

### Summary of Pharmacokinetics/Toxicokinetics

Study # R2.67 examined the pharmacokinetics and biodistribution of intravenously administered technetium Tc 99m apcitide (200 $\mu$ Ci/animal; 2 $\mu$ g peptide/kg ) in male and female rats. Tc 99m was cleared rapidly from the blood with about 97% been cleared within the first 30 minutes. Biodistribution data showed that overall recovery of radioactivity averaged 92.1 $\pm$ 8.7 % ID (Injected Dose) and 96.7 $\pm$ 6.8% ID in male and female rats respectively. The 4-hour total urinary excretion was higher in female than in males (91 vs 79% ID). Male carcass contained on average 8% ID compared to 0.7% ID for female rats. It was concluded that intravenously administered Technetium Tc 99m apcitide disappears rapidly from the blood in a biexponential manner with average distribution and elimination half life of 1.8 and 21 minutes respectively.

Study # R2.53 utilized \_\_\_\_\_ to determine whether technetium Tc 99m apcitide injection (20 mCi per animal, 40 $\mu$ g peptide per kg) undergoes metabolism following intravenous injection. Urinary elimination was fitted unto a single exponential model. Approximately 70% of ID was eliminated within the 4 hour observation period. The study identified 2 radiolabeled species in urine in a constant ratio of 1:3; intact Tc 99m apcitide and an unidentified TC 99m metabolite which was characterized as been more hydrophilic and smaller in size compared to Tc 99m apcitide. This metabolite was not present in the plasma and was therefore concluded to be generated by the kidneys. Rapid plasma clearance could also be responsible for lack of identification in plasma.

Study # R2.75 examined the distribution, metabolism and excretion of Technetium Tc 99m apcitide in rats with experimental renal dysfunction (ERD) produced by bilateral renal artery and vein ligation. Bilateral renal vessels ligation was produced in rats (4 male and 3 female) by tying of the renal artery and vein under surgical anesthesia. Another group of rats had the bile duct cannulated for passive collection of bile in addition to renal vessels ligation. Four rats were used as sham operated controls while a rat had its bladder cannulated to provide analysis of urine metabolites.  $T_{1/2\beta}$ , MRT,  $AUC_{0\rightarrow\infty}$   $K_{elim-half}$  values from ERD rats were increased significantly ( $p < 0.001$ , 2 tail difference of means) compared to normal or sham operated controls. % ID disappearing from the blood in 4 hours was 46 $\pm$ 5.5 in ERD rats compared to 98.6 $\pm$ 0.8 and 99.0 $\pm$ 0.5 in controls and sham-operated rats. In ERD rats, the GI was stated to show an increase in uptake suggestive of increased hepatobiliary activity. This was attributed to increase in hepatobiliary secretion. Two metabolites (one hydrophilic and one lipophilic ) were detected in the bile of ERD rats in addition to Technetium Tc 99m apcitide. The hydrophilic metabolite seen in bile was believed to be identical to the previously identified urine metabolite of normal rats. It was also detectable in the plasma. Its appearance in plasma was attributed to reabsorption of the metabolite following introduction into the gastrointestinal tract via bile. The lipophilic metabolite was not identified in the plasma of ERD rats. The lack of identification was attributed to low relative abundance. It was concluded that renal dysfunction significantly affected systemic clearance of Technetium Tc 99m apcitide.

Study # R2.77: The stated objective of this study was to assess the distribution, metabolism, and excretion of the peptide components of Technetium Tc 99m apcitide injection in rats. [ $^3$ H]-bibapcitide (8 $\mu$ Ci/ $\mu$ g) was labeled with a non exchangeable tritium label in order to preserve the radiolabeling on apcitide, P1007 and P1008 following the conversion of bibapcitide to the peptide components of Technetium Tc 99m apcitide injection. The study had five groups of 3-5

animals each. All the groups received approximately the same dose of peptide,  $40.6 \pm 0.2 \mu\text{g}/\text{kg}$  ( $\sim 80 \mu\text{Ci}/\text{rat}$ ) intravenously. Biodistribution data showed that none of the organs examined showed uptake of more than 1% ID by 4 hours post-injection. Most of the injected dose was contained in the carcass (11%) or was excreted in the urine (84%). By 24 hours, 15% of the injected dose remained in the carcass and organs. No metabolite was detected in plasma, and with the exception of  $[^3\text{H}]$ -P1007, the peptides were not detectable beyond 15 minutes post injection. The injected  $[^3\text{H}]$ -peptide components were not present in urine at any time point. Up to seven  $[^3\text{H}]$ -labeled metabolites were identified in the urine and the sponsor concluded that they were generated by metabolism in the kidney. Pharmacokinetics results were similar to those obtained in study # R2.53. The sponsor concluded that the distribution, metabolism and excretion of  $[^3\text{H}]$ -peptide components of technetium Tc99m apcicide injection were very similar to those of technetium Tc 99m apcicide.

Study # R2.96: This study compared the metabolism profile of  $[^3\text{H}]$ -peptide components of Technetium Tc 99m Apcicide injection by rat, rabbit and human kidney and liver slices in vitro by methodology.  $[^3\text{H}]$ -bibapcicide ( $8 \mu\text{Ci}/\mu\text{g}$ ) was labeled with a non exchangeable tritium label in order to preserve the radiolabeling on apcicide, P1007 and P1008 following the conversion of bibapcicide to the peptide components of Technetium Tc 99m apcicide injection. Tissues slices prepared from rat, rabbit and human liver and kidney were incubated in media containing  $[^3\text{H}]$ -peptide components at 3 dose levels 100, 316, and 1000ng total peptide/mL, for 0.5, 2 and 4 hours. The experiments included appropriate controls. The positive control utilized the ability to metabolize 7-ethoxycoumarin (7-EC) as a test of tissue viability. All tissues remained viable. A single, more hydrophilic metabolite (14% of applied radioactivity) was generated by the human liver while the human kidney produced multiple hydrophilic metabolites (totaling 23.9% of applied radioactivity (AR)). The rabbit liver and kidney produced one (5.1% AR), and two (4.6 and 10.7% AR) metabolites respectively. The rat liver generated one major (16.4% AR) and two minor (3.2% AR total) metabolites respectively. The rat kidney showed extensive metabolism with 47% and 67% of AR metabolized to a major, more hydrophilic metabolite by 30 minutes and 4 hours respectively. The sponsor concluded that the parent peptides are metabolized to more hydrophilic metabolites with proportions varying from 5-25 % for human, rabbit and rat liver and human and rabbit kidney to 76% for rat kidney. The results suggested that some of these metabolites may be similar.

Study R2.50: examined the distribution characteristics of Tc 99m apcicide in whole human blood. Citrated blood samples obtained from adult volunteers were used for the experiments. Standard biochemical techniques including differential centrifugation, blood cell fractionation using neutrophil isolation medium, plasma and plasma protein fractionation using were employed to obtain required samples. Distribution of Tc 99m apcicide (with or without ADP stimulation) in the various fractions was investigated. The sponsors stated that the majority of radioactivity (86%) was recovered in cell free plasma and was not associated with plasma protein. Less than 1% of the radioactivity was associated with either white or red blood cells. Unstimulated platelets contained 7%, this value increased to 21% following platelets activation with ADP. The sponsors concluded that the majority of apcicide was contained in cell free plasma.

28: Toxicology:

## Single Dose Toxicology

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Study # R2.59 Update 1: Single-Dose Intravenous Toxicity Study of Technetium Tc 99m Apcitide Injection Prepared with Decayed Generator Eluate in Mice with 48 Hour and 14 Day Observation Periods.

In-life 09/30/96-10/14/96 Report Dated 12/20/96, Amended Report Dated 07/31/97. Final commercial formulation Lot No. 9603M01 was used for this study. The study is located in volume 1.12 pages 2-61. This study was in compliance with GLP.

The objective of the study was to assess the potential for acute toxicity of Technetium apcitide injection following a single intravenous bolus injection at doses representing 300X and 1000X MHD (2 µg total peptide /kg).

