

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125511Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: October 10, 2014
From: Timothy J. McGovern, PhD, ODE Associate Director for
Pharmacology and Toxicology, OND IO
BLA: 125511
Agency receipt date: October 24, 2013
Drug: Natpara (rhPTH[1-84])
Sponsor: NPS Pharmaceuticals, Inc.

Indication: Replacement for endogenous parathyroid hormone(1-84) indicated
for long-term treatment of hypothyroidism

Reviewing Division: Division of Metabolism and Endocrinology Products

The primary pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data for recombinant human parathyroid hormone [PTH(1-84)] support approval for the indication listed above.

The recommended pharmacologic class for PTH(1-84) is a bone anabolic agent; PTH(1-84) is synthesized and secreted by the parathyroid glands and is the principal endocrine hormone regulating systemic calcium and phosphorous homeostasis.

The majority of nonclinical data for this BLA was submitted and reviewed under NDA (b) (4) from NPS for the treatment of osteoporosis. (b) (4)

(b) (4) it was eventually withdrawn in 2011. A complete nonclinical program was conducted including chronic toxicology studies in rats and monkeys, a genetic toxicology battery, a two-year carcinogenicity study in rats, and a battery of reproductive and developmental toxicology studies. Newly submitted studies include a peri-/post-natal developmental study and a lactation study in rats. In the general toxicology studies the primary toxicities included kidney and bone marrow effects. Hypercalcemia was observed in monkeys at all doses tested and could be inferred in rats. The identified NOAELs in the toxicity studies provided exposure margins of < 1- to 3-fold (based on AUC) compared to the anticipated clinical exposure at the maximum recommended dose.

PTH(1-84) was negative in a battery of genetic toxicity studies. PTH(1-84) dosing in a 2-year carcinogenicity was associated with increases in the incidence of bone neoplasms, especially osteosarcomas. Although the increase at the low dose was not statistically significant, the findings do not represent a negligible risk for humans given the relatively low exposure margins. Natpara demonstrated similar potency to teriparatide (FORTEO) for initiating bone tumor formation. The clinical significance of these findings is unknown.

Fertility, embryo-fetal development (EFD), and pre/postnatal development studies of nintedanib were conducted in rats and an EFD study was conducted in rabbits. PTH(1-84) administration resulted in no findings of significance in these studies. Developmental

effects in a peri-/post-natal study in pregnant rats included a stillborn litter in the mid- and group and an entire litter in the high-dose group was dead by postnatal day 4. Other findings included an increased incidence of morbidity associated with dehydration, broken palate and palate injuries, kidney dilatation, extra liver lobe, and diaphragmatic hernia. The Division recommended a Pregnancy Category “C” for this product based on the above findings. Low levels of PTH(1-84) were observed in milk of rats at levels 42-fold below those observed in plasma.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that nintedanib can be approved from the pharmacology/toxicology perspective. I have discussed and am in agreement with labeling revisions proposed by the Division that include a box warning for bone neoplasms similar to that included for teriparatide and a modification of the proposed text for Section 13.1 of the label regarding carcinogenesis.

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/s/

TIMOTHY J MCGOVERN
10/10/2014

To: BLA 125-511 Natpara PTH 1-84/NPS

From: Karen Davis-Bruno PhD; DMEP

NPS has developed Natpara, a full-length recombinant human parathyroid hormone (PTH 1-84) as a replacement therapy to treat hypoparathyroidism resulting from inadequate endogenous PTH secretion. PTH (1-84) is synthesized and secreted by the parathyroid glands and is the principal endocrine hormone regulating systemic calcium and phosphorus homeostasis. Physiological actions of PTH include regulation of bone and renal tubular reabsorption of calcium and phosphate, and intestinal calcium absorption. This is mediated through binding to specific high affinity cell surface receptors.

In the studies summarized below, PTH 1-84 is known as Natpara and ALX 1-11. The majority of nonclinical data for this BLA is supported by reference to ALX 1-11 NDA (b) (4) from NPS for osteoporosis which was reviewed in 2006 but not approved for marketing (approvable). This NDA was withdrawn in 2011. In BLA 125-511, there are a total of 9 nonclinical studies submitted. Some are validation assays, and final reports of drafts already reviewed in NDA (b) (4). Four studies are new to the application and provide additional safety information. These include: a peri-/post-natal rat developmental study, 4-week SC rat toxicity study comparing drug substance from two manufacturers, 4-week SC rat toxicity study qualifying drug substance/product impurities and a rat lactation transfer of drug study. Details on these studies are contained in the primary review of BLA 125-511. PTH 1-34, teriparatide (Forteo) is a synthetic peptide, approved under NDA 21-318 for the treatment of osteoporosis with a high risk of fracture as well as for increasing bone mass.

PHARMACOLOGY

Natpara is identical to endogenous PTH (1-84) and binds PTH-1 receptors in bone, kidney and indirectly affects calcium reabsorption in the intestine. In bone, PTH liberates calcium by increasing the rate of calcium absorption from the bone by osteocyte/osteoblast mediated osteolysis when administered acutely and by activation of osteoclasts when given chronically. In the kidney, PTH acts on the distal tubule and collecting ducts to increase reabsorption of calcium. PTH increases the conversion of 25-hydroxyvitamin D3 to 1, 25-dihydroxyvitamin D3, which increases absorption of calcium from the intestines. When PTH is delivered intermittently (e.g. once daily) it acts on bone as an anabolic agent by preferentially activating osteoblasts over osteoclasts. This forms the basis of increasing bone mass and strength in osteopenic, ovariectomized female rats and monkeys. This anabolic effect of intermittent PTH exposure contrasts with the net bone catabolism that can occur with continuous exposure to PTH. The stimulatory effects of PTH on osteoblasts are thought to be direct, since these cells express PTH-receptors. The stimulatory effect on osteoclasts is thought to be indirect and mediated through osteoblasts. Osteoblasts presumably integrate the strength and duration of the PTH signal in determining whether paracrine activation of osteoclasts will occur. It is important to appreciate

that Natpara treatment in hypoparathyroid patients does not replace physiological conditions, as drug delivery achieves a Cmax of 300 pg/ml whereas the normal PTH plasma range is 15-50 pg/ml. Rats used in the carcinogenicity evaluation were exposed to pharmacologic doses of rh-PTH.

PTH is removed from circulation by the liver and kidney. Kupffer cells in the liver, take up PTH via receptor mediated internalization. PTH is cleaved into N- and C-terminal fragments by peptidases inside these cells. The N-terminal fragment is further degraded within the Kupffer cells while the C-terminal fragments are released back into circulation. The C-terminal fragments of PTH (CPTH) are cleared by the kidney, being hydrolyzed to amino acids during tubular resorption. Because hydrolysis of intact PTH in Kupffer cells occurs more rapidly than renal hydrolysis of the CPTH fragments, the CPTH fragments typically circulate at levels several fold higher than that of full-length PTH.

There is speculation that the CPTH fragments may be biologically active at physiological or pharmacologic exposure, especially in modulating the physiological response to the signal delivered via PTHR1 by PTH 1-84 and by stimulating osteoblast apoptosis (at least in vitro). However CPTH fragments do not compete for binding at the PTHR1 receptor. The existence of a specific CPTH receptor has been hypothesized.

The osteoblast is the major target cell for PTH in bone. The cell possesses high-affinity surface membrane PTH receptors which are coupled to adenylyl cyclase and phospholipase C. Activation of the two associated signal transduction pathways leads to alterations in osteoblastic function and gene expression. Most likely the anabolic action of PTH on bone is mediated by a combination of intracellular responses triggered specifically by intermittent receptor occupation.

BONE EFFECTS

Hypoparathyroidism is an uncommon, condition in which parathyroid hormone secretion is deficient or absent. Hypocalcemia and hyperphosphatemia are usually present. Bone in hypoparathyroidism differs from osteoporosis in that bone turnover is low and BMD is high, whereas the converse is the case for osteoporosis. PTH 1-84 treatment in both diseases results in an increase in BMD and bone turnover. The increase in BMD is primarily trabecular bone (i.e. lumbar spine), however BMD decreases primarily at cortical bone sites (i.e. distal radius). Treatment with PTH is expected to improve the biomechanical properties of bone and decrease vertebral fractures in hypoparathyroidism as it does in osteoporosis. Therefore the effects of PTH on the skeletal of ovariectomized animal models are relevant to its effect in hypoparathyroidism.

Parathyroid hormone (PTH) promotes absorption of calcium from the bone in two ways. The acute phase results in a rise in serum calcium within minutes and appears to occur at the level of the osteoblasts and osteocytes. The same cells that promote deposition of bone are involved in

resorption, as these cells form an interconnected network known as the osteocytic membrane overlying the bone matrix, with a small layer of interposed fluid termed bone fluid. When parathyroid hormone (PTH) binds to receptors on these cells, the osteocytic membrane pumps calcium ions from the bone fluid into the extracellular fluid.

The slow phase of bone resorption occurs over several days and has two components. First, osteoclasts are activated to digest formed bone, and second, proliferation of osteoclasts occurs. Mature osteoclasts lack parathyroid hormone (PTH) membrane receptors; activation and proliferation appear to be stimulated by cytokines released by activated osteoblasts and osteocytes or by differentiation of immature osteoclast precursors that possess parathyroid hormone (PTH) and vitamin D receptors. Additional research has revealed additional complexities in the control of parathyroid hormone dynamics in vivo, including circadian and pulsatile patterns in parathyroid hormone as well as hysteresis and rate dependence in the relationship between intact parathyroid hormone levels and calcium.

When PTH is administered at closer than daily intervals or infused continuously, the balance shifts and the net response is bone resorption rather than formation. Sustained exposure to PTH activates the osteoblast PTH receptor leading to an indirect paracrine effect on osteoclasts. The result is an increase in bone turnover and a net effect of accelerated bone resorption, increased calcium release, and reduced bone mineral density. In bone, PTH 1-84 increases calcium release from an exchangeable pool near bone surfaces and increases the activity of osteoblasts and osteoclasts thereby increasing bone turnover. With sustained elevations in PTH 1-84, osteoclastic activity exceeds that of osteoblasts resulting in a net release of calcium from bone. However when the increase in PTH 1-84 is transient, bone formation exceeds resorption leading to a marked decrease in bone mineral density at trabecular bone sites such as the spine and hip.

Preclinical and clinical studies have shown that intermittent treatment with PTH injections increases vertebral bone mass and strength. However, the effect of this treatment on non-vertebral, predominantly cortical bone sites is not as clear. Various studies were performed by the sponsor on osteopenic rats and monkeys. In the 18-month monkey bone quality study, daily administration of PTH 1-84 at 10 ug/kg/day optimally increased trabecular and cortical bone mass, increased trabecular bone mineral density, improved trabecular bone architecture, quality and strength, and increased cortical bone area/thickness as well as cortical bone strength (work to failure). Similar findings were seen in the 1-year rat bone quality study except that in the rat the improvement is in cancellous bone. Bone volume was primarily a consequence of increased thickness of existing trabeculae, rather than the formation of new trabeculae as seen in the monkeys. PTH 1-84 improved cortical bone intrinsic strength parameters in the rat, while these parameters worsened in the monkey even while overall cortical bone strength in the monkey remained unchanged as a consequence of increased cortical area. The new bone formed in both species was of normal lamellar structure and there was no evidence of osteoid accumulation or

mineralization defects. Neither species exhibited pathologic changes in serum calcium, marrow fibrosis or the presence of abnormal bone cells.

Bone forming agents, including Natpara, affect calcium and phosphorus metabolism in a pattern consistent with the known actions of endogenous PTH (e.g., increases serum calcium and decreases serum phosphorus). The skeletal effects depend on the pattern of systemic exposure. Once daily Natpara stimulates new bone formation on trabecular and cortical bone surfaces by

preferential stimulation of osteoblasts over osteoclast activity. In rats, dose-related increases in bone mass and strength were observed consistently in both trabecular and cortical bone. In monkeys, Natpara treatment improved trabecular microarchitecture and increased bone mass and strength by stimulating new bone formation in both cancellous and cortical bone. In cortical bone it increased Haversian remodeling. While high doses of Natpara decreased cortical bone mineral density (BMD), further analysis showed that bone mineral content (BMC) was either unaffected or was increased by treatment as was cortical bone strength. At sites containing both trabecular and cortical bone, e.g. femoral neck, Natpara also increased bone strength. Continuous excess of endogenous PTH, as occurs in hyperparathyroidism may be detrimental to the skeleton, because bone resorption may be stimulated more than formation.

In both rat and monkey, Natpara treatment was associated with increased bone mineral content in trabecular and cortical bone, with a concomitant increase in biomechanical indicators of trabecular bone strength and some biomechanical indicators of cortical bone strength. In monkey, the only cortical bone strength parameter to show improvement was work to failure. In both rat and monkey, improvements in work to failure at cortical sites was primarily a consequence of increased cortical area/thickness. Increases in trabecular bone strength were associated primarily with increased trabecular thickness in the rat and increased trabeculae number in monkey.

Stimulation of new bone formation on trabecular and cortical bone by preferential stimulation of osteoblastic over osteoclastic activity whose mechanism is incompletely understood but thought to involve incomplete differentiation of osteogenic precursor cells (bone lining cells) into osteoblasts and possibly an inhibition of osteoblast apoptosis. The precise mechanism by which PTH exerts this effect has not been elucidated.

TOXICOLOGY

The toxicologic profile of Natpara associated with chronic subcutaneous (SC) dosing has been assessed in the rat and monkey animal models. In both models the primary toxicological targets were the kidney and bone marrow. Post-dose transient hypercalcemia was apparent for 3-6 hours in the monkey at all tested doses, with excursions as high as 1.6 mg/dL (~17% increase) at 10

$\mu\text{g}/\text{kg}/\text{day}$ (3 times the Maximum Recommended Human Dose; MRHD, based on AUC ratios). Transient serum calcium excursions were not assessed in the rat in the chronic SC dosing study, but can be inferred from the dose-dependent calciuria that was observed. At toxic doses of Natpara, repetitive kidney exposure to high serum calcium levels resulted in the formation of renal calculi, mineralization, and damage to the renal tubules and occasionally the parenchyma. This was associated with increases in serum alkaline phosphatase and BUN. At the highest dose tested in the rat, 1000 $\mu\text{g}/\text{kg}/\text{day}$ (100 times the MRHD, based upon a mg/m^2 comparison), 30% of the male rats died or were sacrificed moribund. The cause of death/morbidity was determined

to be severe kidney damage. All had moderate to severe renal tubular mineralization, and most had calcification of the major vessels, heart and/or stomach. The other consistent toxicological finding with Natpara was a dose-dependent reduction in the level of blood cells (all types) that occurred as a consequence of an exaggerated bone-anabolic effect, which lead to osteosclerosis and occlusion of the marrow space, causing a reduction in blood cell precursors. In the rat, significant increases in blood cells were seen at doses $\geq 300 \mu\text{g}/\text{kg}/\text{day}$ (19 times human AUC at the MRHD).

There is species variability in the sensitivity to PTH-induced renal failure and vascular mineralization with the dog being highly sensitive based on the high capacity of the dog kidney to reabsorb calcium. Thus doses in the dog as low as 9 $\mu\text{g}/\text{kg}/\text{day}$ (3 times MRHD) causes morbidity in 30% male dogs after 3 doses. Rats are relatively resistant to the pathological effects of hypercalcemia where a NOAEL of 50 $\mu\text{g}/\text{kg}/\text{day}$ (11 times human AUC at the MRHD) was established for hypercalcemic renal pathology. The monkey NOAEL was 2 $\mu\text{g}/\text{kg}/\text{day}$ (less than the human AUC at the MRHD) for renal hypercalcemic associated pathology.

All nonclinical test species developed antibodies to Natpara. However, only a small percentage of test animals developed detectable levels of antibody, and these were generally not neutralizing. Being a native human protein, Natpara is not expected to be highly immunogenic in humans, and animal immunoreactivity is not necessarily predictive of human clinical immune response.

REPROTOXICITY

A rat fertility study and both rat and rabbit embryo-fetal developmental toxicity studies found no significant effects outside of the laboratory's range of the historical control variation for measures of fertility, and early embryonic development and development of the embryo and fetus following exposure of the pregnant dam from implantation through closure of the hard palate. Exposure of the pregnant dam from implantation through lactation in a pre- and post-natal development study, had some findings at exposures >25 times systemic exposure (AUC) at a 100 $\mu\text{g}/\text{day}$ clinical dose. Developmental effects in a peri-/post-natal study in pregnant rats

given subcutaneous doses of 100, 300, 1000 µg/kg/day from organogenesis through lactation an entire litter was stillborn in the 300 µg/kg/day group (34 times the 100 µg/day clinical dose based on AUC) and an entire litter from the 1000 µg/kg/day (123 times the 100 µg/day clinical dose based on AUC) was dead by postnatal day 4. Increased incidence of morbidity associated with dehydration, broken palate and palate injuries related to incisor misalignment were found in pups from litters given ≥ 100 µg/kg/day (10 times the 100 µg/day clinical dose based on AUC). At 300 µg/kg/day there was a litter with kidney dilatation and another with an extra liver lobe. There was a single pup with a diaphragmatic hernia from a litter exposed to 1000 µg/kg/day.

