



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

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**Applicant:** Octapharma

**Product:** Panzyga

**Subject:** Preclinical Pharm-Tox Review

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## **Contents**

Background.....	2
Main Findings, Nonclinincal Studies.....	3
Conclusions.....	5
Complete Review.....	5
Primary Pharmacology (Proof-of-Concept Study).....	5
Safety Pharmacology .....	5
Thrombogenicity.....	5
Cardiovascular safety.....	8
Local Tolerance .....	10
Study on Local Tolerance after Single Intravenous, Intra-Arterial and Paravenous Administration in Rabbits with NewGam (IVIG10%), Study 30211 .....	10
"NewGam (IVIG 10 %)": Study on Local Tolerance after Intravenous, Intra-arterial and Paravenous Administration in Rabbits, Study (b) (4)-L-2848 .....	10
Toxicology, Single Dose Toxicity Studies .....	11

Single Dose Toxicity Study of NewGam (IVIG 10 %) in (b) (4) Rats, # 170.110.1260.....	11
Single dose toxicity study of NewGam in (b) (4) Mice, Study 170.110.1259.....	12
Toxicology, Repeated Dose Toxicity Studies.....	13
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 .....	13
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 .....	13
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.....	13
13-week subchronic toxicity study of TNBP+Triton X-100 (b) (4) by intravenous administration to (b) (4) Dogs, Study 5569/1/89.....	14
Other GLP Toxicity Studies.....	15
Genotoxicity.....	16
Mutagenicity Study of TNBP + Triton x-L00 (b) (4) in the Ames Salmonella, Microsome Plate Test (in vitro), Study 6088/90.....	16
Mutation Study of TNBP + Triton X-100 (b) (4) in Mammalian Cells (b) (4) in vitro, Study 6089/90 .....	18
Micronucleus Test of TNBP + Triton X-100 (b) (4) in Bone Marrow Cells of the (b) (4) Rat, study 6091/90 .....	19
In Vivo Bone Marrow Cytogenetic Test of TNBP + Triton X-L00 (b) (4) by Intravenous Administration to (b) (4) Rats (chromosomal analysis), Study 6090/90.....	19
Other Genotoxicity Studies.....	20
Pharmacokinetic Studies.....	20
"NewGam (IVIG 10%)": Pharmacokinetic Study in Rabbits, Study (b) (4) --L-2920 .....	20
Pharmacokinetics of TNBP+Triton X-100 .....	21

## Background

Panzyga is an immune globulin intravenous (human) preparation manufactured by Octapharma. The sponsor is seeking approval for Panzyga when used for two indications: the treatment of primary humoral immunodeficiency (PI) and treatment of chronic immune thrombocytopenia (ITP) in adults. Doses for these indications are shown in Table 1.

Panzyga is a 10% liquid formulation containing glycine as a (b) (4) (Table 2). Impurities in final preparation include TNBP and octoxynol (Triton X-100) (the solvent and detergent used for viral clearance) and (b) (4) (used during purification process).

Table 1: Panzyga, Proposed Doses

Indication	Dose
PI	300-600 mg/kg every 3-4 weeks
Chronic ITP in adults	1 g/kg daily for 2 consecutive days

Table 2: Panzyga, Select Specifications

Ingredients	Concentration	Function
Total protein	(b) (4) mg/ml	
Immunoglobulins	(b) (4) mg/mL or 96% of total protein	Active ingredient
pH	4.5-5.0	
Glycine	15.0 – 19.5 mg/ml	(b) (4)
(b) (4)	(b) (4)	(b) (4)
Octoxynol (Triton X-100)	(b) (4)	Detergent, Impurity
TNBP	(b) (4)	Solvent, Impurity

### Main Findings, Nonclinical Studies

Nonclinical studies submitted in support of this BLA were performed with two batches of Panzyga final product, a nonclinical material with the same formulation as Panzyga (manufactured with an earlier process that did not include (b) (4)), and with the solvent/detergent (b) (4) of the same proportion (b) (4)) as in final product. The main findings from these studies are listed.