Male and female Swiss albino mice were assigned to groups as shown in the table (Vol. 1.12 pp.7)

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Group	Test Material	Dose Route	No. M/F <sup>1</sup>	Duration of Observation Period	Dose Level (µg peptide/kg) <sup>3</sup>	Multiple of MHD <sup>4</sup>	Dose Volume (mL/kg)
Control	Saline	IV	3/3 5/5 10/10	Pre-dose 48 hours 14 days	0	0	20
Dose Level 1	Test Article <sup>2</sup>	IV	3/3 5/5 10/10	Pre-dose 48 hours 14 days	600	300	6
Dose Level 11	Test article	IV	3/3 5/5 10/10	Pre-dose 48 hours 14 days	2000	1000	20

<sup>1</sup> Swiss Albino mice, M= males, F= females

<sup>2</sup> Technetium apcitide Injection

<sup>3</sup> Peptide refers to the amount of total peptide present in Technetium apcitide Injection

<sup>4</sup> Multiple of maximum human dose (=2 µg peptide/kg for a 50 kg patient)

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Clinical signs were evaluated daily and body weights measured on the first day of dosing and on every other day thereafter. Hematology and blood chemistry parameters were assessed from blood taken prior to terminal sacrifice. Complete necropsy was performed on all animals. Liver and spleen, were weighed. Liver, heart, spleen, lung and kidneys were preserved, fixed and examined microscopically from high dose and control groups.

**Results:** No test-related changes were seen in mice observed for 48 hours. In the 14-Day observation group, there was a single mortality in a low dose female group on day 8. Necropsy was reported to be normal, and death was considered unrelated to test article by the contractor. The animals did not exhibit any sign of toxicity during the study. There were no significant

differences for absolute or relative spleen or liver weights between test and control animals at necropsy (relative to body weight). Spleen demonstrated microscopic evidence of lymphoid depletion in 4 of 10 high-dose and 5 of 10 low-dose males, 5 of 10 high-dose females, 1 of 10 control males, and 1 of 10 control females after 14 day observation. The histopathological findings of lymphoid hypoplasia corresponded with slightly absolute smaller weights in some of the treated groups, the changes were not significant. According to the contractor, a number of the spleens in the high and low dose males were grossly atrophic. The contractor surmised that although this effect on the spleen had minimal biological or toxicological significance, it was observed more frequently in test article-treated mice and therefore attributed to treatment with the test article. Test and control animals did not demonstrate significant differences in hematological parameters.

Other histopathological findings such as perivascular chronic inflammation (lungs), focal necrosis (liver) and chronic inflammation (kidney) were incidental and unrelated to treatment.

**Conclusion:** The sponsor stated that "Technetium Apcitide injection was well tolerated in male and female mice after i.v. administration of 300X and 1000X MHD". The biological significance of the changes in spleen was questioned. "A qualified acute no-observable effect-level (NOEL) was thus assigned in this study at 1000X MHD"

**Reviewer's comments:** The term "qualified NOEL" is new to this reviewer. Qualified or not, no NOEL was demonstrated in this study. Decreases in the weight of the spleens (absolute or relative to body weight) did not reach statistical significance level despite what the contractor considered as "grossly atrophic appearance" in some of the groups. Moreover, lymphoid hypoplasia was observed at both dose levels. I agree with the contractor's conclusion that the lymphoid hypoplasia effect seen in the spleen should be attributed to the test article. As to the sponsor's statement that the finding has minimal biological or toxicological significance, I submit that the biological or toxicological significance of such an effect has to be evaluated in the context of overall toxicological studies. It should be noted that an effect on spleen weights or histopathology was observed in more than one study in this NDA. Because of the fact that the effect on spleen was conserved, an overall assessment of the possible biological significance of this observation will be discussed later in this section. Although other organs were evaluated in this study, data on the weights were not provided. Hematological data did not reveal any treatment-related changes. I agree that other histopathological findings were incidental to study.

**Study # R2.73, Update 1: Single-Dose Evaluation of Technetium Tc 99m Apcitide Injection Prepared with Decayed Eluate with Nonpeptide Formulation Excipient In Mice with 48 Hour and 14 Day Observation Period.**

**In-life 07/01/97-07/21/97 Final Report Dated 05/21/97. Final commercial formulation Lot No. 9603M01 used for this study. The study is located in volume 1.12 pages 62-143. This study was in compliance with GLP.**

This study is essentially a repeat of study #R2.59 Update 1 with two additional groups; an intermediate group at 200µg peptide/kg corresponding to 100X MHD, and the dose of nonpeptide formulation excipient that was equivalent to 1000X MHD.

Male and female Swiss albino mice were assigned to groups as shown in the table (Vol. 1.12 pp.68).

Group	Test Material	Dose Route	NO. M/F <sup>1</sup>	Duration of Observation period	Dose Level $\mu\text{g}$ peptide/kg <sup>4</sup>	Multiple of MHD <sup>5,6</sup>
Control	Saline	IV	5/5 10/10	48 hours, 14 days	0	0
Low Dose	Test Article <sup>2</sup>	IV	5/5 10/10	48 hours, 14 days	60	30
Mid Dose	Test Article <sup>2</sup>	IV	5/5 10/10	48 hours, 14 days	200	100
High Dose	Test Article <sup>2</sup>	IV	10/10	14 days	2000	1000
Formulation Dose	Formulation excipient <sup>3</sup>	IV	10/10	14 days	0	1000

<sup>1</sup>Albino Swiss mice, m= Males, F= females. <sup>2</sup>Technetium Apcitide Injection prepared with decayed generator eluate.

<sup>3</sup>Nonpeptide components (excipients) of technetium Apcitide Injection prepared with decayed generator eluate. Referred to as mock kit. The excipients were: sodium glucoheptonate and stannous chloride.

<sup>4</sup>Peptide refers to the amount of total peptide present in Technetium Apcitide Injection

<sup>5</sup>Multiple of maximum human dose (= 2  $\mu\text{g}$  peptide/kg for a 50 kg patient)

<sup>6</sup>All mice were injected at a dose volume of 20mL/kg

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Body weights were measured on first day of dosing, and on every other day thereafter and the animals observed for clinical signs during the course of the study. Following completion of the study, blood samples were taken for the evaluation of hematology parameters. Necropsies were carried out at pre-scheduled times. Liver and spleen from all animals were weighed. The spleen, liver, heart, lung, thymus, mesenteric lymph nodes and kidneys from all animals were examined microscopically.

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**Results:** With the exception of one male in the control group, all the animals sacrificed at the end of 48 hours exhibited weight loss over the time period. The loss was significant with the males in low and mid dose groups. The animals sacrificed at the end of 14 days gained weight except for 1 female each in the control, mid dose and formulation excipient groups, and two females in the high dose group. The body weight changes were not considered by the contractor to be of biological significance. The animals did not exhibit any sign of toxicity during the study.

No significant findings for the blood chemistry. The spleen and liver absolute weights were within normal ranges for 48 hour group. The absolute liver weights of the high dose and excipient dose female groups were statistically significantly different from controls ( $p < 0.05$ ). All histopathological findings were considered to be of no biological significance.

The hematological profile of both 48 hours and 14-Day groups showed statistically significant changes ( $p < 0.05$ ) as listed in the table below

Parameter	Test Group	Control	Test Group
<b>48 hours</b>			
Neutrophil, %	Male/Low	20.0 ± 1.6	12.8 ± 3.0
	Male/Mid	20.0 ± 1.6	8.8 ± 1.9
	Female/Low	19.4 ± 3.8	12.6 ± 2.4
	Female/Mid	19.4 ± 3.8	8 ± 2.9
Neutrophil, number	Male/Low	1069 ± 263	536 ± 364
	Male/Mid	1069 ± 263	549 ± 122
lymphocyte, %	Male/low	75 ± 1.96	83.4 ± 2.2
	Male/Mid	75 ± 1.96	88.6 ± 2.1
	Female/Low	77.2 ± 3.9	83.4 ± 2.5
	Female/Mid	72.2 ± 3.9	89.2 ± 2.4
<b>14-DAY</b>			
total WBCs, K/ul	Male/Low	5.6 ± 1.74	17.1 ± 4.1
Neutrophil, %	Male/Low	8.8 ± 1.7	21.6 ± 2.67
	Male/excipient	8.8 ± 1.7	6.9 ± 0.43
	Female/Low	6.6 ± 1.0	23 ± 2.23
	Female/Excipient	6.6 ± 1.0	18.8 ± 1.14
Neutrophil, No.	Male/Low	523 ± 235	3770 ± 1329
	Male/Excipient	523 ± 235	1292 ± 157
	Female/Low	541 ± 277	2228 ± 627
	Female/excipient	541 ± 277	1672 ± 361
Monocyte, %	Female/Low	1.6 ± 0.48	3 ± 0.88
Monocyte, No.	Male/Low	102 ± 46.5	523 ± 216
	Male/Excipient	102 ± 46.5	166 ± 30.8
Lymphocyte, %	Male/Low	88.0 ± 2.2	72.2 ± 3.9
	Male/Mid	88.0 ± 2.2	77 ± 1.75
	Female/Low	90.0 ± 1.6	71.8 ± 3.2
	Female/Mid	90.0 ± 1.6	77.8 ± 1.14
Lymphocyte, No.	Male/Low	4890 ± 1466	12261 ± 2443

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**Conclusions:** Diatide concluded that 'Technetium apcitide injection prepared with decayed generator eluate was well tolerated, with minimal findings in the liver and white blood cell counts. A NOEL was assigned as 2,000 µg peptide/kg (1000X MHD)'.

