

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

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**Date:** 07/19/2018  
**To:** BLA 125668/0  
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**Through:** Dorothy E. Scott, M.D., Chief, PDB, DPPT, OTAT  
**Applicant:** Octapharma Pharmazeutika Produktionsges.m.b.H  
**Product:** Cutaquig, immune globulin subcutaneous (human) 16.5% liquid solution  
**Subject:** Preclinical Pharm-Tox Review

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## Background and Summary

Cutaquig (also referred with its IND name, (b) (4) in this review) is a 16.5% immune globulin preparation intended for subcutaneous administration and indicated for the treatment of Primary Immunodeficiency (PI) in adults.



Cutaquig is a liquid formulation; the most abundant constituents, including active ingredients, excipients and process related impurities are shown in Table 1.

Table 1. Constituents of Cutaquig

Ingredients/Impurities	Highest amount in Cutaquig (from final specifications)	Highest amount in other IG approved products
Total protein	(b) (4)	(b) (4), Hizentra
Maltose	(b) (4)	(b) (4), WinRho
Polysorbate 80, PS80	NMT* (b) (4)	NMT* (b) (4), Flebogamma
Tri(n-butyl)phosphate, TNBP (impurity)	(b) (4)	(b) (4), Flebogamma (b) (4), Octagam 5 and 10%
Octoxynol-9, Triton X-100 (impurity)	(b) (4)	(b) (4), Octagam 5 and 10%

\*NMT=No more than

The recommended dose for Cutaquig is individualized for each patient based on the previous IGIV or IGSC dose. A potential high dose administration would be ~375 mg/kg every week<sup>1</sup>. The single highest dose administered in the clinical trial was 390 mg/kg or 2.4 mL/kg; this dose is used to calculate the potential exposures for all ingredients in the final product (Table 2).

Table 2. Potential Exposures after one Administration of Cutaquig

Ingredients	Highest amount in Cutaquig (from final specifications)	High dose from Cutaquig <sup>2</sup>	Exposure from a high dose of Cutaquig <sup>3</sup>
Total protein	(b) (4)		
Maltose			
Polysorbate 80, PS80			
Tri(n-butyl)phosphate, TNBP (impurity)			
Octoxynol-9, Triton X-100 (impurity)			

## Recommendation

This reviewer did not identify any animal pharmacology and toxicology studies that would prevent this BLA from being approved.

## Main Findings, Nonclinical Studies

Given the existing safety database for IGIV products, the toxicology program for Cutaquig was tailored to the new subcutaneous preparation and consisted of two safety pharmacology and a local tolerance study.

<sup>1</sup> Weekly subcutaneous dose derived from a high IGIV dose of 1 g/kg would be: (b) (4) =375 mg/kg

<sup>2</sup> Calculated for a 2.4 mL/kg administration volume, the highest single dose volume administered in the supporting clinical trial

<sup>3</sup> Calculated for a 75 kg subject

Additional studies performed with the solvent/detergent (b) (4) used during manufacturing and present as impurity were also submitted as supportive information and summarized here. The main findings from these studies are listed below.

- 1) There were no adverse effects attributed to Cutaquig in any of the safety studies.
  - a) When administered to rabbits at a dose volume of 2.4 mL/kg, ~1x highest human dose used in the clinical trial, Cutaquig did not display any thrombogenic properties.
  - b) When administered to dogs at 3 mL/kg, 1.25x highest human dose used in the clinical trial, Cutaquig did not show cardiovascular adverse effects or potential for QT interval prolongation.
  - c) A single subcutaneous injection of 5 mL Cutaquig in rabbits did not cause any adverse effects systemically or at the local injection site. This dose volume is lower than the label recommended administration volume for each single site (25 – 40 mL/site). Clinical trial data (please refer to the clinical review) was used to support the label.
- 2) The efficacy/proof of concept study performed in mice indicated dose-related improvement in survival in a sepsis model in (b) (4) mice.
- 3) Cutaquig contains maltose and polysorbate 80 (PS80) as excipients. These substances are present in other approved IGIV products and their presence and quantity in Cutaquig do not raise toxicologic concerns.
- 4) Cutaquig contains TNBP and Triton X-100 (Octoxynol-9), two process related impurities deriving from the solvent detergent treatment. These impurities are present at amounts equal to those in the approved product Octagam. In acute and sub-chronic toxicology and genotoxicity studies exposure to these impurities at doses multiple times higher than human dose was determined to be a No-Observed-Effect-Level (NOEL) (see [Formulation](#) for more details).

## Complete Review

### Local Tolerance

Study No. 29970

Title: Local Tolerance Test of (b) (4) 16.5% and a Comparator in Rabbits after a Single Subcutaneous Administration

Date: 26 Sep 2013

Performing Laboratory: (b) (4)

Animal species: (b) (4) rabbits

The aim of the study was to obtain information on the local tolerance of (b) (4) in comparison with a reference item (Hizentra) in rabbits.