CARCINOGENICITY

In a 2-year rat carcinogenicity study, daily SC dosing of F344 rats with doses of NATPARA > 50 µg/kg/day were associated with significant increases in the incidence of bone neoplasms, especially osteosarcomas. The incidence of osteosarcoma in males was 22% at 50 mcg/kg/day (26 times the MRHD, based on AUC ratios) and 45% at 150 µg/kg/day (71 times the MRHD, based on AUC ratios), and somewhat lower in females at 8.3% and 22%, respectively (at 19- and 55-times MRHD based on AUC ratios). Osteoblastomas and osteomas were also increased. The highest dose tested, 150 µg/kg/day (71 times the expected clinical exposure at 100 µg/day), caused significantly increased mortality, especially in males, because of increased osteosarcoma-related mortality. At the lowest tested dose of 10 µg/kg/day, there was no significant increase in bone neoplastic incidence. This dose gave mean exposures in the rat that are 4 times the expected clinical exposure at 100 µg/day at the MRHD. Despite the lack of statistically significant increases in neoplasms in the low dose group, the relatively low safety margin that this dose provides (5 times in males and 3 times in females the clinical exposure at the MRHD of 100 µg/day) does not suggest a negligible risk for developing bone tumors in humans at clinical exposure levels. The Sponsor suggests that this indicates NATPARA has a significantly reduced clinical risk of causing bone tumors compared to teriparatide, as teriparatide is associated with an increase in osteosarcomas (~6%) at 5 µg/kg/day; a dose equivalent to 11µg/kg/day of PTH on a molar basis.

Comparison of Bone Neoplastic Potential of PTH(1-84) and PTH(1-34):

Table 1 -- Incidence (Animals Affected) of Bone Neoplasms in Male F344 Rats: Comparison of ALX1-11 to Teriparatide									
	ALX1-11					Teriparatide			
Number examined	60	60	60	60	60	60	60	60	60
Dose Group	C1	C2	LD	MD	HD1*	C	LD	MD	HD
Dose (µg/kg/day)	0	0	10	50	150	0	5	30	75
Exposure Ratio (AUC _{rat} /AUC _{human} **)	-	-	5	26	71	-	3	21	58
Osteoma (n)	0	0	0	1	2	0	0	2	1

(%)	0	0	0	1.67	3.33	0	0	3.33	1.67
Osteoblastoma (n)	0	0	0	2	4	0	0	2	7
(%)	0	0	0	3.33	6.67	0	0	3.33	11.67
Osteosarcoma (n)	0	0	1	13	27	0	3	21	31
(%)	0	0	1.67	21.67	45.00	0	5.00	35.00	51.67
All Bone Neoplasms (n)	1	0	2	17	28	0	3	24	36
(%)	1.67	0	3.33	28.33	46.67	0	5.00	40.00	60.00

*Male HD1 only dosed for 94 weeks and necropsied after 101 weeks.

**AUC_{human} at 100 µg/day= 0.924 ng h/ml (Clinical study C09-002)

Table 2 -- Incidence (Animals Affected) of Bone Neoplasms in Female F344 Rats: Comparison of ALX1-11 to Teriparatide

	ALX1-11					Teriparatide			
	C1	C2	LD	MD	HD1	C	LD	MD	HD
Number examined	60	60	60	60	60	60	60	60	60
Dose Group	C1	C2	LD	MD	HD1	C	LD	MD	HD
Dose (µg/kg/day)	0	0	10	50	150	0	5	30	75
Exposure Ratio (AUC _{rat} /AUC _{human} **)	-	-	3	19	55	-	3	21	58
Osteoma (n)	0	0	0	1	1	0	0	0	1
(%)	0	0	0	1.67	1.67	0	0	0	1.67
Osteoblastoma (n)	0	0	0	3	9	0	1	1	3
(%)	0	0	0	5.00	15.00	0	1.67	1.67	5.00
Osteosarcoma (n)	2	0	0	5	13	0	4	12	23
(%)	3.33	0	0	8.33	21.67	0	6.67	20.00	38.33
All Bone Neoplasms (n)	2	0	0	11	20	0	5	13	25
(%)	3.33	0	0	18.33	33.33	0	8.33	21.67	41.67

**AUC_{human} at 100 µg/day= 0.924 ng h/ml (Clinical study C09-002)

Table 3 -- Incidence (Animals Affected) of Bone Neoplasms in F344 Rats: Comparison of ALX1-11 to Teriparatide (male & female combined)

	ALX1-11					Teriparatide			
	C1	C2	LD	MD	HD1*	C	LD	MD	HD
Number examined	120	120	120	120	120	120	120	120	120
Dose Group	C1	C2	LD	MD	HD1*	C	LD	MD	HD
Dose (µg/kg/day)	0	0	10	50	150	0	5	30	75
Exposure Ratio (AUC _{rat} /AUC _{human} **)	-	-	4	23	63	-	3	21	58
Osteoma (n)	0	0	0	2	3	0	0	2	2
(%)	0	0	0	1.67	2.50	0	0	1.67	1.67
Osteoblastoma (n)	0	0	0	5	13	0	1	3	10
(%)	0	0	0	4.17	10.83	0	0.83	2.50	8.33
Osteosarcoma (n)	2	0	1	18	40	0	7	33	54
(%)	1.67	0	0.83	15.00	33.33	0	5.83	27.50	45.00
All Bone Neoplasms (n)	3	0	2	28	48	0	8	37	61
(%)	2.50	0	1.67	23.33	40.00	0	6.67	30.83	50.83

*Male HD1 only dosed for 94 weeks and necropsied after 101 weeks.

**AUC_{human} at 100 µg/day= 0.924 ng h/ml (Clinical study C09-002)

pre-neoplastic activity of the N-terminal fragment of PTH, acting at the PTH-1 receptor is not substantiated by data. Furthermore when normalizing the osteosarcoma incidence by a marker of intrinsic activity (i.e. bone formation rate) we found no difference in the bone formation-rate-normalized osteosarcoma incidence between PTH 1-34 and PTH 1-84. While intriguing the CPTH theory lacks substantiation.

CPTH fragments are generated by the liver and released back into circulation. There is some speculation that these fragments may be biologically active at physiological or pharmacological exposure levels, especially in modulating the physiological response to the signal delivered by PTH-1 receptor by PTH 1-84, however CPTH fragments do not compete for binding at the

PTH-1 receptor. The existence of a specific PTH receptor (CPTH_R) has been hypothesized based on bimodal Scatchard plots for PTH 1-84 but not PTH 1-34 in cell homogenates from renal cells, in addition to renal and osteosarcoma cell lines. CPTH fragments can compete with radioligand binding to these high capacity, low affinity CPTH_R sites. The addition of CPTH fragments to osteosarcoma cell lines has been shown to activate alkaline phosphatase and stimulate the uptake of extracellular calcium,. On the basis of these findings the sponsor hypothesizes that PTH 1-84 may be expected to have a unique therapeutic profile compared to other structurally related peptides such as PTH 1-34. It is noteworthy that CPTH_R has not been identified or characterized to date so this remains speculative at best. In contrast when concurrently compared in osteopenic OVX rats, PTH 1-84 is a less potent bone anabolic agent than PTH 1-34 consistent with the idea that CPTH fragments negatively modulate the activity of the N-terminal region of PTH. This does not rule out the possibility that the C-terminal portion of PTH 1-84 could negatively affect the potency or efficacy of the N-terminal portion of PTH 1-84 at the PTH-1 receptor.

CONCLUSIONS

The toxicology profile of Natpara is consistent with exaggerated pharmacodynamics. The anticipated elevations in serum calcium levels following PTH administration can lead to pathology i.e. renal failure and mineralization of multiple organ systems particular the cardiovascular and GI. The magnitude and duration of the calcium elevation is best correlated with whether or not PTH-induced hypercalcemia results in detectable pathology.

The rat carcinogenicity data indicates a dose-dependent increase in the incidence of bone neoplasms in Natpara treated F344 rats. This effect was similar to that seen with teriparatide (PTH 1-34). There are some numerical differences between the two studies, with Natpara being associated with fewer bone tumors than teriparatide dosing when the tumor incidence is normalized to the AUC exposure ratio between rats and humans. The sponsor has suggested that this is a consequence of the pro-apoptotic activity of CPTH fragments and thus is an indication

that Natpara is less likely to cause bone tumors than teriparatide. It is likely that the molecular events associated with the bone anabolic effects of PTH peptides are the same. The differences in the outcomes of the carcinogenicity studies (osteoblast induced neoplasia) may be explained by the lower bone anabolic potency of Natpara. When osteosarcoma incidence is normalized to the drug-related increase in bone formation rate there is little difference between the two peptides. Based on bone forming unit of activity, Natpara and teriparatide have similar potencies for initiating bone tumor formation in F344 rats. The clinical significance of this ability of PTH peptides to induce bone tumors in rats remains unknown. It is important to appreciate that Natpara treatment in hypoparathyroid patients does not mimic physiological conditions in that

drug delivery achieves a Cmax of 300 pg/ml whereas the normal PTH range is 15-50 pg/ml. Normal rats used in the carcinogenicity evaluation were exposed to pharmacologic doses of rh-PTH.

RECOMMENDATION: Approval pending labeling changes for a box warning for osteosarcomas similar to teriparatide and modifications to Sections 8, 12 and 13.

LABELLING CHANGES: The following is a clean copy of the recommended labeling changes.

Highlights Section to contain a box warning for osteosarcomas :

(b) (4)

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/s/

KAREN L DAVIS BRUNO
07/31/2014
Supervisory P/T Review

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: BLA 125511

Supporting document/s: STN: BL 12551/0 (NDA (b) (4)
Serial No.: 0000

Applicant's letter date: October 23, 2013

CDER stamp date: October 24, 2013

Product: Natpara® (Proposed) (rhPTH[1-84]) for Injection

Indication: Replacement for endogenous parathyroid hormone (1-84) indicated for the long-term treatment of hypoparathyroidism.

Applicant: NPS Pharmaceuticals Inc.

Review Division: DMEP

Reviewer: Robert Maher

Supervisor/Team Leader: Karen Davis-Bruno

Acting Division Director: Jean-Marc Guettier

Project Manager: Meghna Jairath

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of [NDA number] are owned by [name of applicant] or are data for which [name of applicant] has obtained a written right of reference.

Any information or data necessary for approval of [NDA number] that [name of applicant] does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling.

Any data or information described or referenced below from a previously approved application that NPS Pharmaceuticals Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125511.

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Recommendation on approvability: Approval (AP), pending acceptance of recommended changes in labeling.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

Sponsor's BLA submission labeling states the following:



(b) (4)

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1.2 Brief Discussion of Nonclinical Findings

The toxicologic profile of NATPARA associated with chronic subcutaneous (SC) dosing has been assessed in the rat and monkey animal models. In both models the primary toxicological targets were the kidney and bone marrow. Post-dose transient hypercalcemia was apparent for 3-6 hours in the monkey at all tested doses, with excursions as high as 1.6

mg/dL (~17% increase) at 10 mcg/kg/day (4.6- to 6.6-fold the Maximum Recommended Human Dose; MRHD, based on AUC ratios). Transient serum calcium excursions were not assessed in the rat in the chronic SC dosing study, but can be inferred from the dose-dependent calciuria that was observed. At toxic doses of Natpara, repetitive kidney exposure to high serum calcium levels resulted in the formation of renal calculi, mineralization, and damage to the renal tubules and occasionally the parenchyma. This was associated with increases in serum alkaline phosphatase and BUN. At the highest dose tested in the rat, 1000 µg/kg/day (~100X the MRHD, based upon a mg/m² comparison), 30% of the male rats died or were sacrificed moribund. The cause of death/morbidity was determined to be severe kidney damage. All had moderate to severe renal tubular mineralization, and most had calcification of the major vessels, heart and/or stomach. The other consistent toxicological finding with Natpara was a dose-dependent reduction in the level of blood cells (all types) that occurred as a consequence of an exaggerated bone-anabolic effect, which lead to osteosclerosis and occlusion of the marrow space, causing a reduction in blood cell precursors. In the rat, significant increases in blood cells were seen at doses ≥300 µg/kg/day (~19X human AUC at the MRHD).

In a 2-year rat carcinogenicity study, daily sc dosing of F344 rats with doses of ALX1-11 > 50 mcg/kg/day were associated with significant increases in the incidence of bone neoplasms, especially osteosarcoma. The incidence of osteosarcoma in males was 22% at 50 mcg/kg/day (~20x the MRHD, based on AUC ratios) and 45% at 150 mcg/kg/day (~50x the MRHD, based on AUC ratios), and somewhat lower in females at 8.3% and 22%, respectively. There was no increase in the incidence of osteosarcoma or other bone neoplasms at a dose of 10 mcg/kg/day (~4x the MRHD, based on AUC ratios). These findings are qualitatively similar to what was seen in the initial 2-year carcinogenicity study conducted by Eli Lilly & Company to support the marketing of teriparatide. The clinical significance of this class effect of PTH bone anabolic agents in rats remains unclear, as does the significance of the Sponsor's analysis showing a lower incidence of osteosarcoma in ALX1-11-treated F344 rats, as compared to teriparatide-treated F344 rats when the results are normalized on the basis of human AUC ratios. Since it is likely that the molecular events that are associated with the bone anabolic effects of PTH peptides are the same that, when exaggerated, cause osteoblast neoplasia, the differences in the outcomes of the carcinogenicity studies of ALX1-11 and teriparatide may be explainable by the drug-related increase in bone formation rate, there is little difference between the two peptides.

Nonclinical safety issues relevant to clinical use:

Hypercalcemia

Bone neoplasia (relevance uncertain)

2 Drug Information

2.1 Drug

- 2.1.1 CAS Registry Number 68893-82-3
- 2.1.2 Generic Name Parathyroid hormone (PTH)
- 2.1.3 Code Name ALX1-11, PTH, hPTH, rhPTH, rPTH, NPSP558
- 2.1.4 Chemical Name Parathyroid hormone (human recombinant)

Previous Names/Synonyms

Primary Name	Previous Names/Synonyms
rhPTH(1-84)	PTH, hPTH, NPSP558 PTH, PTH(1-84), hPTH, hPTH(1-84), rhPTH, rhPTH(1-84), rPTH, and rPTH(1-84), ALX1-11, Preotact [®] , Preos [™]
Natpara [®]	rhPTH(1-84)
Natpara [®] Q-Cliq [™] (pen injector)	Natpara reusable pen, Natpara pen, Haselmeier pen, pen injector
Natpara Mixing Device	(b) (4) Mixing Device, (b) (4) reconstitution device

- 2.1.5 Molecular Formula/Molecular Weight
C408H674N126O126S2, 9425 Daltons

- 2.1.6 Pharmacological Class bone anabolic agent

Composition of Natpara Drug Product (after reconstitution)

Ingredient	Concentration				Function	Quality Standard
	25 mcg/dose strength	50 mcg/dose strength	75 mcg/dose strength	100 mcg/dose strength		
rhPTH(1-84)	(b) (4)				Active Ingredient	NPS In-house Standard
Sodium Chloride					(b) (4)	USP-NF
Mannitol					USP-NF	
Citric Acid Monohydrate					USP-NF	
(b) (4)					USP-NF	
m-Cresol					USP-NF	
(b) (4)					USP-NF	
(b) (4)					USP-NF	

NPS = NPS Pharmaceuticals, Inc.; rhPTH(1-84) = recombinant human parathyroid hormone; q.s. = quantity sufficient; USP-NF = United States Pharmacopoeia-National Formulary

(b) (4)

2.3.1 Comments on Novel Excipients

There are no novel excipients used in the manufacture of Natpara drug product.

2.3.2 Comments on Impurities/Degradants

Quantitative Summary of Product Related Impurities Levels

Regulatory Impurity Method	Impurity Name	Approximate Relative Retention Time to Main PTH Peak (min/min)	Range of Levels Observed	
			Release	Stability (Long-Term)
QC-ANP-PTH-5006 (RP-HPLC-purity)	(b) (4)			

Quantitative Summary of Product Related Impurities Levels

Regulatory Impurity Method	Impurity Name	Approximate Relative Retention Time to Main PTH Peak (min/min)	Range of Levels Observed	
			Release	Stability (Long-Term)
QC-ANP-PTH-2110 (Ion Exchange)	(b) (4)			
QC-ANP-PTH-5018 (HPSEC)				

BLQ = below limit of quantitation; HPSE = high performance size exclusion chromatography; ND = not determined; PTH = parathyroid hormone; RP-HPLC = reverse phase-high performance liquid chromatography

Chemically synthesized preparations of various PTH impurities have been examined for PTH biological activity (QC-ANP-PTH-0400). The (b) (4) substances have biological relative potencies of (b) (4) respectively. These biological potencies were determined using the standard biological activity assay employed for the rhPTH(1-84) Drug Substance. Therefore, by virtue of biological activities divergent from rhPTH(1-84), the (b) (4) would be considered a product related impurity, while the (b) (4) is categorized as a product related substance. Two other impurities (b) (4) were chemically synthesized and then tested for PTH biological activity. The (b) (4) has been detected in trace levels in rhPTH(1-84) Drug Substance. The (b) (4) was a speculated impurity which has not

been found in the rhPTH(1-84) Drug Substance but was also examined in the PTH biological activity assay for better understanding of structure/function relationship. Both of these impurities have no biological activity above baseline and showed no dose response.

2.4 Proposed Clinical Population and Dosing

Natpara® (rhPTH[1-84]) for the treatment of adult hypoparathyroidism.

2.5 Regulatory Background

NPS initiated the development of rhPTH(1-84) in 1995 under IND (b) (4) for the treatment of osteoporosis. This IND is currently inactive.

In 2005, NDA (b) (4) Preos (ALX1-11) was submitted for osteoporosis and was approvable pending clinical and device deficiencies in 2006. This NDA was withdrawn in 2011.

In August 2007, FDA granted NPS Orphan Drug Designation for rhPTH(1-84) for the treatment of hypoparathyroidism.

On September 19, 2008 an initial IND application was submitted (IND 76514) under which Natapara (rhPTH[1-84]) was being developed for the treatment of hypoparathyroidism and remains the only active IND.