**Reviewer's comments:** Disagree with the NOEL assigned at 1000X MHD. Hematological profile changes were observed at all dose levels. This precluded the assignment of NOEL at 1000X MHD. I attribute the finding of weight loss in the 48-hour groups to stress resulting from handling. Agree that there were no significant histopathological findings.

Study No: R2.76, Update 1: A Single Dose Evaluation of Intravenous Toxicity of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate In Rabbits With 48-hour and 14 day Observation Periods.

In-Life 03/12/97-03/27/97. Final commercial formulation Lot No. 9603B04 was used for this study. The study is located in volume 1.12 pages 153-332. This study was in compliance with GLP.

The objective of the study was to evaluate the potential single-dose acute toxicity of Technetium apcitide injection in rabbits.

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Male and female rabbits were assigned to the following groups ( Vol. 1.12 pp. 158).

Group No	Test Material	Dose Route	No. M/F	Duration of Observation	Dose Level µg peptide/kg	Multiple of MHD <sup>2</sup>
1	Saline control	IV	3/3 5/5	2 days 14 days	0	0
2	Test article <sup>1</sup>	IV	3/3 5/5	2 days 14 days	300	150
3	Test article	IV	3/3 5/5	2 days 14 days	1000	500

<sup>1</sup> Technetium Tc 99m Apcitide Injection prepared with synthetic decayed generator eluate

<sup>2</sup> Multiple of maximum human dose (2µg peptide/g for a 50 kg patient)

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Each rabbit received the test or control article once via a single intravenous injection on day 1. Body weights were measured prior to the first dose and every other day, and the animals were observed for clinical signs during the course of the study. Following completion of the study, blood samples were taken from the aorta for the evaluation of clinical pathology parameters. Necropsies were carried out at pre-scheduled times and gross observations were made on all animals. For all the groups, selected organs (adrenal, brain, liver and spleen) were weighed and organs to body or organ/brain weight ratios calculated. Tissues from all animal groups were preserved in appropriate media, however, only tissues from control and high-dose groups were examined microscopically.

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**Results:** There was no mortality during the course of this study and there was no biologically significant changes in body weight. Spleen histology was normal, although significant differences were noted in mean spleen and adrenal absolute and relative to body and brain weights compared with control values for the low dose group. No such changes were noted for any of the high-dose groups. The changes were attributed to the small numbers of animals used for the study and were considered to be within normal biological variation. There were no significant microscopical findings. Bone marrow smear evaluation were normal. No biologically significant findings was reported for serum chemistry and hematology.

**Conclusion:** Technetium apcitide injection was well tolerated and there was no evidence of toxicity at doses of 300 and 1000 µg peptide/kg administered intravenously as a single dose in rabbits. NOEL was 1000µg peptide/kg, or 500X MHD.

**Reviewer's comments:** Although the spleen weight showed statistically significant difference for the low-dose group compared with control, my examination of the data did not convince me that we are dealing with a biologically significant effect. However my position is not helped by

the fact that there were no detailed pathology report for individual animals in this group. I agree that NOEL was demonstrated at 500X MHD.

**Study No: R2.82, Update1: A 14 Day Repeated Dose Intravenous Toxicity Study Of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate Administered To Rats.**

**In-Life 12/5/96-12/20/96. Final commercial Formulation Lot No. 9603B03 was used in this study. The study is located in volume 1.13 pages 2-178. This study was in compliance with GLP.**

The objective of the study was to investigate the potential toxicity of Technetium apcitide given for fourteen consecutive days intravenously in rats.

Sixty rats (male and female) were assigned to the following groups (Vol. 1.13 pp.07)

Group No.	No. of M/F	Test Material	Dose route	Dose level (µg/kg)	Dose volume	Multiple of MHD
1	10/10	Saline	IV	0	5.0 ml/kg	0
2	10//10	Tc-Apcitide	IV	150	1.5 ml/kg	75
3	10/10	Tc-Apcitide	IV	500	5.0 ml/kg	250

Each rat received the test or control vehicle (saline) intravenously once daily for 14 consecutive days. Body weights were measured before the beginning of the study, and every other day during the study period. The animals were observed for clinical signs during the course of the study. Following completion of the study, blood samples were taken via the abdominal aorta for hematological and serum chemistry evaluation. Necropsies were carried out at day 15 on all the animals. A bone marrow smear was collected from the sternum of all animals at necropsy. All abnormalities were described completely and recorded. Selected tissues were weighed before fixation; adrenal, brain, liver and spleen, and organ to brain or body weight ratios calculated. Aorta, heart, the gastrointestinal tract, liver, pancreas, trachea, lung, spleen, thymus, lymph node, urogenital system, adrenal, pituitary, thyroid, parathyroids, skin/musculoskeletal system, eyes, sciatic nerve, brain, spinal cord and injection site were preserved. For all animals from control and high-dose groups, these tissues were examined microscopically. A one-way ANOVA with Dunnett's t-test was applied to all numerical data.

**Results:** No animal died during the course of the study and there were no biologically significant changes in body weight. Clinical signs were unremarkable. For serum chemistry, there were statistically significant decreases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at 500 µg/kg compared to control. Albumin/globulin ratios were increased significantly at both doses. These changes were considered due to biological variation, and not treatment related. No biologically significant treatment-related effects were observed on any of the hematology parameters. Brain, liver and adrenal weights did not show any treatment-related changes. However spleen weight (absolute and relative to body or brain) were decreased significantly compared to control. The effect on the spleen was considered to be of no biological significance since no histopathological correlation or concomitant changes in serum chemistry and hematological parameters were observed

Findings that showed statistically significant changes ( $p < 0.05$ ) are listed in the table.

Parameter	Test Group	Mean± S.E.	
	Sex/ Dose	Control Group	Test Group
<b>Hematology</b>			
Neutrophil, %	Male/Low	10.1±2.2	14.5±3.9
Lymphocytes No.	Male/Low	10680±1932	8478±2372
<b>Organ/Body weight x (10<sup>-2</sup>)</b>			
Spleen, g	Male/Low	0.27±0.03	0.21±0.01
Spleen, g	Male/High	0.27±0.03	0.23±0.02
Spleen, g	Female/Low	0.30±0.04	0.26±0.04
Spleen, g	Female/High	0.30±0.0	0.26±0.03

**Conclusion:** It was concluded that Technetium Apcitide injection was well tolerated in male and female rats. NOEL was stated to be at 500 µg/kg peptide (250X MHD)

**Reviewer's comments:** Spleen from both high- and low-dose groups showed a statistically significant decreases in weight (13-20% reduction). However the effect on the spleen was not dose-related, and was not accompanied by concomitant changes in serum chemistry or hematological parameters. I agree that the biological significance is debatable.

**Study No. R2.83, A Single-Dose and 14-Day Repeated-Dose Evaluation of Intravenously Administered Technetium Tc 99m Apcitide Injection (Original Formulation) Prepared with Decayed Generator Eluate in Rats.**

**In-Life 01/19/93-02/08/93.** This study used the original formulation kit. The study is located in volume 1.13 pages 179-304. This study was in compliance with GLP

A comparison of the formulation used in this study and the final commercial preparation is given in the table<sup>a</sup> below.

Formulation	Component	Quantity per vial (1mL)
<b>Final</b>	Sibapcitide Trifluoroacetate Tin Chloride, Dihydrate Sodium Glucoheptonate Dihydrate	100 µg 89 µg 75 mg

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Original formulation contains a much smaller amount of SnCl<sub>2</sub> per µg of apcitide plus does not contain sodium glucoheptonate. (From Table 1, Vol.1.13 pp.182)

The study assessed the potential for acute or subacute toxicity of technetium Tc 99m Apcitide injection given as a single dose or after 14 daily intravenous administrations in male and female rats. The animals were assigned to the following groups as shown in the table below

Group No.	No. of M/F rats	Test Material	Sacrifice Day	Dose Level (µg peptide/kg)	Multiples of MHD
1 (Control)	10M/10F	Saline	15	0	0
2 (Single-dose)	10M/10F	Test Article	5M/5F, 48 Hr 5M/5F, 15	500	250
3(Repeated dose)	10M/10F	Test Article	15	150	75
4 (Repeated dose)	10M/10F	Test Article	15	500	250

<sup>1</sup>Table from Vol.1.13 pp. 184 Test article = Original kit formulation.