1. In single dose toxicity study in mice a preclinical preparation of Panzyga did not cause dose-dependent toxicity when administered at doses up to 16.7 times the intended human dose or 5 times higher than a single dose of 2 g/kg. There were dose independent findings such as spleen and lymph node enlargement, sometimes associated with red discoloration. These findings could be related to the host immune response to the human biologic.
2. In single dose toxicity study in rats, a dose of a preclinical preparation of Panzyga 3.3 times higher than human dose for PI (or equal to the total 2 g/kg dose given over two days for ITP) was tolerated. The only toxicities noted were unqualified lung discolorations. Similar findings were also seen in vehicle and in saline controls.
3. There was no thrombogenic activity of clinical or preclinical preparations of Panzyga in the Wessler rabbit model when dosed at 1 g/kg, equal to 1.7 times maximum human dose for PI or equal to the one –day dose (half of the total dose) for ITP.
4. A nonclinical preparation of Panzyga caused no effects in blood pressure in spontaneously hypertensive rats or bronchospastic effects in guinea pigs at doses similar to human dose.
5. There was no local toxicity in rabbits when 10 mL Panzyga was injected intravenously.
6. Panzyga 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.

- a. The maximal exposure to TNBP + Triton X-100 from (b) (4) Panzyga would be (b) (4) from a dose of 600 mg/kg or (b) (4) from a single dose of 2 g/kg given over two days for ITP. In animal studies, when administered at doses equivalent to these human doses, TNBP+Triton X-100 (b) (4) was safe locally and systemically in rats, and systemically in dogs.
- b. Safety margins following single-dose toxicity studies in rats with TNBP + Triton X-100.
  - i. No observable effect level (NOEL) was 3,160 mcg/kg, corresponding to an equivalent human dose (b) (4) times higher the highest dose from Panzyga given for ITP or PI, respectively.
  - ii. The lowest toxic (LOEL) dose was 10 mg/kg, or corresponding to equivalent human dose approximately (b) (4) times higher than the highest human exposure from Panzyga. At this dose, toxicity in rats included dyspnea, mydriasis, ataxia, and reduced muscular tone.
- c. Safety margins 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 equivalent human dose (b) (4) times higher than exposure from Panzyga for PI (chronic) 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 Panzyga, 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. No causal relationship to the administered article was established.
  - ii. NOEL in dogs for systemic toxicity was 13+65 µg/kg/day TNBP+Triton X-100, corresponding to equivalent human dose (b) (4) times higher than the exposure form weekly administration of Panzyga.
    1. LOEL in dogs was 300 µg/kg, corresponding to equivalent human dose approximately (b) (4) times higher than highest chronic human exposure; only local injection site adverse effects, likely due to the high injection rate were seen at this dose.
    2. At human equivalent doses (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 embryotoxic or teratogenic in pregnant rats and rabbits at equivalent doses multiple times higher than expected human exposure following use of Panzyga. These impurities, when administered daily during organogenesis in rabbits at an equivalent dose approximately 7 -24 times higher than the highest exposure following single or repeated administration of Panzyga, respectively caused a slight increase in fetal resorption and a moderate but statistically significant reduction in fetal body weight. These effects were not observed in pregnant rats.
- 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) of TNBP/kg and (b) (4) Triton X-100/kg. 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.

## Conclusions

There are no pharmacology/toxicology issues that would prevent this BLA from being approved.

## Complete Review

### Primary Pharmacology (Proof-of-Concept Study)

Study Number: 023613

Performing Laboratory: (b) (4)

Study Title: **Evaluation of Two Batches of NewGam (IVIG 10 %) in a Dose Response Study Design against *Streptococcus pneumonia* in the Mouse Sepsis Model**

#### Non-GLP

Study Design: 7-weeks-old female (b) (4) mice weighing 19 – 27 g (6 animals/group) received either of two batches of the non-clinical preparation of Panzyga (IND name NewGam) at doses of 150, 300 and 600 mg IgG/kg or negative control (12 mL/kg Albumin 5%) intravenously via the tail vein 20 hours before intraperitoneal challenge (0.5 mL) with one of the five 10-fold dilutions of *Streptococcus pneumoniae*. Animals were observed for six days following inoculation and mortality was recorded.

Outcome measure: mortality

Results: All of the mice treated with the human albumin died. A significant dose response was observed between the Panzyga treatment arms. The log<sub>10</sub>LD<sub>50</sub> for the 150, 300 and 600 mg/kg dose of NewGam was 0.5; < 1.5 and 3.5 (batch 081006) and 0.9, 3.1 and 4.9 (batch 081122), respectively.

**Table 3.** Mean lethal dose (LD<sub>50</sub>) of *S. pneumoniae* in mice treated with two batches of Panzyga, 081006 and 081122.

