

MEMORANDUM



Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research

Pharmacology / Toxicology Review Memorandum

Date: September 12, 2007

From: Paul W. Buehler [REDACTED] **APPROVED**
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By buehlerp at 4:38 pm, Sep 12, 2007

Through: Abdu Alayash, Basu Goiding and Susan Abbondanzo

To: Cheryl Campbell and Elena Karnaukhova

Subject: STN – BN070012/0 Voluven®, (6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride infusion)

Sponsor: Fresenius Kabi

Receipt Date: March 15th, 2007

Finalized Review: September 12, 2007

Proposed Indication and Clinical Dosing:

6% hydroxyethyl starch 130/0.4 (HES 130/0.4) is indicated for volume replacement as a maximum dose of 50 mL/kg/day. In the compiled clinical trial data studying volume replacement in sepsis, cardiac surgery, traumatic brain injury, abdominal aortic aneurism, ischemic stroke and orthopedic surgery. Most subjects (27% of 1315) of subjects received > 40 mL/kg with a maximum dose of 70 mL/kg/daily corresponding to a maximal exposure of 4900 mL and 294 grams of HES 130/0.4 daily.

Background:

6% hydroxyethyl starch 130/0.4 (HES 130/0.4) is a lower molecular weight (weighted average molecular weight = 130 kDa) starch mixture than the currently marketed HES 450/0.7 (weighted average molecular weight = 450 kDa). Additionally, HES 130/0.4 contains a lesser degree of hydroxyethyl groups. Safety of starch solution may depend on the lower

weighted average molecular weight and the lesser degree of hydroxyethyl group substitution which allow for more rapid plasma clearance. While this provides the potential for lesser impairment of hemostasis (inhibition of FVIII activity and [vWF antigen]) compared to HESpan 6% there is a potential for renal impairment induced by more extensive renal versus non-renal clearance of low molecular weight starch.

List of non-clinical studies

Primary safety pharmacology studies

- (1) 8932/3/94- Examination of HES 130/0.4 infusion solution in a shock model on long – term survival in conscious Sprague Dawley rats.
- (2) 9002/2/94- Examination of HES 130/0.4 infusion solution in a shock model on long – term survival in anesthetized beagle dogs.

Pharmacokinetic and toxicokinetic studies

- (3) # 18168/04- Toxicokinetic study of hyperbranched starch after single intravenous administration to rats
- (4) # 8871/94- Examination of ¹⁴C-labelled HES 130/0.4 after intravenous bolus injection for 18 days in rats

Toxicology studies

Single dose

- (5) # 18168/04- Toxicokinetic study of hyperbranched starch after single intravenous administration to rats (also covers single dose toxicity)

Repeated dose

- (6) # 8993/1/94 – 13 week subchronic toxicity study of HES 10% 130/0.5 in administered to rats by intravenous infusion over three hours each day
- (7) # 8993/1/94 - 13 week subchronic toxicity study of HES 10% 130/0.5 in administered to dogs by intravenous infusion over three hours each day

Genotoxicity

- (8) #9004/94- Mutagenicity study of HES 130/0.4 10% infusion solution in the salmonella typhimurium reverse mutation assay in vitro
- (9) #9005/94- Mutagenicity study of HES 130/0.4 10% infusion solution in mammalian cells (b) in the in vitro gene mutation assay (b)
- (10) #9005/94 – In vivo bone marrow cytogenetic test of HES 130/0.5 10% infusion solution by intravenous administration in Sprague Dawley rats.

Reproductive and developmental toxicity

- (11) #9008/94 – Examination of the influence of HES 10% (130/0.5) on the pregnant rat and rabbit and fetus by intravenous administration

Overview of Pharmacology and Toxicology:

Primary safety pharmacology

The primary pharmacodynamic studies with HES 130/0.4 has been evaluated in study # 8932/3/94 to evaluate the long term survival of rats following shock induced by a controlled hemorrhage. In brief HES 130/0.4 resulted in complete survival following a controlled blood loss of 50% and 67%. Additionally, HES 130/0.4 has been evaluated in study #9002/2/94 using beagle dogs following hemodilution to a hematocrit of 20% using a rate of exchange transfusion equal to 10 mL/kg/5min. Cardiovascular parameters were such as MAP, heart rate, LVP, PAP and PCWP remained unchanged following exchange transfusion. CO increased in HES transfused animals and remained elevated for 2.5 hours compared to the higher molecular weight starches which maintained an elevated CO over the course of the three hour study. The increase in CO was also associated with an increased SV as heart rate did not change and an increased left ventricular stroke work index.