Study design: In this GLP study, n=2/group/sex rabbits weighing 4.3-4.7 kg and aged 6-7 months received a single dose of 5 mL of either (b) (4) or reference article (Hizentra diluted to 165 mg/kg with saline) subcutaneously at the left dorsal flank; all animals received 0.8% saline in the right dorsal flank as the negative control as shown in Table 3.

Table 3: Groups and administration articles

Group	Administration		Animal no.
	Left side Test item / Reference	Right side Control	
1	(b) (4) 16.5%	0.9% NaCl solution	1, 2 m 3, 4 f
2	Hizentra 16.5%	0.9% NaCl solution	5, 6 m 7, 8 f

m = male  
f = female

Outcome measures. Clinical signs and mortality were checked and recorded daily until 4 days post administration. Local reactions were inspected macroscopically 2, 24, 48, and 96 hours after administration and scored based on Draize scoring (Table 4).

Table 4: Scoring of local reactions

<b>Erythema and eschar formation</b>	<b>Value</b>
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth) preventing erythema reading	4

  

<b>Edema formation</b>	<b>Value</b>
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined By definite raising)	2
Moderate edema (raised approx. 1 millimeter)	3
Severe edema (raised more than 1 millimeter And extending beyond area of exposure)	4

After sacrifice 4 days post administration, the injection sites for both test items and 0.9% NaCl control, were collected, fixed in 10% buffered formalin, sectioned (3 and 5 µm), stained with H&E and assessed by histopathology.

Results: There were neither systemic nor local toxicities observed in this study.

## Safety Pharmacology

Study No. 29972

Title: Examination of (b) (4) 16.5% Formulations for Thrombogenic Properties in Rabbits after Intravenous Administration

Date: 25 Oct 2013

Performing Laboratory: (b) (4)

Animal species: (b) (4) rabbits

The aim of the study was to assess the test items for thrombogenic properties in rabbits after intravenous administration.

Study design: In this GLP study, n=3 male rabbits/group weighing 1.8-2 kg and aged 3 months received a single dose of (b) (4) 2.4 mL/kg at a rate 10 mL/min in the marginal ear vein contralateral to the ligated jugular vein. After a stasis of 10 min the content of the vein was emptied and scored. Design of the study is shown in Table 5 and the scoring sheet on table 6.

Table 5: Study design

Group	Test item	Stasis time	Dose level	Application volume	Strain	Animal nos. and sex
1	(b) (4) 16.5% batch no. DgG-13-0004	10 min	0.4 g/kg	2.4 mL/kg	(b) (4)	1 - 3 m
2	(b) (4) 16.5% batch no. DgG-13-0005	10 min	0.4 g/kg	2.4 mL/kg	(b) (4)	4 - 6 m
3	FEIBA (positive control)	10 min	30 U/kg	2.4 mL/kg	(b) (4)	7 - 9 m
4	Physiological saline (negative control)	10 min	0 g/kg	2.4 mL/kg	(b) (4)	10 - 12 m

Table 6: Scoring Sheet

Score	Observation
0	no clot
0.5	a few macroscopic strands of fibrin are barely visible
1.0	a few macroscopic strands of fibrin
1.5	one or several thrombi $\leq 1.5$ mm in length or diameter
2.0	one or several thrombi $> 1.5$ mm in length or diameter
2.5	several thrombi $> 2$ mm $\leq 3$ mm in length or diameter
3.0	one large thrombus $> 3$ mm in length or diameter
3.5	two or more large thrombi $> 3$ mm in length or diameter
4.0	a single thrombus forming a cast of the isolated segment

Results: (b) (4) administered IV had no thrombogenic effect; the positive control item FEIBA (Factor Eight Inhibitor Bypassing Activity) produced score of 4 at a dose level of 30 U/kg.

Reviewer's conclusions: The intended route of administration was not used in this study, However, IV administration can be considered a "worst case scenario" for thrombogenic effects, thus it is acceptable. The deviations from the study design (no statistical analysis due to 0 scores, shorter acclimatization and a higher volume of pentobarbital used for euthanasia) did not affect the integrity of the study.

#### Study No. 29971

Title: Assessment of cardiovascular effects and potential for QT interval prolongation after single s.c. administration of SCIG (b) (4) 16.5% in telemetered conscious dogs

Date: 25 Sept 2013

Performing Laboratory: (b) (4)

Species: (b) (4) dogs

Aim: to assess the cardiovascular effects and the potential for QT interval prolongation of (b) (4) 16.5% (batch no. DgG-13-0004) in telemetered (b) (4) dogs following single subcutaneous administration of 500 mg/kg.

Study design: n=4 male (b) (4) dogs weighing 10-12 kg and aged 33-36 months were surgically fitted with radiotelemetry device and allowed to acclimatize for 4 weeks then administered 500 mg/kg or 3.03 mL/kg (b) (4). Each animal served as its own control via the baseline data available.