<i>S. pneumoniae</i>		% mortality						
CFU/mouse	Log <sub>10</sub> CFU/mouse	Human albumin	Batch 081006			Batch 081122		
			150 mg/kg	300 mg/kg	600 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
340000	5.5			100.0	100.0		100.0	100.0
34000	4.5	100.0	100.0	100.0	83.3	100.0	66.7	33.3
3400	3.5	100.0	100.0	100.0	50.0	100.0	50.0	0.0
340	2.5	100.0	100.0	100.0	16.7	100.0	50.0	0.0
34	1.5	100.0	100.0	83.3	0.0	100.0	16.7	0.0
3.4	0.5	100.0	50.0			16.7		
<b>LD<sub>50</sub> (CFU/mouse)</b>			3	<34	3,162	7	1,301	88,269
<b>Log<sub>10</sub>LD<sub>50</sub></b>			0.5	<1.5	3.5	0.9	3.1	4.9

### Safety Pharmacology

Safety pharmacology studies to assess possible thrombogenic potential, hypotensive activity and anaphylactoid reactions of Panzyga were conducted in rats, guinea pigs, and rabbits.

Thrombogenicity

**TITLE: SAFETY PHARMACOLOGY STUDY ON THE THROMBOGENIC POTENTIAL IN RABBITS, STUDY NO 30210**

Performing laboratory: (b) (4)

GLP

Design: 20 male (b) (4) rabbits (5 animals/group) aged ~3 months, weighing 1.8 - 2.3 kg at start of administration were randomized and anesthetized via a cannula in a marginal ear vein. 1 to 2 cm segment of the contralateral external vena jugularis was exposed, its tributaries were ligated. Either 1 g/kg Panzyga clinical batch #B321A821/U, B325B826/U, positive (FEIBA) or negative (buffer) controls were administered within 15 minutes into the contralateral ear via the cannula in place as a bolus with injection speed ~10 ml/min. Within 25 seconds after completion of the injection, the previously exposed vena jugularis was gently ligated and stasis maintained for 10 minutes. The vein segment was then removed from the animal, its contents were emptied into a Petri dish containing 30 mL of a 5% sodium citrate solution and the clotting status scored based on a scoring table. At the end of the experiment all animals were terminated.

Group	Test item	Stasis time	Test item dose	Application volume	Strain	Animal # and sex
1	NewGam batch no. B321A821/U	10 min	1 g/kg	10 mL/kg	(b) (4)	1 - 5 m
2	NewGam batch no. B325B826/U	10 min	1 g/kg	10 mL/kg	(b) (4)	6 - 10 m
3	FEIBA (positive control)	10 min	100 U/kg	2 mL/kg	(b) (4)	11 - 15 m
4	Glycine buffer (negative control)	10 min	0 g/kg	10 mL/kg	(b) (4)	16 - 20 m

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: The total score of both tested batches was 0 indicating no thrombogenic risk. FEIBA served as positive control item to ensure the sensitivity of the test system, and showed the maximum possible score of 4. The negative control isotonic saline had a total score of 0.

Conclusion: Two batches of Panzyga when administered at a dose 1 g/kg (1.7x chronic dose) in (b) (4) rabbits did not reveal any thrombogenic effect.

#### **SAFETY PHARMACOLOGY STUDY ON THE THROMBOGENIC POTENTIAL IN RABBITS, STUDY 25046/1 GLP, study performed by the same lab and with the same design as the study before.**

Design: 20 male rabbits (5 animals/group) aged 2.5 - 4.5 months, weighing 1.9 - 2.5 kg at start of administration were randomized and anesthetized. 1 to 2 cm segment of the external vena jugularis was exposed, its tributaries were ligated. Either NewGam<sup>1</sup> batch A946C851/U, A944D851/U or positive (FEIBA) or negative (buffer) controls were administered within 15 seconds into a marginal vein of the

<sup>1</sup>NewGam batches used in this and some other animal studies were preclinical preparations manufactured with an older version of the manufacturing process that did not include (b) (4).

ear. Within 25 seconds after completion of the injection, the previously exposed vena jugularis was gently ligated and stasis maintained for 10 minutes. The vein segment was then removed from the animal, its contents were emptied into a Petri dish containing 30 mL of a 5% sodium citrate solution and the clotting status scored based on a scoring table. At the end of the experiment all animals were terminated.

Results and conclusions: The total score of both tested batches was 0 indicating no thrombogenic risk. FEIBA treated animals had thrombi with the maximum score of 4. Two non-clinical batches of Panzyga when administered at a dose 1 g/kg in (b) (4) rabbits did not reveal any thrombogenic effect.