Secondary safety pharmacology

No specific secondary safety pharmacology studies have been performed with HES 130/0.4. Both HES 200/0.5 and HES 450/0.7 have been used with relative safe clinical profiles. Given the considerably shorter terminal half-life (26 fold) and equivalent fold lower clearance compared to HES 450/0.7 it would be unlikely that the starch composition of HES 130/0.4

would cause demonstrate safety pharmacology issues which would exceed that of currently marketed products. As a result it is agreeable that further safety pharmacology on HES 130/0.4 will provide minimal information.

Toxicokinetics

The toxicokinetics of HES 130/0.4 versus hyperbranched starch (HBS) was performed in study # **18168/04** following single intravenous administration in 44 male rats. Rats were dosed at volume 90 mL/kg of HES (10%) or HBS (10%) over 3 hours (22.5 mL) for 13 days. Toxicokinetic analysis revealed a terminal half-life, AUC and clearance slightly less in the HBS dosed animals. In brief, the toxicology evaluation analysis performed in this study revealed the following: no injection site intolerability, no influence on body weight, no influence on hematology parameters, increased **α -amylase levels after 8 hours returning to baseline at 24 hours**. Similar findings were associated with both HES and HBS in this study. The elevation in amylase appears not to create issue in normal animals. This study represents the primary single dose toxicology study in rodents.

Distribution and Metabolism

The distribution and metabolism of ^{14}C -labelled HES 130/0.4 was performed in study # **8871/94** following 18 days of intravenous administration. The dose studied was 1.4 mL of HES 130 (10%) or HES 200 (10%) per 200 g body weight per day. A majority of radioactivity was concentrated in the liver and carcass (all organs excluding liver, lymph nodes kidney and spleen). Excretion occurred in the urine and feces within the first three days after the 18th dose. Trace amounts of radioactivity were detectable until 52 days following the last dose.

Single and Repeat-dose toxicology

Single dose toxicology was carried out with HES 130/0.4 as a part of study # **18168/04** and no other single toxicity studies have been conducted. Repeated-dose toxicity was performed in study # **8993/1/94**. This study was conducted as a 13 week subchronic toxicity study of HES 10% 130/0.4 with daily infusions of 60 mL/kg/day or 90 mL/kg/day. Thus the maximum dose received by animals based on the rats 200 g start weight is 1.8 g of starch. Additionally, 4 dose levels were evaluated in beagle dogs in study # **9003/94** at doses of 20, 40, 60 and 90 mL/kg/day for 13 weeks. Thus repeated dose toxicology studies in rat and dog demonstrate a dose equivalent of 630 g/day in a 70 kg human and a total 13 week exposure of

57 kg HES 130/0.4. When taking into account the fact that 6% HES 130/0.4 is proposed for human use this represents a 3 fold excess of the clinical dose evaluated in the rat and dog.

Genotoxicity

The genotoxic potential of HES 130/0.4 was evaluated in studies #9004/94, #9005/94, and #9007/94 using the Salmonella typhimurium reverse mutation assay, [REDACTED] gene mutation assay ([REDACTED]) and the in vivo bone marrow cytogenetic test in Sprague Dawley rats. None of the assays demonstrated evidence for HES 130/0.4 genotoxicity. As a result long term carcinogenicity studies have not been conducted and are not necessary.

Reproductive and developmental toxicity

The reproductive and developmental toxicity was evaluated in study #9008/94 using pregnant rats dosed at 12.5, 25 and 50 mL/kg/day for 20 days of gestation. In this study the no observed effect level (NOEL) for the dams was 12.5 mL HES 130/0.4 10%. At 25 mL a slight decrease in food consumption and dyspnoea were observed. The NOEL for the fetal organism was 25 mL/kg whereas 50 mL HES 130/0.4 resulted in decreased body weight and skeletal malformations. Thus the fetal effects occurred only at the dosing levels of maternal toxicity.

Summary of Safety Pharmacology Reports:

Study # 8932/3/94- Examination of HES 130/0.4 infusion solution in a shock model on long-term survival in conscious Sprague Dawley rats.

Objective – The aim of the study was to investigate the effect of HES 6% 130/0.5 in and artificial state of hemorrhagic shock in conscious rats.

Methodology – 36 female Sprague Dawley rats were catheterized at the position of the left and right femoral artery and the femoral vein. Following recovery rats were partially restrained such that systemic blood pressure could be monitored from one femoral artery and 50% or 67% of the total blood volume was removed in two steps over a 3 hour period.

