



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

Pharmacology/Toxicology Review
Division of Hematology
Office of Blood Research & Review

Product: Octaplas LG™, pooled (human) plasma, solvent detergent treated
Indication: Management of perioperative bleeding and substitution of intentionally removed plasma
Applicant: Octapharma USA, BL 125416/0 (x-ref, Octagam, STN # 125062)
Date received: December 22, 2011 (Pharm/Toxicology, MODULE 4)
Reviewer: M. Keith Wyatt, Ph. D., Pharmacologist, CBER\OBRR\DH
Through: Paul Mintz, M.D., Deputy Director for Medical Affairs, CBER\OBRR\DH

Executive Summary

Octapharma (the Applicant) has developed Octaplas LG™, a solvent detergent treated plasma-derived product for the management of several indications including bleeding during fibrinolytic therapy, replacement of coagulation factors and treatment of thrombotic thrombocytopenic purpura. An approved solvent detergent procedure previously used with Octagam (STN # 125062) which includes tri (n-butyl) phosphate (TNBP) and octyl phenyl ethoxylate (Triton x-100), will be used to reduce the viral load in the product. A ---(b)(4)--- gel column, called (b)(4), comprised of -----
--(b)(4)----- will also be used to reduce prions associated with Creutzfeldt-Jakob disease. While chromatographic procedures to remove TNBP, Triton and the prion binding ligands have been optimized, residual levels of TNBP and Triton, permissible at 2 and 5 µg/ml, respectively, are still detectable in the final Octaplas LG™, product.

To assess the potential risk associated with these residuals, TNBP and Triton were evaluated separately or in combination for: (1) acute, subchronic and development/reproductive (DART) toxicity in animals; (2) mutagenicity using (b)(4) and other *in vitro* assays; (3) clastogenicity using the -----(b)(4)-----
----- assays and; (4) hemolytic potential using human blood. The toxicity and mutagenicity of ----- (b)(4) column extract was also evaluated in rats during a repeat-dose study and by the method of (b)(4), respectively. Importantly, the toxicity of the final Octaplas LG™ product has not been evaluated pre-clinically in animals.

The results from these studies indicate the NOAELs for the TNBP + Triton combination, using the clinically relevant intravenous route of administration, are far greater than the expected residual levels in the final Octaplas LG™ product. Results from the ----- (b)(4)----- and hemolysis assays suggest TNBP and Triton residuals at the expected human doses of -----(b)(4)-----, respectively, administered with the final product are not mutagenic, clastogenic, teratogenic or hemolytic. Moreover, results from the repeat-dose toxicity study in rats and the (b)(4) assay suggest that --- (b)(4)--- ligands present in the final Octaplas LG™ product may also be safe. However, an additional (b)(4) assay and a risk assessment based on an analytical analysis of impurities actually imparted by the (b)(4) column is still needed to ensure the safety of Octaplas LG™. A summary of all these results, which have been previously submitted by the Applicant to support licensure of Octagam (STN # 125062), are presented in Table 1 that follows.

Table 1, Summary of results from toxicity and mutagenicity studies with the TNBP + Triton combination present in Octaplas LG™

Species	Study (time)	TNBP (NOAEL)	Triton (NOAEL)	----- ---(b)(4)--- (NOAEL)
rat	repeat (2 wks)			1.4
rat	repeat (6 wks)	0.06	0.3	
rat	acute	0.53	2.62	
rat (neonate)	acute	0.021	0.1	
dog	repeat (6 wks)	0.05	0.25	
mouse	acute	10 ⁺	10 ⁺	
---(b)(4)---	mutagenicity	0.158	0.79	
----- (b)(4)-----	mutagenicity	0.1 µg/ml		
----- (b)(4)-----	clastogenicity	5	25	
rabbit	developmental	0.05	0.25	
rat	developmental	≤ 0.1	≤ 0.5	
human RBCs	hemolysis	16 µg/ml	83 µg/ml	

Values are in mg/kg unless specified otherwise, + intraperitoneal route of administration

Octaplas LG™ was previously licensed in Europe and Canada in 2005. Since licensure, the product has only been linked with non-serious adverse events, such as transient urticaria, and other minor allergic reactions that can be easily managed. More important, these non-serious events have not been attributed to residual TNBP, Triton or ----- (b)(4)----- in the product. While the good clinical safety record and results from pre-clinical toxicity, mutagenicity, DART and hemolysis studies suggest residual TNBP and Triton levels present in Octaplas LG™ are safe, a full risk assessment based on an analytical analysis of ligands and impurities imparted by the ----- (b)(4) column is still needed prior to licensure. (b)(4) ----- should also be evaluated for its potential mutagenicity using a -----(b)(4)----- assay.

A. Introduction

B. Recommendation

C. Letter ready (IR) comments

D. Letter ready (general) comments

E. Indication

F. List of studies

G. Summary of pre-clinical studies

1. Toxicity of (b)(4) extract following repeat-dosing

2. TNBP/Triton, acute toxicity

3. TNBP/Triton, mutagenicity

4. TNBP/Triton, DART

5. TNBP/Triton, PK

6. TNBP/Triton, Hemolysis

H. List of studies not conducted

A. Introduction

Octaplas LG is a blood-group specific solvent/detergent treated plasma-derived product that can be used for many different surgical or clinical indications. Viral inactivation of the plasma-derived product is achieved by using a combination of tri-n-butyl-phosphate (TNBP), 1% and octyl phenyl ethoxylate (Triton X-100), 1% followed by incubation and removal chromatographically. Prion removal from plasma will be achieved using a (b)(4) column comprised of -----(b)(4)-----
----- . While chromatographic procedures to remove TNBP, Triton and the prion binding ligands have been optimized, residual levels of TNBP and Triton, permissible at 2 and 5 µg/ml, respectively, are still detectable in the final Octaplas LG™ product.

To address the potential toxicity and risk associated with these residuals, TNBP + Triton were evaluated for (1) acute, subchronic and development and reproductive toxicity in animals; (2) mutagenicity by the (b)(4) and the (b)(4) assays; (3) clastogenicity by the (b)(4) assay and; (4) hemolytic potential using human blood. The toxicity and mutagenicity of a (b)(4) was evaluated during a repeat dose study in rats and by the (b)(4) assay, respectively. Of note, the toxicity of the Octaplas LG™ product itself has not been evaluated pre-clinically in animals.

Herein is a review of the pre-clinical studies examining TNBP, Triton and -----
(b)(4) extracts and resulting data that will be used to support licensure of Octaplas LG™.

B. Recommendation

While the good clinical safety record and results from pre-clinical toxicity, mutagenicity, DART and hemolysis studies suggest residual TNBP and Triton levels present in Octaplas LG™ are safe, a full risk assessment based on an analytical analysis of ligands and impurities imparted by the ----(b)(4)----- column is still needed prior to licensure.

The requirement for an additional repeat-dose toxicity study in rats could be waived provided results from (b)(4) assay and risk assessment conducted on extracts ----- (b)(4) column, and not the --(b)(4)-- extract, are acceptable.

C. Letter-ready (IR) comments**1. Regarding the extraction procedures and toxicity results using -----(b)(4)--- ---
(b)(4)---- extract (#116-006-EMD)**

- a) The Applicant should conduct an extractable/leachable study on the (b)(4) column according to methods described in --(b)(4)--. The identity and concentration of chemical compounds and impurities in the extracts should be determined according to methods described in ---(b)(4)---. These analytical results should then be used to perform a risk assessment based on acute and chronic Octaplas LG™ dosing by the clinically relevant intravenous route of administration. The extracts should also be evaluated for their mutagenic potential based on the method of -----(b)(4)----- . Following submission of these results, FDA will determine if an additional repeat-dose study in rats is still needed to further evaluate the potential toxicity of the (b)(4) column extracts.