3 Studies Submitted

3.1 Studies Reviewed

1. Subcutaneous Developmental Toxicity Study of ALX1-11 in Rats. (b) (4) Study No. XGW00013. (Report Amendment 1. Bioanalytical and Toxicokinetic Analyses Report.)
2. Subcutaneous Developmental Toxicity Study of ALX1-11 in Rabbits. (b) (4) Study No. XGW00015. (Report Amendment 1. Bioanalytical and Toxicokinetic Analyses Report.)
3. Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of ALX1-11 in Rats, Including a Postnatal Behavioral/Functional Evaluation. (b) (4) Study Report No. XGW00020.
4. Validation of an ImmunoRadiometric assay (IRMA) for the Quantitative Determination of human PTH (1-84) in Rabbit K2EDTA Plasma. (b) (4) Study No. 320292.
5. Validation of an Immunoradiometric Assay (IRMA) for the Quantitative Determination of Full Length Human PTH (1-84) in Rat Milk. (b) (4) Study No. 341608.
6. Validation of an ImmunoRadiometric Assay (IRMA) Method for the

Quantitative Determination of Full Length human PTH (1-84) in Rat K2EDTA Plasma. (b) (4) Study No. 341907.

7. Validation of an ImmunoRadiometric (IRMA) Method for the Determination of Recombinant Human Parathyroid Hormone (hPTH 1-84) in Sprague Dawley Rat K2 EDTA Plasma. (b) (4) Study No. AA74443.
8. NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers. (b) (4) No. 1150-003.
9. NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substance and Drug Product. (b) (4) No. 1150-004.
10. Subcutaneous Lactation Transfer Study of ALX1-11 in Rats. (b) (4) Study No. 20008518.

3.2 Studies Not Reviewed None.

3.3 Previous Reviews Referenced

NDA (b) (4) Pharmacology/Toxicology Review, Serial No. 000, 5/10/2005, R. Wange, PhD. (Studies listed above are reviewed and the summary findings of previously submitted studies are excerpted from the NDA (b) (4) P/T Review. Disclaimer: Tables and figures from the Sponsor's submission have been incorporated into this document.)

4 Pharmacology

Natapara is purified recombinant human parathyroid hormone (rhPTH), and as such would be expected to have effects comparable to PTH. PTH is an 84 amino acid protein that is synthesized and secreted by the parathyroid gland, and is the principal regulator of extracellular calcium and phosphorus homeostasis.

Physiologically, PTH is secreted into the bloodstream in response to low extracellular calcium levels. It acts directly on bone and kidney and indirectly on the intestine to increase serum calcium levels. At bone, PTH liberates calcium by increasing the rate of calcium absorption from the bone by osteocyte/osteoblast mediated osteolysis (acutely) and by activation of osteoclasts (chronically). In the kidney, PTH acts on the distal tubule and collecting ducts to increase reabsorption of calcium. Also in the kidney, PTH increases conversion of 25-hydroxyvitamin D₃ to 1, 25-dihydroxyvitamin D₃, which increases absorption of calcium from the intestines. The known pharmacology of PTH indicates that expected adverse events associated with high doses of Natapara would include hypercalcemia and hypercalciuria, and their sequelae.

Action of PTH in bone

The overall effect of PTH on bone depends on whether it is administered continuously or intermittently. Sustained exposure to PTH activates the osteoblast PTH-receptor which leads to an indirect paracrine activation of osteoclasts. This results in an increase in bone

turnover and a net effect of accelerated bone resorption, increased calcium release, and reduced bone mineral density. Since the first experiments done in the 1930's by Hans Selye in the rat, however, it has been confirmed by numerous experiments in animal species and humans that intermittent (e.g. once daily) dosing with bioactive PTH fragments predominantly stimulates the osteoblast, resulting in an anabolic effect on bone, i.e., bone growth. When PTH is administered at closer than daily intervals or infused continuously, the balance shifts and the net response is bone resorption rather than formation. The precise mechanisms involved in the catabolic and anabolic effects of PTH are unknown. The stimulatory effects of PTH on osteoblasts are thought to be direct, since these cells do express receptors for PTH. However, the stimulatory effect on osteoclasts is thought to be indirect and mediated through osteoblasts. Osteoblasts presumably integrate the strength and duration of the PTH signal in determining whether paracrine activation of osteoclasts will occur. The different actions may be explained by a differential expression of osteoblastic genes such as those encoding for OPG, FGF-2, IGF-1, IGF-binding proteins, IL-6, or IL-11.

Multiple studies were carried out by the Sponsor in vitro or in vivo in osteopenic rats or monkeys to assess the effects of ALX1-11 on bone formation, mass, architecture and strength. These studies showed generally favorable effects of ALX1-11 on these parameters. Several studies compared ALX1-11 with other PTH variants, in particular teriparatide (PTH1-34). These studies generally found ALX1-11 to be less potent than teriparatide as a bone anabolic/remodeling agent. In the pivotal long term, 18-month, monkey bone quality study, daily administration of ALX1-11 at 10 µg/kg/day optimally increased trabecular and cortical bone mass, increased trabecular bone mineral density, improved trabecular bone architecture, quality and strength, and increased cortical bone area/thickness as well as cortical bone strength (work to failure). Overall, similar findings were seen in the 1-year rat bone quality study, except that in the rat the improvement in cancellous bone volume was primarily a consequence of increased thickness of existing trabeculae, rather the formation of new trabeculae, as was seen in the monkeys. Also, ALX1-11 tended to improve cortical bone intrinsic strength parameters in rats, while these parameters worsened in the monkey even while overall cortical bone strength in the monkey remained unchanged as a consequence of increased cortical area. The new bone formed in both species was of normal lamellar structure, and there was no evidence of osteoid accumulation or mineralization defects. Neither species exhibited pathologic changes in serum calcium levels, marrow fibrosis, or the presence of abnormal bone cells.

As indicated below in the discussion of PTH metabolism, C-terminal PTH (CPTH) fragments are generated by the liver and released back into circulation. There is some speculation that these fragments may be biologically active at physiological or pharmacological exposure levels, especially in modulating the physiological response to the signal delivered via PTHR1 by full-length PTH. However, CPTH fragments do not compete for binding at the PTHR1 receptor. The existence of a specific CPTH receptor (CPTHR) has been hypothesized primarily based upon the finding of bimodal Scatchard plots for the binding of PTH(1-84), but not PTH(1-34) to some intact and broken cell preparations, including renal plasma membranes, kidney cell lines and osteosarcoma cell lines. These data indicate the presence of high-capacity, low-affinity ($K_d = 200$ to 600 nM) binding sites in these tissues. Radioligand binding to these sites could be specifically competed by

unlabeled CPTH fragments. Furthermore, the addition of CPTH fragments to osteosarcoma cell lines has been shown to activate alkaline phosphatase and stimulate the uptake of extracellular calcium. The Sponsor has hypothesized on the basis of these and related findings that PTH(1-84) may be expected to have a unique therapeutic profile compared to other, structurally-related peptides, such as PTH(1-34), that do not result in the formation of CPTH fragments. However, no CPTHRs have been molecularly identified or characterized to date, and the existence of such receptors remains speculative at this time.

The question of whether or not CPTH fragments are active modulators of physiology following endogenous release of PTH, or pharmacological administration of rhPTH also remains open to debate, and awaits the molecular identification of the CPTH fragments that circulate in the blood and their effects on appropriate experimental systems. It is interesting to note however that when concurrently compared in osteopenic OVX rats, PTH(1-84) is a less potent bone anabolic agent than PTH(1-34), consistent with the idea that CPTH fragments negatively modulate the activity of the N-terminal region of PTH. However, these results do not rule out the possibility of the C-terminal portion of PTH(1-84) being able to negatively affect the potency (or efficacy) of the N-terminal portion of PTH(1-84) at PTHR1. In both rat and monkey, ALX1-11 treatment was associated with increased bone mineral content in trabecular and cortical bone, with a consequent increase in biomechanical indicators of trabecular bone strength and some biomechanical indicators of cortical bone strength. In monkey, the only cortical bone strength parameter that showed improvement (n.s.s.) was work to failure (AUC). In both rat and monkey, improvements in work to failure at cortical bone sites were primarily a consequence of increased cortical area/thickness. Increases in trabecular bone strength were associated primarily with increased trabecular thickness in the rat and increased trabeculae number in the monkey.

Drug activity related to proposed indication: Stimulation of new bone formation on trabecular and cortical bone surfaces by preferential stimulation of osteoblastic activity over osteoclastic activity. The precise mechanism is incompletely understood, but is thought to involve increased differentiation of osteogenic precursor cells (bone-lining cells) into osteoblasts, and possibly an inhibition of osteoblast apoptosis. The precise molecular mechanism by which PTH exerts this effect has not yet been elucidated.

4.3 Safety Pharmacology

CARDIOVASCULAR EFFECTS:

ALX1-11, rhPTH(1-84): Effect on Cloned hERG Channels Expressed in Mammalian Cells

The effect of ALX1-11 on hERG current was studied in vitro in HEK293 cells stably expressing hERG. Nominal concentrations of 0, 0.3, 3, 30 and 300 ng/mL of ALX1-11 were tested. 60 nM terfenadine was used as a positive control to validate the test system. All concentrations were tested in triplicate.

ALX1-11 does not affect hERG channel currents in the nominal concentration range of 0.3 to 300 ng/mL. The Sponsor notes that 0.3 ng/mL is approximately equal to the maximal concentration observed in humans following a dose of 100 µg.

Effects of ALX1-11, rhPTH(1-84) on Action Potentials in Isolated Canine Cardiac Purkinje Fibers

Three different concentrations of ALX1-11 (nominally 0.3, 3 and 300 ng/mL) were assessed with regards to their ability to affect cardiac action potential in isolated canine Purkinje fibers. Increasing concentrations of the test item were added sequentially to each of four fiber preparations (n of 4) at three stimulus intervals (basic cycle lengths of 2, 1 and 0.5 s). The effects of ALX1-11 on action potential parameters were compared to time-matched vehicle controls. A positive control of 100 µM dl-Sotalol was used a positive control to validate the assay system.

Quantitation of the actual amount of ALX1-11 used in the assay, found substantially lower amounts of the test item than the nominal amount:

nominal	measured
0.3 ng/mL	0.14 ng/mL
3 ng/mL	0.93 ng/mL
300 ng/mL	68 ng/mL

ALX1-11, at nominal concentrations of 0.3, 3 and 300 ng/mL, does not prolong action potential repolarization in isolated canine Purkinje fibers.

Acute Hemodynamic Effects of the Intravenous Administration of ALX1-11 in the Open-Chest Anesthetized Dog

The intravenous administration of ALX1-11 at doses between 1.0 and 10 µg/kg, inclusive, mildly increased cardiac contractile force in the fasted, open-chest anesthetized dog model. This effect of ALX1-11 is likely to be secondary to its ability to raise serum calcium levels, since the contractile strength of cardiac muscle is sensitive to changes in extracellular free ionized calcium levels.

Acute Effects of Intravenous Injection of Parathyroid Hormone, Teriparatide and C-terminal Parathyroid Hormone Fragments on Blood Pressure in Conscious Female Rats.

Neither C-terminal peptide had any measurable effect on MAP. Both ALX1-11 and Teriparatide transiently decreased MAP in a dose-dependent manner. Teriparatide had greater efficacy in decreasing MAP at all doses and all timepoints. At the HD the maximum decrease in MAP 1 min was ~22 mmHg and 34 mmHg with ALX1-11 and Teriparatide, respectively.

Teriparatide is ~50% more potent than ALX1-11 in inducing acute hypotension when administered i.v. to the conscious female rat. The clinical significance of this difference is unclear given that the proposed clinical dose of ALX1-11 (100 mcg per day) is 2.2x higher than the recommended clinical dose of Teriparatide (20 mcg per day) on a molar basis.

The following safety pharmacology studies were not assessed, see NDA (b) (4) or Toxicology Section: neurologic, pulmonary, renal, GI, abuse liability.

PHARMACODYNAMIC DRUG INTERACTIONS

No preclinical studies were performed to assess pharmacodynamic drug interactions.

5 Pharmacokinetics/ADME/Toxicokinetics

The following assumptions were made by the Sponsor in the design of their program for the pharmacokinetic characterization of ALX1-11 in the species used in the nonclinical pharmacological and toxicological investigations: 1) the basic processes of polypeptide catabolism and filtration are species independent, 2) ALX1-11 should behave similarly to comparably-sized polypeptides irregardless of test species, and therefore should have little to no permeability across the intestinal mucosa, a volume of distribution, following parenteral administration, that is comparable to blood volume, and should be relatively rapidly removed from the plasma. These assumptions seem reasonable.

Both single-dose and repeat dose pharmacokinetics were evaluated in rats (SD and F344) and monkeys (Cynomolgus and Rhesus), as these were the primary species used for both the nonclinical safety evaluations and pharmacology studies. PK in the dog was only superficially studied, since this species was deemed to be undergo an exaggerated calcemic response to ALX1-11, and was not therefore a suitable test species. The nonclinical PK studies were carried out with ALX1-11 formulations containing mannitol and citrate buffer that is similar to the formulations used in the clinical trials. The exception was one study in rats (DM04-005), in which various prototype formulations were tested.

In general, the results indicate that ALX1-11 is rapidly absorbed from the sc site of injection, and rapidly removed from systemic circulation. There were no consistent differences in the PK response based on sex. ALX1-11 levels reached peak levels in approximately 15 min in rats and 40 min in monkeys, and had an elimination half-life of about 20 to 30 min and 30 to 60 min in rats and monkeys, respectively. Except for at the higher doses used in the toxicology studies, the PK parameters of ALX1-11 were generally linear.

Absorption

Subcutaneously administered PTH was rapidly absorbed in both rats and monkeys, reaching peak plasma levels generally by 15 and 30 minutes, respectively. The absorption rate (k_a) in the rat was $\sim 2 \text{ h}^{-1}$. The absolute bioavailability of a single sc dose in the rat was 46%, with $\sim 95\%$ of the bioavailable dose being absorbed from the subdermal depot by 1.5 h. In rats, but not in monkeys, exposure was significantly greater following multiple daily doses, as compared to single doses.

Distribution

The distribution of ALX1-11 throughout the body was not specifically investigated. As for most polypeptides, its distribution would be expected to be primarily restricted to the systemic circulation, with limited distribution into extracellular water at capillary pores and fenestrations. This supposition is supported by the estimated volume of distribution (V_{dss} between 50-100 mL/kg), which approximates the blood volume, following intravenous administration in the rat (notably volume of distribution was not calculated in the dog or non-human primate). PTH-specific receptors have also been described in the Kupffer cells of the liver. Logically there may also be other specific receptors in other tissues that may facilitate PTH access to other target tissues.

Metabolism

No specific studies were conducted by the Sponsor during the nonclinical development program for the purposes of measuring peptide metabolite levels, nor were mass-balance studies carried out. However, the metabolic fate of ALX1-11 should largely mirror that of endogenous hPTH. The published literature indicates that PTH is metabolized sequentially in the liver and kidney. Kupffer cells take up intact PTH via a receptor that recognizes a sequence between amino acids 28 to 42 of PTH. PTH is cleaved into N-terminal and C-terminal fragments by non-specific peptidases either during uptake into the Kupffer cells or once internalized. The N-terminal fragment is subsequently further degraded within the Kupffer cells, while the C-terminal fragments are released back into circulation. The C-terminal fragments of PTH are cleared exclusively by renal processes. After glomerular filtration and peritubular secretion, the C-terminal fragments are further hydrolyzed during tubular resorption, presumably into component amino acids or dipeptides. Because hydrolysis of intact PTH in Kupffer cells occurs more rapidly than renal hydrolysis of the C-terminal fragments, the C-terminal fragments typically circulate at levels that are several fold greater than that of full-length PTH. Whether the higher circulating levels of rhPTH that result from sc injection with ALX1-11 are subject to different metabolic processes than the relatively lower circulating levels of endogenous hPTH has not been evaluated. Excretion of PTH, and therefore presumably ALX1-11 is eliminated primarily through hydrolysis to component amino acids, which are then salvaged for use in polypeptide synthesis or energy metabolism. The elimination of ALX1-11 was rapid in all species studied, with a $t_{1/2}$ between 0.4 to 3 h. The apparent $t_{1/2}$ generally increased with dose and with prolonged dosing in both rats and monkeys. These changes in $t_{1/2}$ were frequently reflected in the AUC. The increase in AUC was proportional to dose, at all tested doses in rats, but only up to 10 $\mu\text{g}/\text{kg}/\text{day}$ in monkeys. Above this dose the increase in AUC was hyperproportional.