The following groups were evaluated:

1. HES 6% 130/0.5 - (67%) shed blood volume replacement

2. HES 6% 130/0.5 - (50%) shed blood volume replacement
3. Ringer lactate – (67%) shed blood volume replacement
4. Ringer lactate – (50%) shed blood volume replacement
5. HES 6% 200/0.5 - (67%) shed blood volume replacement
6. HES 6% 200/0.4 - (50%) shed blood volume replacement

The end points of the study were mean arterial pressure and 14 day survival.

Results – In all groups MAP was approximately 120 mmHg at baseline and reduced to 80 ± 10 mmHg in the 50% shock groups and 50 ± 13 mmHg in the 67% shock groups. Blood pressures returned to normal in the HES treated groups. 14 day Survival: HES 130 = 100% in the 50% blood loss group and 83 % in the 67% blood loss group. 14 day survival HES 200 = 100% in the 50% blood loss group and 100 % in the 67% blood loss group. Ringer lactate animals survived to a maximum 24 hours.

The hematocrit was reduced to from 38.3% to approximately 25.1 (50% blood loss) and 23.2 (67% blood loss). No differences were observed in the complied blood withdrawal volume or infusion volumes.

Conclusions: It appears that low molecular weight starches are equally effective to mid-range molecular weight starches at restoring volume after blood loss.

9002/2/94- Examination of HES 130/0.4 infusion solution in a shock model on long –term survival in anesthetized beagle dogs.

Objective- The primary objectives of this study were to obtain safety pharmacology information on cardiovascular parameters following hemodilution with HES 130 (6%) compared to HES 200 (6%).

Methodology – The hematocrit of splenectomized dogs under anesthesia with nembutal was reduced in one step to 20%, representing approximately a 50% volume exchange (blood for starch). The exchange occurred over at a rapid rate 10 mL/kg/5 min to obtain a set HCT of 20%. The amount of blood withdrawn from each animal was recorded. The following cardiovascular parameters were evaluated: MAP, PAP, HR, CO, SV LVP dp/dt max, CVP and hematology parameters.

Results – MAP, HR, LVP, PAP and PCWP remained unchanged during the exchange and for the three hour monitoring period. CO and SV increased by 68% in both HES (130 and 200) groups. The CO and SV decreased over the 3 hour monitoring period to baseline levels in

the HES 130 but both parameters remained elevated over the entire 3 hour monitoring period in the HES 200 group. This was accompanied by an increase in left ventricular stroke index. As would be expected hemoglobin content and the number of thrombocytes decreased, the fibrinogen content decreased and thromboplastin / activated thromboplastin time increased. There were no notable influences on blood gases between the two starch groups. The amount of blood exchanged ranged from 385-440 mL in the HES 130 (6%) group and 315-395 mL in the HES 200 (6%). While exact exposures are unknown based on continuous exchange a mean of 412.5 mL (HES 130/0.5) and 355 mL (HES 200/0.5) were circulated through dogs. This accounts for 21.3 g (2.13 g/kg) of HES 130/0.5 and 24.75 g (2.48 g/kg) of HES 200/0.5. Human equivalent dose of HES 130/0.5 would be approximately 149.1 g (2485 mL).

Conclusions – There were no pharmacological efficacy differences between HES 130/0.5 versus HES 200/0.5. Both HES 130/0.5 and HES 200/0.5 demonstrated similar cardiovascular responses however HES 130/0.5 was shorter lived. Both HES 130/0.5 and HES 200/0.5 demonstrated increases in thromboplastin and activated thromboplastin time.

18168/04- Toxicokinetic study of hyperbranched starch after single intravenous administration to rats

Objectives – The primary objective of this study was to determine the toxicokinetic profile of hyperbranched starch following single intravenous administration to rats in comparison to a control containing 10% HES 130/0.4. Hyperbranched starch contains more hydroxyethyl units and is analogous in this regard to HESpan 6%.

Methodology - 44 male animals were dosed with 90 mL/kg via the tail vein over an infusion period of three hours. Blood samples were obtained from separate rats 0, 1, 2, 3, 8, 24 hours. A separate group of animals were dosed with 90 mL/kg for toxicology evaluation.

Results – Pharmacokinetics:

Dose (90 ml/kg)	C _{max}	T _{max}	t _{1/2}	AUC 0-24 hr	AUC 0-inf.
HBS	21.86	End of infusion	1.31	40.60	40.69
10% HES	16.70	End of infusion	1.19	32.61	32.81

Hematology – Hematocrit values were not changed. No other hematology parameters were evaluated in this study.