Each rat in group 2, single-dose group received 500 µg peptide /kg on day 1 intravenously. Rats in the remaining groups received 0, 150, or 500 µg/kg once daily for 14 consecutive days. Body weights were measured before the beginning of the study, and daily during the study period. The animals were observed for clinical signs during the course of the study.

Half of the animals in group 2 were bled from the abdominal aorta 48 hours post-dosing, for evaluation of hematological and clinical chemistry parameters prior to necropsy. The remaining half from group 2, together with all the animals in groups 1, 3, and 4 were anesthetized on day 15 and blood samples were taken via the abdominal aorta for hematological and serum chemistry evaluation. Necropsies were carried out, and gross pathological observations made. The following tissues were weighed from all the animals: adrenals, brain, kidneys, liver, lungs, ovaries, pituitary gland, spleen, testes, thyroid and parathyroid. T- and adjusted t-test were used to determine significant differences. Only tissues from groups 1 and 4 rats were evaluated for histopathology.

**Results:** There was no mortality during the course of this study. Clinical observations were normal. On day 14, the percentage weight gain for the control male rats was +17%. Groups 2 and 3 male rats gained 25% and 23% . these values reached statistical significant level, however they were attributed to wide range of weight gain commonly seen in male rats as there was no evidence of edema, congestion or hypertrophy. Clinical chemistry and hematological values were comparable between treated rats and controls. Spleen weights were comparable among groups. However, mean organ weights for group 3 male left kidneys, lungs, testes and hearts and group 4 male lungs and thyroid were different from control males.

**Conclusion:** It was concluded that Technetium Apcitide injection (original formulation) has a NOEL of greater than or equal to 500µg peptide/kg in Sprague-Dawley rats after either a single intravenous administration or after 14 consecutive daily intravenous administration.

**Reviewer's comments:** With the exception of the formulation differences, it is noted that the present study, and study R2.82 conducted also in rats are essentially very similar. The only reason it was reviewed was to examine whether the 48 hour component of the study can stand on it's own merit since the submission lacked a study devoted to this component of acute toxicity in rat. While there was not a proper time matched control, it is noted that clinical observation,

clinical chemistry, hematology and gross pathological were normal for the 48 hour study. In view of lack of significant findings, I recommend that we accept the 48 hour study as adequate for submission. I agree that a NOEL was demonstrated at 500µg peptide/kg for the 14-day repeated-dose study.

**Study No: R2.80, Update 1: A 14 Day Repeated Dose Intravenous Toxicity Study Of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate Administered To Rabbits.**

**1. In-Life 12/03/96-12/18/96. Final commercial formulation Lot No. 9603B03 was used in this study. The study is located in volume 1.14 pages 1-140. This study was in compliance with GLP.**

The objective of the study was to investigate the potential toxicity of Technetium apcitide given for fourteen consecutive days intravenously in rabbits.

Thirty-six rabbits (male and female) were assigned to the following groups ( table from Vol.1.14 pp.06).

Each rabbit received the test or control vehicle (saline) intravenously once daily for 14 consecutive days. Body weights were measured before the beginning of the study, and every other day during the study period. The animals were observed for clinical signs during the course of the study. Following completion of the study, blood samples were taken via the abdominal aorta for hematological and serum chemistry evaluation.

Group No.	No. of M/F	Test Material	Dose route	Dose level <sup>1</sup> (µg/kg)	Dose volume ml/kg	Multiple of MHD
1	6/6	Saline	IV	0	2.4	0
2	6/6	Tc-Apcitide Injection	IV	72	0.72	36
3	6/6	Tc-Apcitide Injection	IV	240	2.4	120

<sup>1</sup>Gravimetric dose refers to total amount of peptide present in injection

A bone marrow smear was collected from the sternum of all animals at necropsy. Necropsy were carried out at day 15 and gross observations made on all the animals, selected tissues were weighed before fixation; adrenal, brain, liver and spleen, and organ to brain or body weights ratios calculated. Microscopical examination of tissues was performed on all control and high-dose groups. A one-way ANOVA with Dunnett's t-test was applied to all numerical data.

**Results:** All animals survived the study and there was no biologically significant changes in body weight. Clinical signs included one case of transient loss of consciousness and apnea in a high dose female on day 14 of dosing. The pathologist report did not reveal any significant finding for this animal. It was concluded that the loss of consciousness was treatment unrelated and may have been caused by restraint used during injection. There were cases of loose stool and diarrhea which were more common in the 240µg/kg male group but did not occur in the 240µg/kg female or 72µg/kg male and female groups. There was no treatment related changes in

bone marrow smears. Findings that showed statistically significant changes ( $p < 0.05$ ) are listed in the table below.

Parameter	Test Group	Mean ± S.E.	
	Sex/ Dose	Control Group	Test Group
<b>Organ/Body weight x (10<sup>-3</sup>)</b>			
Spleen, g	Male/Low	0.65±0.09	0.49±0.07
Spleen, g	Male/High	0.65±0.09	0.42±0.09
Spleen, g	Female/Low	0.71±0.27	0.44±0.0.04
Spleen, g	Female/High	0.71±0.27	0.51±0.07
<b>Clinical chemistries</b>			
Globulin, g/dl	Male/Low	1.9±0.2	1.6±0.1
Albumin/globulin Ratio	Male/Low	2.0±0.2	2.3±0.1
AST, U/L	Female/High	22.5±5.4	18.2±3.1
Calcium, mg/dl	Female/High	12.4±0.3	11.9±0.5

**Conclusion:** It was concluded that Technetium Apcitide injection was well tolerated in male and female rabbits. Since there were no histopathological correlation or concomitant changes in serum chemistry and hematological parameters, the effect on the spleen was concluded to be of no biological significance. NOEL was stated to be at 240 µg/kg peptide (120X MHD).

**Reviewer's comments:** Effect on spleen weight has been a consistent finding. I am not sure of the biological significance of such a change especially since it was not accompanied by changes in serum chemistry and hematological parameters.

**Study R2.63: Safety of Tin chloride and Sodium glucoheptonate - Non-Peptide Components of Technetium Tc 99m Apcitide Injection. Literature survey and Inferences drawn from studies conducted by Diatide. The literature review is located in volume 1.15 pages 73-88.**

The objective of this literature review was to address the safety of tin chloride and sodium glucoheptonate, the two non-peptide components of Technetium Tc 99m Apcitide injection. A number of published articles demonstrated that large multiples of the MHD of tin chloride and sodium glucoheptonate were well tolerated in several acute and subacute animal toxicology studies. Moreover, the sponsor emphasized that all preclinical GLP toxicity studies on Technetium Tc 99m Apcitide injection prepared with decayed generator eluate contained these

two components at high multiples of MHD. It concluded that "it is unlikely that clinically, the single intravenous administration of one vial of technetium <sup>99m</sup>Tc Apcitide injection will produce any untoward effects attributable to SnCl<sub>2</sub> or sodium glucoheptonate".

**Reviewer's comments:** Agree, I do not anticipate the two non-peptide components to contribute to the acute and subacute toxicity of the preparation. Moreover, the GLP toxicity studies conducted with the final formulation contained these two components.

### Summary of Toxicology

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The only effect of relevance that seems to be conserved across species is a reduction in the absolute or relative weight of the spleen that was noted in the following studies.

- Single-dose intravenous study in rabbits, 14-day observation (study R2.76)
- 14-day repeated dose study in rabbits (R2.80)
- 14-day repeated dose study in rats (R2.82)
- Moreover, microscopic evidence of lymphoid depletion was observed in a single dose, 14-day observation study in mice (R2.59).

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Study # R2.59 (pp. 32-33): This was a single dose acute intravenous study in mice to assess the potential for acute toxicity of technetium Tc <sup>99m</sup> apcitide injection following doses representing 300X and 1000X MHD (2 µg total peptide /kg). The major findings occurred in the 14-day observation group. Spleen demonstrated microscopic evidence of lymphoid depletion in 4 of 10 high-dose and 5 of 10 low-dose males, 5 of 10 high-dose females 1 of 10 control males and 1 of 10 control females after 14 day observation. The histopathological findings corresponded with lower weights in some of the treated animals. The contractor attributed the observation to the test article. Test and control animals did not demonstrate significant differences in hematological parameters. The sponsor questioned the biological significance of the changes in spleen. A **qualified** acute no-observable effect-level (NOEL) was assigned in this study at 1000X MHD.