Outcome measures: Clinical signs, physical activity, body temperature, body weight, hemodynamic measurements including direct blood pressure, BP (systolic, diastolic and mean arterial pressure, derived), and ECG for 72 hours. Baseline measurements for BP were recorded for 24 hours prior to dosing. The ECG was visually assessed for any abnormalities of the electrical complexes (i.e. arrhythmias, interval prolongations) and heart rate (beats/min), QRS interval (ms), QT interval (ms), PQ interval (ms) (equivalent to PR interval), RR interval (ms); QTc values were calculated according to the van de Water and Fridericia formulas. Blood was collected at 0, 0.5 and 72 hours post dose to ascertain exposure.

Results: Concentration of human IgG is shown in Table 7; the IG concentration data ascertains that exposure to the administered drug occurred at 72 hrs following administration of 500 mg/kg (b) (4).

Table 7: IgG concentration in dog serum in study #29971 (from Octapharma Validation Report #020STD81x.200/00)

Samples	Serum Clarity	Degree of Hemolysis*	Ig-G content (mg/ml)	Remarks
<b>Dog Serum 1:</b>				
Pre-dose	clear	±	<0.001	
0.5 h p.a	clear	±	0.17	
72 h p.a	clear	±	5.4	
<b>Dog Serum 2:</b>				
Pre-dose	clear	±	<0.001	
0.5 h p.a.	clear	+	0.01	Dilution effect noted!
72 h p.a.	clear	±	3.9	
<b>Dog Serum 3:</b>				
Pre-dose	clear	±	<0.001	
0.5h p.a.	clear	±	0.06	
72 h p.a	clear	±	4.6	
<b>Dog Serum 4:</b>				
Pre-dose	clear	±	<0.001	
0.5 h p.a	clear	±	0.05	
72 h p.a	clear	±	4.9	

\*: ± (trace of hemolysis noted)

+ (slightly hemolyzed)

No test item-related local or systemic toxicity were noted. No test item-related influence was measured on systolic, diastolic or mean arterial blood pressure, heart rate, RR interval, QRS interval, QT interval, QTc value (van de Water), QTc value (Fridericia), PQ interval, physical activity or body temperature. No signs were noted for a prolongation of the QT interval or the QTc values.

### Primary Pharmacodynamics

Study Number: RR-059-13

Title: Efficacy of (b) (4) 16.5 % in a dose response study design against *Streptococcus pneumoniae* in the mouse sepsis model, Study No.: RR-059-13,

Performing Laboratory: (b) (4)

Date: 16 Sept 2013

Aim: To test efficacy of (b) (4) 16.5% given subcutaneously in three different dose levels, in the mouse sepsis model induced by *Streptococcus pneumoniae* (b) (4) in (b) (4) female mice.

Study Design: This non-GLP proof-of-concept study 1) determined the mortality in (b) (4) mice following IP administration of *Streptococcus pneumoniae* bacteria in a preliminary study and 2) assessed the effect that administration of (b) (4) had in mortality.

Either negative control (660 mg/kg human albumin) or (b) (4) (batch DgG-13-004) at doses of 165, 330 and 660 mg/kg, were administered to 6 female (b) (4) mice/group weighing 24-26 by subcutaneous injection (site not specified). 48 hours following test article administration, one of six dilutions of *Streptococcus pneumoniae* were inoculated intraperitoneally at a inoculum of 0.5 mL/mouse. Animals were observed for six days following inoculation and mortality was recorded.

Results: Following (b) (4) administration, a dose dependent improvement in mortality was observed, up to 144 hrs or 6 days post-infection (Table 8).

Table 8: Mortality from *S. pneumoniae* of mice on day 6 post-infection

<b>S. pneumoniae</b>		<b>% mortality</b>			
<b>CFU/mouse</b>	<b>Log<sub>10</sub> CFU/mouse</b>	<b>Human albumin 660 mg/kg</b>	<b>(b) (4) 16.5%</b>		
			<b>165 mg/kg</b>	<b>330 mg/kg</b>	<b>660 mg/kg</b>
390 000	5.59	100		100	83
39 000	4.59	83	100	66	16
3 900	3.59	100	83	50	0
390	2.59	83	16	16	0
39	1.59	100	0	0	0
3.9	0.59	100	0	0	0
0.39	-0.40		0		
<b>LD<sub>50</sub> (CFU/mouse)</b>		<3.9	1 266	5 923	126 683
<b>Log<sub>10</sub>LD<sub>50</sub></b>		<0.59	3.10	3.77	5.10