Clinical chemistry – The most notable clinical chemistry finding was a trend toward an increase in serum α -amylase (U/L) level for both hyperbranched and HES 130. Baseline HES 130 values were 4180.33 ± 613.05 (U/L) the mean increased to 5446.3 ± 1511 (U/L) at 2 hours. The difference was not significant and is heavily weighted to a single animal at the 24 hour time point all values returned to baseline or below.

Conclusions - The maximal concentration of hyperbranched versus HES 130/0.4 is the same following a three hour infusion of 90 mL/kg starch. However the overall exposure based on AUC_{0-inf} was approximately 25% less with HES 130/0.4. No apparent signs of gross toxicity were observed and the influence on amylase appears to be present but likely not important based on pathophysiology.

Note: No other toxicological evaluations were performed in this study.

8871/94- Examination of ¹⁴C-labelled HES 130/0.4 after intravenous bolus injection for 18 days in rats

Objectives – The purpose of this study was to compare the storage characteristics of ¹⁴C-labelled HES 130 and ¹⁴C-labelled HES 200 after daily intravenous bolus injection in Sprague Dawley rats.

Methodology - ¹⁴C-labelled HES 130 and ¹⁴C-labelled were administered daily to rats by intravenous injection for 18 consecutive days at a dose of 1.4 mL/200g body weight. Blood was sampled at 3, 10, 24 and 52 days. On day 18 of dosing 3 animals treated with ¹⁴C-labelled HES 130 and ¹⁴C-labelled HES 200 respectively were euthanized. Urine and feces was collected and tissues were harvested for determination of radioactivity.

Results – Radioactivity was presented as percent of the total dosage. The liver as well as collected urine and feces displayed the greatest amounts of radioactivity. The half-life of the compounds in each tissue ranked as follows: HES 130 and HES 200 (spleen > kidney > carcass > plasma > liver). However, longer half-lives were observed in liver, spleen and carcass for HES 200 compared to HES 130. At the time of necropsy no gross abnormalities were observed. Lymph node, liver, kidney and spleen weights were within normal limits.

Conclusion: Both HES 130 and 200 appear to accumulate in liver kidney and spleen; however it is unclear what the differential renal and non renal clearance is. Clearly HES 200 persists longer in the tissues examined particularly the spleen and liver.

8993/1/94 – 13 week subchronic toxicity study of HES 10% 130/0.5 in administered to rats by intravenous infusion over three hours each day

Objective – This study represents the primary toxicity study in rodents following repeated daily dosing for 13 weeks. The primary end points were to determine the presence of dose limiting toxicity to all organ systems.

Methodology – The following groups were evaluated: (Group1) – 90 mL isotonic solution/kg over 3 hours; 60 mL HES 130/0.4 10%/kg over 3 hours; 90 mL HES 130/0.4 10%/kg over 3 hours daily for 13 days. The distribution of animals evaluated was Group 1 (15 male/15 female), Group 2 (10 male/10 female), Group 3 (15 male/15 female) and 20 additional rats were allocated to the evaluation of recovery (4 weeks) of any treatment induced toxicity. The dose was determined during a 14 day pilot study in which the sponsor anticipated a 2 g HES 130/0.4 (6%) in humans. Therefore the maximum dose of 90 mL/kg used in this study is approximately 4.5 times higher than the human dose.

List of observations

Clinical signs (daily)

Mortality

Body weight (daily)

Food and water consumption (daily)

Hematology (6th week, 13th week and at 28 days following dosing for recovery animals)

Differential blood count

RBCs

WBCs

Hemoglobin

Reticulocytes

Platelets

Thromboplastin time

Activated partial thromboplastin time

MCV

MCHC

Clinical chemistry (6th week, 13th week and at 28 days following dosing for recovery animals)

Electrolytes

α-amylase

Total bilirubin

Total cholesterol

Albumin

Creatinine

Total protein

Glucose

Blood urea nitrogen

ALT

Alkaline phosphatase

ASAT/GOT

LDH

Urinalysis (6th week, 13th week and at 28 days following dosing for recovery animals)

Color

Specific gravity

Protein

Glucose

Bilirubin

Hemoglobin

Ketones

pH

urobillogen

nitrite

Pathology/Histopathology (13th week and at 28 days following dosing for recovery animals)

All organs

Results – Local tolerance of infusion at the tail vein indicated induration of the tail tissues with both the 60 and 90 mL/kg dose this occurred from week four onward. Normal behavior

and appearance was observed in all animals throughout the treatment period. No treatment induced changes in body weight were observed.