D. Additional letter-ready comments**1. Regarding the extraction procedures and toxicity results using -----
(b)(4)-- extract (#116-006-EMD)**

- a) The Applicant should justify why rats administered low and mid doses of -----
(b)(4) extract were not allowed a recovery period to rule out any potential delayed toxicities.
- b) The Applicant should justify why rats tissues were not evaluated histopathologically following the 7-day repeat dose toxicity study.
- c) The Applicant should explain why the rat toxicity study is referred to as a maximum tolerated dose study (MTD) when the highest dose was pre-determined before the study began.
- d) The Applicant should justify why the potential toxicity of -----
----- (b)(4) ----- was not evaluated by an implantation study according to procedures described in --- (b)(4) ---.
- e) The Applicant should justify why clinical chemistry and urinalysis was not performed in rats repeatedly administered ----- (b)(4) extract.

2. Regarding the extraction procedures used during the --- (b)(4) --- studies (#116-006-EMD)

- a) The Applicant indicates the (b)(4) ligands are tolerated at levels 3 to 4 order magnitude higher than those expected in the final product, but this result does not account for other impurities imparted by the ----- (b)(4) column. Please comment.
- b) The Applicant should indicate the level of ---(b)(4)---, imparted by the column, present in the final product. The Applicant should provide a protocol explaining how these ---(b)(4)--- levels were validated.

3. Regarding the (b)(4) mutagenicity studies conducted with ----- (b)(4)--- extract (#AFY 0017/074120)

- a) The Applicant should justify why the -----(b)(4)--- extract was not evaluated for mutagenicity or clastogenicity using -----(b)(4)-----).
- b) The Applicant should explain why the mean number of -----(b)(4)----- from positive control -----(b)(4)----- were only slightly larger than the historical controls and not larger than results using the ---(b)(4)--- extract reported on page 37/80 of Study Report AFY 0017/074120. In these cases, the Applicant should justify why the more sensitive -----(b)(4)----- was not used to evaluate mutagenic potential of the -----(b)(4)--- extract.

4. Regarding the 13-week sub-chronic TNBP + Triton toxicity study in rats, Study 5568-1-89

- a) The Applicant should explain why the standard deviation for the ALAT and LDH results, presented on page 157 and page 170, respectively, are larger than generally accepted for these assays.
- b) Reticulocyte levels in dogs (Study 5569-1-89) and rats repeatedly administered TNBP + Triton were both reduced compared with levels in control treated animals. The Applicant should elaborate on why reduced reticulocyte levels in two species should not be considered a safety signal.

5. Regarding (b)(4) assays, Study 6088/90

- a) The Applicant should justify why the mutagenic potential of Triton alone was not evaluated during these studies.
- b) Results published in the literature and data generated during this study suggest TNBP is mutagenic but probably not carcinogenic. Please comment.

6. Regarding assays for hemolytic activity, Study 6346/90

- a) The experimental procedure indicated that RBCs were -----(b)(4)----- minutes at (b)(4). This contrasts with a -----(b)(4)----- time normally used during hemolysis studies. Please comment.

7. Regarding the -----(b)(4)----- assay, no study # provided, Nov 5, 1986

- a) The Applicant should calculate the final concentration of TNBP used during these experiments.

8. Regarding IP routes of administration used during several acute toxicity studies in rodents

- a) The Applicant has provided results from a large number of acute toxicity studies conducting in rodents using an IP route of administration. However, IP does not mimic the IV route used to administer Octaplas LGTM. In the absence of adequate PK data for both TNBP + Triton using an IV route, the Applicant should explain how acute toxicity data generated by IP can be extrapolated and used to predict toxicity, safety and exposure to TNBP + Triton in patients administered Octaplas LGTM by IV.

9. Regarding the cytogenic assay, Study 6090/90

- a) The Applicant should provide the number of cells in ----(b)(4)---- per 1000 cells which were omitted from Table 7b, page 30 and Table 9b, page 34 of the study report.

10. Regarding developmental toxicity studies in rats (6086-90) and rabbits (6087-90)

- a) The Applicant should justify why a satellite group of pregnant rats that went full term was not included in the study design.
- b) The Applicant should justify why a reproductive toxicity study was not conducted with F1 rats and F1 rabbits exposed to TNBP + Triton during their gestation.
- c) Slight teratogenic effects were observed in rabbits and rats at all TNBP + Triton dose amounts during the DART studies so a true NOAEL could not be established. Please comment.

11. Regarding acute toxicity study, Study 6344/90

- a) The acute toxicity of TNBP and Triton was only evaluated separately by IV. The Applicant should justify why the acute toxicity of the TNBP + Triton combination was not evaluated in rats or mice using an IV route of administration.

E. Indication

Octaplas LG is used for peri-operative management of bleeding or for patients who require replacement of multiple plasma coagulation factors. Indications are also sought for substitution of intentionally removed plasma which includes but is not limited to plasma exchange in patients with thrombotic thrombocytopenic purpura (TTP).

Under normal clinical scenarios, adult patients receiving Octaplas LG at 20 ml/kg by an intravenous route of administration will also receive doses of TNBP and Triton at (b)(4) - --(b)(4)---, respectively.

Additional excipients present in Octaplas LG which include sodium citrate dihydrate, 4.4-7.4 mg/ml, sodium dihydrogen phosphate dihydrate, 0.3 -1.2 mg/ml and glycine, 4-6 mg/ml, are considered safe based on historical use in the clinic.

F. List of Studies

(b)(4) column extraction and toxicology/mutagenicity studies

1. Toxicological Studies on ---(b)(4)--- Leachates, Study 116-006-EMD, 2007-2008

Repeat-dose toxicity

1. 13-week subchronic toxicity study of TNBP + Triton x-100 (1+5) by IV in (b)(4) rats, Study 5568-1-89, LPT, 1990
2. 13-week subchronic toxicity of TNBP + Triton (1 + 5) by iv in (b)(4) Dogs, Oct 31, 1990, 5569-1-89

Acute toxicity

1. EXAMINATION OF THE ACUTE TOXICITY OF TNBP by INTRAPERITONEAL ADMINISTRATION TO (b)(4) MICE, 5123/88, LPT, August 28, 1989
2. EXAMINATION OF THE ACUTE TOXICITY OF TRITON X-100 BY INTRAPERITONEAL ADMINISTRATION TO (b)(4) MICE, 5124/88, LPT, August 28, 1989
3. Examination of the acute toxicity of TRITON X-100 BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5127/88, LPT, August 28, 1989
4. Examination of the acute toxicity of TNBP + TRITON X-100 (1 + 20) BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5128/88, LPT, August 28, 1989

5. Examination of the acute toxicity of TNBP BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5136/88, LPT, August 28, 1989
6. EXAMINATION OF THE ACUTE TOXICITY OF TNBP + TRITON X-100 (1 + 5) BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6343/90, LPT, Feb 6, 1991
7. EXAMINATION OF THE ACUTE TOXICITY OF TNBP BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6344/90, LPT, Feb 6, 1991
8. Examination of the acute toxicity of TRITON X-100 BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6345/90, LPT, January 22, 1991
9. ACUTE TOXICITY STUDY OF TNBP + TRITON X-100 (RATIO 1 + 5) BY INTRAPERITONEAL ADMINISTRATION, 7724/92, Sept 9, 1993
10. ACUTE TOXICITY STUDY OF TNBP + TRITON X- 100 (RATIO 1+ 5) BY INTRAPERITONEAL ADMINISTRATION TO NEW-BORN -----(b)(4)----- RATS, 7725/92, LPT, May 17, 1993
11. EXAMINATION OF THE ACUTE TOXICITY OF TNBP + TRITON X-100 (1 + 20) BY INTRAPERITONEAL ADMINISTRATION TO (b)(4) MICE, 5125/88, August 28, 1989, LP

Mutagenicity

1. MUTAGENICITY STUDY OF TNBP + TRITON X-100 (1 + 5) IN THE -----(b)(4)----- TEST (IN VITRO), 6088/90, LPT Nov 12, 1990
2. MUTATION STUDY OF TNBP + TRITON X-100 (1 + 5) IN -----(b)(4)----- IN VITRO, 6089/90, LPT, Nov 12, 1990
3. TNBP -----(b)(4)----- ASSAY IN VITRO IN -----(b)(4)-----, November 5, 1986
4. -----(b)(4)----- TEST WITH THE TEST COMPOUND TRIBUTYLPHOSPHATE - CALLED TNBP --- (b)(4)----- CELLS OF TREATED (b)(4)-MICE, December 24, 1986
5. IN VIVO -----(b)(4)----- CYTOGENETIC TEST OF TNBP + TRITON X-100 (1 + 5) BY INTRAVENOUS ADMINISTRATION TO