Group	Test item	Dose level	Application volume	Animal # and sex
1	Glycin buffer (negative control)	10 mL/kg	10 mL/kg	1 - 5 m
2	NewGam, batch A946C851/U	1 g/kg	10 mL/kg	6 - 10 m
3	NewGam, batch no. A944D851/U	1 g/kg	10 mL/kg	11 - 15 m
4	FEIBA (positive control)	100 U/kg	2 mL/kg	16 - 20 m

score of 0	no clot
score of 1	a few macroscopic strands of fibrin
score of 2	several small thrombi
score of 3	two or more large thrombi
score of 4	a single thrombus forming a cast of the isolated segment

#### EXAMINATION OF NEWGAM (IVIG 10%) ON THROMBOGENIC RISK IN RABBITS AFTER INTRAVENOUS ADMINISTRATION - BASED ON WESSLER ET AL., STUDY (b) (4) 22200

Performing Laboratory: (b) (4)

Animal model: (b) (4) Rabbit

Design: 10 M rabbits/dose/group weighing 2-2.5 kg aged 2.5 - 8 months were dosed IV (ear vein) with two different batches of NewGam, KIOVIG, formulation buffer or Factor VIII as positive control; 25 sec after dosing, exposed vena jugularis was ligated, kept in situ for 10 minutes then emptied in a Petri dish and examined and scored for thrombi.

score of 0	no clot
score of 1	a few macroscopic strands of fibrin
score of 2	several small thrombi
score of 3	two or more large thrombi
score of 4	a single thrombus forming a cast of the isolated segment

Group	Test item	Dose level	Number and sex of animals	Animal nos.
1	Formulation buffer (negative control)	0 (5 mL/kg)	10 male	1 - 10
2	NewGam 10% solution, (Batch no. A745D851/D)	500 mg/kg	10 male	11 - 20

3	NewGam 10% solution (Batch no. A746C851/L)	500 mg/kg	10 male	21 - 30
4	KIOVIG 10% solution (active control)	500 mg/kg	10 male	31 - 40
5	FEIBA Batch VNF2F004 (positive control)	40 U/kg	10 male	41 - 50

Results: The test items NewGam (IVIG 10%) (Batches A745D851/D and A746C851/L), the active control KIOVIG 10% solution and the negative control did not show any thrombogenic effect. The mean score was 0.0 (n = 10 animals per group).

The mean score of the positive control group treated with FEIBA (Factor Eight Inhibitor Bypassing Activity) was 3.7 (n = 10 animals) with two or more large thrombi or a single thrombus forming a cast of the isolated segment.

#### Cardiovascular safety

#### ANTI-HYPERTENSIVE EFFECT OF NEWGAM (IVIG 10%) FOLLOWING INTRAVENOUS ADMINISTRATION TO SPONTANEOUS HYPERTENSIVE (SH) RATS, STUDY <sup>(b) (4)</sup> 22202

Performing Laboratory: <sup>(b) (4)</sup>

Aim: To examine the anti-hypertensive effects of NewGam (IVIG 10%) following intravenous administration to spontaneous hypertensive rats.

Animal Model: spontaneous hypertensive rats

Design: 60 M rats aged 8-9 weeks and weighing 208-276 g were dosed IV via a vena jugularis catheter with two batches of NewGam, KIOVIG, Vivaglobin (positive control) and saline and NewGam formulation buffer controls at 10 ml/kg for 45 sec. The arterial pressure was monitored for 20 minutes after the injection via the carotid artery and recorded.

Dose:

Group	Test item	Dose level [mg/kg]	Number and sex of animals	Animal no.
1	0.9% NaCl solution (control)	0	10 M	1 – 10
2	NewGam (IVIG 10%) Batch 1: A 745A851/D	1000	10 M	11 – 20
3	NewGam (IVIG 10%) Batch 2: A 746A851/L	1000	10 M	21 – 30
4	KIOVIG Batch LE12G062AF (active control)	1000	10 M	31 – 40
5	Vivaglobin (positive control)	100	10 M	41 – 50
6	NewGam Formulation buffer (negative control)	0	10 M	51 – 60

#### Outcome measurements:

Positive reaction was a decrease of more than 30% lasting for more than 1 minute. Mean arterial pressure is also recorded and reported at -10; 0; 2; 5; 10; 15; and 20 min after administration.

No test item-related influence was noted on the mean arterial blood pressure for the ten animals treated intravenously with 1000 mg NewGam (IVIG 10%) (A 745A851 ID)/kg or the ten animals treated intravenously with 1000 mg NewGam (IVIG 10%) (A 746A851 IL) per kg, i.e. no anti-hypertensive effects were noted.