Food intake of the 60 and 90 mL/kg dose groups declined significantly compared to the control group, which was likely the cause of the high caloric content of the starch since body weight did not change. Drinking water consumption was not influenced by treatment. Food consumption trended toward normalization by the end of the 4 week study period.

Ocular changes did not occur in the HES 130/0.5 treated animals.

Hematology parameters were minimally changed, however the total hemoglobin decreased by 12.2% compared to the control at week 6 in the 90 mL/kg female animals, while all other HES 130/0.5 treated animals showed decline that did not reach statistical significance. This corresponded to an increase in reticulocytes and a decline RBCs and hematocrit. These findings are potentially caused by mass dosing of HES causing a continuous hemodilution effect.

Certain clinical chemistry parameters were directly influenced by treatment and dose of HES 130/0.4. Blood glucose (breakdown product) increased by a maximum of 23.1% compared to control at the 6th week (males and females alike) and declined to 11% of control values at 13 weeks in the 90 mL/kg group. The recovery period allowed for the return of blood glucose to baseline levels. Significant, but lesser increases in blood glucose levels were seen in the 60 mL. Blood urea increased by a maximum of 82 % versus control in the 90 mL/kg group and declined to 33.4% by week 13. The 4 recovery allowed for resolution to control levels in female but male animals. Total cholesterol in 90 mL/kg group increased to a maximum of 59.9% compared to control which declined to 39.3% at week 13. Complete resolution was not observed at 4 weeks of recovery and total cholesterol remained 28.7% higher than control values. The same pattern of increase was seen in the 60 mL/kg dose group but to a lesser degree. α -amylase activity increased dose dependently to a maximum of 45.6% in the 90 mL/kg group then declined to 15.9 % higher than control at week 13 and completely resolved over the 4 week recovery period. The remaining biochemical marks were not influenced by treatment.

Urinalysis revealed a decrease in urine volume in the low dose compared to the control group, but in general the urine decreased in all the HES 130/0.4 treated animals. It is unclear if this is a volume effect or an ADH dependent effect. Urine specific gravity increased in all HES 130/0.4 treated animals, while pH decline. The affected urinary parameters returned to control levels after 4 weeks of recovery.

Macroscopic examination of tissue indicated no substance related pathological changes after 13 weeks of repeated dose treatment with HES 130/0.5 60 mL/kg or 90 mL/kg. The weight of heart, liver, kidney and adrenals increased significantly in all starch treated animals. These findings are likely the result of prolonged exposure rather than short term exposure. This indicates that the properties of the starch as it influences volume and as it is metabolized likely caused these effects.

Microscopic evaluation demonstrated vacuolation in the liver, kidneys, urinary bladder and mammary glands. The histopathology was a dose dependent finding. Evidence of recovery from vacuolation in the liver, kidneys, urinary bladder and mammary glands was potentially occurring at 4 weeks of recovery.

Conclusions - The human equivalent dose derived from this study demonstrates a 630 g/day dose in a 70 kg human and a total 13 week exposure of 57 kg HES 130/0.4. Most of the findings in this study were resolvable, however the increase in heart, liver, kidney and adrenals weight was not and the histopathologic findings of liver, kidneys, urinary bladder and mammary glands vacuolation was revealed minor recovery after 4 weeks. This study suggests a minimal risk from HES 130/0.4 with short term use.

8993/1/94 - 13 week subchronic toxicity study of HES 10% 130/0.5 administered to dogs by intravenous infusion over three hours each day

Objective – This study represents the primary toxicity study in a large animal species (Beagle dog) following repeated daily dosing for 13 weeks. The primary end points were to determine the presence of dose limiting toxicity to all organ systems.

Methodology – The following groups were evaluated: (Group1) – 90 mL isotonic solution/kg over 3 hours; 60 mL HES 130/0.4 10%/kg over 3 hours; 90 mL HES 130/0.4 10%/kg over 3 hours daily for 13 days. The distribution of animals evaluated was Group 1 (4 male/4 female), Group 2 (3 male/3 female), Group 3 (4 male/4 female) and 4 additional dogs were allocated to the evaluation of recovery (4 weeks) of any treatment induced toxicity. The dose was determined during a 14 day pilot study in which the sponsor found a decrease in food consumption at 90 mL/kg, therefore 90 mL/kg was taken as the maximum dose.