----- (b)(4) ----- RATS (CHROMOSOMAL ANALYSIS),
6090/90, Sept 13, 1990

6. ----- (b)(4) ----- TEST OF TNBP + TRITONX-100 (1+5) IN ----- (b)(4) -----
----- CELLS OF THE ----- (b)(4) ----- RAT, 6091/90, Sept
7, 1990

Developmental and reproductive toxicity

1. EXAMINATION OF the INFLUENCE OF TNBP + TRITON X-100 (1 + 5 ON THE PREGNANT RAT AND THE FOETUS BY INTRAVENOUS ADMINISTRATION, 6086/90, Oct 17, 1990, LPT
2. EXAMINATION OF THE INFLUENCE OF 1NBP + TRITON X-100 (1 + 5) ON THE PREGNANT RABBIT AND THE FOETUS BY INTRAVENOUS ADMINISTRATION, 6087/90, Oct 17, 1990, LPT
- 3.

Pharmacokinetics

1. PHARMACOKINETIC STUDY OF TNBP + TRITON X-100 (1 + 5) IN
----- (b)(4) ----- RATS AFTER SINGLE INTRAVENOUS
ADMINISTRATION, 6149/90, LPT, January 28, 1991

Hemolysis assay

1. EXAMINATION OF TNBP + TRITON X-100 (1 + 5) ON
HAEMOLYTIC PROPERTIES (IN VITRO STUDY), 6346/90, LPT, Dec
17, 1990

G., Summary review of pre-clinical studies

G.1., Summary of repeat dose toxicity studies

Toxicological Studies on --- (b)(4) --- Leachates, 2007-2008,

**Rat repeat dose toxicity study conducted ----- (b)(4) ----, Study 116-006, 2007 and
(b)(4) assay by ---- (b)(4) ---- Study AFY 0017/074120 performed in 2007,
GLP**

Purpose: To determine the maximum tolerated dose (MTD) and Estimated Maximum dose (EMD) of the ----- (b)(4) ----- used to replicated (b)(4) column leachates at a dose of 1400 µg/kg following 7 days of repeat dosing. To evaluate the potential mutagenicity ----- (b)(4) ----- used to replicate (b)(4) column leachates the (b)(4) assay.

Methods: The 7-day repeat-dose toxicity study performed by ----- (b)(4) ----
----- rats, 5M/5F/group, were administered a reconstituted
mixture of ----- (b)(4) ----- at a ratio of 1:1:1 called --- (b)(4) --- or vehicle in a
dose-escalation format. This ---- (b)(4) --- extraction mixture was formulated to replicate
the worst case amount of --- (b)(4) --- leaching that could occur during the Octaplas LC

manufacturing processing. The composition and the amount of ---(b)(4)--- were determined during leach studies previously conducted by ----- (b)(4) column in -----(b)(4)----- . Leachate actually derived from the column was not used in the 7-day repeat-dose or (b)(4) assays.

Rats in Group 1 received vehicle. Rats in Group 2 were administered a single dose of ---(b)(4)--- at 350 µg/kg and observed for 2 days. The same rats were then administered a single dose of (b)(4) at 1050 µg/kg followed by 2 days of observation which was then followed by administration of another single dose of ---(b)(4)--- at 1400 µg/kg followed by two day of observation. Rats in Group 3 were administered ---(b)(4)--- at 700 µg/kg for 2 days and then followed by single doses of 1400 µg/kg for an additional 7 days (14 day total study). After seven days, rats in Groups 2 and 3 both received the maximum doses of 1.4 mg/kg once a day for 7 days.

At the end of the 14 day study, blood was collected for hematologic evaluation. Rats were euthanized and subjected to necropsy 24 hrs after the final dose. Large organ weights were recorded but histopathology was not performed.

Column extraction and estimation of dose amounts:

Leachate used in the dose escalation and repeat dose studies were generated by -----(b)(4)----- ligand in -----(b)(4)----- the total ligand amount in the extract was determined to be of 0.07 µg/ml. This amount represents approximately --(b)(4)-- of the total ligand which corresponds to an estimated dose of 2.1 µg/kg received by patients administered Octaplas. The maximum dose of 1.4 mg/kg used in the toxicity studies was calculated with uncertainty factors of 10 and 6.7 for intra-species differences and differences in the amount of plasma administered, respectively.

A ----(b)(4)---- assay, performed by -----(b)(4)-----, using the appropriate -----(b)(4)----- and commonly accepted negative and positive controls were conducted on directly on ---(b)(4)--- at 1:1:1 ratio with and without metabolic activation. The mutagenic potential of (b)(4) alone was tested during the first study, Study 1, with -----(b)(4)----- at a final concentration of 10%. The second confirmatory study was modified to include (b)(4) concentration of 20%. (b)(4) leachate at 2 to 5000 µg total was also evaluated by (b)(4). The amount of ---(b)(4)--- used in (b)(4) represents a 30-70,000 fold increase of --(b)(4)-- compared with the amount that can leach from columns following extraction with -----(b)(4)-----.

Results: No rats died and no adverse effects or overt signs of toxicity were observed following administration of ---(b)(4)--- leachate during the dose escalation and repeat-dose studies at a maximum dose of 1.4 mg/kg. (b)(4) leachate was not associated with any mutagenicity based on results from an (b)(4) assay.

However, histopathology revealed a few red spots in the lungs of two males (no's 16 and 27) at the mid and high dose while similar effects were not observed in vehicle treated rats. No significant changes in relative lung weight in either male were observed. The

Applicant stated these were typical background findings. No significant changes in relative organ weights or hematology were reported. The Applicant claims an MTD of 1400 µg/kg which represents an amount of leachate is 3 to 4 orders of magnitude greater than the expected amounts of exposure.

Results from both (b)(4) assays, with and without activation using (b)(4) at 10 and 20% final concentration, indicate (b)(4) amounts ranging from 2-5000 µg were not mutagenic. There was no evidence of bacterial toxicity observed during these assays.

Reviewer concerns:

The ---(b)(4)--- extract was formulated by -----(b)(4)-----
----- at a 1:1:1 ratio. The ratio was based on results from an extraction study previously performed on the (b)(4) column using -----(b)(4)----- . By using the
---(b)(4) extract, impurities imparted by the -----(b)(4)-----
----- procedure were not included in the mutagenicity and toxicology studies. As expected, results from these studies yielded negative results which were not acceptable to establish extract safety. .

G.1.2., 13-week sub-chronic toxicity study of TNBP + Triton x-100 (1+5) by IV in (b)(4) rats, Study 5568-1-89, LPT, 1990

Purpose: To evaluate the potential toxicity of TNBP + Triton X-100 in rats.

Methods: Initially, an 8-day pilot study in rats, 2F/2M, administered TNBP+ Triton (1+5 ratio, dose based on Triton concentration at 156, 312 and 625 µg/kg/day was performed.

Based on intolerance at injection sites observed during the initial study, rats, 20M/20F/group, were administered TNBP + Triton combination at 12 + 60 (Group 2), 60 + 300 (Group 3) and 300 + 1500 µg/kg (Group 4) daily. Groups 1, 2 and 3 were administered TNBP + triton for continuously for 13 weeks followed by necropsy. Rat numbers 21-25 (in Group 1), 46-50 (in Group 2), 151-155 (in Group 3) and 176-180 (in Group 4) were administered the combination for 6 weeks followed by a 4 week recovery period. Data from Groups 2 and 3 collected during recovery were not presented in the study report.

Hematology, Pt, clinical biochemistry, urinalysis and pathology were evaluated in all rat groups on week 6 and 10. Only rats in Groups 1, 2 and 3 were evaluated on week 13 (high dose rats in Group 4 were excluded).