Study #R2.73 (pp.33-35) This study was essentially a repeat of study #R2.59, a single dose acute intravenous toxicity study in male and female albino mice conducted by a different contractor. The groups were as follows: 30X, 100X, 1000X and the dose of nonpeptide formulation excipient that was equivalent to 1000X MHD. The animals did not exhibit signs of toxicity. Lymphocytes, monocytes and eosinophil counts showed variable differences. No significant findings was reported for blood chemistry. The spleen and liver weights were within normal ranges. All other findings were considered by the contractor to be of no biological significance. Diatide concluded that "Technetium Tc <sup>99m</sup> apcitide injection prepared with decayed generator eluate was well tolerated, with minimal findings in the liver and white blood cell counts". A NOEL was assigned as 2,000 µg peptide /kg (1000X MHD).

Study # R2.76 (pp. 35-36) the objective of the study was to evaluate the potential single-dose acute toxicity of technetium Tc <sup>99m</sup> apcitide injection in rabbits. Males and females rabbits were divided into groups that received 0, 150X and 500X of human MHD once and were observed for 48 hours or 14 days. There was no mortality, and there was no biologically significant changes in body weight. Spleen histology was said to be unremarkable. Histological and bone marrow smear evaluation were said to be unremarkable. No biologically significant findings was reported for serum chemistry. It was concluded that Technetium Tc <sup>99m</sup> apcitide injection was well

tolerated and there was no evidence of toxicity at doses of 300 and 1000 µg peptide/kg administered intravenously as a single dose in rabbits. NOEL was 1000µg peptide/kg, or 500X MHD. --

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Study # R2.82 (pp. 36-37). This was a 14 day repeated dose intravenous toxicity study of technetium Tc 99m apcitide in rats . There were 3 groups M/F (10 each) that received 0, 150 or 500 µg/kg once daily for 14 consecutive days. No animal died, and there was no biologically significant changes in body weight. Clinical signs were unremarkable. There were statistically significant decreases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at 500 µg/kg compared to control. A/G ratios were increased significantly at both doses. These changes were considered not treatment related. No biologically significant treatment-related effects were observed on any of the hematology parameters. Spleen weight (absolute and relative to brain) were decreased significantly compared to control. The contractor considered the effect on the spleen to be of no biological significance since no histopathological correlation or concomitant changes in serum chemistry and hematological parameters were seen. It was concluded that Tc Apcitide injection was well tolerated in male and female rats. NOEL was stated to be at 500 µg/kg peptide (250X MHD).

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Study # R2.83 (pp.37-38). A single dose and 14-Day repeated evaluation of intravenously administered technetium Tc 99m apcitide injection (original formulation) in rats. With the exception of the formulation differences, this study and R2.82 are similar. Results are not significantly different from study # R2.82.

Study # R2.80 (pp. 39-40). The objective of the study was to investigate the potential toxicity of technetium Tc 99m apcitide given for fourteen consecutive days intravenously in rabbits. There were 3 groups M/F (6 each) that received 0, 72 or 240 µg/kg once daily for 14 consecutive days. All animals survived the study. Clinical signs included one case of transient loss of consciousness and apnea in a high dose female. The contractor felt that the loss of consciousness was treatment unrelated. For serum chemistry, there were statistically significant decreases in creatinine and sodium at 72µg/kg and decreased globulin and increased A/G ratios at 72 and 240µg/kg compared to controls. These changes were considered due to biological variation, and not treatment related. Hematology parameters were said to be unremarkable. Spleen weight (absolute and relative to brain) were decreased significantly in male (low and high doses) compared to control. The contractor considered the effect on the spleen to be of no biological significance since no histopathological correlation or concomitant changes in serum chemistry and hematological parameters were seen. There was no treatment related changes in bone marrow smears. It was concluded that Tc Apcitide injection was well tolerated in male and female rabbits. NOEL was stated to be at 240 µg/kg peptide (120X MHD).

Study # R2.63 (pp. 40). The objective of the study was to assess the safety of tin chloride and sodium glucoheptonate. The two non-peptide components of acutect. It reviewed a number of published articles that supported the assertion that large multiples of the MHD of tin chloride and sodium glucoheptonate is well tolerated in several acute and subacute animal toxicology studies. It concluded that "it is unlikely that clinically, the single intravenous administration of one vial of technetium <sup>99m</sup>Tc Apcitide injection will produce any untoward effects attributable to SnCl<sub>2</sub> or sodium glucoheptonate".

**29. Carcinogenicity:**

Carcinogenicity studies were not conducted.

Diatide claimed that "consistent with the notice issued in March 1996 by the ICH entitled, Final guideline on the need for long term rodent carcinogenicity studies of pharmaceuticals" no carcinogenicity or reproductive toxicity tests were performed with Technetium Tc 99m apcitide injection.

**30. Immunotoxicology:**

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**Study No. R2.61; Update 2: Evaluation of the Immunogenicity of Mock-Labeled Technetium Tc 99m Apcitide Injection in Guinea Pigs.**

**In-Life 10/9/96-11/18/96 Final Commercial formulation Lot No. 9603M01 used for this study. The study is located in vol. 1.15 pages 2-33. This study was in compliance with GLP.**

The objective of the study was to examine the antigenic potential of Technetium Tc 99m.

10 male guinea pigs were used for the study. The animals were divided into two groups. Six animals were used for the test substance. Each received the entire content of 1 unit of mock-labeled Tc 99m apcitide as intraperitoneal (I.P) injection three times a week for two weeks. The remaining animals received equal volume of normal saline in the same dosing regime. Fifteen days after the last I.P. injection, three of the six guinea pigs received i.v. injection of the test preparation. They were observed for unusual behavior such as face pawing, fur ruffling, labored breathing, sneezing or coughing, retching, abnormal respiratory sound, prostration, convulsion and death. The remaining animals received identical treatment twenty-nine days following the last induction.

**Results:** No animal demonstrated any sign of antigenicity in this test.

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**Conclusion:** It was concluded that Technetium Tc 99m Apcitide injection is non-antigenic.

**Reviewer's comments:** Agree. There was no indication that apcitide elicited a systemic anaphylaxis response in this model. However, the experimental design is inadequate. A positive control group should have been done concurrently with the study. It is noted that measurement of hematologic indicators of immunogenicity were not part of this study.

**Study No: R2.60, Update 1: Evaluation of the Potential for Producing Perivascular Irritation by Mock-Labeled Technetium Tc 99m Apcitide Injection in Rabbits.**

**In-Life 10/02/96-10/05/96. Final commercial formulation Lot No. 9603M01 was used in this study. The study is located in vol. 1.15 pages 34-63. The study was in compliance with GLP.**

The study examined the potential of Technetium Tc 99m apcitide injection to produce tissue irritation after a single perivascular injection.

Six New Zealand white rabbits were used for the study. Each animal received 0.25 mL (25µg peptide/animal) of the test article perivascularly into a marginal ear vein. An equal volume of saline was injected into the contralateral ear vein. The animals were evaluated for tissue irritation by gross visualization and by histopathology, and scored according to the method of Draize at 24 hours n = 3, and 3 days n = 3.

**Results:** On a scale of 0-16 (none to severe irritation), the primary irritation index of apcitide injection was 0.8.

**Conclusion:** Technetium Tc 99m Apcitide injection was considered a non-irritant.

**Reviewer's comments:** Agree. There was no indication that Technetium Tc 99m Apcitide is an irritant in the test model.

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**Study No. R2.49: An Evaluation of the Compatibility of Mock-Labeled Technetium Tc 99m Apcitide Injection with Human Blood or Serum.** The study was conducted by Diatide Inc. The study used the final commercial formulation Lot No. 9603M01. The study is located in vol. 1.15 pages 64-72 The study was not in compliance with GLP.

The study examined the compatibility of mock-labeled Tc 99m Apcitide injection with human blood or serum.

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Technetium Tc 99m apcitide injection was mixed with serum or anticoagulated blood obtained from two volunteers in a ratio of 1:4 or 1:8. Controls received equal amount of saline in place of mock-labeled Technetium Tc 99m apcitide injection. Blood and serum samples with no additions were included to determine dilution effect. The final concentrations of the kit component in the 1:4 dilution were 25µg/mL, 19mg/mL and 11µg/mL for P280 peptide, sodium glucoheptonate and SnCl<sub>2</sub>.2H<sub>2</sub>O respectively. The following tests were performed by New Hampshire Medical Laboratories: erythrocyte sedimentation rate, plasma and serum protein precipitation, white blood cell WBC count and morphology including Rouleaux formation, mean cell hemoglobin concentration and platelets count

**Results:** The sponsor stated that there was no evidence of hemolysis or protein precipitation in either subject plasma to which saline or mock-labeled apcitide injection was added. Both were stated to show either "slight or present" Rouleaux formation in the 1:4 dilution samples. This was absent in 1:8 dilution.