6 General Toxicology

Overall Toxicology Summary:

6.1 Single-Dose Toxicity

Single-Dose Toxicity

Test Article: parathyroid hormone

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Doses (µg/kg)	Gender and No. per Group	Observed Maximum Non-lethal Dose (µg/kg)	Approximate Lethal Dose (µg/kg)	Noteworthy Findings	Study Number
Mice/CD-1	Subcutaneous Injection (5% mannitol, 10 mM citrate, pH 6.0/ lyophilized drug substance)	5000 10,000	5M; 5F 5M; 5F	10,000	NA	Decreased activity, ptosis, and dyspnea were observed in 10,000-µg/kg/day group 1 hour postdosing. All signs absent 24 hours postdose.	PH 416- ALX-002-93
Rat/ Sprague- Dawley	Subcutaneous Injection (5% mannitol, 10 mM citrate, pH 6.0/ lyophilized drug substance)	10,000	5M; 5F	10,000	NA	Decreased activity was observed in 1 male at 1 and 4 hours postdosing.	PH 416- ALX-001-93

F = female

M = male

NA = not applicable

6.2 Repeat-Dose Toxicity

Repeat-Dose Toxicity: Pivotal Studies

Report Type: 180-Day Subcutaneous Toxicity Study in Rats

Test Article: parathyroid hormone

Species/Strain: Rat/Sprague-Dawley	Duration of Dosing: 180 days	Study Number: PH 460-ALX-001-93						
Initial Age: 43 days old	Duration of Postdose: None	(Toxicokinetic results reported in CTBR Study 43812)						
Date of First Dose: 02 December 1993	Method of Administration: Subcutaneous Injection	GLP Compliant: Yes						
Vehicle/Formulation: 5% mannitol, 10 mM citrate, pH 6.0/lyophilized drug substance								
Special Features: None								
No Observed Adverse -Effect Level: 50 µg/kg/day								
Daily Dose (µg/kg)	0 (Control)	50 (Low)	300 (Mid)	1000 (High)				
Number of Animals	Male: 30 Female: 30	Male: 30 Female: 30	Male: 30 Female: 30	Male: 30 Female: 30				
Toxicokinetics								
AUC _{0-t} (ng-hr/mL)								
Day 1	ND	ND	4.19	4.42	22.5	17.3	ND	ND
Day 7	ND	ND	8.11	9.40	82.0	48.0	ND	ND
Day 180	ND	ND	ND	ND	76.8	66.3	ND	ND
C _{max} (ng/mL)								
Day 1	ND	ND	7.62	7.54	26.9	21.6	ND	ND
Day 7	ND	ND	14.9	13.2	96.2	84.8	ND	ND
Day 180	ND	ND	ND	ND	77.3	64.0	ND	ND
t _{max} (h)								
Day 1	ND	ND	0.03	0.08	0.33	0.03	ND	ND
Day 7	ND	ND	0.17	0.33	0.17	0.17	ND	ND
Day 180	ND	ND	ND	ND	0.33	0.08	ND	ND

ND = not determined

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)	0 (Control)	50 (Low)	300 (Mid)	1000 (High)				
Number of Animals	Male: 30 Female: 30	Male: 30 Female: 30	Male: 30 Female: 30	Male: 30 Female: 30				
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	1 ^a	0	9 ^a	1 ^b
Body Weight (g)								
Day 1	164.5	140.4	168.5	140.9	166.3	139.2	163.1	139.5
Day 181	602.3	335.3	607.1	351.5	615.9	376.0**	584.4	367.0**
Differences (Day 181-Day 1)	437.8	194.9	438.6	210.6	449.6	236.8	421.3	227.5
% Differences ^c	602.3 g	335.3 g	+ 0.8	+ 4.8	+ 2.3	+ 12.1	- 3.0	+ 9.4
Food Consumption (g)								
Total ^d	844.4	640.4	895.4	671.5	905.0	684.2	925.8	735.3
Day 181	31.4	25.5	35.5**	26.7	35.8**	26.1	31.3	27.1
Water Consumption	NP	NP	NP	NP	NP	NP	NP	NP
Clinical Observations								
Vasodilatation of								
Extremities ^e (%)	Day 1	0	0	0	0	10	66	16
Day 181	0	0	0	0	100	100	100	100
Decreased Activity (%)	Day 181	0	0	0	3	0	57	24
Abnormal Gait (%)	Day 181	0	0	0	0	0	9	6
Abnormal Stance (%)	Day 181	0	0	0	0	0	23	17
Body Drop (%)	Day 181	13	3	6	6	17	100	62
Ophthalmoscopy	-	-	-	-	-	-	-	-

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

^a The deaths or moribundity of males were attributed to severe kidney damage.^b The cause of death was not determined.^c Numbers indicate percent differences from the control groups at the end of the dosing period. Means are shown for control groups.^d Number indicates the total of the group mean daily food consumption by weekly interval from Day 8 to Day 181.^e Percent of animals with vasodilatation of hindpaws, forepaws and ears. In addition, on Day 1, 6% male and 33% female at 1000 µg/kg/day dose group showed vasodilatation of hindpaws. 10% male and 3% female at 300 µg/kg/day dose group, and 26% male and 43% female at 1000 µg/kg/day dose group showed vasodilatation of forepaws, and 3% male at 1000 µg/kg/day dose group showed vasodilatation of ears.

- No noteworthy findings. NP = not performed

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)		0 (Control)		50 (Low)		300 (Mid)		1000 (High)	
Number of Animals		Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30
Electrocardiography		NA	NA	NA	NA	NA	NA	NA	NA
Hematology									
Leukocytes (WBC) (10 ³ /mm ³)	Day 28	17.6	8.0	14.9	9.7	13.3*	8.8	13.3*	7.9
	Day 90	11.6	6.5	9.6	4.9	6.5**	4.6	9.6	7.2
	~ Day 181	9.3	7.8	8.1	4.7**	6.2**	5.0*	8.3	7.9
Erythrocytes (RBC) (10 ⁶ /mm ³)	Day 28	7.18	7.18	6.85	7.11	6.42**	6.75	6.09**	5.92**
	Day 90	8.21	7.93	7.87	7.57	6.73**	6.45**	6.41**	6.35**
	~ Day 181	7.93	7.23	7.46**	6.62*	6.26**	5.98**	6.29**	5.80**
Hemoglobin (g/dL)	Day 28	16.3	16.0	16.0	15.9	15.0**	15.3	14.6**	13.9**
	Day 90	15.9	16.2	15.7	15.7	14.5**	14.9**	14.5**	15.0*
	~ Day 181	15.1	15.0	14.6*	14.3	13.4**	13.6**	13.7**	13.5**
Hematocrit (%)	Day 28	40.6	41.0	39.6	41.2	36.8**	40.2	36.0**	35.0**
	Day 90	40.9	41.4	41.0	40.0	37.1**	37.8**	37.2**	37.9*
	~ Day 181	39.7	40.0	38.1	38.2	34.6**	36.8*	36.2**	35.5**
Mean Corpuscular Volume (µM ³)	Day 28	56.6	57.2	57.9	58.0	57.3	59.5**	59.2**	59.2*
	Day 90	49.8	52.3	52.1**	52.9	55.1**	58.7**	58.1**	59.7**
	~ Day 181	50.1	55.3	51.2	57.8*	55.2**	61.6**	57.7**	61.2**
Mean Corpuscular Hemoglobin (µg)	Day 28	22.8	22.3	23.4	22.4	23.4	22.8	24.0**	23.5**
	Day 90	19.4	20.4	20.0	20.7	21.5**	23.2**	22.6**	23.5**
	~ Day 181	19.1	20.7	19.6	21.6	21.5**	22.8**	21.9**	23.3**
Mean Corpuscular Hemoglobin Concentration (%)	Day 28	40.2	39.1	40.4	38.6	40.9	38.2*	40.5	39.6
	Day 90	39.0	39.1	38.4	39.2	39.1	39.5	38.9	39.4
	~ Day 181	38.1	37.5	38.2	37.5	38.9*	37.0	38.0	38.1
Prothrombin Time (sec.)	~ Day 181	16.0	15.4	16.0	16.2	15.4	16.8**	15.1	16.4*

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

NA = not applicable

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)		0 (Control)		50 (Low)		300 (Mid)		1000 (High)		
Number of Animals		Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	
Hematology (continued)										
Reticulocytes (%)	Day 28	4.1	3.7	6.4*	4.9	5.6	5.3	7.5**	7.0**	
	Day 90	2.7	1.1	3.0	1.8	2.8	2.7**	3.3	2.6**	
	~ Day 181	1.1	1.3	1.2	1.4	1.8*	1.5	1.5	1.0	
Segmented Neutrophils (10 ³ /mm ³)	Day 28	3.0	1.0	2.0	0.9	1.6	0.6	1.8	0.9	
	Day 90	3.3	2.0	0.9*	0.6	0.6*	0.4	2.2	2.3	
	~ Day 181	1.8	1.6	1.0*	0.7*	0.8**	0.5**	1.5	2.2	
Lymphocytes (10 ³ /mm ³)	Day 28	14.4	7.0	12.8	8.7	11.6	8.1	11.4	7.0	
	Day 90	8.0	5.2	8.4	4.4	5.7	4.4	7.0	5.0	
	~ Day 181	7.4	6.0	6.8	3.8*	5.2	4.4	6.6	5.5	
Eosinophils (10 ³ /mm ³)	Day 28	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	
	Day 90	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	
	~ Day 181	0.1	0.1	0.1*	0.0**	0.0	0.0**	0.1	0.0*	
Monocyte (10 ³ /mm ³)	Day 28	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	
	Day 90	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	
	~ Day 181	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2*	
Serum Chemistry										
Creatinine (mg/dL)	~ Day 181	0.7	0.8	0.6	0.7	0.6	0.7	0.7	0.7**	
	Blood Urea Nitrogen (mg/dL)	Day 28	13	15	15	16	16*	17	16*	16
		Day 90	12	12	12	13	14*	14	14*	13
~ Day 181	15	16	16	15	17	15	18	14		
ALT (IU/L)	~ Day 181	45	82	37	48	33*	41*	30**	26**	
AST (IU/L)	~ Day 181	94	159	78	99	71	98	98	84	
Bilirubin (mg/dL)	~ Day 181	0.19	0.22	0.17	0.21	0.15**	0.18	0.19	0.17	

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)		0 (Control)		50 (Low)		300 (Mid)		1000 (High)	
Number of Animals		Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30
Serum Chemistry									
Alkaline Phosphatase (IU/L)	Day 28	182	136	211	120	245*	162	289**	172*
	Day 90	84	46	103	55	130**	73**	172**	93**
	~ Day 181	75	33	96*	48	110**	63**	145**	75**
Sodium (mEq/L)	~ Day 181	147	147	144**	148	145*	147	145**	146
	Chloride (mEq/L)	~ Day 181	102	101	101	100	103	98**	103*
Calcium (mg/dL)	~ Day 181	11.4	12.1	11.2	11.9	11.3	11.5**	11.6	11.6**
Phosphorus (mg/dL)	Day 28	10.6	8.6	10.4	8.7	9.7	8.4	8.8**	7.1
	Day 90	8.1	6.6	8.6	6.4	7.6	6.6	7.1**	6.3
	~ Day 181	7.5	6.8	7.7	6.9	7.3	7.1	7.5	7.1
Albumin (g/dL)	~ Day 181	4.7	5.8	4.5	5.4	4.4	4.9**	4.6	4.8**
Globulin (g/dL)	~ Day 181	2.5	2.5	2.5	2.7	2.8	2.7	3.0**	2.6
Albumin/Globulin Ratio	~ Day 181	1.9	2.3	1.9	2.1	1.6**	1.8**	1.6**	1.9**
Urinalysis (S.G., pH)									
Urine Chemistry									
Calcium (md/dL)	~ Day 181	3.6	21.6	4.9	20.3	9.2**	34.2	11.4**	28.0
Phosphorus (mg/dL)	~ Day 181	77.4	120.4	42.9*	140.1	12.2**	88.9	6.4**	32.6
Organ Weights									
Kidney ^f (g)		4.20 g	2.49 g	+2.4	+0.8	+16.7**	+10.0**	+26.4**	+20.1**
Kidney to Body Weight Ratio ^f (%)		0.73	0.79	+1.4	-3.8	+15.1**	-2.53	+30.1**	+8.9*
Kidney to Brain Weight Ratio ^f (%)		189.43	125.85	+2.17	+0.41	+21.5**	+9.4*	+27.5**	+18.2**

^f Values for the control groups are the means and for the treated groups are the percent difference from the controls.

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

- No noteworthy findings.

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)		0 (Control)		50 (Low)		300 (Mid)		1000 (High)	
Number of Animals		Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30
Gross Pathology									
Kidney	Number Examined	30	30	30	30	29	30	21	29
	No Gross Finding ^e (%)	93	100	100	100	90	100	62	93
Injection site	Number Examined	30	30	30	30	29	30	21	29
	No Gross Finding ^b (%)	37	33	13	17	14	20	10	14
Histopathology									
Femur	Number Examined	30	30	30	30	30	30	30	30
	Osteosclerosis, Epiphysis (No.)	0	0	30	30	30	30	30	30
	Osteosclerosis, Diaphysis (No.)	0	0	++	++	++++	++++	+++	++++
	Epiphyseal disk dysplasia (No.)	0	0	+	+	++	+++	++	+++
		0	0	30	30	30	30	30	30
Sternum	Number Examined	30	29	30	30	30	30	30	29
	Osteosclerosis (No.)	0	0	30	30	30	30	30	29
		-	-	+	+	++	+	++	++
		0	0	0	0	6	2	25	22
	Bone marrow cellularity; Minimal to No Marrow present (No.)	+++	+++	++	++	++	+++	(No marrow present: 19)	(No marrow present: 21)

^e Findings included bilateral or single enlarged, mottled, pale, granular, pitted or rough surface, dilated pelvic or cysts.^b Findings included large, small, or scattered area of subcutaneous hemorrhage and/or thickening.

- No noteworthy findings. + Minimum or Slight ++ Moderate +++ Moderately Severe ++++ Severe

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: PH 460-ALX-001-93 (continued)

Daily Dose (µg/kg)		0 (Control)		50 (Low)		300 (Mid)		1000 (High)	
Number of Animals		Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30	Male: 30	Female: 30
Histopathology (continued)									
Kidney	Number Examined	30	30	30	30	30	30	30	30
	Tubular mineralization (No.)	2	9	1	6	11	10	28	23
		+	+	+	+	+	+	++	+
	Pelvic mineralization (No.)	0	7	5	3	18	19	8	16
		-	+	+	+	++	++	++	+
	Pelvic epithelial hyperplasia (No.)	0	1	3	1	12	8	7	9
		-	+	+	+	+	+	+	+
	Tubular regeneration (No.)	10	4	22	2	29	4	30	17
		+	+	+	+	+	+	+++	+
	Tubular dilatation (No.)	6	5	8	1	20	5	29	5
	+	+	+	+	+	+	++	+	
Nephritis, Nonsuppurative, Multifocal (No.)	5	2	7	0	18	1	25	6	
	+	+	+	-	+	+	+	+	
Injection site	Number Examined	30	30	0	0	0	0	30	30
	Hemorrhage, Focal (No.)	22	22	NA	NA	NA	NA	26	28
		+	++					++	++
	Fibrosis, Interstitial (No.)	19	3	NA	NA	NA	NA	26	28
		+	+					++	++
	Cellulitis, Subacute (No.)	6	1	NA	NA	NA	NA	0	5
	+	+					-	+	

- No noteworthy findings. + Minimum or Slight ++ Moderate +++ Moderately Severe ++++ Severe

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

NA = not applicable

Repeat-Dose Toxicity: Pivotal Studies

Report Type: A 26-Week Toxicology Study of rhPTH (1-84) in the Cynomolgus Monkey

Test Article: parathyroid hormone

Species/Strain: Primate/Cynomolgus Monkeys		Duration of Dosing: 26 weeks		Study Number: CTBR 86142					
Initial Age: Exact age unknown; reported to be young adults		Duration of Postdose (Recovery): 4 weeks							
Date of First Dose: 17 May 1994		Method of Administration: Subcutaneous Injection							
Special Features: None		Vehicle/Formulation: 5% mannitol, 10 mM citrate, pH 6.0/lyophilized drug substance		GLP Compliant: Yes					
No Observed Adverse -Effect Level: 30 µg/kg/day									
Daily Dose (µg/kg)		0 (Control)		2 (Low)		10 (Mid)		30 (High)	
Number of Animals		Male: 5	Female: 5	Male: 4	Female: 4	Male: 4	Female: 4	Male: 5	Female: 5
Toxicokinetics									
AUC _{0-t} (ng·h/mL)	Day 1	0.102	0.104	0.735	0.794	3.337	2.255	9.749	21.186
	Week 4	0.085	0.187	0.674	0.602	2.822	2.105	10.858	17.088
	Week 13	0.118	0.096	0.689	0.620	2.987	2.332	15.576	15.301
	Week 26	0.125	0.121	0.401	0.338	2.492	2.814	12.745	18.281
C _{max} (ng/mL)	Day 1	0.0432	0.0480	0.6205	0.9208	1.9316	2.0100	6.2987	14.4800
	Week 4	0.0341	0.0502	0.6680	0.7985	1.7000	1.8725	7.5520	12.6080
	Week 13	0.0387	0.0433	0.5918	0.6228	1.9850	1.6925	7.8860	8.9500
	Week 26	0.0407	0.0631	0.3833	0.3613	1.2650	1.9075	6.2920	9.0140
t _{max} (h)	Day 1	3.81	0.80	0.45	0.40	0.50	0.32	0.55	0.39
	Week 4	3.16	5.42	0.33	0.25	0.38	0.38	0.31	0.46
	Week 13	5.42	3.79	0.39	0.34	0.52	0.33	0.45	0.51
	Week 26	1.88	0.48	0.44	0.25	0.63	0.50	0.75	0.56
Noteworthy Findings									
Died or Sacrificed Moribund		0	0	0	0	0	0	0	0
Body Weight (%) ^a		4.04 kg	3.30 kg	-3.47	-3.03	-2.97	-3.03	-1.49	-2.42

^a Numbers indicate percent differences from the control group at Week 26. Means are shown for control groups. There were no changes in body weights that were considered related to treatment with PTH.

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: CTBR 86142 (continued)

Daily Dose (µg/kg)		0 (Control)		2 (Low)		10 (Mid)		30 (High)	
Number of Animals		Male: 5	Female: 5	Male: 4	Female: 4	Male: 4	Female: 4	Male: 5	Female: 5
Food Consumption (%) ^b		-	-	-	-	-	-	-	-
Water Consumption		NA	NA	NA	NA	NA	NA	NA	NA
Clinical Observation ^c		-	-	-	-	-	-	-	-
Ophthalmoscopy ^d		-	-	-	-	-	-	-	-
Electrocardiography ^e		-	-	-	-	-	-	-	-
Hematology									
Hemoglobin (g/dL)	Week 25	12.3	11.4	12.1	12.1	11.8	11.3	10.3**	11.1
	Recovery	11.9	11.3	NA	NA	NA	NA	12.0	11.6
Hematocrit (%)	Week 25	38.7	35.5	37.4	38.1	36.6	35.8	33.0**	35.0
	Recovery	37.6	35.8	NA	NA	NA	NA	39.0	36.9
Serum Chemistry									
Blood Urea Nitrogen (mg/dL)	Week 12	19.5	19.9	22.0	21.1	22.7	23.9	21.7	22.8
	Week 25	20.0	21.7	24.0	26.2	25.0	25.7	28.0**	22.4
	Recovery	18.6	17.2	NA	NA	NA	NA	15.9	18.2
Phosphorus (mg/dL)	Week 12	6.82	5.80	6.21	5.13	5.45**	5.36	5.69*	4.97
	Week 25	6.01	5.23	5.48	4.91	4.72*	4.68	4.71*	3.93
	Recovery	6.55	4.70	NA	NA	NA	NA	6.78	5.38
Calcium (mg/dL)	Week 12	9.8	9.5	9.8	9.9	9.6	10.0	9.6	9.8
	Week 25	9.8	9.4	9.4	9.9	9.5	9.7	9.6	9.7
	Recovery	9.0	8.9	NA	NA	NA	NA	9.2	9.4

^b Appetency observations indicated that food consumption was not affected by treatment with ALX1-11.^c There were no clinical signs observed which were considered related to treatment with ALX1-11.^d There were no treatment-related ocular changes observed at Week 24. Minor findings were observed in a few animals and were considered incidental in origin and unrelated to treatment since they have been routinely seen in comparable populations.^e There was no electrocardiographic evidence of test article effect in this study.