Results: Overt toxicity was observed in a low dose female (#20) on day 88, in a high dose male (#23) on day 12 and in high dose female (#25) on day 66 which dead prematurely. Pathology revealed pulmonary oedema and sinusoidal congestion of the liver in the dead male at the high dose. The female that died prematurely on day 66 at the high dose had some fatty deposits in the liver overall pathology was not remarkable. Pathology revealed spongy pulmonary tissue with vascular congestion in the low dose female. Importantly, 4 control rats in group 1 also died prematurely during the study.

Extreme local intolerance characterized by necrosis and discolored tissue in Group 4 necessitated a reduction in the dosing schedule from 13 to 6 weeks. Group 2 rats also exhibited swollen and discolored injection sites beginning by about week 6. Rats in group 3 began swelling at injections during week 3. The localized swelling and lesions were somewhat reversed during the recovery period.

Hematology was normal in all groups but significant decreases in reticulocyte counts in males at the high dose were reported. Neutrophil values increased 2-fold in several cases but the significance of this occurrence is unknown to the reviewer. Basophil and Eosinophil levels were elevated from 0 to 4 or 0 to 3, respectively, in high dose females by week 6. Eosinophil levels remained elevated in these female during week 10 of the recovery period.

Reticulocyte levels in both male and females at the high dose were reduced to 27 and 28 (% of erythrocytes) compared with 39 and 33 in controls, but the differences were not significant by week 6 of the study. By week 10, normal reticulocyte levels had not been restored at the highest dose. Reticulocytes levels at the low and intermediate dose levels compared with levels in control treated rats throughout the entire study.

Platelet levels in both male and female rats at the high dose were reduced but not significantly on week 6. Platelet levels were increased and compared with levels in control rats by week 10.

ALAT level trended upward in a dose dependent fashion at week 6 in all test groups, but the results were not significant. ALAT levels in high dose rats were similar to levels in control animals during recovery on week 10.

Pathology in Group 3 male and females that went full term, 13 weeks, did not reveal any significant findings. In some instances, the site of injection on the tail was blackish and inflamed. Pathology of Group 4 male and females rats that survived full term, 43 day, did not reveal any significant findings although pulmonary granulomas and infiltrate and hemorrhaging in tail tissue was observed in some males. Female rats that went full term in groups 2, 3 and 4 (6 week treatment + 4 week recovery) had similar findings in lungs, liver and at the injection site as males and none were considered significant.

Reviewer concerns:

Liver damage evidenced by increased LDH and ALAT levels were reported but only at the highest dose.

A moderate level of hemolysis was observed in a male, 1/10, in Groups 2 and 3 and males, 3/10, in Group 4 at week 6. One female, 1/10, in group 4 also had elevated Hb levels suggestive of a slight hemolytic effect.

G.1.3., 13 week subchronic toxicity of TNBP + Triton (1 + 5) by iv in (b)(4) Dogs, Oct 31, 1990, 5569-1-89

Purpose: To evaluate the potential toxicity of TNBP + Triton X-100 in dogs.

Methods: Initially a 7-day pilot study in dog, 1 dog /dose, was conducted at 0.250 mg TNBP + 1.25 mg triton , 0.5 mg TNBP + 2.5 mg triton , 0.83 mg TNBP + 4.150 mg triton, 5 mg TNBP + 25 mg triton by iv.

At the conclusion of the dose finding study dogs, 4M/4F were administered control (group 1) or 0.5 + 2.5 mg/kg (Group 4). Dogs, 3M/3F, were administered 0.013 + 0.065 (Group 2) and 0.05 + 0.25 mg/kg (Group 3) by iv. Dog, 1M/1F in Groups 1 and 4 were assigned to a 4 week recovery period were administered TNBP + Triton daily by iv. Dogs in group 1, 2 and 3 were administered TNBP + Triton for 13 weeks. Dogs, 1M/1F, in group 4 were sacrificed prematurely at week 8 and week 12. Dogs in group 4 were administered TNBP + Triton for 8 weeks followed by a 4 week recovery according to the regimen detail below:

Dogs were examined daily for signs and symptoms. Hematology, clinical biochemistry, urinalysis, ECG, BP (measured 10 minutes after dosing) was performed before the study and on weeks 6, 8 and 11 and on surviving dogs in week 13. Histology was performed on dogs sacrificed in week 8, 12 and 13.

Results:

Pilot study: Acute toxicity including ataxia, tonoclonic, convulsions and hemorrhagic feces were observed in dogs administered the combination at 5 mg TNBP + 25 mg triton. Increased respiratory rate was observed in dogs administered 0.83 mg TNBP + 4.150 mg triton.

Sub-chronic study: None of the dogs died prematurely. No local inflammation observed in dogs in groups 1 and 2. Local intolerance was observed in dogs in groups 3 and 4 beginning at week 3.

Dogs in all treatment groups remained lively and their behavior was unchanged during the study although occasionally feces in most groups were soft but discharged normally.

The thyroid in a single male dog in group 4 was observed to have squamous epithelial metaplasia during the 4 week of recovery.

Slight decreases were observed in hematocrit 39 verses 35, hb, and erythrocytes levels but only in dogs in group 4 beginning at week 6 and 8. These decreases were partially reversed by week 11 in the recovery period. Thromboplastin and partial thromboplastin times did not change significantly in the Group 4 dogs during the study. No changes in PT were observed in dogs receiving lower doses.

Average platelet and reticulocyte counts were lower in Groups 4 dogs, 2/4, in the recovery period 261 verses 312 in the control group. Erythrocyte sedimentation rate were dramatically higher in Group 4 dogs during recovery in week 11, 7.5 verses 1.5 mm/1h, and during week 6 compared with levels observed in controls.

LDH levels were elevated but not significantly compared with control levels in group 4 dogs during week 8. LDH levels of 24 verses 30 reported in vehicle treated control dogs and Group 4 dogs, 2/4, respectively, were reported during the recovery period.

No significant changes in clinical biochemistry, ECG or BP results were observed in any groups. ALT and ASAT level remained normal in all treatment groups.

Potassium levels were increased from 4.41 to 4.9 in one female dog (#8) in recovery at week 8 compared with control. The same dog total bilirubin was increased but blood urea was normal which suggests kidney function was not affected.

Globulin α_2 levels were increased in a male and female dogs in Group 4, 6.9 compared with 3.4 in control treated dogs during the recovery period.

Hb levels in urine were high Group 2 male dogs, 2/3, Group 3 dogs, 3/7, but lower levels were observed in Group 4 dogs compared with values reported in the urine of control dogs.

The absolute and relative spleen and liver weights on average in Group 4 dogs were larger than absolute and relative spleen and liver weights in control treated dogs on week 12.

Pathology revealed that lungs and livers in Group 4 female dogs, 2/4, had mononuclear infiltrates but these findings were not considered significant. Group 4 male dogs did not appear to have lung or liver infiltrates.

Reviewer concerns:

Reticulocyte levels in dogs and rats repeatedly administered TNBP + Triton were both reduced compared to control treated dogs and rats. Should this be considered a meaningful safety signal?

Was diarrhea a common AE observed in patients? This might be consistent with adverse effects observed in dog administered TNBP + Triton.

G.2., Acute toxicity studies

F.2.1., EXAMINATION OF THE ACUTE TOXICITY OF TNBP by INTRAPERITONEAL (IP) ADMINISTRATION TO (b)(4) MICE, 5123/88, LPT, August 28, 1989

Purpose: To evaluate acute toxicity of TNBP in mice.

Methods: (b)(4) mice, 3M/5F/group, were administered a single dose of TNBP at 0.4, 0.1, 0.4, 0.5, 0.6, 0.8 and 0.97 g/kg using an ip route of administration.

Results: Intolerance characterized by ataxia and dyspnea were first observed with TNBP at a dose of 0.144g/kg. The lowest lethal dose reported was 0.5 g/kg. The TNBP LD₅₀ in males of 0.66 g/kg at 24 hrs and 0.60 g/kg at 14 days were reported. The TNBP LD₅₀ in reported in females was 0.73 g/kg at 24 hrs and 0.669 g/kg at 14 days.