**Conclusion:** Technetium Tc 99m Apcitide injection is compatible with blood.

**Reviewer's comments:** Agree.

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**Summary of Immunotoxicology**

Study # R2.61 (pp.45) The objective of the study was to examine the antigenic potential of Technetium Tc 99m in guinea pigs. Repeated intraperitoneal injection of Tc 99m apcicide for three times a week for two weeks followed by challenge administration fifteen or twenty nine days later did not produce anaphylactoid type antigenic response in the animals as measured by behavioral observation indicators..

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Study # R2.60 (pp.45-46) The study examined elicitation of tissue irritation reaction after a single perivascular injection in rabbits. Each animal received 25µg peptide (in 0.25 ml) perivascularly into marginal ear vein. An equal volume of saline was injected into the contralateral ear vein. The animals were evaluated for tissue irritation. No significant irritation was produced.

Study # R2.49 (pp.46) The study examined the compatibility of mock-labeled Tc 99m Apcicide injection with human blood or serum. Technetium Tc 99m apcicide injection was mixed with serum or anticoagulated blood in a ratio of 1:4 or 1:8. Concentrations of the kit component in the 1:4 dilution were 25µg/mL, 19mg/mL and 11µg/mL for P280 peptide, Sodium glucoheptonate and SnCl<sub>2</sub>.2H<sub>2</sub>O respectively. Controls received equal amount of saline. Erythrocyte sedimentation rate, plasma and serum protein precipitation, white blood cell count and morphology including Rouleaux formation, mean cell hemoglobin concentration and platelet count were determined. There were no evidence of hemolysis or protein precipitation in plasma to which saline or mock-labeled apcicide injection was added. The samples were stated to show either "slight or present" Rouleaux formation in the 1:4 dilution samples. It was concluded that technetium Tc 99m apcicide injection is compatible with blood.

**31. Reproductive Toxicology:**

Reproductive studies were not conducted. Diatide requested for a waiver in accordance with 21 CFR 314.90.

**32. Genotoxicity:**

Study No. R2.84. Update1: Evaluation of Mutagenicity of Technetium Tc 99m Apcicide Injection Prepared With Decayed Generator Eluate in the Salmonella/E. coli Mamalian Microsome Reverse Mutation (Ames) Assay.

In-Life 03/06/96-05/05/96.

The study used the final commercial formulation Lot No. 9603M01. The study is located in vol. 1.15 pages 171-242. This study was in compliance with GLP.

This study evaluated the ability of Technetium Tc 99m apcicide prepared with decayed generator eluate to induce reverse mutation at the histidine locus of S.typhimurium tester strain (TA98, TA100, TA1535, and TA1537), and at the tryptophan locus in the E. coli strain WP2uvrA with and without metabolic activation by rat liver S9 fraction. Both positive and negative controls were evaluated concurrently in the study. The assay was conducted in three phases; a dose range finding phase/cytotoxicity test ( 37-18800 µg of kit per plate corresponding to 0.049-25µg peptide/plate) using tester strains TA100 and WP2urv, the definitive mutagenicity assay and an independent confirmatory assay. The tests were done in triplicate. All phases of the study were

conducted using the preincubation method whereby the test article is mixed with buffer or S9 microsomal enzymes plus bacteria, incubated for 20 minutes at 37°C, and combined with 2 mL of melted top agar for plating. Following incubation at 37 ± 2 °C for 48 ± 8 hr, revertant colonies were counted.

**Results:** The test substance was found not to be cytotoxic at concentrations up to 25 µg peptide/plate in the dose range/cytotoxicity test. Moreover, it did not increase the number of revertant colonies with and without metabolic activator in all the tests conducted.

**Conclusion:** Technetium Tc 99m apcptide injection prepared using decayed generator eluate was deemed non mutagenic in the Ames Assay.

**Reviewer's comments:** Agree. Although the highest concentration of peptide used for the experiment was not cytotoxic, technetium apcptide injection is not considered mutagenic in this assay.

**Study R2.85, Update1: Mutagenicity Test of Technetium Tc 99m Apcptide Injection Prepared With Decayed Generator Eluate in the L5178Y TK± Mouse Lymphoma Forward Mutation Assay.**

**In-Life 03/06/96-04/17/96.** The study used the final commercial formulation Lot No. 9603M01. The study is located in vol. 1.15 pages 243-307. This study was in compliance with GLP.

The study assessed the ability of Technetium Tc 99m Apcptide to induce forward mutation at the thymidine kinase locus in cultured L5178 Y cells.

The assay was performed in three phases: a dose range finding/cytotoxicity phase, the definitive mutagenicity assay, and a confirmatory assay. Appropriate controls were utilized to assure mutagenic response in the cell line. The smallest detectable colony was between 0.2-0.3 mm in diameter, depending on it's position in the agar matrix. As reported by the contractor, the dose range finding assay involved exposing cells to the test article at concentrations ranging from 0.0195 µg peptide/mL to 10 µg peptide/mL with and without S9 activation. No cytotoxicity was observed at 10µg total peptide/ml. For the definitive mutagenicity study, non-cytotoxic peptide dose range of 0.156 µg/mL to 10µg/mL was assessed. Both positive (non-activation control: methyl methanesulfonate; activation control: 3 methyl-cholanthrene) and negative controls (unexposed to test article) were utilized in the presence and absence of S9 activation. The confirmatory assay was performed similarly to the definitive assay. As reported by the sponsors, no cytotoxicity was observed and no increases in mutant frequency were observed. Because the test result was negative, colony sizing was not performed.

**Conclusion:** It was concluded that the preparation of Technetium Tc-99m apcptide was non mutagenic.

**Reviewer's comments:** Agree. Technetium Tc 99m apcptide did not induce mutation at the thymidine kinase locus in L5178Y culture cells.

**Study R2.86: Evaluation of the Clastogenicity of Technetium Tc 99m Apcitide Injection Prepared with Decayed generator**

In-

**Life 03/027/96-05/23/96.** The study used the final commercial formulation Lot No. 9603M01. The study is located in vol. 1.15 pages 308-366. This study was in compliance with GLP.

Induction of micronuclei in bone marrow polychromatic erythrocytes of Clr:CD-1(ICR) BR mice was used to examine the ability of Technetium Tc 99m apcitide injection to induce clastogenic response in this study.

The animals were grouped (5 males & five females) as follows ( From Vol.1.15 pp.313 table 2 )

Treatment Group	Dose	Sacrifice Time
Negative Controls	0.9% saline	24, 48, and 72hrs
Low	500µg/Kg	24, 48, and 72hrs
Medium	1000µg/Kg	24, 48, and 72hrs
High	2000µg/Kg <sup>1</sup>	24, 48, and 72hrs
Positive Controls	CP <sup>2</sup>	24 hrs only

<sup>1</sup> The high dose group had a backup group of 10 additional mice in the event that any from the high dose group died during the study. The maximum dose of mock-labeled technetium Tc 99m apcitide injection tested was equivalent to 1000X MHD. Test substance was given intravenously.

<sup>2</sup> CP, cyclophosphamide. Administered at 80 mg/kg by oral gavage.

Animals were euthanized at stated times, followed by aspiration of the femur marrow and preparation of slides for each animal. The slides were scored for micronuclei, and the polychromatic erythrocyte (PCE) to normochromatic (NCE) ratio for the first 1000 erythrocytes. The criteria for determining a positive response was a statistically significant dose-related increase in micronucleated PCE's or the detection of a reproducible and statistically significant positive response for at least one dose level.

**Results:** Cyclophosphamide induced significant increases in micronucleated PCEs. Technetium Tc 99m apcitide induced no significant increases in micronucleated PCEs or PCE/NCE ratio compared with concurrent or historical control.

**Conclusion:** Technetium Tc 99m apcitide injection is non- clastogenic.

**Reviewer's comments:** Agree. There was no indication that Technetium Tc 99m apcitide is clastogenic in this model.

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ON ORIGINAL**Summary of Genotoxicity**

Study # R2.84 (pp.43): The ability of Technetium Tc 99m apcicide to induce reverse mutation at the histidine locus of *S.typhimurium* tester strain (TA98, TA100, TA1535, and TA1537), and at the tryptophan locus in the *E. coli* strain WP2uvrA with and without metabolic activation (Ames bacterial assay) was conducted. Preliminary assay showed that it was not cytotoxic at concentrations up to 25 µg/plate. Moreover, a primary and confirmatory assay showed that it did not increase the number of revertant colonies. Tc 99m apcicide was considered non-mutagenic in this assay.