- No noteworthy findings.

* P < 0.05 ** P < 0.01 (Analysis of Variance from control means using Dunnett's Procedure)

NA = not applicable

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: CTBR 86142 (continued)

Daily Dose (µg/kg)		0 (Control)		2 (Low)		10 (Mid)		30 (High)	
Number of Animals		Male: 5	Female: 5	Male: 4	Female: 4	Male: 4	Female: 4	Male: 5	Female: 5
Urinalysis									
Calcium (mg/dL)	Week 13	9.9	8.6	18.1	7.2	10.3	22.5	12.1	12.9
	Week 26	16.5	12.7	15.9	13.3	17.3	18.5	14.0	17.4
	Recovery	NA	8.1	NA	NA	NA	NA	8.9	7.6
Organ Weights (%)		-	-	-	-	-	-	-	-
Gross Pathology		-	-	-	-	-	-	-	-
Histopathology ²									
Kidney ^e	Total Animals	3	3	4	4	4	4	3	3
	Examined								
	Nephritis, Interstitial (No.)	0	0	1	1	1	0	2	1
	Tubular Mineralization (No.)	0	0	0	0	2	1	3	1
Injection Sites	Total Animals	3	3	4	4	4	4	3	3
	Examined								
(2/animal) ³	Hemorrhage ¹ (No.)	0	2	3	2	2	1	0	1
Additional Examinations: Bone Mineral Density (BMD)									
Number	End of Study	3	3	4	4	4	4	3	3
	Examined	2	2	0	0	0	0	2	2
LDF BMD (g/cm ²)	End of Study	0.429	0.328	0.374	0.332	0.393	0.394*	0.460	0.423
	Recovery	0.398	0.350	NA	NA	NA	NA	0.475	0.472

¹ Findings were likely resulted from a physiological response, rather than an adverse effect (no regenerative or degenerative changes were seen in the kidneys).² Other single observations included glomerular thrombosis, tubular basophilia, intratubular crystal, cyst, and tubular dilatation. Two of 4 females in the low-dose group showed pyelitis.³ Other single observations included folliculitis and cellulitis.¹ Number indicates total observations of two injection sites per animal.

- No noteworthy findings.

* P < 0.05 ** P < 0.01 (Analysis of Variance using Dunnett's Procedure)

LDF: left distal femur – measured as 15% of the total femur length

NA = not applicable

Repeat-Dose Toxicity: Pivotal Studies (continued)

Study Number: CTBR 86142 (continued)

Daily Dose (µg/kg)	0 (Control)		2 (Low)		10 (Mid)		30 (High)		
Number of Animals	Male: 5	Female: 5	Male: 4	Female: 4	Male: 4	Female: 4	Male: 5	Female: 5	
Additional Examinations: BMD (continued)									
LCF BMD	End of Study	0.473	0.392	0.442	0.397	0.441	0.430	0.504	0.435
(g/cm ³)	Recovery	0.442	0.401	NA	NA	NA	NA	0.511	0.456
LPF BMD	End of Study	0.460	0.361	0.421	0.370	0.427	0.418*	0.496	0.435
(g/cm ³)	Recovery	0.433	0.399	NA	NA	NA	NA	0.490	0.469
LDR BMD	End of Study	0.239	0.211	0.231	0.221	0.241	0.228	0.244	0.240
(g/cm ³)	Recovery	0.230	0.218	NA	NA	NA	NA	0.254	0.245
LDR2 BMD	End of Study	0.222	0.197	0.210	0.204	0.225	0.220	0.278	0.235
(g/cm ³)	Recovery	0.207	0.194	NA	NA	NA	NA	0.238	0.238
LCR BMD	End of Study	0.263	0.234	0.243	0.235	0.246	0.244	0.266	0.248
(g/cm ³)	Recovery	0.247	0.237	NA	NA	NA	NA	0.269	0.261
LPR BMD	End of Study	0.213	0.201	0.195	0.199	0.207	0.214	0.228	0.222
(g/cm ³)	Recovery	0.211	0.195	NA	NA	NA	NA	0.220	0.230
L2-L4 BMD	End of Study	0.577	0.483	0.520	0.531	0.554	0.562*	0.627	0.554
(g/cm ³)	Recovery	0.503	0.515	NA	NA	NA	NA	0.612	0.632
Number of Animals	Male: 2	Female: 2	Male: 10	Female: 0	Male: 0	Female: 0	Male: 2	Female: 2	
Postdose Evaluations									
Test Article-related Changes									
	-	-	-	-	-	-	-	-	

- No noteworthy findings.

* P < 0.05 ** P < 0.01 (Analysis of Variance using Dunnett's Procedure)

LCF: left central femur – measured as 65% of the total femur length

LCR: left central radius – measured as the central one third of the radius

LDR: left distal radius – measured as the distal one third of the radius

LDR2: left distal radius2 – measured as the most distal 1.2-1.3 cm of the radius

LPF: left proximal femur – measured as 20% of the total femur length

LPR: left proximal radius – measured as the proximal one third of the radius

L2-L4: 2nd-4th lumbar vertebrae section

NA = not applicable

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

ALX1-11 given SC at doses of 100, 300, 1000 mcg/kg/day prior to mating and in females continuing through gestation Day 7 (up to 120 times the human dose of 100 mcg/day based on body surface area for a 70 kg person, did not significantly affect fertility or other measures of parental reproductive performance. In the female, small statistically significant, dose-dependent changes in multiple uterine parameters were seen, including reductions in the number of corpora lutea, the number of implantation sites and the number of live embryos; however, all group mean values remained within the range of the historical control data. In the male, there were statistically significant slight reductions in the group mean weight of the prostate and the cauda epididymis; however, these values remained within the historical control range. 1 of 22 males receiving either the middle dose (300 µg/kg/day) or the high dose (1000 µg/kg/day) of ALX1-11 had notable defects in spermatogenesis, including: exceptionally low sperm counts and motility, and an unusually high percentage of morphologically abnormal spermatozoa. The absence of dose-dependency suggests that the group distribution of these latter findings may be coincidental.

Table 2.6.7.12 – Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation

Test Article: parathyroid hormone

Design Similar to ICH 4.1.1? Yes	Duration of Dosing: Male: 28 days prior to mating, during and following mating (a total of 64 to 70 days) Female: 14 days prior to mating, during mating and until gestation day 7 (a total of 23 to 37 days).		Study Number: CTBR 98356	
Species/Strain: Rat / CrI:CD®(SD)IGS BR	Day of Mating: Gestation Day 0		Study Title: A Fertility Study of ALX1-11, rhPTH(1-84) Administered by Subcutaneous Injection in the Rat	
Initial Age at the Initiation of Dosing: Male: 82-86 days Female: 61-65 days	Day of C-Section: Gestation Day 13		GLP Compliant: Yes	
Date of First Dose: 22 October 2003	Method of Administration: Subcutaneous Injection			
Special Features: None	Vehicle/Formulation: Citric Acid (1.1 mg/mL [w/v] in D-Mannitol USP (27 mg/mL [w/v])/ Lyophilized PTH [5% mannitol, 10 mM citrate, pH 5.5]			
No Observed Adverse Effect Level: F ₀ Males: 1000 µg/kg/day F ₀ Females: 1000 µg/kg/day F ₁ Litters: 1000 µg/kg/day				
Daily Dose (µg/kg):	0 (Control)	100	300	1000
Males				
Number Evaluated	22	22	22	22
Number Died or Sacrificed Moribund	0	0	0	1 ^a
Clinical Observations	-	-	-	-
Body Weight (Days 1 to 67) ^b (%)	596 g	2.4	2.3	-3.1*
Food Consumption (Days 1 to 64) ^b (%)	494 g/animal/day	2	3	1

^a Not test article-related. Cause of death unknown.^b For control, group means are shown (mean total for food consumption). For treated groups, percent differences from controls are shown.

- No noteworthy findings.

* Test article-related.

** Significantly different from the control (p ≤ 0.001) (Dunnett). Not test article-related finding since values are within the historical control range of 1.207 to 1.673 g.

*** Significantly different from the control (p ≤ 0.05) (Dunnett). Not test article-related finding since values are within the historical control range of 0.262 to 0.333 g.

Table 2.6.7.12 – Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation (continued)

Study Number: CTBR 98356 (continued)

Daily Dose (mg/kg):	0 (Control)	100	300	1000
Males (continued)				
Necropsy Observations				
Spleen enlargement	0/22	0/22	0/22	1/22*
Mean Organ Weights (absolute) (g)				
Prostate gland (g)	0.337	0.318	0.331	0.304***
Cauda epididymis (g)	1.71	1.65	1.65	1.38**
Mean Number of Days Prior to Mating	2.9	2	3.2	2.4
Number of Males that Mated	22	22	22	22
Number of Fertile Males	20	21	21	21
Male Reproductive Assessments	-	-	-	-
Females				
Number Evaluated	22	22	22	22
Number Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Premating Body Weight ^b (%)	244.3 g	1.4	3.8	3.4
Gestation Body Weight ^b (%)	325 g	-1.8	2	-0.3
Premating Food Consumption ^b (%)	82 g/animal/day	-1.2	1.2	3.7
Gestation Food Consumption ^b (%)	342 g/animal/day	-0.3	5.17	2.6
Mean Number of Estrous Cycles/14 days	2.7	2.5	2.6	2.5
Necropsy Observations				
Spleen enlargement	0/22	0/22	0/22	6/22*

^b For control, group means are shown (mean total for food consumption). For treated groups, percent differences from controls are shown.

- No noteworthy findings.

Total number of animals with incidence/total number examined.

* Test article-related.

** Significantly different from the control (p ≤ 0.001) (Dunnett). Not test article-related finding since values are within the historical control range of 1.207 to 1.673 g.

*** Significantly different from the control (p ≤ 0.05) (Dunnett). Not test article-related finding since values are within the historical control range of 0.262 to 0.333 g.

Table 2.6.7.12 – Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation (continued)

Study Number: CTBR 98356 (continued)

Daily Dose (mg/kg):	0 (Control)	100	300	1000
Mean Number of Days Prior to Mating	2.9	2	3.2	2.4
Number of Females Sperm Positive	22	22	22	22
Number of Pregnant Females	20	21	21	21
Number Aborted or with Total Resorption of Litter	0	0	0	0
Mean Number of Corpora Lutea	18.8	17.6	17.1 ^c	16.9 ^d
Mean Number of Implantations	17.3	14.5 ^a	15.5 ^a	15.3 ^a
Mean % Preimplantation Loss	7.6	18.6 ^f	9.1	9.3
Mean Number of Live Conceptuses	16.3	13.4 ^e	14.5 ^e	14.4 ^e
Mean Number of Resorptions	1	0.9	1	0.9
Number of Dead Conceptuses	0	1	1	0
Mean % Postimplantation Loss	5.9	6.9	6.3	6

^c Significantly different from the control ($p \leq 0.05$) (Wilcoxon). Not test article-related because values are within the historical control range (16.3 to 18.8).

^d Significantly different from the control ($p \leq 0.01$) (Wilcoxon). Not test article-related because values are within the historical control range (16.3 to 18.8).

^e Significantly different from the control ($p \leq 0.01$). Not test article-related because values are within the historical control range (13.8 to 17.1).

^f Significantly different from the control ($p \leq 0.01$) but not test article-related.

^g Significantly different from the control ($p \leq 0.01$). Not test article-related because values are within the historical control range (12.6 to 16.3).

9.2 Embryonic Fetal Development

Study title: Subcutaneous Developmental Toxicity Study of ALX1-11 in Rats

Study no.: XGW00013

Study report location:

(b) (4)

Conducting laboratory and location:

(b) (4)

Date of study initiation: 12 September 2008

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: PTH Drug Substance, lot # 04260R2/005

%purity by RP-HPLC 98.8%, by CEC-

HPLC 99.5%

Key Study Findings

The maternal no-observable-adverse-effect-level (NOAEL) of ALX1-11 is 300 mcg/kg/day. Transient, but statistically significant reductions in body weight gains and feed consumption occurred between DGs 7 and 13 in the 300 and/or 1000 mcg/kg/day dosage groups.

The developmental NOAEL of ALX1-11 is 1000 mcg/kg/day. A transient delay in skeletal ossification (i.e. significant reduction in the average number of ossified hindlimb phalanges) was observed in the 1000 mcg/kg/day dosage group, however the frequency of this finding was within the historical control range of the Testing Facility.

Methods

Doses

Dosage Group	Number of Rats	Dosage ^a (mcg/kg/day)	Concentration (mcg/mL)	Dosage Volume (mL/kg)
I	25	0 (Vehicle)	0	2
II	25 + 6 ^c	100	50	2
III	25 + 6 ^c	300	150	2
IV	25 + 6 ^c	1000	500	2

- a. The test article was considered 100% active/pure for the purpose of dosage calculations.
c. Six rats assigned to toxicokinetic sample collection

Rats were administered the test article and/or the vehicle once daily on DGs 7 through 17 at approximately the same time each day by subcutaneous injection into the dorsal region.

Species/Strain Crl:CD(SD) rat.

Number/Sex/Group Refer to Table above.

Route, Volume, and Infusion Rate Subcutaneous, 2 mg/kg, bolus.

Dosing Solution Analyses/Drug Stability and Homogeneity

The concentrations of the prepared formulations were within the acceptable limits ($\pm 15\%$ of the targeted concentration) with the exception of the 50 mcg/mL formulation on the first day of dose administration and the 500 mcg/mL formulation on the last day of dose administration. The low dose (Group II) formulation on the first day of dose administration was 22% lower than the targeted volume. This out of specification result did not adversely affect the outcome of the study because it occurred in the lowest dose evaluated. The high dose (Group IV) formulation on the last day of dose administration was 17% greater than the targeted values.

Satellite Groups Used for Toxicokinetics Refer to Table above.

Study Design

The purpose of this study was to detect adverse effects of ALX1-11 on Crl:CD(SD) presumed-pregnant female rats and development of the embryo and fetus consequent to exposure of the dam from implantation to closure of the hard palate. This study was designed to evaluate ICH Harmonized Tripartite Guideline stages C and D of the reproductive process.

Parameters and Endpoints Evaluated

The following parameters were evaluated: viability, clinical observations, body weights, body weight changes, feed consumption, necropsy observations, Caesarean-sectioning and litter observations, fetal body weights, fetal sex and fetal gross external, visceral and skeletal alterations.

Results

Mortality (Dams) All rats survived to scheduled sacrifice.

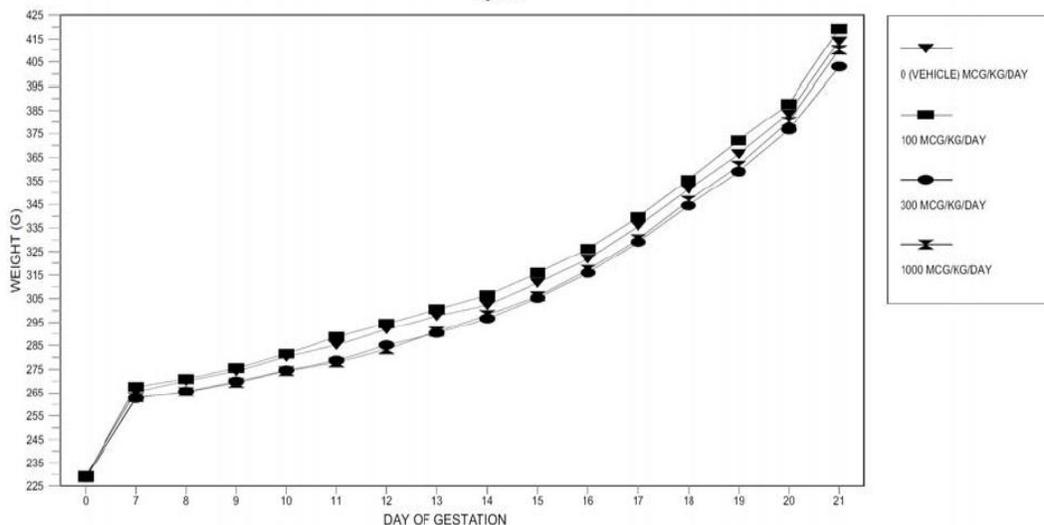
Clinical Signs (Dams) No clinical or necropsy observations related to administration of ALX1-11 occurred.

Body Weight (Dams) Body weights and body weight gains were unaffected by the 100 mcg/kg/day dosage of ALX1-11. Transient, but statistically significant reductions in body weight gains occurred in the 300 and 1000 mcg/kg/day dosage groups during the first three days of the dosage period (DGs 7 to 10), and again on DGs 10 to 12 in the 1000 mcg/kg/day dosage group, relative to the vehicle group values. Thereafter, body weight gains were comparable to the vehicle group values.