Pathology revealed pale liver tissue but in general was not remarkable.

Reviewer comments:

The IP route of administration used during these studies does not mimic the iv administration that will be used clinically with Octaplas LG. Without PK data using the IP route, extrapolating data from this study to predict toxicity by an iv route of administration cannot be accomplished with confidence.

TNBP administered by IP may be subjected to first pass effects which could change its toxicity profile. First pass would not be an issue during iv delivery.

G.2.2., EXAMINATION OF THE ACUTE TOXICITY OF TRITON X-100 BY INTRAPERITONEAL ADMINISTRATION TO (b)(4) MICE, 5124/88, LPT, August 28, 1989

Purpose: To evaluate the toxicity of Triton x-100 in mice by IP.

Methods: (b)(4) mice, 5M/5F, were administered single doses of Triton x-100 at 0.01, 0.03, 0.1, 0.12, 0.157, 0.19, 0.22 g/kg in sesame oil by IP. Mortality data collected over a 14 day evaluation period were used to calculate LD₅₀ values. Necropsy and macroscopic evaluation was performed on all mice used in the study.

Results: A NOAEL of 0.01 g/kg for Triton X-100 was claimed. First signs of intolerance, ataxia and dyspnea were observed at Triton doses of 0.03 g/ml. The Triton LD₅₀ was 0.145 and 0.145 g/kg in male and females mice, respectively, at day 14. A Triton LD₅₀ was not obtained 24 hrs after dosing. A Triton LD_{50 low} of 0.129 g/kg was reported. No significant pathologic findings were identified or reported at 0.229 g/kg.

Reviewer concerns:

The significance of LD₅₀ values obtained at 24 hr and LD_{50 low} are unknown to the reviewer. It is assumed the LD₅₀ reported at 2-weeks is generally lower and the preferred measure of lethality.

G.2.3., EXAMINATION OF THE ACUTE TOXICITY OF TRITON X-100 BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5127/88, LPT, August 28, 1989

Purpose: To evaluate the acute toxicity of Triton x-100 in rats.

Methods: Rats, 5M/5F, were administered a single injection of Triton x-100 at a dose of 0.004, 0.01, 0.04, 0.07, 0.1, 0.15, 0.23, 0.33 g/kg in sesame oil by ip. All rats were subjected to necropsy and microscopic evaluation.

Results: A NOAEL of 0.004 for Triton was claimed. The first signs of intolerance to Triton were observed at a single dose of 0.015 g/kg. The Triton LD_{50 low} of 0.7 g/kg was reported. The Triton LD₅₀ at 14 days was 0.1 g/kg and 0.125 g/kg in males and female rats, respectively.

G.2.4., EXAMINATION OF ACUTE TOXICITY of TNBP + TRITON X-100 (1 + 20) BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5128/88, LPT, August 28, 1989

Purpose: To evaluate the acute toxicity of TNBP + TRITON X-100.

Methods: Rats, 5M/5F, were administered single doses of TNBP + Triton x-100 (1+20) at 0.004, 0.01, 0.04, 0.07, 0.1, 0.155, 0.228 and 0.335 g/kg in sesame oil by ip. Rats were monitored for 14 days and then subjected to necropsy.

Results: A NOAEL of 0.004 g/kg for the TNBP +Triton combination was claimed. First intolerance (LOAEL) was observed at a dose of 0.015 g/kg. The TNBP + Triton LD_{50 low} of 0.106 g/kg was reported. The TNBP + Triton LD₅₀ at 14 days were 0.126 and 0.128 g/kg in male and female rats, respectively.

Reviewer comments:

The “first intolerance” classification used by the Applicant to characterize toxicity may correspond to LOAEL used in modern toxicology.

The term LD_{50 low} has been used by the reviewer to describe the lowest lethal dose reported by the Applicant.

The significance of toxicity observed using an IP route is unknown when an IV route will be used to administer Octaplas LG in the clinic.

G.2.5., EXAMINATION OF ACUTE TOXICITY of TNBP BY INTRAPERITONEAL ADMINISTRATION TO -----(b)(4)----- RATS, 5136/88, LPT, August 28, 1989

Purpose: To evaluate the acute toxicity of TNBP in rats.

Methods: Rats, 5M/5F/group, were administered single doses of TNBP in oil at 0.04, 0.14, 0.45, 0.54, 0.66, 0.80 and 0.977 g/kg by ip.

The TNBP LD₅₀ data was calculated by regression analysis (dose-mortality curve) using the method of Litchfield and Wilcoxon. Mortality observed after 24 hrs but before 14 days was used in the analysis.

All rats were subjected to pathologic analysis including macroscopic and microscopic evaluation following the study.

Results: A NOAEL of 0.045 g/kg for TNBP was claimed. First intolerance (LOAEL) was observed at a dose of 0.144 g/kg. An LD_{50 low} of 0.549 g/kg for TNBP was also reported. The TNBP LD₅₀ at 14 days was 0.615 g/kg for males. LD₅₀ values of 0.803 g/kg at 24 hrs and 0.610 g/kg at 14 days were reported in male and female rats.

Ataxia and dyspnea were observed in male and female rats 15 minutes after administration of TNBP at a dose of 0.549 g/kg. At 6 hrs, the same rats exhibited reduced muscle tonus and other systemic toxicities. At 24 hrs, rats exhibited slight reductions in motility. At day 4, one female died while the other animals survived until euthanization at day 14.

G.2.6., EXAMINATION OF THE ACUTE TOXICITY OF TNBP + TRITON X-100 (1 + 5) BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6343/90, LPT, Feb 6, 1991

Purpose: To evaluate the acute toxicity of TNBP + Triton.

Methods: Rats, 3M/3F/group, were administered single doses of TNBP, 0.53 mg + Triton, 2.63 mg, TNBP, 1.67 mg + Triton, 8.33 mg, TNBP, 5.27 mg + Triton, 26.33 mg, TNBP, 16.67 mg + Triton, 83 mg/kg by iv. Signs and symptoms were recorded periodically for 14 days. Rats were euthanized and subjected to macroscopic inspection. The LD₅₀ values for the combination were determined at 24 hrs and at 14 days.

Results: The claimed NOAEL for the combination was of TNBP, 0.53 mg + Triton, 2.63 mg/kg. First intolerance (LOAEL), presented as ataxia, was observed at doses of TNBP, 1.67 mg + Triton, 8.33 mg/kg. The LD₅₀ for TNBP, 5 mg + Triton, 25 mg/kg at 14 days was reported. No significant changes in weight gain or decreases in weight gain were reported. No pathological findings were reported in rats that died prematurely.

G.2.7., EXAMINATION OF THE ACUTE TOXICITY OF TNBP BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6344/90, LPT, Feb 6, 1991

Purpose: To determine the acute toxicity of TNBP in rats.

Methods: Rats, 3F/group, were administered single doses of TNBP at 2.15, 4.64, 10, 21.5 and 46.4 mg/kg by IV. Rats were monitored periodically for signs and symptoms and changes in weight over a 14 day period and then subjected to euthanization and necropsy.

Results: A NOAEL of 2.15 mg/kg for TNBP was claimed. First intolerance (LOAEL) was observed at a TNBP dose of 4.64 mg/kg. The TNBP LD_{50 low} of 21.5 mg/kg was also reported. Although several rats died in 2 to 6 minutes after administration, the LD₅₀ for TNBP was 22 mg/kg at day 14. At doses equal to or less than 21.5 mg/kg no decreases in weight gain were observed. No significant findings were observed by necropsy in rat that died prematurely or survived full term.

G.2.8., EXAMINATION OF ACUTE TOXICITY of TRITON X-100 BY INTRAVENOUS ADMINISTRATION TO -----(b)(4)----- RATS (orientating study), 6345/90, LPT, January 22, 1991

Purpose: To evaluate the toxicity of Triton in rats.