Study # R2.85 (pp.43-44): This study assessed the ability of Technetium Tc 99m Apcicide to induce forward mutation at the thymidine kinase locus in cultured L5178 Y cells. The dose range finding assay involved exposing cells to the test article at concentration ranging from 0.0195 µg/mL to 10 µg/mL with and without S9 activation. A primary and confirmatory assay was conducted with concentration . No cytotoxicity was observed and no increases in mutant frequency were observed. It was concluded that the preparation of Technetium Tc-99m apcicide was non mutagenic.

Study # R2.86 (pp. 44): Induction of micronuclei in bone marrow polychromatic erythrocytes of Clr:CD-1(ICR) BR mice was used to examine the ability of Technetium Tc 99m apcicide injection to induce clastogenic (chromosome breaking) response. Peptide dose range of 500-2000µg/kg was used. Animals were sacrificed at 24, 48, and 72 hours. The polychromatic erythrocyte (PCE) to normochromatic (NCE) ratio for the first 1000 erythrocytes was scored. Tc 99m apcicide did not increase micronucleated PCEs or PCE/NCE ratio compared with control. Technetium Tc 99m apcicide injection was considered non- clastogenic.

33. Special Toxicology: None

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**34. Overall Summary and Evaluation:****Introduction:**

In a Journal of Nuclear Medicine editorial submitted with this NDA and aptly titled "Thrombus-Specific Imaging: Approaching The Elusive Goal", Prantika Som and Zvi Oster declared that "the importance of developing a thrombus-specific imaging method is a goal of undisputed clinical importance". Som and Oster opined further that small peptides with platelet affinity labeled with 99m Tc may soon become an alternative to the use of labeled antibodies. In their view, "These small peptides with specific amino acid sequences are more advantageous than complex, large molecular weight antibodies or fragments since they can be produced synthetically and therefore less expensive. They are minimally antigenic and there is little danger of viral contamination, resulting in less stringent procedural requirements".

This reviewer agrees with these statements. The diagnostic drawbacks inherent in currently available techniques were discussed in the introduction and drug history section of this review. Diatide, INC. believes that an imaging agent that binds to activated platelets being incorporated into a thrombus, but which, if not bound clears rapidly from the blood, leading to improvement in target-to-background ratios would have great potential as an imaging agent for acute venous thrombosis. As described by Diatide INC., Technetium Tc 99m Apcitide is a peptide-based receptor imaging agent which offers potential utility in the detection and localization of acute venous thrombosis. The main indication as proposed is for *Detection and Localization of Acute Venous Thrombosis*.

This summary examines the NDA submission from the following perspectives:

**Safety Evaluation**

The major safety issue raised by apcitide's primary effect is its ability to inhibit platelet aggregation. This antiaggregatory action is implicit in the mechanism of action, and therefore may not be separable. In an in vitro study of human platelets (study # R2.51), the peptide components of Acutect; P1007, apcitide and P1008 inhibited platelet aggregation elicited by ADP with IC<sub>50</sub>s of 52, 382 and 689 nM respectively. In another study (#R2.74) examining ex vivo platelet aggregation in dogs administered 1X, 30X and 100X MHD of Tc apcitide injection, aggregatory responses to ADP declined by 43% and 98% in 30X and 100X groups respectively. The aggregatory response regained over half of the deficits observed within two hours in both groups. The 1X group showed no antiaggregatory effect. The study did not examine intermediate doses between 1X and 30X. It would have been useful to know the maximum dose at which no platelet aggregatory effect occurs (NOEL). This would have provided a margin of difference between the diagnostic dose and the NOEL for aggregatory response.

This effect on platelets was recognized by the sponsor. However they posited that for the in vitro study, the IC<sub>50</sub> of the most potent peptide was substantially higher than the maximum plasma concentration of any peptide component that could occur in vivo. However, in the formulation, there are three peptides not one, capable of inhibiting platelet aggregation. For the ex vivo study they reasoned that "as would be expected from in vitro studies of platelet aggregation, no inhibition of ex vivo platelet aggregation was observed at 1X MHD, even in the 3 minute post-

*injection plasma sample. Thus the administration of technetium 99m apcitide injection at the current MHD of 2µg peptide/kg is unlikely to perturb hemostasis".* Overall the sponsor maintained that the rapid elimination of the tritium labeled peptide components of technetium Tc 99m apcitide injection from the blood mitigates against actually attaining, and certainly not maintaining, the calculated maximal system plasma concentration.

From the pharmacology perspective, the rapid elimination of technetium Tc 99m apcitide injection from the circulation will certainly curtail the achievement of plasma concentration sufficient to inhibit platelet aggregation in vivo. What I am not sure of is the clinical probability of exposing the same patient to repeated diagnostic imaging within a short period of time. However, the platelet inhibiting action must be given considerable attention in such a scenario. I recommend limiting the number of diagnostic imaging procedures on the same patient within a given period in line with the known human pharmacokinetics profile of Acutect.

The animal pharmacokinetics data did not reveal any serious safety concern issue. Study # R2.53 showed that technetium Tc 99m apcitide is either excreted unchanged in urine or undergoes metabolism to an unidentified, more hydrophilic metabolite. This metabolite was not present in the plasma and was therefore concluded by the sponsor to be generated by the kidneys. An alternative explanation is that rapid plasma clearance or peptide below detection limits of the analytical system was responsible for lack of identification in plasma.

Study # R2.67 examined gender differences in pharmacokinetics and biodistribution of intravenously administered technetium Tc 99m apcitide in rats. Tc 99m apcitide was cleared rapidly from the blood with about 97% cleared within the first 30 minutes. The 4-hour total urinary excretion was higher in female than in male (91 vs 79% ID). I agree with the sponsor that the clinical significance of the apparent gender differences in handling of injected apcitide is uncertain, especially in light of the fact that elimination was almost completed during the 4 hour observation period in both male and female rats. Nevertheless, this observation of faster elimination in females is intriguing given the fact that gender differences (including humans) in platelet reactivity are well documented. Males are reported to show enhanced platelet aggregatory activity compared to females through testosterone-dependent mechanisms. If kinetics is different, one wonders whether dynamics of apcitide injection will also demonstrate subtle gender differences.

In rats with experimental renal dysfunction (ERD) produced by bilateral renal artery and vein ligation (Study # R2.75), it was demonstrated that renal dysfunction significantly affected systemic clearance of Tc 99m apcitide. It seems that hepatobiliary excretion does take over when renal elimination is impaired. However, a major criticism of this study is that the pharmacokinetics profile was monitored for a four hour period at the end of which only about 50% of the injected radioactivity had disappeared from the blood in ERD rats compared with 98% for normal rats during the same interval. Thus it was impossible to determine whether the entero-hepatic system is quantitatively large enough to compensate fully in the event of complete renal failure.

Acutect administration resulted in minimal toxicological effects. The only effect of relevance that seems to be conserved across species is a reduction in the absolute or relative weight of the spleen that was noted in the following studies.

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- Single-dose intravenous study in rabbits, 14-day observation (study R2.76)
- 14-day repeated dose study in rabbits (R2.80)
- 14-day repeated dose study in rats (R2.82)
- Moreover, microscopic evidence of lymphoid depletion was observed in a single dose, 14-day observation study in mice (R2.59).

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From these toxicological studies, it is obvious that the effect on the spleen deserves some comments. The reduction in the weight of the spleen observed in rats and rabbits did not exhibit a dose-dependent profile, and was sometimes not accompanied by changes in hematological profile. Mice appear to be sensitive to the toxicological effect of apcitide. Lymphoid hypoplasia observed in study R2.59 may be an isolated finding or part of a generalized pathophysiological process. It is invariably associated with autosplenectomy and increased susceptibility to infection due to loss of immunoglobulin generating cells. Since there was no evidence of lymphoid hypoplasia in other studies, the questions then arise. Why are mice sensitive? Overly so, or appropriately. How do we know that humans are not like mice? Even if it happens that the effect on spleen is devoid of biological significance, why is the spleen the target of this effect? I do not have answers to these questions. The medical reviewer informed me that safety assessment of Acutect is continuing. From the preclinical safety perspective, it is appropriate to request a phase 4 commitment from the sponsor to monitor the hematological profile of the patients undergoing diagnostic imaging with Acutect.

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The sponsor did not conduct any reproductive or carcinogenicity toxicity study. They requested for a waiver (21 CFR 314.90) for reproductive toxicity testing based on the following:

There was no histopathological evidence of any toxic effect of Technetium Tc 99m apcitide prepared with decayed generator eluate on reproductive organs in male or female rats and rabbits.

There was no evidence of possible mutagenicity or clastogenicity of Technetium Tc 99m apcitide injection in any of the in vitro or in vivo assays conducted.