No test item-related influence on the mean arterial blood pressure was noted for the ten animals treated intravenously with the active control KIOVIG at 1000 mg/kg.



All ten tested animals treated intravenously with the positive control Vivaglobin at 100 mg/kg, revealed a positive reaction, i.e. a decrease in the mean blood pressure by  $\geq 30\%$ , compared to the predose value, within 10 minutes after administration lasting for  $\geq 1$  minute.

Reviewer conclusions: There were no hypotensive effects within 20 minutes of dosing with 1.7X human dose in anesthetized rats.

#### EXAMINATION OF BRONCHOSPASTIC EFFECTS OF NEWGAM BY INTRAVENOUS ADMINISTRATION TO GUINEA PIGS STUDY <sup>(b) (4)</sup> 22201

Performing Laboratory: <sup>(b) (4)</sup>

Aim: to examine the bronchospastic effects of the test item NewGam (IVIG 10%) following single intravenous administration to male guinea pigs.

Design: N=60M anesthetized guinea pigs, 10/group were dosed aged 6-15 weeks and weighing 351-553 g were dosed IV via a bolus in vena jugularis catheter with two batches of NewGam, KIOVIG, Vivaglobin (positive control) and saline and NewGam formulation buffer controls at 10 ml/kg for 45 sec. The trachea was cannulated and animals ventilated. The respiratory pressure was monitored via a Y connector and the arterial pressure was monitored through a carotid catheter.

Group	Test item	Dose level [mg/kg]	Number of animals and sex	Animal no.
1	0.9% NaCl solution (negative control)	0	10 m	1 – 10
2	Formulation buffer (negative control)	0	10 m	11 – 20
3	NewGam (IVIG 10%) Batch A745B851/D	1000	10 m	21 – 30
4	NewGam (IVIG 10%) Batch A746B851/L	1000	10 m	31 – 40
5	KIOVIG 10% solution (active control)	1000	10 m	41 – 50
6	Vivaglobin (positive control, batch no. 02140611A)	100	10 m	51 – 60

#### Outcome Measurements:

The pulmonary inflation pressure and the blood pressure were recorded (the systolic blood pressure was only used to decide on the entry of the animals into the study. Animals with a systolic blood pressure  $< 40$  mmHg would have been excluded from the study).

The initial value of the pulmonary inflation pressure (immediately before administration of the test item) and the pulmonary inflation pressure at 10 and at 20 minutes p. a. were documented. A positive reaction was defined as an increase of  $\geq 30\%$  which lasted for  $\geq 1$  minute in pulmonary inflation pressure. In addition, the pulmonary inflation pressure at 10 and at 20 minutes p. a. were evaluated.

Results: No test item-related influence was noted on the pulmonary inflation pressure of the ten animals treated intravenously with 1000 mg NewGam (A745B851/D)/kg or 1000 mg NewGam (A746B851/L)/kg. No test item-related influence was noted on the pulmonary inflation pressure of the ten animals treated with the active control KIOVIG 10 solution at 1000 mg/kg. No test item-related influence on pulmonary inflation pressure was noted for the animals treated with the negative controls 0.9% NaCl solution or Formulation buffer at 10 mL/kg. All ten tested animals treated with the positive control Vivaglobin at 100 mg/kg revealed an increase in the pulmonary inflation pressure by more than 30% at 10 and 20 minutes after the injection, compared to the predose value. In addition, the positive reaction lasted more than 1 minute within the first 10 minutes post administration in all ten tested animals.

Conclusion: There were no bronchospastic reactions associated with the use of NewGam in guinea pigs at a dose 1.7X human dose.

### Local Tolerance

Study on Local Tolerance after Single Intravenous, Intra-Arterial and Paravenous Administration in Rabbits with NewGam (IVIG10%), Study 30211

Performing Laboratory: (b) (4)

Test article: Panzyga Clinical Batches B321B821/U and B325A826/U; stability data provided.

Design: 12 male (2.2 - 2.8 kg) and 12 female (2.3 - 2.8 kg) (b) (4) rabbits aged ~10 weeks were randomized and acclimated (34 days). Two animals/sex/group received the test item (Panzyga batch B321B821/U or batch B325A826/U) on the right ear, and the reference item (Glycine buffer) on the left ear. The administration was a) single intravenous infusion of 10 mL into the marginal vein of the ear b) single intra-arterial infusion of 10 mL into the central artery of the ear and c) single paravenous injection of 0.1 mL beside the marginal vein of the ear. The routes b) and c) were chosen to account for erroneous administration of Panzyga.