Methods: Rats, 3F/group, were administered a single dose of Triton x-100 at 4.64, 10, 21.5 and 46.4 mg/kg and observed for 14 days followed by necropsy. Triton LD₅₀ was determined at 24 hr and at 14 days.

Results: A NOAEL of 4.64 mg/kg for Triton was claimed. An LD_{50 low} for Triton of 21.5 mg/kg was also reported. The Triton LD₅₀ at 14 days was 22 mg/kg. Although, slight ataxia, dyspnoea and reduced motility were observed in rats administered 10 mg/kg, surviving rats recovered 60 minutes at administration. Pathology yielded no significant findings.

G.2.9., ACUTE TOXICITY STUDY OF TNBP + TRITON X-100 (RATIO 1 + 5) BY INTRAPERITONEAL ADMINISTRATION, 7724/92, Sept 9, 1993

Purpose: To determine the acute toxicity of the combination TNBP + Triton x-100 in rats by IP.

Methods: Rats, 3M/3F/group, were administered single doses of TNBP at 21.5, 46.4, 100, 147, and 215 mg/kg + Triton at a ratio of 1:5. A separate group of male rats, 3M, were administered the combination at a TNBP dose of 316 mg/kg + Triton at a 1:5 ratio by IP. Rats were observed for signs and symptoms periodically for 14 days. Rats were then euthanized and subjected to necropsy and gross macroscopic pathological analysis.

LD₅₀ was determined using the method of FINNEY at 24 hrs and 14 days after administration.

Results: A NOAEL of 21.5 mg for TNBP + Triton was claimed. First intolerance (LOAEL) was observed at a TNBP dose of 46.4 mg/kg + Triton. The TNBP LD_{50 low} reported was 100 mg/kg and 215 mg/kg for female and male rats, respectively. The combination LD₅₀ was 212 mg/kg for males and 177 mg/kg for females at 24 hrs. At 14 days, the combination LD₅₀ was 155.5 mg/kg for both males and female rats. Slight ataxia was observed at doses of 46 mg/kg. No decreases in weight gain were report at any dose including lethal doses.

Pathology revealed swollen inflamed spleen and liver tissues in males and females administered the combination dose of 100 mg/kg.

**G.2.10., ACUTE TOXICITY STUDY OF TNBP + TRITON X- 100 (RATIO 1+ 5) BY INTRAPERITONEAL ADMINISTRATION TO NEW-BORN -----(b)(4)-----
----- RATS, 7725/92, LPT, May 17, 1993**

Purpose: To determine the acute toxicity of TNBP +Triton in neonatal rats.

Methods: Rats, 3M/3F/group were administered TNBP at doses of 10, 21.5, 46.4, 100, 147, 215 and 316 mg/kg + Triton at a 1 + 5 ratio by IP. Rats were observed for signs and symptoms periodically for 14 days and then subjected necropsy and macroscopic inspection.

Results: A NOAEL of 21.5 mg/kg for TNBP + Triton for males and 100 mg/kg TNBP + Triton for females was claimed. First intolerance (LOAEL) was observed at the combination dose of 46.4 mg/kg in males and 146 mg/kg in female rats. An LD_{50 low} of 46.4 mg/kg for males and 147 mg/kg for females was reported. The LD₅₀ at 24 hrs was 154 mg/kg in males and 148 mg/kg in females. The LD₅₀ at 14 days was 102 mg/kg for both males and female rats.

Reviewer comments:

Data generated from this study suggest newborn rats are somewhat resistant to the toxic effects of the TNBP + Triton combination. While these findings are acknowledged, data from adult rats administered the combination at the same dose by an IP route was not provided for comparative purposes.

G.2.11., EXAMINATION OF THE ACUTE TOXICITY OF TNBP + TRITON X-100 (1 + 20) BY INTRAPERITONEAL ADMINISTRATION TO (b)(4) MICE, 5125/88, August 28, 1989, LP

Purpose: To determine the acute toxicity of TNBP + Triton x-100 (1+20) in (b)(4) mice.

Methods: (b)(4) mice, 5M/5F/group, were administered TNBP + Triton in sesame oil at doses of 0.01, 0.03, 0.1, 0.12, 0.16, 0.19 and 0.22 g/kg by IP. Signs and symptoms were evaluated periodically over 14 days followed by euthanization, necropsy and macroscopic evaluation. Mice that died prematurely were subjected to necropsy and inspection.

LD₅₀ was determined by regression analysis (dose-mortality curve) using the method Litchfield and Wilcoxon.

Results: A NOAEL of 0.01 g/kg for the combination was claimed. The LD_{50 low} for the combination was 0.128 g. The LD₅₀ for males was 0.141 and 0.144 g/kg for females at day 14. First intolerance (LOAEL) observed as ataxia occurred at a dose of 0.03 g/kg. Autopsy and results from pathologic examination from deceased and surviving animals were not remarkable.

G.3., Summary of mutagenicity studies

F.3.1., MUTAGENICITY STUDY OF TBP + TRITON X-100 (1 + 5) IN THE -----(b)(4)----- TEST (IN VITRO), 6088/90, LPT Nov 12, 1990

Purpose: To evaluate the potential for TNBP + Triton x-100 (1+5) to cause for -----(b)(4)-----.

Methods: The mutagenicity of the TNBP + Triton combination, with and without metabolic activation was evaluated at 7 concentrations ranging from 5 µg + 25 µg to 5000 µg + 25000 µg. The combination was evaluated in -----(b)(4)----- using the method of (b)(4).

Results: No mutagenic effects or mutagenicity were observed at concentrations of the combination TNBP + Triton below 158 + 790 µg/plate, respectively. Bacterial cell toxicity occurred beginning with combination concentrations of 500 µg for TNBP and 2500 µg for Triton.

Reviewer comments:

It should be confirmed that bacterial colony counts reported at combination doses of 150 µg + 750 µg/plate in -----(b)(4)----- of 145 ± 9.6 are not statistically different from colony counts of 108 reported in the negative control. The Applicant indicated that results from this study were still under discussion when the report was finalized in 1990.

TNBP is probably a mutagenic but is not carcinogenic based on brief search of the EPA website.

G.3.2., MUTATION STUDY OF TNBP + TRITON X-100 (1 + 5)**----- (b)(4) IN VITRO, 6089/90, LPT, Nov 12, 1990**

Purpose: To evaluate the mutagenic potential of TNBP + Triton x-100 (1+5) in (b)(4) ----
----- (b)(4) -----

Methods: ----- (b)(4) -----, were treated with TNBP + Triton x-100 doses over a range of concentrations from 1.56 + 7.81 µg to 25 to 125 µg /ml, without metabolic activation, for 24 hrs. In a separate experiment, --- (b)(4) --- were treated with TNBP + Triton doses ranging from 6.25 + 31.25 µg/ml to 100 to 500 µg, for 24r hrs followed by treatment with metabolically active (b)(4) for an additional 2 hrs. Separate - (b)(4) - were exposed to standard positive and negative control compounds.

Results: The results from -- (b)(4) -- suggest TNBP + Triton at dose amounts of 12.5 µg/ml + 62.5 µg/ml are not mutagenic compared with cells treated with negative control compounds. A slight increase in the frequency of point mutations was observed but was not considered dose-dependent or significant. -- (b)(4) -- treated at the highest TNBP + Triton dose did exhibit some cytotoxic effects. Additional results from experiment 1 are provided in the table below.

----- (b)(4) -----

(b)(4)

Increases in the mutation frequency were reported at TNBP + Triton doses of 12 + 62 µg and at the highest dose during the second ---- (b)(4) ---- experiment that included metabolic activation. However, the increased frequency result correlated with a decrease in plating efficiency and an increase in cytotoxic so was not considered valid. At low doses no increase in mutational frequency was observed. Additional results from the second experiment, which included metabolically activated TNBP + Triton, are provided in the following table.

----- (b)(4) -----

(b)(4)

Reviewer concerns:

The Applicant should explain why lower frequencies of mutation occurred without metabolic activation compared with metabolically activated TNBP + Triton at the lower doses used during the study.