Moreover, the sponsor is of the opinion that this request is consistent with the notice issued in March 1996 by the ICH entitled, "Final guideline on the need for long term rodent carcinogenicity studies of pharmaceuticals".

From my perspective, granting of the waiver will be consistent with historical decisions taken by the Division.

**Pharmacological proof of concept and demonstration of efficacy**

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The pharmacology of apcitide is linked to the mechanism whereby fibrinogen combines with the glycoprotein receptors. The glycoprotein 11b/111a (GP11b/111a) receptor is a member of the integrins family of receptors present on platelets and endothelial cell surfaces. Upon activation, this family of receptors binds a number of naturally occurring proteins including fibrinogen. Platelets must become activated before GP11b/111a receptor undergoes the required conformational changes needed for binding fibrinogen; so called "inside-out" signaling. The pharmacophore of apcitide is a mimetic of the tripeptide arginyl-glycyl-aspartic acid (RGD in

single-letter amino acid nomenclature) sequence present in fibrinogen which mediates the binding of fibrinogen to GP11b/111a receptor. Since platelet activation is required for thrombus formation, Diatide reasoned that a radiolabeled GP11b/111a-binding peptide could be used to detect and localize acute thrombi, in particular, deep venous thrombi.

One of the keys to examining the proof of concept is to break the proposed mechanism of action into discreet modules, and to analyze the available evidence for each module. The core module, that fibrinogen binds to activated glycoprotein 11b/111a receptor is well documented. As expected Diatide provided pertinent literature information in support of the fact. That the amino acid sequence RGD represents the active pharmacophore needed for binding of fibrinogen to activated platelets is perhaps less well realized. However the whole submission is based on this rationale. Secondly a table from the study by *Edward Plow et. Al. Proc. Natl. Acad.Sci. 82 pp. 8057-8061* showed the structure-activity relationship that defined the pharmacophore.

Plow's study examined the effects of Arg-Gly-Asp containing peptides on fibrinogen binding to platelets. They concluded that the key sequence for inhibition of the platelet-fibrinogen interaction was the Arg-Gly-Asp since reversal of the sequence resulted in inactive peptide. This study elegantly and adequately addressed the issue of structure-activity relationship (question 3) raised by me in the introduction/drug history section of the NDA.

Table: Structure-functional relationships for the inhibition of fibrinogen binding to ADP-stimulated platelets by Arg-Gly-Asp-containing peptides

Peptide	ID <sub>50</sub> μM
GRGDSP	35
GRGESP	>1000
GRADSP	800
GRADSPC	420
GKGDS	450
RGDS	18
RGDV	15
CPSDGRD	>1000
AGDV	>1000

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The single letter amino acids are as follows: G, glycine; R, arginine; D, aspartic acid; S, serine; P, proline; E, glutamic acid; A, alanine; C, cysteine; K, lysine; and V, valine.

In this NDA, Diatide Inc., advanced as proof of concept 5 pivotal studies. Study #R2.79, examined the specificity and affinity of rhenium complex of apcitide for purified human GP11b/111a receptor. The study compared Re-apcitide's ability to inhibit the binding of fibrinogen to purified  $\alpha_2\beta_3$  with its ability to inhibit the binding of vitronectin to  $\alpha_5\beta_3$  receptor. Re-apcitide inhibited the binding of fibrinogen by 50% at 1.8nM while it required  $\geq 100$ nM of re-apcitide to inhibit vitronectin binding by  $\sim 20\%$ . Diatide concluded that "This is a stringent demonstration of specificity, since vitronectin, like fibrinogen, contains the RGD sequence and because the vitronectin and GP11b/111a receptors share a common  $\beta$  strand."

I agree that this study can be taken as proof of demonstration of the relative selectivity of re-apcitide for the fibrinogen receptor over vitronectin receptor.

Implicit in the ability of RGD-like peptides to bind to GP11b/111a receptor is the fact that these peptides are capable of blocking the binding of fibrinogen to the same receptor, and consequently of inhibiting platelet aggregation induced by global stimulants such as ADP. Study #R2.52 quantified the binding of Technetium Tc 99m apcitide to resting and activated human platelets or platelet-rich plasma in vitro. It was concluded that technetium Tc 99m apcitide binds specifically to washed human platelets and that technetium Tc 99m apcitide bound 2.4 to 3.5 times more avidly to activated platelets than to resting platelets. My main concern about this study is the low level of specific binding to platelets. "Binding" in the absence of activation reveals non-receptor mediated adsorptive processes, and it illustrates the practical difficulty of obtaining a "fibrinogen free" non-activated platelet. The reader is again reminded that these results are indicative of the fact that the specificity of Technetium Tc 99m apcitide binding to activated platelets is not absolute, and that Technetium Tc 99m apcitide can bind to unstimulated platelets as well as to endothelial cells perhaps through interaction with closely related integrins receptor sites. Be as it may, I concur that Technetium Tc 99m apcitide showed 2-3 fold increase in binding in activated washed platelets compared with non activated platelets.

Both the in vitro inhibition of platelets aggregation by peptides P280, P1007, P1008 and technetium Tc 99m apcitide injection examined in study # R2.51 and the ex vivo platelet aggregation study in dogs administered 30X and 100X MHD of Tc apcitide injection (Study #R2.74) could also be taken as proof of concept studies, since it can be concluded that the dynamic activated platelet peptide interaction led to the antiaggregatory effect.

As a proof of efficacy, the ability of <sup>99m</sup>Tc-P280 to detect thrombi in vivo in a canine venous thrombosis model was examined using 24 hour old venous thrombi produced in canine by insertion of dacron-entwined stainless steel embolization coil in the right femoral vein (Journal of Nuclear Medicine 1996, 37: 775-781 ). Technetium Tc 99m apcitide (185-370 MBq <sup>99m</sup>Tc and 0.2-0.4 mg peptide,) was administered intravenously, and the animals imaged for 4 hours. Positive control and negative control animals received <sup>99m</sup>Tc-HMPAO-labeled autologous platelets (260 MBq) or <sup>99m</sup>Tc-glucoheptonate (290 MBq) respectively . Uptake of technetium Tc 99m apcitide by thrombi was quantified by excision and counting. It was concluded that <sup>99m</sup>Tc-P280 provided in vivo visualization of thrombi and good thrombus-to blood (4.4), and thrombus-to-muscle (11) ratios. It was also noted that HMPAO-labeled autologous platelets provided a better visualization of the thrombi compared with <sup>99m</sup>Tc-P280.

However, from a pharmacologist perspective, it is my opinion that the present formulation is less than optimal. During kit formulation and subsequent radiolabeling, bibapcitide is converted to apcitide, and two related peptides, P1008 and P1007 in the approximate ratio 20.15.65. Although these three peptides are present in the final injection, it is believed that only apcitide undergoes labeling with technetium. Nevertheless both P1007 and P1008 are potential competitors for apcitide binding site on platelets. Theoretically the presence of both P1007 and P1008 in the injectate will reduce the clinical efficacy of apcitide imaging. It is my considered opinion that apcitide represented a more rationale choice.

Taken together, these results provided sufficient evidence of pharmacological prove of concept and demonstration of efficacy.

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**Conclusion**

Having considered all the evidence submitted by the sponsor, I recommend that this NDA be approved subject to phase 4 commitment by the sponsor.

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**Internal Comments:**

A conference call with the sponsor was set up by C.T. Viswanathan, Ph.D., Associate Director, Division of Scientific Investigations (HFD-345) to resolve the issue. Drs. Viswanathan and Meyers accepted Diatide's explanation which centered on the fact that the required information were provided in the chemistry section of the submission (CMC Vol. 1.3 Sec 3.d.7. pp. 48-99). Both concluded that the studies are acceptable, and that no further action is warranted.

**Reviewer's comments:** Agree. The fact that no sample was retained for analysis was noted and considered during my primary review of the data. However, my understanding of the chemistry and pharmacology of the formulation, as well as my interaction with the reviewing chemist convinced me that the composition and stability of the reconstituted dosing formulation (according to clinical protocol) could be confirmed indirectly according to CMC analytical and stability data, as long as the reconstitution of the lyophilized drug product with the decayed technetium eluate was also according to clinical protocol. I confirmed that the GLP compliance statement included an inspection of a dosing formulation preparation phase. Moreover, I used the effect of apcitide on the spleen as a biological marker of whether the test substance was administered to the animal or not. This effect was conserved in all the toxicology studies conducted by the contract laboratories.

**36. NDA Issues:**

**37. Labeling Review:**

Deletions are indicated by "Strikeout"

Additions are indicated by "Redline"

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