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MATERNAL BODY WEIGHTS

Figure 1



PROTOCOL XGW00013: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RATS

TABLE 4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) a		0 (VEHICLE)	100	300	1000
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	24	25
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+36.0 ± 8.5	+38.7 ± 9.6	+33.7 ± 7.3	+34.0 ± 6.3
DAYS 7 - 10	MEAN±S.D.	+14.8 ± 3.8	+13.9 ± 3.3	+12.0 ± 4.0*	+11.4 ± 4.9*
DAYS 10 - 12	MEAN±S.D.	+11.5 ± 3.0	+12.9 ± 4.3	+10.6 ± 3.5	+9.2 ± 4.4*
DAYS 12 - 15	MEAN±S.D.	+19.8 ± 5.9	+21.4 ± 4.7	+20.2 ± 3.4	+22.5 ± 5.5
DAYS 15 - 18	MEAN±S.D.	+39.8 ± 6.8	+39.6 ± 5.4	+39.2 ± 6.9	+40.6 ± 7.3
DAYS 7 - 18	MEAN±S.D.	+86.0 ± 13.2	+87.8 ± 9.4	+82.0 ± 10.0	+83.7 ± 10.4
DAYS 18 - 21	MEAN±S.D.	+62.6 ± 11.6	+63.8 ± 8.1	+58.7 ± 7.9	+63.7 ± 6.6
DAYS 7 - 21	MEAN±S.D.	+148.6 ± 23.1	+151.7 ± 15.1	+140.7 ± 13.8	+147.4 ± 11.8
DAYS 0 - 21	MEAN±S.D.	+184.6 ± 28.1	+190.3 ± 17.7	+174.4 ± 17.6	+181.4 ± 14.6

DAYS = DAYS OF GESTATION

a. Dosage occurred on days 7 through 17 of gestation.

* Significantly different from the vehicle control group value (p≤0.05).

** Significantly different from the vehicle control group value (p≤0.01).

Food Consumption (Dams) Absolute (g/day) and relative (g/kg/day) feed consumption values were unaffected by dosages of ALX1-11 of 100 mcg/kg/day. Corresponding to reductions in body weight gains, absolute and relative feed consumption values in the 300 and 1000 mcg/kg/day dosage groups were significantly lower on DGs 7 to 10, as compared to the vehicle group values. Absolute and relative feed consumption values remained significantly lower in the 1000 mcg/kg/day dosage group on DGs 10 to 12, as compared to the vehicle group values. As a result, absolute and relative feed consumption values in the

1000 mcg/kg/day dosage group were significantly lower for the dosage period, as compared to the vehicle group values. During the postdosage period (DGs 18 to 21), rats in the 1000 mcg/kg/day dosage group consumed more feed than rats in the corresponding vehicle group. This can be attributed to a rebound phenomenon common in these types of studies.

Toxicokinetics

The toxicokinetics of ALXI-11 was characterized in presumed pregnant female Sprague-Dawley rats when delivered by subcutaneous injection once daily from Days 7 to 17 of presumed gestation at dose levels of 100, 300 and 1000 mcg/kg/day. Peak concentrations were generally achieved within 5 minutes of administration, and the terminal elimination half-life was estimated to be approximately 1 hour or less. Exposure of the test article generally increased in a proportional manner with ascending dose level. Although the test article was detected at the predose occasion at dose levels of 300 and 1000 mcg/kg/day on presumed Gestation Day 17, this observation was not consistently associated with higher C_{max} and $AUC_{(0-t_{last})}$ parameters on that occasion.

Terminal and Necroscopic Evaluations-Section Data (Implantation Sites, Pre- and Post Implantation)

Pregnancy occurred in 24 to 25 rats in the four dosage groups. No Caesarean-sectioning or litter parameters were affected by dosages of ALX1-11 as high as 1000 mcg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among the four dosage groups and did not significantly differ. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

There were no test item-related effects at any dose on any parameter.

Offspring (Malformations, Variations, etc.)

No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of ALX1-11 as high as 1000 mcg/kg/day. There were no dosage-dependent or significant differences in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations. A significant decrease occurred in the average number of ossified phalanges in the hindlimb in the 1000 mcg/kg/day dosage group, relative to the vehicle group value. This decrease in hindlimb phalanges was not considered adverse because the average value was within the historical range of the Testing Facility. All other fetal ossification site averages were comparable among the four dosage groups and did not significantly differ.

There were no s.s. test item-related effects on gross external, soft tissue or skeletal fetal alterations (malformations or variations) at any dosage tested (up to 1000 mcg/kg/day). There was a small n.s.s. ↑ in the % of fetuses with any alteration per litter at HD (2.8% in C & 3.3% in HD). Findings that showed an increased incidence at HD compared to C are indicated in summary tables below (red circles). In all instances, these findings were within the historical control range of the testing facility and/or are documented commonly occurring variations in this species, and are not considered to be toxicologically significant.

There was a s.s. ↓ in the average number of ossified phalanges in the hindlimb in the HD group, relative to C; however this finding was not considered adverse, since the average value was within the historical control range of the testing facility.

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TABLE 9 (PAGE 1): FETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	100	300	1000
LITTERS EVALUATED	N	24	24	24	25
FETUSES EVALUATED	N	340	334	322	355
LIVE	N	340	334	322	355
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	5(25.0)	8(33.3)	7(29.2)	9(36.0)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	9(2.6)	9(2.7)	10(3.1)	12(3.4)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	2.8 ± 5.9	2.8 ± 4.2	3.0 ± 5.2	3.3 ± 5.4

a. Dosage occurred on days 7 through 17 of gestation.

PROTOCOL XGW00013: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RATS

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	100	300	1000
LITTERS EVALUATED	N	24	24	24	25
FETUSES EVALUATED	N	340	334	322	355
LIVE	N	340	334	322	355
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3) ^b	0(0.0)	0(0.0)
EYES: LIDS OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3) ^b	0(0.0)	0(0.0)
EYE: BULGE DEPRESSED					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
BODY: EDEMA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)
BODY: UMBILICAL HERNIA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)	0(0.0)

a. Dosage occurred on days 7 through 17 of gestation.

b. Fetus 226-9 had other gross external alterations.

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TABLE 11 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	100	300	1000
LITTERS EVALUATED	N	24	24	24	25
FETUSES EVALUATED	N	165	161	156	171b
LIVE	N	165	161	156	171b
EYES: MICROPHthalmIA					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
EYES: RETINA FOLDED					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
VESSELS: INNOMINATE ARTERY ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)

a. Dosage occurred on days 7 through 17 of gestation.

b. Excludes values for fetus 291-14, which only the head was examined at soft tissue examination.

PROTOCOL XGW00013: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RATS

TABLE 12 (PAGE 1): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	100	300	1000
LITTERS EVALUATED	N	24	24	24	25
FETUSES EVALUATED	N	175	173	166	183
LIVE	N	175	173	166	183
SKULL: FRONTAL, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
SKULL: PARIETAL, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
SKULL: SUPRAOCCIPITAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
SKULL: INTERPARIETAL, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
SKULL: PARIETAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(8.3)	1(4.2)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	2(1.1)	1(0.6)	0(0.0)	1(0.5)
SKULL: POSTERIOR FONTANELLE LARGE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRAE					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	2(8.3)	2(8.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	2(1.2)	2(1.1)
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	2(8.3)	4(16.7)	2(8.3)	5(20.0)
FETAL INCIDENCE	N(%)	2(1.1)	4(2.3)	3(1.8)	6(3.3)c,d
LUMBAR VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)c

a. Dosage occurred on days 7 through 17 of gestation.

b. Fetus 225-9 had other skeletal alterations.

c. Fetus 283-8 had other skeletal alterations.

d. Fetus 292-3 had other skeletal alterations.

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TABLE 12 (PAGE 2): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 100	III 300	IV 1000
LITTERS EVALUATED	N	24	24	24	25
FETUSES EVALUATED	N	175	173	166	183
LIVE	N	175	173	166	183
RIBS: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6) ^b	0(0.0)	0(0.0)
RIBS: SHORT					
LITTER INCIDENCE	N(%)	2(8.3)	1(4.2)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	3(1.7)	1(0.6) ^b	1(0.6)	0(0.0)
MANUBRIUM: DUPLICATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5) ^c
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	2(8.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)	2(1.2)	0(0.0)
STERNAL CENTRA: DUPLICATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5) ^c
STERNAL CENTRA: NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
XIPHOID: DUPLICATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5) ^c

a. Dosage occurred on days 7 through 17 of gestation.

b. Fetus 226-9 had other skeletal alterations.

c. Fetus 292-3 had other skeletal alterations.

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TABLE 13 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) a		0 (VEHICLE)	100	300	1000
LITTERS EXAMINED	N	24	24	24	25
FETUSES EXAMINED	N	175	173	166	183
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.98 ± 0.06	0.96 ± 0.08	0.99 ± 0.04	0.94 ± 0.10
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.03 ± 0.07	13.02 ± 0.07	13.08 ± 0.22	13.08 ± 0.16
LUMBAR	MEAN±S.D.	5.96 ± 0.09	5.97 ± 0.08	5.91 ± 0.23	5.92 ± 0.16
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	7.30 ± 0.63	7.30 ± 0.70	7.50 ± 0.80	7.34 ± 0.72
RIBS (PAIRS)	MEAN±S.D.	13.02 ± 0.05	13.02 ± 0.08	13.06 ± 0.18	13.06 ± 0.12
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	4.00 ± 0.02	3.99 ± 0.04	3.99 ± 0.04	3.97 ± 0.07
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.00	3.99 ± 0.06	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	8.05 ± 0.74	7.44 ± 0.87*	7.90 ± 0.70	7.61 ± 0.76
HINDLIMB b					
TARSALS	MEAN±S.D.	0.00 ± 0.02	0.01 ± 0.03	0.01 ± 0.03	0.00 ± 0.02
METATARSALS	MEAN±S.D.	4.88 ± 0.16	4.76 ± 0.28	4.84 ± 0.23	4.78 ± 0.24
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	6.18 ± 0.93	5.82 ± 0.89	6.10 ± 0.84	5.51 ± 0.57*

a. Dosage occurred on days 7 through 17 of gestation.

b. Calculated as average per limb.

* Significantly different from the vehicle control group value (p≤0.05).

** Significantly different from the vehicle control group value (p≤0.01).

Study title: Subcutaneous Developmental Toxicity Study of ALX1-11 in Rabbits

Study no.: XGW00015

Study report location:



Conducting laboratory and location:



Date of study initiation: 12 SEP 2008

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity:

ALX1-11 (Parathyroid Hormone 1-84, NPSP 558) Lot # 04260R2/005, 98.8% by RP-HPLC, 99.5% by CEC-HPLC

Key Study Findings

- Maternal toxicity was observed at HD (50 mcg/kg/day), as evidenced by markedly reduced food consumption and a 56% decrement in body weight gain during the treatment period in this dose group, compared to control. The MD (10 mcg/kg/day) is considered to be the maternal NOAEL (HED = 194 mcg/day) by both the Sponsor and the PT reviewer.
- Test item had no effect on uterine or litter observations.
- The few findings noted below as being elevated in the HD group are likely to have emerged secondary to reduced food consumption and decreased weight gain during the period of organogenesis.

- Of note is the finding of a single HD fetus with spina bifida. Given the known relationship between malnutrition (specifically folic acid) and neural tube defects, it is reasonable to hypothesize that any contribution of the test item towards the development of spina bifida in the affected fetus is likely to be secondary to maternal malnutrition, and not an indication of teratogenic potential. Nonetheless, the developmental NOAEL is considered to be 10 mcg/kg/day (HED = 194 mcg/day) on the basis of the single incidence of spina bifida. Sponsor considers the developmental NOAEL to be 50 mcg/kg/day.
- **In conclusion**, the maternal no-observable-adverse-effect-level (NOAEL) of ALX1-11 is 10 mcg/kg/day. The 50 mcg/kg/day dosage caused reductions in body weight gains, body weights and feed consumption values during the dosage period.
- The developmental NOAEL is 50 mcg/kg/day. There were no frank malformations produced by maternal treatment with ALXI-11. However, the 50 mcg/kg/day dosage of ALX 1-11 was associated with increases in the occurrence of supernumerary thoracic ribs with concurrent changes in the average number of ossified thoracic and lumbar vertebrae, respectively, a common variation observed at maternally toxic dosages.

Methods

Doses

Dosage Group	Number of Rabbits	Dosage ^a (mcg/kg/day)	Concentration (mcg/mL)	Dosage Volume (mL/kg)
I	20	0	0	1.0
II	20 + 3 ^c	5	50	0.1
III	20 + 3 ^c	10	50	0.2
IV	20 + 3 ^c	50	50	1.0

- The test article was considered 100% active/pure for the purpose of dosage calculations.
- NA is an abbreviation for not applicable.
- Three rabbits assigned to toxicokinetic sample collection.

Species/Strain New Zealand White [Hra:(NZW)SPF] rabbit

Number/Sex/Group 20 rabbits/main study gp

Route, Formulation, Volume, and Infusion Rate

Subcutaneous route; formulation contained citric acid, monohydrate, USP, D- Mannitol, USP, Sodium Hydroxide 1N, and R.O. Deionized Water; dose volume was 0.1 – 1.0 ml/kg; and infusion rate was by injection.

Dosing Solution Analyses/Drug Stability and Homogeneity

SGS #	LOT#	SAMPLE DESCRIPTION	Content
137842	04260R2/005	B-XGW00015-A (15/SEP/2008), 0 mcg/mL	Not Detected
137843	04260R2/005	B-XGW00015-B (15/SEP/2008), 50 mcg/mL	36 mcg/mL*
137844	04260R2/005	B-XGW00015-A (30/SEP/2008), 0 mcg/mL	Not Detected
137845	04260R2/005	B-XGW00015-B (30/SEP/2008), 50 mcg/mL	47 mcg/mL

*This sample was outside the allowable 15% variance, (OOS# 09-095) i.e 28% variance. The sample was not reanalyzed at the discretion of the Study Director in consultation with the Study Monitor.

SUITABILITY/COMMENTS:

The expiry had been updated on the basis of current stability data (48 months) supporting drug substance from (b) (4)

The test article, ALX1-11, was stored (b) (4).

Solutions of the test article were prepared once daily at the Testing Facility, stored at ambient conditions and used within 12 hours of formulation. The vehicle was prepared at least once weekly at the Testing Facility, stored at room temperature and used within seven days of formulation. Preparation was conducted in a biological safety cabinet using aseptic technique.

Satellite Groups Used for Toxicokinetics 3 rabbits/TK test group

Study Design

Parameters and Endpoints Evaluated

The following parameters were evaluated: viability, clinical observations, body weights, body weight changes, feed consumption, necropsy observations, Caesarean-sectioning and litter observations, fetal body weights, fetal sex and fetal gross external, visceral and skeletal alterations.

Results

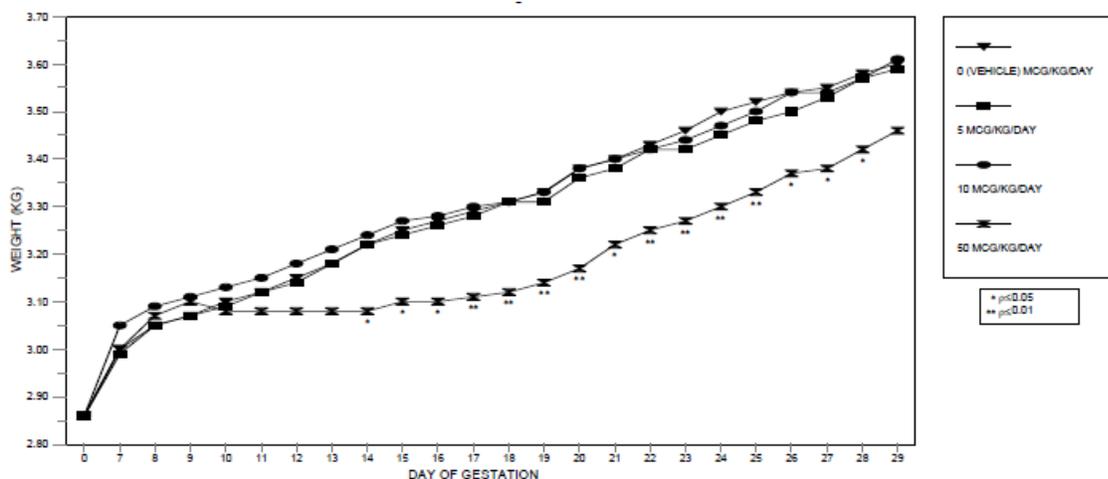
Mortality (Dams) All rabbits survived until scheduled sacrifice.

Clinical Signs (Dams)

No clinical or necropsy observations related to treatment with ALX1-11 occurred.

Body Weight (Dams)

Body weights and body weight gains were unaffected by dosages of ALX1-11 ≤ 10 mcg/kg/day. A significant loss in body weight gain occurred in the 50 mcg/kg/day dosage group on DGs 10 to 13, as compared to the vehicle group value. Thereafter, body weight gains in the 50 mcg/kg/day dosage group were significantly lower on DGs 13 to 16.



PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 3 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	16	20
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.13 ± 0.07	+0.13 ± 0.06	+0.20 ± 0.09**	+0.14 ± 0.06
DAYS 7 - 10	MEAN±S.D.	+0.10 ± 0.03	+0.10 ± 0.04	+0.08 ± 0.06	+0.08 ± 0.07
DAYS 10 - 13	MEAN±S.D.	+0.08 ± 0.04	+0.09 ± 0.04	+0.08 ± 0.05	-0.01 ± 0.07**
DAYS 13 - 16	MEAN±S.D.	+0.09 ± 0.03	+0.08 ± 0.04	+0.07 ± 0.03	+0.03 ± 0.05**
DAYS 16 - 20	MEAN±S.D.	+0.12 ± 0.05	+0.10 ± 0.05	+0.10 ± 0.05	+0.07 ± 0.04**
DAYS 7 - 20	MEAN±S.D.	+0.39 ± 0.07	+0.37 ± 0.07	+0.33 ± 0.10	+0.17 ± 0.12**
DAYS 20 - 24	MEAN±S.D.	+0.11 ± 0.05	+0.09 ± 0.06	+0.08 ± 0.04	+0.12 ± 0.06
DAYS 24 - 29	MEAN±S.D.	+0.10 ± 0.06	+0.15 ± 0.07	+0.14 ± 0.04	+0.16 ± 0.07**
DAYS 20 - 29	MEAN±S.D.	+0.21 ± 0.08	+0.23 ± 0.08	+0.22 ± 0.07	+0.29 ± 0.08*
DAYS 7 - 29	MEAN±S.D.	+0.60 ± 0.10	+0.60 ± 0.10	+0.56 ± 0.10	+0.46 ± 0.13**
DAYS 0 - 29	MEAN±S.D.	+0.74 ± 0.10	+0.74 ± 0.10	+0.76 ± 0.08	+0.60 ± 0.14**

DAYS = DAYS OF GESTATION

a. Dosage occurred on days 7 through 19 of gestation.