List of observations

Clinical signs (daily)

Mortality

Body weight (daily)

Food and water consumption (daily)

Hematology (6th week, 13th week and at 28 days following dosing for recovery animals)

Differential blood count

RBCs

WBCs

Hemoglobin

Reticulocytes

Platelets

Thromboplastin time

Activated partial thromboplastin time

MCV

MCHC

Clinical chemistry (6th week, 13th week and at 28 days following dosing for recovery animals)

Electrolytes

α-amylase

Total bilirubin

Total cholesterol

Albumin

Creatinine

Total protein

Glucose

Blood urea nitrogen

ALT

Alkaline phosphatase

ASAT/GOT

LDH

Urinalysis (6th week, 13th week and at 28 days following dosing for recovery animals)

Color
Specific gravity
Protein
Glucose
Billirubin
Hemoglobin
Ketones
pH
urobillogen
nitrite

Electocardiography (day 1 before and after dosing, 6th week, 13th week and at 28 days following dosing for recovery animals)

Circulatory functions (day 1 before and after dosing, 6th week, 13th week and at 28 days following dosing for recovery animals)

Pathology/Histopathology (13th week and at 28 days following dosing for recovery animals)

All organs

Results –

Local tolerance of infusion at the vein indicated macroscopically visible induration of tissue occurring from 8 weeks onward. Microscopic evaluations of the tissue surrounding the injection site indicate inflammatory and degenerative changes with a similar incidence and severity compared to control dogs with both the 60 and 90 mL/kg dose.

Normal behavior and appearance was observed in all animals throughout the treatment period.

No treatment induced changes in body weight were observed.

Food intake of the 60 and 90 mL/kg dose groups declined significantly (28% reduction at 6 weeks post dosing) 6 weeks compared to the control group, which was likely the cause of the high caloric content of the starch since body weight did not change (the same observation was made in the rodent study). Drinking water consumption was not influenced by

treatment. Food consumption trended toward normalization by the end of the 4 week study period.

Ocular changes did not occur in the HES 130/0.5 treated animals.

No treatment related influence was found in the ECG recordings of dogs treated for 13 weeks with HES 130/0.5. The heart rate, P-Q, Q-T, and QRS intervals were within the normal range.

Peripheral arterial systolic blood was not affected by treatment.

hematological parameters were influenced in dose dependent fashion in the 60 mL/kg and the 90 mL/kg dose groups compared to the control group:

Hematology parameters were minimally changed, however the total hemoglobin decreased by 15.6% compared to the control at week 6 in the 90 mL/kg female animals, while all other HES 130/0.5 treated animals showed decline that did not reach statistical significance. This corresponded to an increase in reticulocytes and a decline RBCs and hematocrit. These findings are potentially caused by mass dosing of HES causing a continuous hemodilution effect (similar to the findings seen in the rat). Erythrocyte sedimentation increased to a maximum of 168% in the 90 mL/kg HES 130/0.4 dosed animals versus control animal values by week 13. Sedimentation rates declined to normal over the four week recovery period.

Certain clinical chemistry parameters were directly influenced by treatment and dose of HES 130/0.4. The profile affected parameters differ quite substantially compared to the rat. Albumin decreased by a maximum of 14.2% compared to control at the 6th week (males and females alike) and declined further to 23% of control values at 13 weeks in the 90 mL/kg group. The recovery period allowed for the return of albumin to baseline levels. Significant, but lesser increases in albumin levels were seen in the 60 mL and followed the same pattern of decline from week 6 to week 13 to recovery. ALAT activity increased by a maximum of 123% of control at 13 weeks, but returned to control levels over 4 week recovery period. Like in the rat α -amylase activity increased dose dependently, but to maximum of 82% in the 90 mL/kg group and remained the same (79.4 %) higher than control at week 13. The increase in α -amylase activity did not completely resolve over the 4 week recovery period. The 4 week recovery period value remained 33.8% higher than the control. The remaining biochemical marks were not influenced by treatment.

Urinalysis in the dog revealed no treatment related effects

Macroscopic examination of tissue indicated numerous foci, pale discoloration and enlargement. The surface of the spleen was described as coarse and grained in two low and two high dosed animals no substance related pathological changes after 13 weeks of repeated dose treatment with HES 130/0.5 60 mL/kg or 90 mL/kg. The weight of liver, kidney and adrenals increased significantly in all starch treated animals. These findings are likely the result of prolonged exposure rather than short term exposure. This indicates that the properties of the starch as it influences volume and as it is metabolized likely caused these effects.