Group	No. of animals, sex	Animal nos.	Dose volume per animal	Duration of the administration	Route
Groups 1-3: New Gam batch no. B321B821/U (left) and reference (right)					
1	2 m	1, 2	10 mL	60 min	Intravenous
	2 f	101, 102			
2	2 m	3, 4	10 mL	10 min	Intra-arterial
	2 f	103, 104			
3	2 m	5, 6	0.1 mL	approx. 10 sec	Paravenous
	2 f	105, 106			
Groups 4-6: New Gam batch no. B325A826/U (left) and reference (right)					
4	2 m	7, 8	10 mL	60 min	Intravenous
	2 f	107, 108			
5	2 m	9, 10	10 mL	10 min	Intra-arterial
	2 f	109, 110			
6	2 m	11, 12	0.1 mL	approx. 10 sec	Paravenous
	2 f	111, 112			

### Outcome measures

Clinical signs and mortality were checked and recorded daily.

Local reactions were inspected macroscopically 30 min, 1 h, 2 h, 6 h, 24 h, 48 h, 72 h and 96 h after administration in the appropriate animals and reactions scored based on DRAIZE scoring. Four days after administration all animals were sacrificed and infusion/injection sites for test and reference items were collected, fixed in Bouin's solution and paraffin sections (3 to 5 µm; two per animal) were prepared, stained with haematoxylin-eosin, and examined histologically.

### Results

Intraarterial infusion of 10 mL NewGam caused moderate perivascular hemorrhage in one female and dermatitis in another female. Similar results were seen in controls. Animals that received intravenous Panzyga injections did not show any findings macro- or microscopically.

"NewGam (IVIG 10 %)": Study on Local Tolerance after Intravenous, Intra-arterial and Paravenous Administration in Rabbits, Study (b) (4)-L-2848

Performing Laboratory: (b) (4)

GLP Study

Test substances used: NewGam (IVIG 10 %), batch A746C851/L and A745B851/D; these batches were manufactured with an older iteration of the process that did not include (b) (4).

KIOVIG, batch No. LE12G062AF

Model: (b) (4) Rabbits weighing 1.6 – 2.2 kg

Design: two batches of NewGam, KIOVIG (active control), NewGam formulation buffer and isotonic saline were administered once intravenously, intraarterially or paravenously to auricular vessels of 2 M and 2F per substance and per route.

Doses:

Intravenous: 10 mL test substance per animal, given as an infusion within 60 min (app. 0.08 ml/kg/min). This corresponds to 450 mg/kg or more; it does not cover the whole range of the dose intended for human use.

Intra-arterial: 10 mL test substance per animal, given as infusion within 10 min (app 0.5 ml/kg/min).

Paravenous: 0.5 mL test substance per animal, given as an injection.

Outcome measurements:

Local reactions and signs and were necropsied 72 h after administration and subjected to a histopathological examination of the injection sites.

Results:

Intravenous and intraarterial administration:

No local changes were found in life and no histopathological abnormalities in both NewGam batches.

Paravenous administration of NewGam, mild local inflammation was found in 4/8 animals. This effect was less than observed with KIOVIG (4/4 animals).

Reviewer Conclusion:

There are no local reactions to NewGam or its formulation when used at this dose. Paravenous use may cause local inflammation.

### ***Toxicology, Single Dose Toxicity Studies***

Single Dose Toxicity Study of NewGam (IVIG 10 %) in (b) (4) Rats, # 170.110.1260

Performing Laboratory: (b) (4)

Aim: To evaluate the acute toxicity of 2 different batches of NewGam (IVIG 10 %) and potential differences in toxicity between the two batches and between the NewGam (IVIG 10 %) batches and the reference item KIOVIG administered intravenously at a dose of 2000 mg IgG/kg to (b) (4) rats.

Stability of test item

Date of test item application: February 12, 2008 (males) February 13, 2008 (females) Expiration in April 2008

Design: N=25 M and 25 F rats, Randomized, 2 control groups, a dose group of each test item and one reference item (saline) group. The animals were dosed once IV and observed for 14 days.

Dose:

Group	Nr	Treatment	Dose (mg/kg)
1	5M, 5F	Isotonic Saline	0
2	5M, 5F	Formulation Buffer	0
3	5M, 5F	NewGam No 746C851/L	2000
4	5M, 5F	NewGam (IVIG 10 %) batch no. A745D851/D	2000
5	5M, 5F	KIOVIG batch no. LE12G062AF	2000

Outcome measurements: Mortality and clinical observation; body mass development; necropsy on all animals at the end of the observation period (study day 14). During necropsy a macroscopic examination was performed and any abnormalities were recorded.