## Formulation

- Cutaquig contains maltose and polysorbate 80 (PS80) as excipients.
  - Maltose is present at NMT (b) (4). This amount is (b) (4) the amount of maltose contained in Octagam 5% and 10%. Due to the higher concentration of IG in Cutaquig, patients receive lower volumes of administration, and thus lower amounts of maltose after receiving Cutaquig compared with the approved IGIV products. Based on the existing safety database, there are no toxicologic concerns due to the presence of maltose in Cutaquig.
  - PS80 is present at (b) (4). This amount is similar to other approved IG products, such as Flebogamma (NMT (b) (4)). Based on existing safety database, the amount of PS80 in Cutaquig does not raise toxicologic concerns.
- Cutaquig contains TNBP and Triton X-100 (Octoxynol), two impurities that are process related (deriving from the solvent detergent treatment) and present at amounts equal to those in the approved product Octagam. Acute and sub-chronic toxicology and genotoxicity studies were performed with these impurities at multiple doses and the conclusions are listed below.
  - The maximum exposure to TNBP + Triton X-100 from ((b) (4)) Cutaquig would be (b) (4) from a high dose of 390 mg/kg (2.4 mL/kg). In animal studies, when administered at doses multiple times higher than this human dose, TNBP+Triton X-100 (b) (4) was safe locally and systemically in rats, and systemically in dogs.
  - Acceptable safety margins were seen following single-dose toxicity studies in rats with TNBP + Triton X-100.

- i. NOEL was 3,160 mcg/kg, corresponding to a human equivalent dose, HED (b) (4) times higher the highest dose from Cutaquig.
- ii. The lowest toxic (LOEL) dose was 10 mg/kg, or corresponding to HED ~ (b) (4) times higher than the highest human exposure from Cutaquig. At this dose, toxicity in rats included dyspnea, mydriasis, ataxia, and reduced muscular tone.
- c. Acceptable safety margins were seen following repeated daily intravenous administration of TNBP + Triton X-100 in sub-chronic (13-week) studies in rats and dogs.
  - i. NOEL for systemic effects in rats was 360 µg/kg/day, corresponding to HED (b) (4) times higher than exposure from Cutaquig administration.
    1. At a daily dose of 1800 µg/kg in rats, corresponding to equivalent to human dose of (b) (4) times higher than weekly exposure from Cutaquig, there was one mortality due to pulmonary edema. Multiple thrombi at the injection site were also seen at this dose and attributed to the very high rate of injection. A decrease in reticulocytes was also seen at this dose.
  - ii. NOEL in dogs for systemic toxicity was 78 µg/kg/day TNBP+Triton X-100, corresponding to HED (b) (4) times higher than the exposure form weekly administration of Cutaquig.
    1. LOEL in dogs was 300 µg/kg, corresponding to HED (b) (4) or approximately (b) (4) times higher than highest chronic human exposure. Only local injection site adverse effects (“hardened veins” and multiple thrombi), likely due to the high injection rate were seen at this dose.
    2. At a dose 3000 µg/kg/day, corresponding to a HED of (b) (4) or more than (b) (4) times higher than human exposure a decrease in hematocrit, hemoglobin and RBC numbers, associated with increased RBC sedimentation were seen.
- d. TNBP:Triton X-100 (b) (4) was not teratogenic in pregnant rats and rabbits at equivalent doses multiple times higher than expected human exposure following use of Cutaquig.
  - i. NOEL during organogenesis in rabbits was 900 µg/kg/day corresponding to HED (b) (4) times higher than exposure following repeated administration of Cutaquig.
  - ii. LOEL during organogenesis in rabbits was 2700 µg/kg/day. This dose corresponds to a HED approximately (b) (4) times higher than the highest exposure following repeated administration of Cutaquig. These impurities caused a slight increase in fetal resorption and a moderate but statistically significant reduction in fetal body weight.
  - iii. No test-article related effects were observed in pregnant rats at doses up 5400 µg/kg/day or a HED (b) (4) times higher than highest expected human dose.
- e. TNBP:Triton X-100 (b) (4) was not genotoxic or mutagenic *in vitro* in bacterial or mammalian cells, and *in vivo* in rats.
- f. A pharmacokinetic study was performed in rats following intravenous administration of (b) (4) µg of TNBP/kg and (b) (4) µg Triton X-100/kg corresponding to equivalent to human dose of (b) (4) times higher than weekly exposure from Cutaquig. The elimination half-life of TNBP was approximately 20 minutes; 2 hours after injection, TNBP was no longer detectable. Triton X-100 was not detected at any time.

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<sup>4</sup> Human Equivalent Dose, HED calculations based on “Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers”, Table 1.



## Impurities, Repeated Dose Toxicity Studies

### *Examination of the influence of TNBP + Triton X-100 (b) (4) on the pregnant rabbit and the fetus by intravenous administration, Study (b) (4) 6087/90*

Performing Laboratory: (b) (4); study performed under GLP.