Microscopic evaluation demonstrated vacuolation in the liver, kidneys and urinary bladder. Extramedullary hematopoiesis was seen in the spleen and hypertrophy was noted in the zona glomerulosa of the adrenal gland. The histopathology was a dose dependent finding. After 4 weeks of recovery vacuolation in the liver, kidneys, urinary bladder vacuolation was less widespread.

Conclusions: The human equivalent dose derived from this study demonstrates a 630 g/day dose in a 70 kg human and a total 13 week exposure of 57 kg HES 130/0.4. Most of the findings in this study were resolvable, however the increase in liver, kidney and adrenals weight was not and the histopathologic findings of liver, kidneys, urinary bladder and mammary glands vacuolation was revealed minor recovery after 4 weeks. The increase in extra medullary hematopoiesis was likely the cause of long term hemodilution. This study suggests a minimal risk from HES 130/0.4 with short term use.

#9004/94- Mutagenicity study of HES 130/0.4 10% infusion solution in the salmonella typhimurium reverse mutation assay in vitro

#9005/94- Mutagenicity study of HES 130/0.4 10% infusion solution in mammalian cells [REDACTED] in the in vitro gene mutation assay [REDACTED]

#9005/94 – In vivo bone marrow cytogenetic test of HES 130/0.5 10% infusion solution by intravenous administration in Sprague Dawley rats.

Objective - Determine the potential for HES 130/0.4 to induce mutation in vitro and in vivo

Methodology - The three standard methods of evaluating genotoxicity were evaluated using the Salmonella typhimurium reverse mutation assay, [REDACTED] gene mutation assay [REDACTED] and the in vivo bone marrow cytogenetic test in Sprague Dawley rats

Results - Maximal concentrations of HES 130/0.4 did not induce mutagenic effects beyond the acceptable fold response in any of the three assays.

Conclusion - The genotoxic potential of HES 130/0.4 was evaluated in studies using the Salmonella typhimurium reverse mutation assay, [REDACTED] gene mutation assay [REDACTED] and the in vivo bone marrow cytogenetic test in Sprague Dawley rats. None of the assays demonstrated evidence for HES 130/0.4 genotoxicity. As a result long term carcinogenicity studies have not been conducted and are not necessary.

#9008/94 – Examination of the influence of HES 10% (130/0.5) on the pregnant rat and fetus by intravenous administration

Objective – Evaluate the effect of HES 10% 130/0.5 on the pregnant rat and the fetus during the critical phase of organogenesis.

Methodology – Four groups of pregnant rats (day 0) were evaluated: Group 1 – 50 mL/kg saline, Group 2 – 12.5 mL/kg HES 130/0.4, Group 3 – 25 mL/kg HES 130/0.4 and Group 4 – 50 mL/kg. Animals were dosed for the first twenty days of pregnancy at which time the uterus and ovaries were removed and thereafter the fetus was collected.

List of observations

Clinical signs (daily)

Body weight (daily)

Food consumption (daily)

Fetal Examination (after day 20)

Clinical signs (daily) – none observed

Body weight (daily) – no change

Food consumption (daily) – significant reduction

Fetal Examination (after day 20) - No effect on the fetuses upto a dose of 12.5 mL HES 130/0.4 10% per kg body weight per day. At 25 mL/kg of HES slight decrease in food

consumption and dyspnea were observed. At 50 mL/kg of HES resulted in a decrease in mean fetal body weight and an increase in the incidence of fetal skeletal retardations.

Conclusion – It is clear both from the repeated dose toxicity studies in rats and dogs that food consumption significantly reduces. It therefore is reasonable to assume that the food consumption influences of long term starch administration have profound effects on fetal development with long use.