**G.3.3., TNBP ----- (b)(4) ----- ASSAY IN VITRO
IN ----- (b)(4) ----- CELLS, November 5, 1986**

Purpose: To determine the clastogenic potential of TNBP with and without metabolic activation.

Methods: ----- (b)(4) ----- were cultured for 26 hrs. TNBP in the proper vehicle at concentrations of 0.01, 0.03, 0.1, 0.15 and 0.3 µl/ml and negative or positive control compounds were added to cells and incubated for 2 hrs. Activated TNBP was prepared with ---- (b)(4) ---- using generally accepted procedures. -----
(b)(4) ----- were used as positive controls.

The exposure period was terminated by -----

----- (b)(4) -----

-----.

Results: The results show that TNBP did not mediate an increase in the number of ----- (b)(4) ----- and is therefore not considered clastogenic. A summary of additional data are provided in the following table.

----- (b)(4) -----

(b)(4)

Results demonstrating that TNBP was not clastogenic, without metabolic activation, were also provided (data not shown).

Reviewer concerns:

The Applicant should indicate the final TNBP amounts used during these experiments. The concentrations of TNBP, expressed in µl/ml, were difficult for the reviewer to understand and interpret.

**G.3.4., -----(b)(4)----- TEST WITH THE TEST COMPOUND
TRIBUTYLPHOSPHATE, CALLED TNBP, ON -----(b)(4)----- OF
TREATED (b)(4)-MICE, December 24, 1986**

Purpose: To determine the mutagenicity and chromosomal aberration potential of TNBP using a ----(b)(4)---- assay.

Methods: Initially, an acute oral toxicity study that demonstrated the TNBP LD₅₀ was 40 mg/kg was performed in mice. Based on these results, (b)(4) mice, 5M/5F, were administered doses of TNBP at 5, 10 and 20 mg/kg by IV. Mice receiving TNBP were euthanized at 16, 48 and 72 hrs. ----(b)(4)----- was extracted. -----

----- (b)(4) -----
----- . Positive control mice were administered -----(b)(4)----- while negative control mice received only vehicle.

Results: The results indicate TNBP is not mutagenic or considered clastogenic. More specifically, -----(b)(4)----- counts remained unchanged after administration of TNBP compared with ----(b)(4)---- from control mice administered vehicle. Rates of ----(b)(4)---- cell formation were similar in treated and control mice but one mouse treated with TNBP at 10 mg/kg did yield 11 ----(b)(4)---- cells per 1000. However, similar counts were not observed at the highest TNBP dose so the mutagenicity was not considered dose-dependent or significant. The cytotoxicity of TNBP did not appear to

confound results from this study. Additional summary data are presented in the table below:

-----**(b)(4)**-----

(b)(4)

G.3.5., IN VIVO -----(b)(4)**----- CYTOGENETIC TEST OF TNBP + TRITON X-100 (1 + 5) BY INTRAVENOUS ADMINISTRATION TO -----**(b)(4)**----- RATS (CHROMOSOMAL ANALYSIS), 6090/90, Sept 13, 1990**

Purpose: To assess the mutagenic and chromosomal aberration potential of TNBP + Triton using a rat ---**(b)(4)**----- assay.

Methods: Rats, 5M/5F, were administered doses of TNBP + Triton at 1.25 mg/kg + 6.25 mg/kg , 2.5 mg/kg + 12.5 mg/kg, 5 mg/kg + 25 mg/kg by iv. Positive control rats were cyclophosphamide. Rats receiving the TNBP + Triton combination or control compounds were euthanized at 6, 24 and 48 hrs after administration. Erythrocytes were extracted from ---**(b)(4)**----- and evaluated microscopically for chromosomal aberration, and mitotic indexes in metaphase cells. Cells were graded for the presence of normal metaphases, 1-2 breaks and interchanges or pulverized metaphases with more than 50% of the chromatin present in the form of point-like fragments.

Results: Results indicate the combination of TNBP + Triton was not mutagenic or capable of depressing mitotic indexes of erythrocytes extracted from treated rats. Increases in cells with total aberrant metaphases including gaps were observed in rats administered TNBP compared with control treated rats at 24 hr but the increases were not dose or time dependent. A table summarizing additional results is presented below:

----- (b)(4) -----

(b)(4)

**G.3.6., ----- (b)(4) ----- TEST OF TNBP + TRITON X-100 (1+5) IN -----
(b)(4) ----- CELLS OF THE ----- (b)(4) -----, 6091/90, Sept 7, 1990**

Purpose: To assess the potential of TNBP + Triton to cause damage to chromosomes or the mitotic apparatus using a rat ---- (b)(4) ---- assay.

Methods: Rats, 2M/2F, were administered doses of the TNBP + Triton combination at 1.25 mg/kg + 6.25 mg/kg, 2.5 mg/kg + 12.5 mg/kg and 5.0 mg/kg + 25 mg/kg by iv. These doses were not cytotoxic based on data from a preliminary dose finding study. ---- (b)(4) ---- samples were collected at 16, 48 and 72 hrs following administration. Erythrocytes in metaphase were prepared using standard methods and evaluated microscopically for chromosomal aberration and mitotic indexes. Cells were graded for normal metaphases, 1-2 breaks or interchanges and pulverized metaphases characterized by more than 50% of the chromatin present in the form of point-like fragments.

Results: Results indicate that administration of TNBP + Triton at 5 mg/kg + 25 mg/kg was not associated with an increase in the number of micronucleated polychromatic erythrocytes or mutagenicity compared with cells from control treated rats. Additional data are summarized in the table that follows:

-----~~(b)(4)~~-----

(b)(4)

G.4., Summary of DART Studies

F.4.1., EXAMINATION OF the INFLUENCE OF TNBP + TRITON X-100 (1 + 5) ON THE PREGNANT RAT AND THE FOETUS BY INTRAVENOUS ADMINISTRATION, 6086/90, Oct 17, 1990, LPT

Purpose: To evaluate the toxicity of TNBP + Triton on pregnant rats and offspring.

Methods: Following a pilot study with non-pregnant females, pregnant rats, 24/group were administered doses of either 300 µg TNBP + 1500 µg Triton, 900 µg TNBP + 4500 µg Triton by iv from between day 6 and 15 of gestation.

Signs and symptoms and body weight of the dam were checked daily. Pregnant rats were euthanized on day 20 of the gestation period. The number of fetuses, runts, corpora lutea, resorptions and placenta were determined during necropsy. The method of Dawson and Wilson was used to assess fetal malformation.

Results: Teratogenic effects and malformations in offspring were observed at the low and mid-doses but the effects did not occur at the highest dose so were considered incidental.

More specifically, 4 malformed fetuses were observed in one liter, 1/4, in dams treated with the low TNBP + Triton dose. Nine malformed fetuses were observed at the mid dose but no malformed fetuses were observed in the highest dose group. Because malformations did not occur in a dose-depend manner, the Applicant considered the occurrence of malformed fetuses as incidental. One rat in group 4 endured a total implantation loss due to stress according to the Applicant. Fetuses with variation increased slightly with dose according to the methods of Dawson and Wilson. A summary of the malformation results are provided in the table that follows.

Summary of fertility and malformation results

	Group 1 Control	Group 2 100 µg/kg TNBP + 500 µg/kg TRITON X-100	Group 3 300 µg/kg TNBP + 1500 µg/kg TRITON X-100	Group 4 900 µg/kg TNBP + 4500 µg/kg TRITON X-100
Resorption rate in %	9.5	5.5	7.6	10.5
Dead foetuses	0	0	0	0
Runts total per dam (2)	3 0.1 ± 0.3	1 0.04 ± 0.2	0 -	0 -
Malformation total per dam (2)	0 -	4 0.2 ± 0.8	9 0.4 ± 1.3	0 -
Malformation rate in %	-	1.3	3.0	-

continued on next page

Reviewer comments:

Effect of TNBP + Triton on fertility was not assessed during this study. The fertility of F1 rats was not assessed by remating these animals.