Date study initiated: 6/1990

Animal Species and study design: 12 Female (b) (4) rabbits per group, each weighing 2.04 – 2.50 kg and aged 4 - 4.5 months received an intravenous injection (ear vein) of (b) (4) of TNBP +Triton X-100 at doses 300, 900, 2700 mcg/kg in 5 ml saline or vehicle daily during organogenesis, i.e. on gestation days (GD) 6-18. On GD29 fetuses were removed via C-sections, and maternal effects were assessed via gross pathology.

Doses were determined on a pilot, dose-finding study.

Sex and viability of fetuses were determined, as well as fetal number, size, weight or resorptions. Fetuses were inspected for external and internal gross malformations, including skeletal malformations.

Statistical calculations: Analysis of variance and Student's t-test were carried out;  $p < 0.01$  was considered significant. Classification measurements were evaluated using  $\chi^2$  test ( $p < 0.05$ ).

Results: There were no local or systemic effects at the low dose which was considered NOEL. At high dose, there were maternal local reactions at the site of injection (swelling, necrosis). 3/12 and 10/12 dams of middle and high dose, respectively, had placenta with discolorations, accompanied in the high dose with hematoma.

There was 1/48 malformed fetus in the study (low dose), which was considered spontaneous and within the reference ranges. At the high dose, resorption rate was slightly increased to 14.4% (control: 7.6%), body weight of fetuses was moderately and significantly decreased, associated with decreased food consumption for the dams, and the number of runts was slightly increased.

#### Conclusions

Under the conditions of this experiment, TNBP/Triton X-100 (b) (4) was not teratogenic. NOEL for fetal development was the middle dose used – 900 mcg/kg/day, or the equivalent of (b) (4) times higher than the highest weekly dose in humans. The lowest observed toxic effect level (LOEL) for dams and embryos was 2,700 mcg/kg/day during organogenesis. The toxicities included increased resorption rate, moderately decreased body weight of fetuses and increased number of runts/litter.

### *Examination of the influence of TNBP + Triton X-100 (b) (4) on the pregnant rat and the fetus by intravenous administration, Study (b) (4) 6086/90*

Performing Laboratory: (b) (4)

GLP

24 female (b) (4) rats, weighing 205-249 g, received 10 mL/kg solution of either negative control, or 600, 1800, or 5400 mcg TNBP + TRITON X-100 /kg from day 6 to 15 of pregnancy. On GD 20 the rats were laparotomised, the ovaries and uterus removed and examined.

Results: Highest dose led to pain reactions, edemas and necrosis of the injection site of almost all dams. None of these effects were observed in the low and middle dose groups.

Four fetuses in 1/24 litters in low dose and 9 fetuses in 2/24 litters in middle dose were found to have skeletal malformations (shifted and fused dorsal, lumbar and coccygeal vertebrae, short tail and, in middle dose, uni- or bilateral crossed legs). No malformed fetuses were detected at high dose. Given the lack of dose-response relationship, the low frequency, and the background occurrence of these malformations, these were not considered test-article related. Under the test conditions, the systemic NOEL for maternal and fetal effects is the highest dose tested.

*13-week Subchronic Toxicity Study of TNBP + Triton X-100 (b) (4) by Intravenous Administration to (b) (4) Rats, Study (b) (4) 5568/1/89*

Performing Laboratory: (b) (4)

Aim: To obtain information on subchronic toxicity of the vehicle of Cutaquig.

Design: N=180 rats, 130 test article and 50 control.

20 or 25 animals/sex/dose (see table below), age 4 weeks, weight 72-83 g, dosed IV at 10 ml/kg for 15 sec. Dose levels used were: (b) (4) µg/kg/day TNBP+Triton X-100. Control, low and medium dose groups were treated for 13 weeks.

Due to high local irritation at week 3, high dose group was treated for 6 weeks, 20 animals prematurely sacrificed and 5 animals allowed to have 4 weeks of recovery and sacrificed week 10.

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1	Control – 10 ml/kg water	20M, 20F 13 weeks 5M, 5F 6 weeks	5M, 5F 4 weeks
2	(b) (4) µg/kg TNBP+Triton X-100	20M, 20F 13 weeks	None
3	(b) (4) µg/kg TNBP+Triton X-100	20M, 20F 13 weeks	None
4	(b) (4) µg/kg TNBP+Triton X-100	25M, 25F 6 weeks	5M, 5F 4 weeks

Outcome measurements:

Local injection site observation, mortality, examination of eyes, hearing, dentition (not clear how were determined), clinical chemistry, hematology, urinalysis, necropsy, organ weighing, microscopic histopathology for the high dose and control group. The middle dose group only the following organs were microscopically examined: injection site, kidneys, liver, and lungs.

Results: NOEL for local and systemic effects: (b) (4) µg/kg, NOEL for systemic effects was (b) (4) µg/kg. Middle dose showed local effects including thrombosis and necroses of the tail. Thrombosis, perivascular fibrosis, and necrosis were observed commonly in the highest dose and the dosing had to be discontinued at week 6. These findings were partially or completely reversed after 4 weeks recovery. One animal in the high dose group dead from pulmonary edema; another in this group and one in the low dose group died on week 12 but no cause of death was given and histopathology was un-remarkable. Males in high dose had a decrease in reticulocyte numbers; causal relationship to dosing is unclear.