Results:

There were no deaths, no changes in body weight. There were lung discolorations in vehicle and test article groups (2, 3, and 4) namely 6/10, 5/10 and 5/10 as compared to 3/10 and 1/10 in groups 1 and 5 respectively. No histopathology pathology performed.

Reviewer conclusions:

At a dose of 2.5X human dose NewGam causes unqualified lung discoloration in (b) (4) rats that seems to be no different than the toxicity of vehicle alone but different from saline or KIOVIG dosed groups. There does not seem to be any difference in the toxicity of the 2 NewGam batches tested.

Single dose toxicity study of NewGam in (b) (4) Mice, Study 170.110.1259

Performing Laboratory: (b) (4)

Aim: To evaluate the acute toxicity of 2 different batches of NewGam (IVIG 10 %) and potential differences in toxicity between the two batches and between the NewGam (IVIG 10 %) batches and the reference item KIOVIG administered intravenously at a dose of up to 10,000 mg IgG/kg to (b) (4) Mice. Design and Dose: The study was performed with 11 groups including 2 control groups, 3 dose groups of each test item and 3 reference item dose groups. Dosing was performed IV in the tail vein and animals were observed for 14 days.

Group	Nr	Treatment	Dose (mg/kg)	Volume (ml/kg)
0	5M, 5F	Isotonic Saline	0	100 (4 x 25)
1	5M, 5F	Formulation Buffer	0	100 (4 x 25)
2	5M, 5F	NewGam No 746C851/L	2500	25
3	5M, 5F	NewGam No 746C851/L	5000	50 (2 x 25)
4	5M, 5F	NewGam No 746C851/L	10000	100 (4 x 25)
5	5M, 5F	NewGam (IVIG 10 %) batch no. A745D851/D	2500	25
6	5M, 5F	NewGam (IVIG 10 %) batch no. A745D851/D	5000	50 (2 x 25)
7	5M, 5F	NewGam (IVIG 10 %) batch no. A745D851/D	10000	100 (4 x 25)
8	5M, 5F	KIOVIG batch no. LE12G062AF	2500	25
9	5M, 5F	KIOVIG batch no. LE12G062AF	5000	50 (2 x 25)
10	5M, 5F	KIOVIG batch no. LE12G062AF	10000	100 (4 x 25)

Outcome measurements: Mortality and clinical observation; Body mass development; Necropsy and macroscopic examination on all animals at the end of the observation period (study day 14).

Results: There were no deaths, no changes in body weight. There were no dose related findings.

Dose independent findings:

There were enlarged spleen and lymph nodes in low and middle dose group, sometimes associated with red discoloration.

Dose level	Nr of subjects with enlarged		Red discoloration of lymph node
	Spleen	Lymph Nodes	
Low	4	1	-
Middle	1	4	3
High	1	2	2

There were lung discolorations in vehicle (1/10) and low group (2/10 of group 2).

No histopathology was performed.

Reviewer conclusions:

Spleen and lymph reactions could be due to immune response to the biologic. This study supports safety of NewGam in mice at doses up to 12.5 times the intended human dose.

### ***Toxicology, 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)

GLP

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 of (b) (4) of TNBP + Triton X-100 at doses 300, 900, 2700 mcg/kg in 5 ml saline or vehicle daily on gestation days (GD) 6-18. On GD29 fetuses were removed via C-sections, and maternal effects were assessed via gross pathology. 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 chi2 test ( $p < 0.05$ ).

Results: There were no local or systemic effects at the low dose. At high dose there were maternal local reactions at the site of injection. 3/12 and 10/12 dams of middle and high dose, respectively, placental with discolorations, accompanied in the high dose with hematoma.

There was 1/48 malformed fetus in the study (low dose), which was considered spontaneous. 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**

There were no signs of teratogenic properties for TNBP/TRITON X-100 (b) (4) at any dose. The lowest observed toxic effect level (LOEL) for dams and embryos was 2,700 mcg/kg/day during organogenesis. The toxicities included slightly increased resorption rate, and moderately decreased body weight of fetuses. This dose corresponds to a dose (b) (4) higher than highest human dose for single or chronic human use, respectively.