* Significantly different from the vehicle control group value (p≤0.05).

** Significantly different from the vehicle control group value (p≤0.01).

Food Consumption (Dams)

Absolute (g/day) and relative (g/kg/day) feed consumption values were unaffected by dosages of ALX1-11 ≤10 mcg/kg/day. Absolute and relative feed consumption values in the 50 mcg/kg/day dosage group were significantly lower at all tabulated intervals within the dosage period, as compared to the vehicle group values. As a result, absolute and relative feed consumption values in the 50 mcg/kg/day dosage group were significantly lower for the entire dosage period, as compared to the respective vehicle group values. During the postdosage period, absolute and relative feed consumption values were comparable between the dosage groups.

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 4 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	16	20
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 7 - 10	MEAN±S.D.	181.2 ± 4.6 [19] ^b	175.9 ± 14.5	180.8 ± 6.1	161.5 ± 23.0*
DAYS 10 - 13	MEAN±S.D.	176.9 ± 8.5	169.1 ± 23.7	169.8 ± 22.1	119.2 ± 41.4**
DAYS 13 - 16	MEAN±S.D.	175.7 ± 13.1	175.4 ± 16.1	168.1 ± 18.8	111.6 ± 40.9** [19] ^b
DAYS 16 - 20	MEAN±S.D.	176.6 ± 12.5	175.6 ± 12.6	169.8 ± 17.5	125.8 ± 28.7**
DAYS 7 - 20	MEAN±S.D.	177.6 ± 8.9	174.2 ± 14.5	172.0 ± 14.9	129.2 ± 28.9**
DAYS 20 - 24	MEAN±S.D.	176.4 ± 10.7	167.1 ± 20.7	173.4 ± 14.6	171.3 ± 14.0
DAYS 24 - 29	MEAN±S.D.	145.4 ± 25.5	151.7 ± 28.1	148.9 ± 20.8	150.1 ± 23.6
DAYS 20 - 29	MEAN±S.D.	159.2 ± 16.6	158.5 ± 22.3	159.8 ± 14.4	159.5 ± 18.4
DAYS 7 - 29	MEAN±S.D.	170.0 ± 9.2	167.8 ± 16.2	167.0 ± 11.3	141.6 ± 20.2**

DAYS = DAYS OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on days 7 through 19 of gestation.

b. Excludes values that were associated with wet/soiled feed or spillage.

* Significantly different from the vehicle control group value (p≤0.05).

** Significantly different from the vehicle control group value (p≤0.01).

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 5 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	16	20
MATERNAL FEED CONSUMPTION (G/KG/DAY)					
DAYS 7 - 10	MEAN±S.D.	59.3 ± 3.5 [19] ^b	57.7 ± 4.6	58.6 ± 4.2	52.8 ± 7.6**
DAYS 10 - 13	MEAN±S.D.	56.6 ± 3.7	53.9 ± 7.0	53.7 ± 7.3	38.7 ± 13.5**
DAYS 13 - 16	MEAN±S.D.	54.5 ± 4.5	54.3 ± 4.6	51.7 ± 5.4	36.2 ± 13.1** [19] ^b
DAYS 16 - 20	MEAN±S.D.	53.3 ± 4.2	53.2 ± 3.7	51.1 ± 4.8	40.1 ± 8.6**
DAYS 7 - 20	MEAN±S.D.	55.7 ± 3.6	54.6 ± 4.1	53.5 ± 4.8	41.7 ± 9.3**
DAYS 20 - 24	MEAN±S.D.	51.5 ± 3.2	49.1 ± 5.8	50.7 ± 4.1	52.8 ± 3.7
DAYS 24 - 29	MEAN±S.D.	40.9 ± 6.6	43.1 ± 7.8	42.1 ± 5.4	44.4 ± 5.8
DAYS 20 - 29	MEAN±S.D.	45.5 ± 4.3	45.7 ± 6.3	45.9 ± 3.6	48.0 ± 4.5
DAYS 7 - 29	MEAN±S.D.	51.3 ± 2.8	50.8 ± 4.7	50.2 ± 3.2	44.4 ± 5.8**

DAYS = DAYS OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on days 7 through 19 of gestation.

b. Excludes values that were associated with wet/soiled feed or spillage.

** Significantly different from the vehicle control group value (p≤0.01).

Toxicokinetics

Summary of mean toxicokinetic parameters*

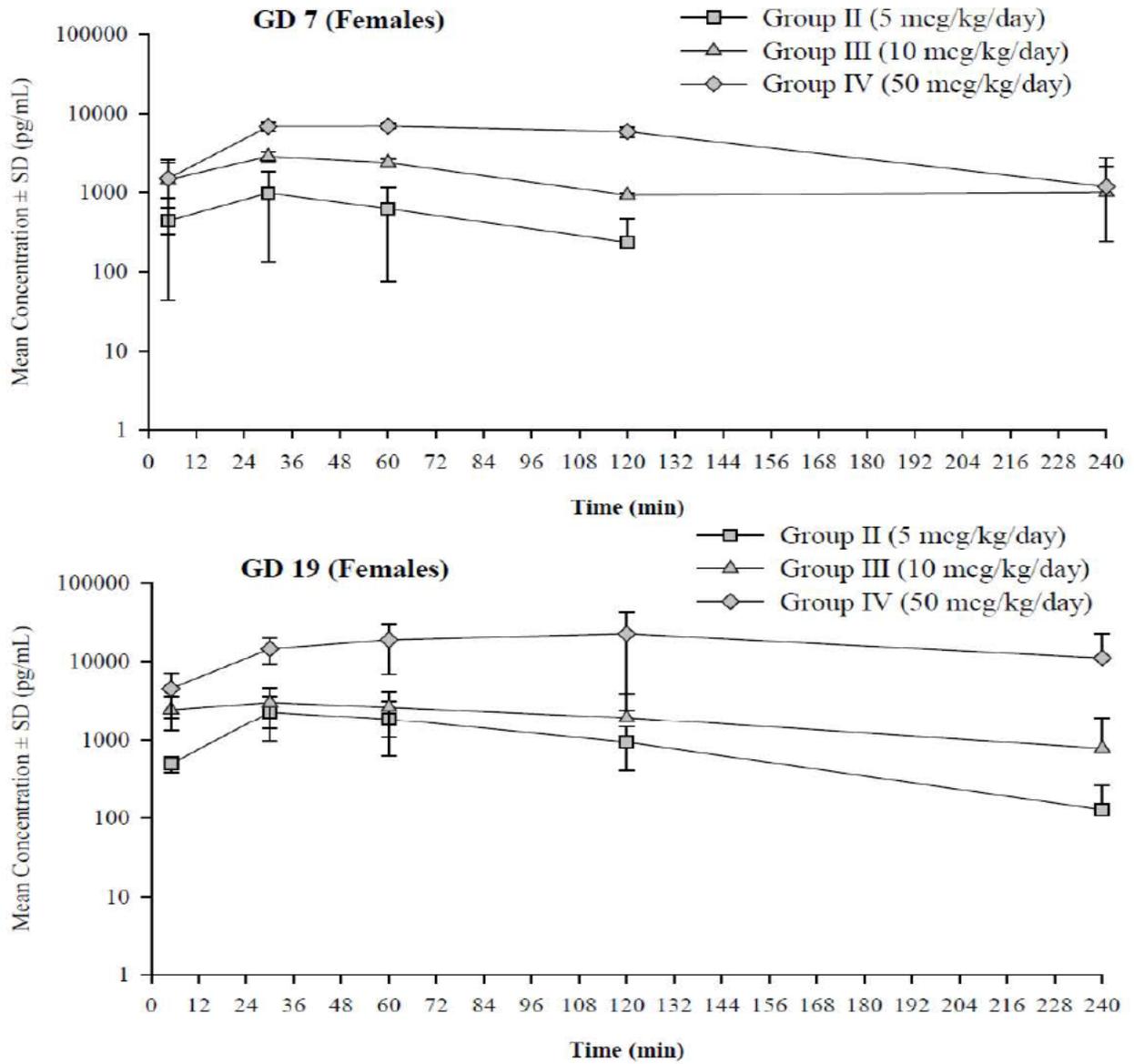
Group No.	Occasion	Tmax (min)	Cmax (pg/mL)	AUC(0-t) (pg*min/mL)	AUCinf (pg*min/mL)
Group II 5 mcg/kg/day	GD 7	30	1477	103107	-
	GD 19	30	2256	243542	252604
Group III 10 mcg/kg/day	GD 7	30	2857	313550	-
	GD 19	30	3769	455951	269314
Group IV 50 mcg/kg/day	GD 7	60	7159	1119836	1208006
	GD 19	60	22991	3970547	-

- Not calculated

*Data is expressed as actual concentrations assuming 100% rabbit K₂EDTA plasma.

Between 5 and 10 mcg/kg/day, the exposure parameters C_{max} and AUC_(0-t) increased with ascending dose level in a dose proportional manner on GD 7 and GD 19. The increase in C_{max} and AUC_(0-t) was less than dose proportional on GD 7 and greater than dose proportional on GD 19 between 10 and 50 mcg/kg/day. There was a larger variability in exposures at the 10 mcg/kg/day dose on GD 19. As shown in the text table above, there was no consistent difference between exposure on GD 19 versus GD 7 in Groups 2 and 3 (accumulation ratios between 1.32 and 2.36). However, despite the predose concentrations on GD 19 being all below LLOQ, there was a greater than 3-fold increase in C_{max} and AUC_(0-t) for Group 4 (accumulation ratios > 3).

Mean Toxicokinetic Profiles of Human PTH 1-84 in New Zealand White Rabbit Plasma Following Subcutaneous Administration of ALX1-11



Accumulation Ratios of Human PTH 1-84 in New Zealand White Rabbit Plasma Following Subcutaneous Administration of ALX1-11

Gender	Group No.	Dose Level (mcg/kg/day)	Mean Cmax (pg/mL)		Ratio
			GD 7	GD 19	
Females	II	5	1477	2256	1.53
	III	10	2857	3769	1.32
	IV	50	7159	22991	3.21

Gender	Group No.	Dose Level (mcg/kg/day)	Mean AUC(0-t) (pg•min/mL)		Ratio
			GD 7	GD 19	
Females	II	5	103107	243542	2.36
	III	10	313550	455951	1.45
	IV	50	1119836	3970547	3.55

Dose Proportionality of Human PTH 1-84 in New Zealand White
Rabbit Plasma Following Subcutaneous Administration of ALX1-11

GD 7 (Females)

Gender	Group No.	Dose Level (mcg/kg/day)	Fold Increase	Mean Cmax (pg/mL)	Fold Increase	Mean AUC(0-t) (pg•min/mL)	Fold Increase
Females	II	5	1.00	1477	1.00	103107	1.00
	III	10	2.00	2857	1.93	313550	3.04
	IV	50	5.00	7159	2.51	1119836	3.57

GD 19 (Female)

Gender	Group No.	Dose Level (mcg/kg/day)	Fold Increase	Mean Cmax (pg/mL)	Fold Increase	Mean AUC(0-t) (pg•min/mL)	Fold Increase
Females	II	5	1.00	2256	1.00	243542	1.00
	III	10	2.00	3769	1.67	455951	1.87
	IV	50	5.00	22991	6.10	3970547	8.71

Terminal and Necroscopic Evaluations-Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnancy occurred in 16 to 20 does in each dosage group. Caesarean-sectioning observations on DG 29 were based on 20, 20, 16 and 20 pregnant does in Groups I through IV, respectively.

No clear effect of test item on any parameter.

Offspring (Malformations, Variations, etc.)

There were no clear toxicologically significant test item-related effects on gross external, soft tissue or skeletal fetal alterations (malformations or variations) at any dosage tested (up to 50 mcg/kg/day). There was a small n.s.s. ↑ in the % of fetuses with any alteration per litter at HD (20.7% in C vs. 27.7% in HD). S.s. findings were limited to an ↑ in the litter and fetal incidences of incompletely ossified sternal centra and small changes (2-4%) in the number of ossification sites per embryo per litter at HD. These findings were all within the historical background range.

1 HD fetus had a gross external finding of spina bifida in the lower lumbar region, along with skeletal malformations consistent with this observation: open arches in the 1st through 3rd sacral vertebrae, open arches in the 4th through 6th lumbar vertebrae and open arches in the 1st through 5th caudal vertebrae. This fetus also had fused caudal vertebrae (8th and 9th) and a slight enlargement of the anterior fontanelle. No

other neural tube defects were seen in any other fetuses, either from control or treated dams. The incidence of the vertebral malformations reported in this fetus appear to be within the historical background rate for studies conducted in this species at this CRO. No incidences of “spina bifida” were reported at the CRO in the 2-year time period of the historical control data reported (appendix C); however the CRO ((b) (4)) had the following to say about this finding:

“A single fetus with the documented observation of spina bifida or meningocele would not be considered related to the test article 1) if the incidence was not dosage-dependent; 2) no increase in the number of resorptions occurred at that dose or a higher dose; and 3) there were no other indications of alterations of the central nervous system.”

In the judgment of this reviewer (R. Wange), to the extent that the test item may have contributed to the finding of spina bifida, it is likely that the effect is indirect via maternal folic acid deficiency as a consequence of reduced food consumption. Other findings that showed an increased incidence at HD compared to C are indicated in summary tables below. Findings outlined in blue fall within the historical background range, while those outlined in red are slightly outside the historical background range. As for the finding of spina bifida noted above, to the extent that the test item contributed to these additional n.s.s. findings, it is likely that the effect of the test item is indirect and related to maternal toxicity.

LITTERS E
FETUSES E
LIVE

LITTERS W
ANY ALTER

FETUSES W
ALTERATIO

% FETUSES
ALTERAT

a. Dosage

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 9 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151

BODY: UMBILICAL HERNIA					
LITTER INCIDENCE	N(%)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
FETAL INCIDENCE	N(%)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)

BODY: SPINA BIFIDA					
LITTER INCIDENCE	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)

a. Dosage occurred on days 7 through 19 of gestation.

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 11 (PAGE 1): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151
SKULL: IRREGULAR OSSIFICATION b					
(SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL c; INDIVIDUAL SUBCATEGORIES CITED BELOW)					
LITTER INCIDENCE	N (%)	14 (70.0)	12 (60.0)	10 (62.5)	16 (80.0)
FETAL INCIDENCE	N (%)	25 (15.5)	23 (15.1)	14 (12.1)	28 (18.5)
SKULL: PARIETALS, SUTURE IRREGULAR					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (6.2)	0 (0.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)
SKULL: NASAL(S), IRREGULAR OSSIFICATION					
(SUMMARIZATION OF MIDLINE SUTURE DISPLACED; CONTAINS AN INTRANASAL; CONTAIN INTERNASAL AND NASAL-FRONTAL SUTURE IRREGULAR)					
LITTER INCIDENCE	N (%)	9 (45.0)	8 (40.0)	9 (56.2)	13 (65.0)
FETAL INCIDENCE	N (%)	12 (7.4)	12 (7.9)	11 (9.5)	21 (13.9)
SKULL: NASALS, MIDLINE SUTURE DISPLACED					
LITTER INCIDENCE	N (%)	9 (45.0)	8 (40.0)	8 (50.0)	12 (60.0)
FETAL INCIDENCE	N (%)	12 (7.4)	12 (7.9) ^h	10 (8.6) ⁱ	18 (11.9) ^{n, p, t}
SKULL: NASAL, CONTAINS AN INTRANASAL					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
SKULL: NASALS CONTAIN INTERNASAL					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7) ^o
SKULL: NASAL-FRONTAL SUTURE IRREGULAR					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (6.2)	2 (10.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (0.9)	2 (1.3)
SKULL: FRONTAL(S), IRREGULAR OSSIFICATION					
(SUMMARIZATION OF SUTURE IRREGULAR; CONTAIN AN INTERFRONTAL AND FUSED)					
LITTER INCIDENCE	N (%)	8 (40.0)	7 (35.0)	2 (12.5)	5 (25.0)
FETAL INCIDENCE	N (%)	13 (8.1)	11 (7.2)	2 (1.7)	8 (5.3)

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 11 (PAGE 2): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151
SKULL: FRONTALS, SUTURE IRREGULAR					
LITTER INCIDENCE	N (%)	4 (20.0)	4 (20.0)	1 (6.2)	4 (20.0)
FETAL INCIDENCE	N (%)	6 (3.7)	4 (2.6) f	1 (0.9)	5 (3.3) r
SKULL: FRONTALS CONTAIN AN INTERFRONTAL					
LITTER INCIDENCE	N (%)	4 (20.0)	6 (30.0)	1 (6.2)	2 (10.0)
FETAL INCIDENCE	N (%)	6 (3.7)	7 (4.6)	1 (0.9)	2 (1.3)
SKULL: FRONTALS FUSED					
LITTER INCIDENCE	N (%)	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.7) m
SKULL - OTHER ALTERATIONS:					
SKULL: FONTANELLE ENLARGED					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (6.2)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	1 (0.9) j	1 (0.7) q
SKULL: BASTOCCIPITAL IRREGULARLY SHAPED					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7) r
SKULL: EXOCCIPITAL FUSED TO ATLAS VERTEBRAE					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7) r
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N (%)	2 (10.0)	7 (35.0)	5 (31.2)	5 (25.0)
FETAL INCIDENCE	N (%)	6 (3.7)	8 (5.3) e, g	8 (6.9) j	7 (4.6)
CERVICAL VERTEBRAE: CENTRUM UNILATERAL OSSIFICATION					
LITTER INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
FETAL INCIDENCE	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7) o
CERVICAL VERTEBRAE: EXTRA OSSIFICATION					
LITTER INCIDENCE	N (%)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
FETAL INCIDENCE	N (%)	0 (0.0)	1 (0.6) g	0 (0.0)	0 (0.0)