Reviewer Conclusions: A dose of (b) (4) µg/kg/day TNBP+Triton X-100 or HED (b) (4) (b) (4) times dose form Cutaquig) shows no effect in rats. A dose of (b) (4) µg/kg/day TNBP + Triton X-100 (HED (b) (4) ) or more than (b) (4) times dose of Cutaquig causes local irritation in rats when injected at 10 ml/kg/day, every day for 13 weeks at a rate 40 ml/kg/min (10 ml/kg for 15 sec). These local effects could be due to the osmotic changes at such a rapid rate of infusion compared to the intended use of the test item (sub-cutaneous administration at a rate not to exceed 20 mL/hr/site). Clinical studies were used to support the volume/speed of infusion in the clinical trial.

*13-week subchronic toxicity study of TNBP+Triton X-100 (b) (4) by intravenous administration to (b) (4) Dogs, Study 5569/1/89*

Performing Laboratory: (b) (4)

Aim: To obtain information on subchronic toxicity of the vehicle of Cutaquig in (b) (4) dogs.

Design: N=28 (b) (4) Dogs, 7-14 months old, weighing 6.4 – 10 kg, dosed with a (b) (4) TNBP and Triton X-100 IV at a volume of 1ml/kg for 15 sec alternately in the legs.

Dose: Dose and control groups are shown in the table below:

Group	Treatment	Number of Animals (#/sex/group)
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		Treatment phase	Recovery phase
1	Control – 1 ml/kg/day water	3M, 3F 13 weeks 1M 1F 8 weeks	1M, 1F 4 weeks
2	(b) (4) µg/kg TNBP+Triton X-100	3M, 3F 13 weeks	None
3	(b) (4) µg/kg TNBP+Triton X-100	3M, 3F 13 weeks	None
4	(b) (4) µg/kg TNBP+Triton X-100	4M, 4F 8 weeks	1M, 1F 4 weeks

Outcome Measurements: Mortality, local tolerance, clinical observation and functional assessment (vision, hearing, dentition), body weight, food consumption, EKG, blood pressure, hematology, clinical chemistry, urinalysis, organ weights, necropsy, histopathology.

Results: At a dose (b) (4) µg/kg TNBP+Triton X-100 4/6 animals showed slight and sometimes moderate local effects such as slight and moderately hardened veins weeks 7-13. At the highest dose, all animals showed slight and moderately hardened veins weeks 2-6 which become markedly hardened on weeks 7 and 8.

Thrombi were observed in histopathology of local injection site in all dose levels - 2/6 in low dose, 1/6 in middle dose, 6/6 in high dose. Histopathology of injection site of recovered animals was unremarkable. There was decrease of hematocrit, hemoglobin and RBC numbers and increase in sedimentation of RBC in the high dose group.

Reviewer Conclusions: NOEL for systemic toxicity was (b) (4) µg/kg/day TNBP+Triton X-100 or <sup>(b) (4)</sup> times higher than the exposure from weekly administration of Cutaquig. There were local injection site thrombotic findings at all doses. The local thrombogenicity become severe at doses between 300 µg/kg TNBP+Triton X-100 and 3,000 µg/kg TNBP+Triton X-100. Given that such findings were not observed in the clinical studies, they are likely due to the rate of infusion - 4 ml/kg/min and an injection artifact.

## Impurities, Other GLP Toxicity Studies

### *Pharmacokinetics of TNBP+Triton X-100*

Study Number 6149/90

This study was performed under GLP conditions

Dose (b) (4) µg/kg TNBP+Triton X-100 administered IV, N=4/dose point (2M and 2F)

Design: 20M and 20F (b) (4) Rats, weighing 150-173 g were treated IV with TNBP+Triton X-100 (b) (4).

Outcome measurements:

Blood for analysis was taken at these time-points: 0, 5, 15 and 30 min, 1, 2, 3, 4, 8, and 24 hr, urine and feces analysis at 0-4 hr, 4-8 hr and 8-24 hr.

Triton X-100 and TNBP were determined by (b) (4) respectively.

Results: There was no Triton X-100 observed in serum, urine or feces.

For TNBP, Tmax was 5 minutes, Cmax 156.2 ng/mL, the elimination half-life was ~ 20 minutes; 2 h after the injection, TNBP was no longer detectable. No TNBP could be detected in urine and very small amounts of TNBP were excreted in the feces – approximately 0.005%-0.96% of the dosage administered.

Study (b) (4) 6343/90

Examination of the Acute Toxicity of TNBP + Triton X-100 (b) (4) by Intravenous Administration to

(b) (4) Rats

Design: N=24 (b) (4) Rats, 3M and 3F /group receiving 3.16, 10, and 100 mg/kg corresponding to (b) (4) mg TNBP and mg Triton X-100 respectively

(b) (4). Volume of injection was 10 ml/kg injected in 15 sec.