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 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. The dose corresponds to equivalent human dose (b) (4) than dose for chronic use and (b) (4) dose for single use. 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 and the low frequency, these occurrences were considered spontaneous and not 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 NewGam

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: (b) (4) /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) TNBP+Triton X-100	20M, 20F 13 weeks	None
3	(b) (4) TNBP+Triton X-100	20M, 20F 13 weeks	None
4	(b) (4) 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), NOEL for systemic effects was (b) (4). 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) /day TNBP+Triton X-100 shows no effect in rats. This dose corresponds to more than (b) (4) dose of NewGam if used at 800 mg/kg every 3-4 weeks. A dose of (b) (4) /day TNBP + Triton X-100 or more than (b) (4) dose of NewGam 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.

This study, when taken into account in conjunction with the results of local tolerance study and to the intended use of the test item – IV every 3-4 weeks at a rate not to exceed 0.08 ml/kg /min – supports the intended dose of this IND.

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 NewGam 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)	
		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) TNBP+Triton X-100	3M, 3F 13 weeks	None
3	(b) (4) TNBP+Triton X-100	3M, 3F 13 weeks	None
4	(b) (4) 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) 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 is (b) (4) /day TNBP+Triton X-100 or (b) (4) dose of NewGam. There are local injection site thrombotic findings at all doses. The local thrombogenicity becomes 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.

#### Other GLP Toxicity Studies

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) 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: dyspnea, mydriasis, ataxia, reduced muscular tone. LD50 (14 days) was calculated at 30.6 mg/kg.

No 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 of low or moderate severity. LD50 (14 days) was calculated at 22 mg/kg.

No findings at pathology.

There are detailed summaries but no individual animal data.

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 in water 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. LD50 (14 days) was calculated at 22 mg/kg.

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 (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.

### **Genotoxicity**

Mutagenicity Study of TNBP + Triton x-L00 (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)





(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)

(b) (4)

Conclusions: TNBP+Triton X-100 are 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 in the absence of metabolic activation showed a very 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.

## Pharmacokinetic Studies

### "NewGam (IVIG 10%)": Pharmacokinetic Study in Rabbits, Study (b) (4)--L-2920

Performing Laboratory: (b) (4)

Aim: to gain information on pharmacokinetics of two batches of NewGam (IVIG 10 %) in comparison with the active control substance KIOVIG after a single intravenous administration in the ear vein.

Substances:

NewGam (IVIG 10 %), batch No. A 746C851/L

NewGam (IVIG 10 %), batch No. A 745B851/D

KIOVIG, batch No. LE12G062AF

Design: N=18 F (b) (4) rabbits weighing 1.6-2.15 kg were dosed with NewGam or KIOVIG at 400 mg/kg, at a dose volume 4 mL/kg body weight via bolus injection. There were 6F/dose group.

Outcome measurements:

Blood samples were taken at the following terms:

0 h, 15 min, 4 h, 24 h, 48 h, 72 h, 96 h, 120 h, 240 h, 360 h and (b) (4) was performed.

The following parameters were calculated from the concentration data obtained:

- Maximum concentration C<sub>max</sub> (mg/mL)
- Time point of the maximum concentration t<sub>max</sub> (h)
- In vivo recovery IVR (%)
- Elimination rate constant  $\lambda_z$  (h<sup>-1</sup>)
- Half-life t<sub>1/2</sub> (h)
- Area under the curve AUC(0-360) (h\*mg/mL) and AUC(0-inf) (h\*mg/mL)
- Clearance CL<sub>0</sub>(0-360) (mL/h) and CL<sub>0</sub>(0-inf) (mL/h)
- Volume of distribution V<sub>z</sub> (mL)

No statistically significant and relevant differences were obtained for the PK parameters between the two batches of the test substance NewGam.

C<sub>max</sub> was determined 15 min p.a. (T<sub>max</sub>) with 9.83 and 10.91 mg IgG/mL for NewGam and 9.13 mg IgG/mL for KIOVIG. The last measured time point was 360 h p.a. (15 d p.a.) with 1.02 and 0.84 mg IgG/mL for NewGam and 1.08 mg IgG/mL for KIOVIG), respectively.

The concentrations vs. time curves of NewGam and KIOVIG were almost identical. The batch of KIOVIG had significantly lower C<sub>max</sub> and IVR than the second batch of NewGam (batch A745B851/D), but not compared to the first NewGam batch (batch A746C851/L).

In summary there are no relevant differences for the pharmacokinetic parameters of the two batches and small differences with the KIOVIG.

### Pharmacokinetics of TNBP+Triton X-100

Dose (b) (4) 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 in water.

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, T<sub>max</sub> was 5 minutes, C<sub>max</sub> 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.