The Applicant claims fetal development was not affected at the maximum dose of TNBP 900 µg + Triton 4500 µg, but one dam experienced total implantation loss at this dose so the claim may not be valid. Malformations observed at the low dose suggest the combination of TNBP + Triton may be teratogenic in rats.

G.4.2., EXAMINATION OF THE INFLUENCE OF TNBP + TRITON X-100 (1 + 5) ON THE PREGNANT RABBIT AND THE FOETUS BY INTRAVENOUS ADMINISTRATION, 6087/90, Oct 17, 1990, LPT

Purpose: To assess the toxicity of TNBP + Triton (1 +5) in pregnant rabbits and their fetuses by iv administration.

Methods: A pilot study with 1 non-pregnant rabbit was conducted with doses of 60 µg TNBP + 300 µg Triton, 300 µg TNBP + 1500 µg triton, 600 µg TNBP + 3000 µg Triton and 900 µg triton + 4500 µg triton. Based on only limited local intolerance reactions and slight increases in respiratory rates, pregnant rabbits, 12F/group, were administered TNBP + Triton at doses of 50 µg + 250 µg, 150 µg + 750 µg and 450 µg + 2250 µg by iv between day 6 and 15 of gestation.

Signs and symptoms were evaluated daily. Fetal malformations, implantation and resorption rates and corpora lutea were determined using standard methods previously described during rat DART studies.

Results: Results indicate that prenatal development was affected in rabbits administered 50 µg TNBP + 250 µg Triton and 450 µg TNBP + 2250 µg Triton. One malformed fetus was observed at the low dose and 6 runts were observed at the highest dose. No malformations were reported at the intermediate dose of TNBP 150 µg + Triton 750 µg. At the highest dose of 450 µg TNBP + 2250 µg Triton, resorption rats increased 14 %, fetal body weight was decreased significantly and placental indurations and hematomas were observed. Increases in the number of variations were observed with increasing doses using the method of Dawes and Wilson. Additional runt and malformation data from the study are summarized below:

Summary of DART studies conducted in rabbit

	Group 1 Control	Group 2 50 µg/kg TNBP + 250 µg/kg TRITON X-100	Group 3 150 µg/kg TNBP + 750 µg/kg TRITON X-100	Group 4 450 µg/kg TNBP + 2250 µg/kg TRITON X-100
Resorption rate in %	7.6	10.7	6.4	14.4
Dead foetuses at laparotomy	0	0	0	0
after 0-6 h total per dam (2)	0 -	5 0.5 ± 0.7	1 0.1 ± 0.3	1 0.1 ± 0.3
after 7-24 h total per dam (2)	1 0.1 ± 0.3	8 0.7 ± 1.2	4 0.4 ± 0.9	7 0.6 ± 0.7
Runts total per dam (2)	0 -	2 0.2 ± 0.4	0 -	6 0.5 ± 0.5
Malformation total per dam (2)	0 -	1 0.1 ± 0.3	0 -	0 -

continued on next page

Local intolerance at injection sites were observed in dams at all doses. White, dispersed foci were observed in placentae in dams at the mid-dose.

Reviewer concern:

TNBP + Triton teratogenicity should be re-evaluated at a lower dose in rabbits to establish a legitimate NOAEL.

Because malformations were only observed at the lowest dose, it is speculated that higher TNBP + Triton doses caused a reduction or down regulation of receptors that result in less placental uptake.

G.5., Pharmacokinetic

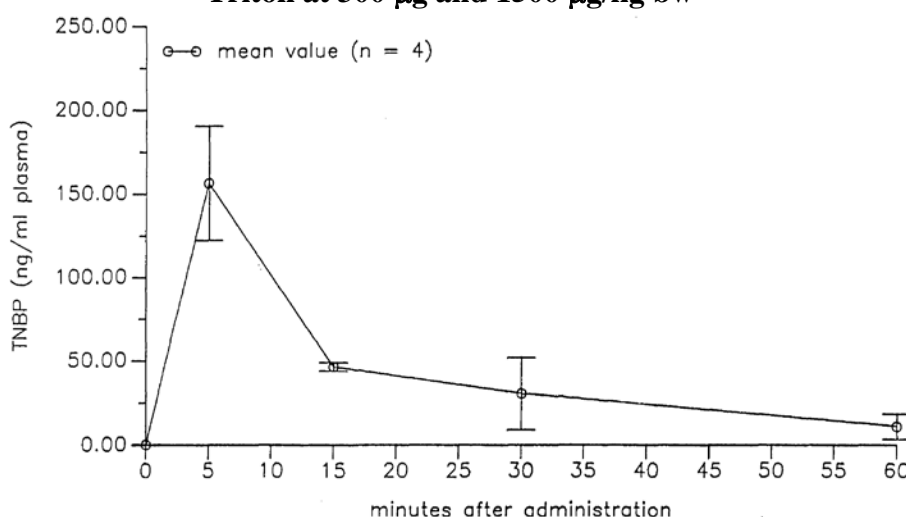
**G.5.1., PHARMACOKINETIC STUDY OF TNBP + TRITON X-100 (1 + 5)
IN -----(b)(4)----- RATS AFTER SINGLE INTRAVENOUS
ADMINISTRATION, 6149/90, LPT, January 28, 1991**

Purpose: To determine of the PK of TNBP + Triton in rats

Methods: Rats, 20M/20F, were administered single doses of TNBP 300 µg + Triton 1500 µg by IV which is approximately 50 fold higher than the expected human dose. Blood was collected over a 24 hr time course. Urine and fecal material was also collected periodically and analyzed for TNBP content by (b)(4) (TNBP) and for Triton by ---- (b)(4)-.

Results: TNBP was detected in only half the rats administered the compound. Approximately 0.005 and 0.96 % of the administered TNBP dose was recoverable. Although the kinetic data limited, a $t_{1/2}$ for TNBP of 20 minutes was calculated. TNBP could not be detected in urine. Triton could not be detected in plasma, urine or in fecal material.

TNBP-level in plasma following a single intravenous administration of TNBP + Triton at 300 µg and 1500 µg/kg bw



Reviewer concerns:

Extrapolating acute toxicity data collected by IP to the clinically relevant IV route was not possible based on the limited PK data produced during this study.

G. 6.0., Summary of hemolysis studies

G.6.1., EXAMINATION OF TNBP + TRITON X-100 (1 + 5) ON HAEMOLYTIC PROPERTIES (IN VITRO STUDY), 6346/90, LPT, Dec 17, 1990

Purpose: To determine the hemolytic potential of TNBP + TRITON.

Methods: Human erythrocytes were diluted 1:9 in saline. A 1 ml amount of the erythrocytes suspension was added to increasing amounts of diluted triton + TNBP and incubated for 20 minutes at 37° C.

The resulting supernatant, following centrifugation of the cell suspension, was evaluated at 540 nm and compared with control supernatant from saline treated blood.

Results: Hemolysis was observed with TNBP + Triton concentrations of 50 µg + 250 µg/ml and above. Additional results are presented in the table below:

			Extinction		Mean Value
			Sample A*	Sample B*	
a)	Control 0.9% NaCl solution		0.008	0.010	0.0090
b)	TNBP + TRITON X-100 (1 + 5) μ g TNBP + TRITON X-100 (1 + 5)/ml 0.9% NaCl solution:				
	0.50	+ 2.5	0.011	0.015	0.0130
	1.67	+ 8.3	0.014	0.015	0.0145
	5.00	+ 25.0	0.012	0.011	0.0115
	16.70	+ 83.3	0.008	0.012	0.0100
	50.00	+ 250.0	2.311	3.264	2.2875
	167.00	+ 833.0	2.324	2.314	2.3190
	500.00	+ 2500.0	2.275	2.272	2.2735

Reviewer concerns:

Hemolysis studies are normally conducted at 37° C for 60 minutes. The procedure used appears too abbreviated.

I. List of pre-clinical studies not conducted

1. PRIMARY PHARMACODYNAMICS
2. SECONDARY PHARMACODYNAMICS
3. SAFETY PHARMACOLOGY
4. PHARMACODYNAMIC DRUG INTERACTIONS
5. CARCINOGENICITY