Outcome measurements: Clinical observation, mortality.

Results: NOEL 3.16 mg/kg; LOAEL was 10 mg/kg and these were the effects: dyspnea, mydriasis, ataxia, reduced muscular tone. There were no adverse findings at pathology.

A similar study was performed using IP administration (number 7724/92) in 18 M and 15 F rats at 21.5, 46.4, 100, 147 and 215 mg/kg in 3M and 3 F and 316 mg/kg only in 3 M rats. LOAEL (ataxia, muscular hypotonia) occurred at 46.4 mg/kg in M and F rats.

Study (b) (4) Number: 6345/90

Title: Examination of the Acute Toxicity of Triton X-100 by Intravenous Administration to (b) (4)

Rats

Design: 12 F (b) (4) Rats receiving triton X-100 (b) (4) at doses 4.64, 10, 21.5, 46.4 mg/kg once IV. Volume of injection was 10 ml/kg injected in 15 sec.

Results: NOEL 4.64 mg/kg; LOAEL was 10 mg/kg; dyspnea, mydriasis, ataxia, reduced muscular tone all described as “low or moderate severity”.

There were no findings at pathology.

Although the study report includes detailed summaries but no individual animal data, this is acceptable for a supporting/dose finding study.

Study Number: (b) (4) 6345/90

Title: Examination of the Acute Toxicity of TNBP by Intravenous Administration to (b) (4)

Rats

Design: 15 F (b) (4) rats, 3/group receiving TNBP (b) (4) at doses 2.15, 4.64, 10, 21.5, and 46.4 mg/kg once IV. Volume of injection was 10 ml/kg injected in 15 sec.

Results: NOEL 2.15 mg/kg; LOAEL was 4.64 mg/kg; reduced motility, dyspnea, and ataxia, all of low severity.

No findings at pathology.

There are detailed summaries but no individual animal data.

Study Number: Triton X-100 (b) (4) 5124/88:

Examination of the Acute Toxicity of Triton X-100 by IP Administration to (b) (4) Mice

Animal model – (b) (4) mice, weight 18-23 g, aged 26-35 days

Design and dose: 5 mice/sex/group were injected IP with 10.7, 33.7, 107, 129, 157, 190, and 229 mg/kg triton X-100 in (b) (4) at a volume of 20 ml/kg.

Outcome measurements: Cageside observation, mortality up to day 14.

Results: NOEL at 10.7 mg/kg; LOAEL at 33.7 (ataxia and dyspnea), Lowest Lethal Dose (LLD) (3/10) - 129 mg/kg, calculated LD<sub>50</sub> in 14 days - 145 mg/kg.

Study Number: (b) (4) 5123/88

Title: Examination of the Acute Toxicity of TNBP by Intraperitoneal Administration to (b) (4) Mice.

Animal Model: (b) (4) Mice

Design and dose: N= 35 M and 35 F mice, 5/sex/group dosed with 45.3, 144, 453, 549, 665, 806, 977 mg/kg TNBP in (b) (4) IP at a volume 20 ml/kg.

Outcome measurements: Cage side observations, mortality up to day 14.

Results:

NOEL at 45.3 mg/kg; LOAEL at 144 mg/kg, LLD was 549 mg/kg, LD<sub>50</sub> in 14 days was 605 and 669 mg/kg in M and F respectively.

Study Number: (b) (4) 5125/88

Title: The Examination of the Acute Toxicity of TNBP + Triton X-100 (b) (4) by Intraperitoneal Administration to (b) (4) Mice

Animal Model: (b) (4) Mice

Design and dose: N=5/group/sex dosed with TNBP+Triton X-100 (b) (4) of 10.6, 33.5, 106, 128, 160, 189, 228, mg/kg IP.

Outcome measurements: Cageside observation, mortality up to day 14

Results: NOEL at 10.6 mg/kg; LOAEL 33.5 (ataxia and dyspnea), LLD is 128 mg/kg (3/10); calculated LD<sub>50</sub> in 14 days is 141 mg/kg.

Study Number: (b) (4) 5128/88

Title: The Examination of the Acute Toxicity of TNBP + Triton X-100 ((b) (4)) by Intraperitoneal Administration to (b) (4) Rats

Animal Model: (b) (4) Rats

Design and dose: N=5/group/sex dosed with 4.9, 15.6, 49.2, 72.3, 106, 156, 228 and 335 mg/kg ((b) (4)) TNBP+Triton X-100 (b) (4) .

Outcome measurements: Cageside observation, mortality up to day 14

Results: NOEL at 4.92 mg/kg; LOAEL was determined at 15.6 mg/kg; LD<sub>50</sub> was calculated at 126 mg/kg in M and 128 mg/kg in F rats.