Reviewer's findings

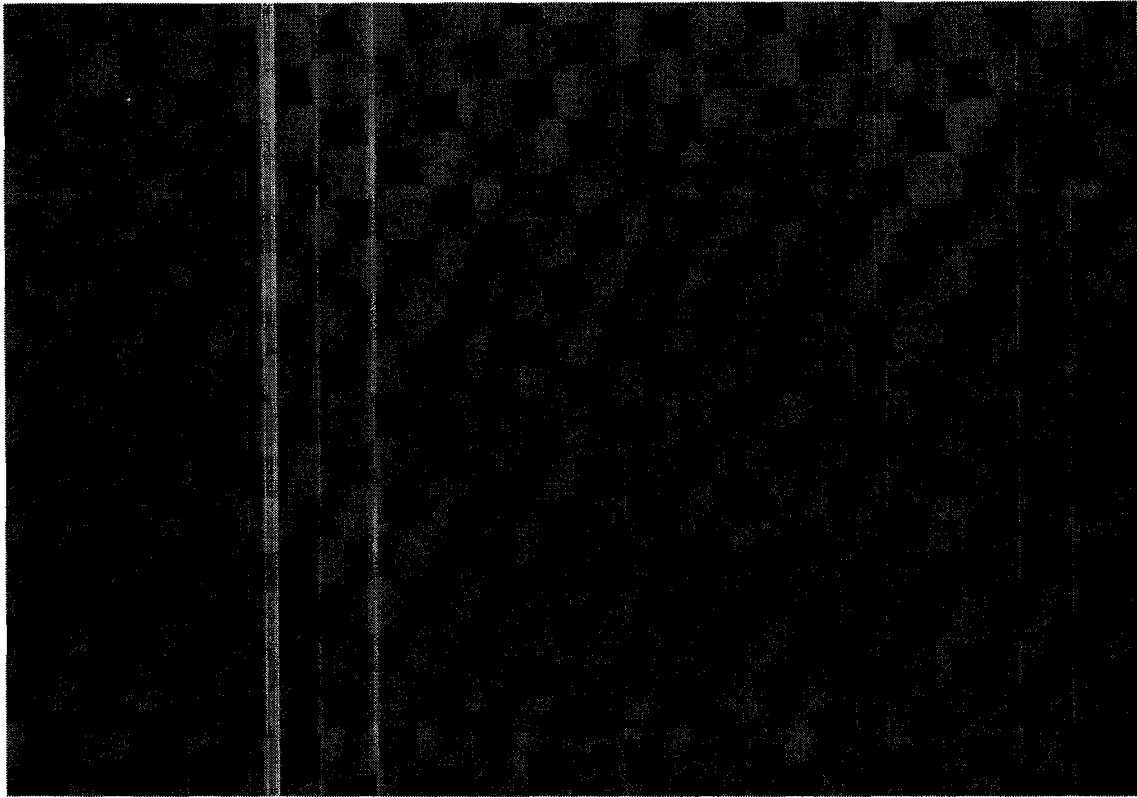
1. The clearance and overall exposure of HES 130/0.4 is less than hyperbranched starch when evaluated in rodents. The differences are primarily seen as a 25% lesser AUC_{0-inf} in HES 130/0.4 dosed animals compared to hyperbranched starch dosed animals. The metabolism of HES 130/0.4 is likely via the liver and spleen and the elimination occurs via the kidneys. The pharmacokinetics translated to pharmacodynamic effects such as less sustained hemodynamic responses.
2. Long term dosing with HES at 3 times the maximum clinical dose (**based on clinical trial dosing**) not anticipated maximal dosing can be deduced from the repeated dose toxicology studies in rat and dog and demonstrate a dose equivalent of 630 g/day in a 70 kg human and a total 13 week exposure of 57 kg HES 130/0.4. When taking into account the fact that 6% HES 130/0.4 is proposed for human use as approximately 2 g/kg the primary toxicology studies in rodent and non-rodent species represents a 3 fold excess of the clinical dose evaluated in the rat and dog.
3. There is a potential for coagulation disorders with starches however the non-clinical data do not clearly support this for HES 130/0.4 and the short half-life should improve the chances of not impairing hemostasis.
4. HES 130/0.4 is not a mutagenic substance and as a result long term carcinogenicity studies are not required
5. Maternal and fetal development are affected by low dose HES 130/0.4 in the critical periods of pregnancy when used long term. This danger to the mother and developing fetus is dependent on daily dosing over a long period of time.

6. In the short term in normal animals HES 130/0.4 demonstrates minimal toxicity. The organs most influenced by dosing are the liver kidney and potentially the heart and spleen with long term administration.
7. Since the intended use of HES 130/0.4 is short term the potential for safety concerns in otherwise normal individuals is reasonable. However there is a known potential risk of kidney failure with excessive dosing. The non clinical studies do not clearly define this risk, however, use of the product in renal and hepatic failure warrants caution.

Recommendation:

Voluven®, (6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride infusion) is approvable from a pharmacology/toxicology standpoint.

Labeling changes-non-clinical toxicology:



food on kidney and liver and the clear signs of uptake and storage of hydroxyethyl starch by the cells of the

