values could not be calculated. They are reported as greater than the maximum administered (that is, LD_{50} > 40 ml/kg or 1320 units of Factor VIII per kilogram of body weight).

4. Effect on the Cardiovascular System in Dogs

The cardiovascular responses to differing rates of infusion of Antihemophilic Factor (Human), Method Four, Hemofil® T were recorded in five pentobarbitone anaesthetised dogs. Groups of five dogs also were given equivalent amounts of a reference material (Antihemophilic Factor (Human), Method Four, Hemofil®), and a control solution (Sodium Chloride 0.9 percent). The parameters recorded were the same as described in study 2 (above).

Three infusions were administered to each dog at rates of 0.5, 1.0, and 5.0 ml/kg/minute. The highest rate used in this study is approximately 10 times the maximum clinical dose.

In this dog study, as in the earlier dog study, no significant changes in mean arterial pressure, heart rate or cardiac output were produced by either the test or reference materials.

CONCLUSIONS

From the four studies reviewed here, it is concluded that Antihemophilic Factor (Human), Method Four, Hemofil® T can be safely administered in several animal species at doses and infusion rates greatly exceeding expected clinical doses, and suggest a reasonable margin of safety for clinical use.

Studies of carcinogenicity, mutagenicity or teratology were not considered appropriate for the Antihemophilic Factor (Human), Method Four, Hemofil® T. Since this is human protein material, it is a certainty that it will produce hypersensitivity reactions in non-human species. Such reactions will make it impossible to do long-term chronic studies needed to evaluate these parameters. Further, vast clinical experience has demonstrated the expected lack of such reactions with a wide array of proteins derived from human blood.

SUMMARY OF HUMAN SAFETY AND EFFICACY STUDIES

The human clinical studies that have been performed emphasise the blood levels of AHF achieved following infusion of Antihemophilic Factor (Human), Method Four, Hemofil® T, when compared to the reference material (Antihemophilic Factor (Human), Method Four, Hemofil®).

Two studies (human clinicals) were conducted to demonstrate the safety and efficacy of Antihemophilic Factor (Human), Method Four, Hemofil® T by evaluating survival and recovery of the AHF.

- In the United States, six patients, each infused with both

  Antihemophilic Factor (Human), Method Four, Hemofil® T
  and
  Antihemophilic Factor (Human), Method Four, Hemofil® product as a control. A normal dosage of 50 AHF units/kg was employed.

- In Europe, six patients, each infused with both Antihemophilic Factor (Human), Method Four, Hemofil® as the control, followed by three injections of Antihemophilic Factor (Human), Method Four, Hemofil® T.

In the U.S. study, statistical comparison of the results (using accepted methods) indicated a nonsignificant difference in the average or median half-lives for the two products, Antihemophilic Factor (Human), Method Four, Hemofil® T concentrate (AHF heat-treated) and Antihemophilic Factor (Human), Method Four, Hemofil® concentrate AHF control.

A statistical comparison of the percent recovery results indicated a nonsignificant difference in the average of median recoveries for the two products ($p = 0.2188$). Detailed analyses are given in Tables X and XI.

TABLE X

HALF-LIFE

Patient	Half-Life (Hours)	
	HEMOFIL® T Concentrate	HEMOFIL® Concentrate
1	8.3	12.6
2	8.5	6.6
3	7.0	8.8
4	8.1	7.8
5	7.2	8.2
6	8.3	7.6
Average	7.9	8.6
Median	8.2	8.0

TABLE XI

RECOVERY

(Maximum % of Normal Over the Post-Infusion Period)

Patient	Recovery (%)	
	HEMOFIL® T Concentrate	HEMOFIL® Concentrate
1	53%	88%
2	92%	114%
3	75%	81%
4	77%	87%
5	127%	107%
6	60%	80%
Average	80.7%	92.8%
Median	76%	87.5%

- In the European study, the patients were treated with both materials and were judged on the basis of clinical response to have been treated effectively. The effectiveness of treatment was also evaluated by comparing the blood levels attained with the Hemofil® T concentrate when compared to the blood level attained with AHF Control in the same hemophilic individual, in order to allow for individual to individual variation. There were six patients treated for spontaneous bleeding. The results of the Factor VIII recovery and half-life are summarised in Table XII. The recovery and half-life values show that the AHF Treated and AHF Control were not distinquishable. No untoward side effects were observed.

These studies were not only successful, but also illustrate that the equivalence of Hemofil® T concentrate and Hemofil® concentrate with regard to recovery and biological half-life support the contention that the two products are therapeutically equivalent.

In addition, no inhibitors or neoantigens were formed, as evidenced by half-life and percent recovery data summarised below in Table XIII for first vs. third injections.

TABLE XII

IN VIVO RECOVERY AND HALF-LIFE OF HEMOFIL®-T CONCENTRATE VERSUS HEMOFIL®- CONCENTRATE (CONTROL)

	F.VIII HALF-LIFE, HRS		IN VIVO F.VIII:C RECOVERY (%)	
PATIENT	HEMOFIL® T CONCENTRATE LOT NO. 1	HEMOFIL® CONCENTRATE LOT NO. 2	HEMOFIL® T CONCENTRATE LOT NO. 1	HEMOFIL® CONCENTRATE LOT NO 2
1	8.5	8.5	96.7	91
2	8.5	7	(>80)*	83
3	11.5	10.5	102	105
4	10.5	10	102	98
5	11	8.5	100	105
6	11	11	94	89

*no data available after 1 hr.

mean	10.2	9.3	mean	95.7	95.2
standard deviation	1.3	1.5	standard deviation	8.34	9.0

The equivalence of Hemofil® T and Hemofil® with regard to recovery and biological half-life support the contention that they are therapeutically equivalent. Furthermore, Hemofil® T was judged to have stopped bleeding in all instances. Specifically, pain when present initially was relieved in all episodes.

TABLE XIII

IN VIVO RECOVERY AND HALF-LIFE OF HEMOFIL T; FIRST VERSUS THIRD INJECTIONS

	F.VIII HALF-LIFE, HRS		IN VIVO F.VIII:C RECOVERY (%)	
PATIENT	HEMOFIL® T CONCENTRATE LOT NO. 1 1st Injection	HEMOFIL® CONCENTRATE LOT NO. 1 1st Injection	HEMOFIL® T CONCENTRATE LOT NO. 1 1st Injection	HEMOFIL® CONCENTRATE LOT NO 2 3rd Injection
1	7	7	83	95
2	8	8	98	98

NCLUSIONS

In summary, both studies indicated that the product subjected to heat treatment is a suitable preparation not only for the treatment of haemophilic patients with uncomplicated haemorrhages, but also for "on demand" therapy and for intensive substitution therapy such as prophylaxis, treatment of inhibitors and for pre-and post-surgical care. These results indicated that no evidence exists to suggest that the two products (HEMOFIL® T and HEMOFIL) differ with respect to half-life or recovery.