#### Impurities, Genotoxicity

*Mutagenicity Study of TNBP + Triton x-100 ((b) (4)) in the Ames Salmonella, Microsome Plate Test (in vitro), Study 6088/90*

Performing laboratory: (b) (4)

GLP

Design: TNBP + TRITON X-100 ((b) (4)) was examined for mutagenic effect in 5 Salmonella typhimurium strains: (b) (4)

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(b) (4)

(b) (4)

(b) (4)

Conclusions: No mutagenic effect was observed for TNBP + TRITON X-100 ((b) (4)) tested up to cytotoxic concentrations in any of the tester strains in two independent experiments with and without metabolic activation.

*Mutation Study of TNBP + Triton X-100 ((b) (4)) in Mammalian Cells ((b) (4)) in vitro, Study 6089/90*

Performing laboratory: (b) (4)

GLP

Study Design: (b) (4)

Government	Percentage
Current government	85%
Previous government	15%

(b) (4)

Conclusions: TNBP+Triton X-100 were not considered mutagenic in this experiment.

*Micronucleus Test of TNBP + Triton X-100 (b) (4) in Bone Marrow Cells of the (b) (4) Rat, study 6091/90*

Five male and five female (b) (4) rats per sampling interval per group (130 rats total) weighing 98 to 141 g were randomized and water for injection or TNBP+Triton X-100 was administered once by intravenously at 10 mL/kg. The doses used were: (b) (4) (the two highest doses were in the range of the maximum tolerated dose level). Cyclophosphamide (25 mg/kg i.p.) was used as a positive control. Three sampling times were used: 16, 48 and 72 hours after administration (except for the positive control, for which only one sampling time, 48 hours, was used). Outcome measures: upon sacrifice, the femur was isolated and the bone marrow was collected, and a smear was prepared on a slide, fixed in solvent methanol, stained and microscopically analyzed. 10,000 polychromatic erythrocytes per animal were scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was also determined for each animal. Results: There were no differences in dose and negative control group on the frequency of micronuclei formation or the frequency of polychromatic RBCs. Positive control significantly increased the frequency of micronuclei and decreased the frequency of polychromatic RBCs.

(b) (4)

*In Vivo Bone Marrow Cytogenetic Test of TNBP + Triton X-100 (b) (4) by Intravenous Administration to (b) (4) Rats (chromosomal analysis), Study 6090/90*

Design: Similar to the micronucleus test with the difference that chromosomal analysis was performed at 6, 24 (the only time point for the positive control) and 48 hours after administration. Bone marrow was fixed overnight with methanol/glacial acetic acid, stained in 10% Giemsa, mounted and analyzed at a magnification of 1000x. The mitotic index was determined by counting the number of metaphases per 1000 cells. The analysis for structural aberrations such as gaps, breaks, chromacity etc. was performed for 50 cells per animal.

Results: For all time points TNBP + TRITON X-100 (b) (4) did not depress the mitotic index. The mean incidence of chromosomal aberrations (excluding gaps) in treated animals ranged from 0.6 to 2.2% at all three sampling time-points and not significantly different than vehicle controls (1.2-1.6%). The number of gaps was also within the range of the controls (treated groups: 2.0 to 5.0%; controls: 2.2 to 3.6%).

The positive control - cyclophosphamide - induced significant levels of chromosomal aberrations.

Reviewer Conclusion: There were no chromosomal aberrations following TNBP/Triton X-100 administration in rats at 48 hours.

*Other Genotoxicity Studies*

*Micronucleus Test with the Test Compound Tributylphosphate - Called TNBP - on Bone Marrow Cells of Treated (b) (4)-Mice*

Performing laboratory: (b) (4)

GLP study

TNBP was administered to (b) (4) mice in dose levels of 5, 10 or 20 mg/kg intravenously. No cytotoxicity could be found in any of the test groups at any time point sampled. The rate of polychromatic RBCs with micronuclei was within the values of the placebo control in all preparations after 16, 48 and 72 hours: 0.17 - 0.29% (controls), 0.13 - 0.28% (test groups). Animals receiving positive control (100 mg methyl methane sulfonate/kg by gavage), had significantly higher cells with micronuclei 16 hours after the administration (3.48% polychromatic erythrocytes with micronuclei).

*TNBP (b) (4) Assay in Vitro in (b) (4) Cells*

(No number, performed Nov 5, 1986)

Performing laboratory: (b) (4)

GLP study



TNBP was tested up to a cytotoxic dose with and without metabolic activation to evaluate its effect in (b) (4) frequency. The mean (b) (4) frequency of the solvent controls ranged from 7.8 to 9.1 (b) (4)/cell. Cells treated with TNBP showed a similar mean (b) (4) frequency ranging from 7.0 to 8.6 (b) (4)/cell without and 8.2 to 9.6 (b) (4)/cell with metabolic activation.