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALK1-11 IN RABBITS

TABLE 11 (PAGE 3): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)h	0(0.0)	0(0.0)
THORACIC VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	1(6.2)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	1(0.9)i	1(0.7)o
THORACIC VERTEBRAE: CENTRA FUSED					
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	1(0.9)i	0(0.0)
THORACIC VERTEBRAE: ARCHES FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9)i	1(0.7)c
LUMBAR VERTEBRAE: ARCH OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)c
SACRAL VERTEBRAE: ARCH OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)c
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	1(5.0)	2(10.0)	1(6.2)	4(20.0)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.3)	1(0.9)	5(3.3)z
CAUDAL VERTEBRAE: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)c
CAUDAL VERTEBRAE: ARCH OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)c

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TABLE 11 (PAGE 4): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151
RIBS: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)	0(0.0)	0(0.0)
RIBS: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9) ⁱ	0(0.0)
RIBS: BRANCHED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9) ⁱ	0(0.0)
RIBS: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7) ^o
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7) ^L
MANUBRIUM: EXTRA OSSIFICATION SITE ANTERIOR TO MANUBRIUM					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6) ^h	0(0.0)	0(0.0)
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	2(12.5)	4(20.0)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6) ^{e,f}	2(1.7) ^j	4(2.6) ^{L,m,p}
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	2(12.5)	2(10.0)
FETAL INCIDENCE	N(%)	2(1.2)	0(0.0)	2(1.7) ^{j,k}	2(1.3) ^L
STERNAL CENTRA: BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9) ^k	0(0.0)

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 11 (PAGE 5): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 50
LITTERS EVALUATED	N	20	20	16	20
FETUSES EVALUATED	N	161	152	116	151
LIVE	N	161	152	116	151
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	4(20.0) ^{**}
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9) ^k	4(2.6) ^{**n,p}
XIPHOID: BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.9)	0(0.0)

** Significantly different from the vehicle control group value (p≤0.01).

a. Dosage occurred on days 7 through 19 of gestation.

b. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.

c. Includes all alterations noted for the skull except hyoid, ala, angulated, fontanelle enlarged, basioccipital irregularly shaped and exoccipital fused to atlas. These categories are excluded because these alterations do not result from irregular ossification.

d. Fetus 7304-4 had other skeletal alterations.

e. Fetus 7331-1 had other skeletal alterations.

f. Fetus 7331-6 had other skeletal alterations.

g. Fetus 7333-5 had other skeletal alterations.

h. Fetus 7338-3 had other skeletal alterations.

i. Fetus 7344-1 had other skeletal alterations.

j. Fetus 7347-4 had other skeletal alterations.

k. Fetus 7351-6 had other skeletal alterations.

L. Fetus 7364-4 had other skeletal alterations.

m. Fetus 7365-2 had other skeletal alterations.

n. Fetus 7367-3 had other skeletal alterations.

o. Fetus 7367-4 had other skeletal alterations.

p. Fetus 7368-5 had other skeletal alterations.

q. Fetus 7374-1 had other skeletal alterations.

r. Fetus 7374-5 had other skeletal alterations.

PROTOCOL XGW00015: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ALX1-11 IN RABBITS

TABLE 12 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	5	10	50
LITTERS EXAMINED	N	20	20	16	20
FETUSES EXAMINED	N	161	152	116	151
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.46 ± 0.29	12.40 ± 0.30	12.52 ± 0.26	12.74 ± 0.25**
LUMBAR	MEAN±S.D.	6.54 ± 0.29	6.62 ± 0.30	6.48 ± 0.26	6.26 ± 0.24**
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.79 ± 0.40	16.74 ± 0.43	16.79 ± 0.34	16.88 ± 0.41
RIBS (PAIRS)	MEAN±S.D.	12.40 ± 0.27	12.34 ± 0.25	12.45 ± 0.26	12.64 ± 0.27**
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.84 ± 0.24	3.90 ± 0.13	3.90 ± 0.12	3.88 ± 0.17
XIPHOID	MEAN±S.D.	0.98 ± 0.06	0.99 ± 0.03	0.96 ± 0.09	0.97 ± 0.06
FORELIMB ^b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.99 ± 0.04	4.98 ± 0.05	4.96 ± 0.11	4.96 ± 0.10
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.90 ± 0.17	13.87 ± 0.25	13.94 ± 0.10	13.85 ± 0.25
HINDLIMB ^b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00

a. Dosage occurred on days 7 through 19 of gestation.

b. Calculated as average per limb.

** Significantly different from the vehicle control group value (p≤0.01).

The only statistically significant variation in the litter and fetal incidences of incompletely ossified sternal centra that occurred in the 50 mcg/kg/day dosage group. It is noteworthy that this variation was limited to four fetuses from four different litters in this dosage group and that the incidences were within the historical range of the Testing Facility. The 50 mcg/kg/day dosage of ALX1-11 was also associated with significant increases in the incidence of supernumerary thoracic ribs and associated increases and decreases in the average number of ossified thoracic and lumbar vertebrae, respectively, a common variation observed at maternally toxic dosages. All other ossification site averages were comparable among the four dosage groups.

No gross external and soft tissue alterations (malformations or variations) or skeletal malformations were caused by dosages of ALX1-11 as high as 50 mcg/kg/day.

For further clarification as requested by the Sponsor, gross observations for Fetus 7374-1 in the 50 mcg/kg/day dosage group were re-evaluated. Specifically, this fetus had the gross external observation of spina bifida in the lower lumbar region. There were no other gross or soft tissue alterations. Skeletal examination of this fetus confirmed the grossly observed malformation which was evident as open arches in the 4th, 5th and 6th lumbar vertebrae, in the 1st, 2nd and 3rd sacral vertebrae and in the 1st through 5th caudal vertebrae. In addition, this fetus had fused caudal vertebrae (8th and 9th) and slight enlargement of the anterior fontanelle. No other alterations occurred in this fetus.

Discussion and Conclusions

Rats: There was no apparent effect of PTH 1-84 on any measured parameter of embryofetal development in the rat at doses up to 1000 mcg/kg/day (~100x the MRHD of 100 mcg/day). No dose elicited significant maternal toxicity (transient reduction in food consumption and weight gain notwithstanding); however the HD is adequate as it represents a limit dose as proposed under ICH M3 (R2). The NOAEL for embryofetal development is considered to be 1000 mcg/kg/day. A NOAEL for the dams was not determined in this study.

Rabbits: Maternal toxicity was seen at the HD of 50 mcg/kg/day (~10x the MRHD of 100 mcg/day), as evidenced by a pronounced ↓ in body weight gain over the entire period of organogenesis (actual body weight loss was seen over the first 4 days of dosing). There were no clear toxicologically significant test item-related effects on any measured parameter of embryofetal development. S.s. findings related to gross external, soft tissue or skeletal fetal alterations were limited to an ↑ in the litter and fetal incidences of incompletely ossified sternal centra and small changes (2-4%) in the number of ossification sites per embryo per litter at HD. These findings were all within the historical background range.

1 HD fetus had a gross external finding of spina bifida in the lower lumbar region, along with skeletal malformations consistent with this observation: open arches in the 1st through 3rd sacral vertebrae, open arches in the 4th through 6th lumbar vertebrae and open arches in the 1st through 5th caudal vertebrae. This fetus also had fused caudal vertebrae (8th and 9th) and a slight enlargement of the anterior fontanelle. No other neural tube defects were seen in any other fetuses, either from control or treated dams. The incidence of the vertebral malformations reported in this fetus appears to be within the historical background rate for studies conducted in this species at this CRO. In the judgment of this reviewer, to the extent that the test item may have contributed to the finding of spina bifida, it is likely that the effect is indirect via maternal folic acid deficiency as a consequence of reduced food consumption. Nonetheless, the NOAEL for embryofetal development is considered to be 10 mcg/kg/day (~2x the MRHD), the same as the maternal NOAEL.

9.3 Prenatal and postnatal development

Study title: Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of ALX1-11 in Rats, Including a Postnatal Behavioral/Functional Evaluation.

Study no.: Testing Facility Study No. XGW00020

Study report location:

(b) (4)

Conducting laboratory and location:

(b) (4)

Date of study initiation: 21 April 2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Lot # 04260R2/005,
% purity 98.8% by RP-HPLC
99.5% by CEC-HPLC

Key Study Findings

Maternal no-observable-adverse-effect-level (NOAEL) for ALX1-11 is 1000 mcg/kg/day. Transient statistically significant reductions in body weight gains occurred between DGs 7 and 10 in the 300 and 1000 mcg/kg/day dosage groups. Corresponding reductions in feed consumption occurred at 1000 mcg/kg/day during the first 3 days of the dosage period. Based on decreases in body weight and feed consumption reduction in previous studies the 1000 mcg/kg/day maternal dose was anticipated to be the limiting dose and was considered appropriate for the high dose in this study.

The reproductive NOAEL in the dams is <100 mcg/kg/day. There were no effects on the numbers of implantations in reproduction at any dose up to and including 1000 mcg/kg/day. A significant increase ($p \leq 0.01$ or $p \leq 0.05$) in the duration of gestation occurred in the 300 and 1000 mcg/kg/day dosage groups. One Dam had no live born pups at 300 mcg/kg/day.

The NOAEL for viability and growth in the offspring was <100 mcg/kg/day. This is based on the findings of one euthanized F1 male rat and one death of F1 female in the 100 mcg/kg/day.

Methods

Doses

Dosage Group	Dosage ^a (mcg/kg/day)	Concentration (mcg/mL)	Dosage Volume (mL/kg)	Number of Rats
I	0 (Vehicle)	0	2	25
II	100	50	2	25
III	300	150	2	25
IV	1000	500	2	25

^aThe test article was considered 100% active/pure for the purpose of dosage calculations.

Species/strain Crl:CD(SD) rat

Number/sex/group 25/mated females/group

Route, formulation, volume, and infusion rate

Subcutaneous, direct dilution of the bulk ALX1-11 solution (7.52 mg/mL) with Citric Acid monohydrate in D-Mannitol USP (pH 5.5) and used within 12 hours of formulation., 2 mL/kg, injection rate

Dosing solution analyses/drug stability and homogeneity

Dose formulation solutions were analyzed by a validated HPLC method. Samples were directly analyzed without further dilutions.

SGS #	LOT#	SAMPLE DESCRIPTION	Content
155806	04260R2/005	B-XGW00020-A (04.MAY.09), 0 mcg/mL	Not Detected
155807	04260R2/005	B-XGW00020-B (04.MAY.09), 50 mcg/mL	37 mcg/mL*
155808	04260R2/005	B-XGW00020-C (04.MAY.09), 150 mcg/mL	161 mcg/mL
155809	04260R2/005	B-XGW00020-D (04.MAY.09), 500 mcg/mL	579 mcg/mL*
155810	04260R2/005	B-XGW00020-A (11.JUN.09), 0 mcg/mL	Not Detected
155811	04260R2/005	B-XGW00020-B (11.JUN.09), 50 mcg/mL	38 mcg/mL*
155812	04260R2/005	B-XGW00020-C (11.JUN.09), 150 mcg/mL	151 mcg/mL
155813	04260R2/005	B-XGW00020-D (11.JUN.09), 500 mcg/mL	561 mcg/mL

*These 3 samples were outside the allowable 15% variance (SGS OOS # 10-073); The 3 samples were not reanalyzed at the discretion of the Study Director in consultation with the Study Monitor .

Satellite groups used for toxicokinetics None

Study design

Parameters and endpoints evaluated

Study was designed to evaluate ICH Harmonized Tripartite Guideline stages C through F of the reproductive process, but does not include an evaluation of Caesarean-delivered fetuses (stages C and D), because this evaluation was performed in a supplementary study. Because manifestations of effects induced during this period may be delayed, observations were continued through sexual maturity of the F1 generation rats.

FO Generation Rats

Viabilities, clinical observations, body weights, feed consumption values, mating performance, maternal behavior and natural delivery observations were recorded. At necropsy, gross pathologic findings, and the number and distribution of implantation sites were recorded. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. Ovaries and uteri of apparently nonpregnant rats were retained for possible future evaluation.

F1 Generation Pups/Rats

Each litter was evaluated for viability at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily during the preweaning period. Pup body weights were recorded on DLs 1 (day of birth), 4, 7, 14, 18 and 21.

Viabilities, clinical observations, body weights, age of vaginal patency (female rats) or age of preputial separation (male rats), passive avoidance test (for learning, short-term retention and long-term retention), watermaze test (for overt coordination, swimming ability, learning and memory) and mating performance were recorded.

At necropsy, gross pathologic findings were recorded. Female rats were sacrificed on DG 21. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. Male rats were sacrificed after completion of the cohabitation period, and testes and epididymides were excised and paired organ weights were recorded.

Results

F₀ in-life

All rats survived until scheduled sacrifice. (Note: In Sponsor Table B5, Footnote c, Dam 782 is listed with an unscheduled sacrifice on Day 9 due to adverse clinical observations. On review of the individual data, Dam 782 is listed as a scheduled sacrifice.)

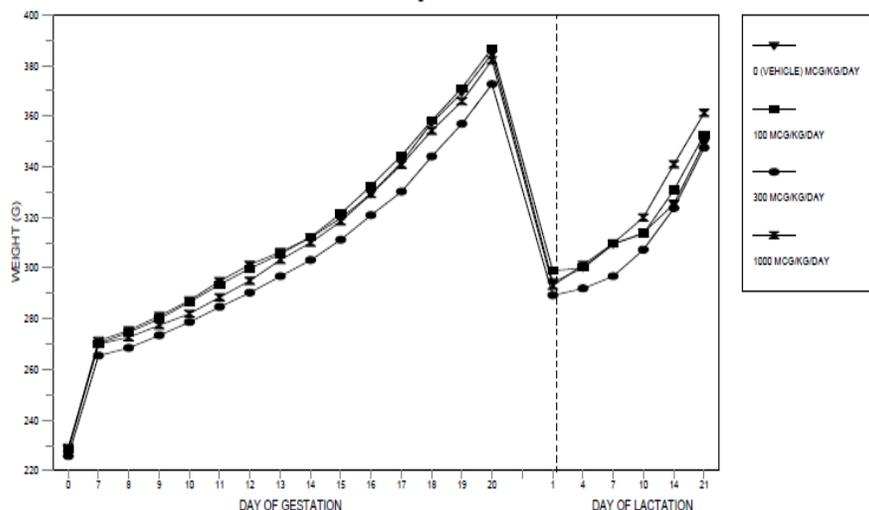
Urine-stained abdominal fur occurred in a significantly increased ($p \leq 0.01$) number of rats in the 1000 mcg/kg/day dosage group during the lactation period, as compared to the vehicle group.

APPEARS THIS WAY ON ORIGINAL

PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS

Figure 1



PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE A3 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	100	300	1000	
RATS TESTED	N	25	25	25	25	
PREGNANT	N	22	23	20	20	
MATERNAL BODY WEIGHT (G)						
DAY	0	MEAN±S.D.	228.2 ± 12.8	228.5 ± 12.6	225.7 ± 10.6	229.0 ± 11.3
DAY	7	MEAN±S.D.	271.3 ± 14.8	270.1 ± 16.5	265.4 ± 16.0	270.0 ± 12.3
DAY	8	MEAN±S.D.	275.3 ± 15.5	274.6 ± 17.0	268.4 ± 17.3	272.6 ± 12.6
DAY	9	MEAN±S.D.	281.0 ± 15.2	279.9 ± 17.8	273.4 ± 18.5	277.4 ± 13.5
DAY	10	MEAN±S.D.	287.2 ± 16.1	286.6 ± 17.9	278.6 ± 19.1	282.0 ± 13.7
DAY	11	MEAN±S.D.	294.8 ± 17.0	293.4 ± 19.2	284.6 ± 20.1	288.5 ± 14.5
DAY	12	MEAN±S.D.	301.3 ± 17.2	299.8 ± 20.2	290.3 ± 20.5	295.0 ± 16.3
DAY	13	MEAN±S.D.	306.3 ± 18.7	305.6 ± 21.7	296.8 ± 21.3	303.3 ± 17.6
DAY	14	MEAN±S.D.	312.0 ± 18.5	312.2 ± 23.1	303.1 ± 22.7	310.0 ± 18.0
DAY	15	MEAN±S.D.	319.7 ± 20.4	321.5 ± 25.2	311.2 ± 23.0	318.4 ± 19.3
DAY	16	MEAN±S.D.	329.2 ± 20.8	332.4 ± 25.7	320.8 ± 24.5	329.2 ± 19.6
DAY	17	MEAN±S.D.	343.8 ± 22.2	344.4 ± 29.1	330.2 ± 25.5	340.8 ± 21.9
DAY	18	MEAN±S.D.	357.3 ± 24.6	358.2 ± 28.6	344.0 ± 28.4	354.2 ± 23.2
DAY	19	MEAN±S.D.	369.1 ± 26.6	371.1 ± 30.2	356.9 ± 29.3	365.8 ± 25.2
DAY	20	MEAN±S.D.	384.6 ± 29.1	386.8 ± 31.0	372.6 ± 31.8	382.0 ± 27.1

DAY = DAY OF GESTATION

a. Dosage administration occurred on day 7 of gestation through day 20 of lactation or day 24 of gestation (rats that did not deliver a litter).

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TABLE A5 (PAGE 1): MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY) ^a		0 (VEHICLE)	100	300	1000
RATS TESTED	N	25	25	25	25
PREGNANT	N	22	23	20	20
DELIVERED A LITTER	N	21	23	20	20
MATERNAL BODY WEIGHT (G)					
DAY		MEAN±S.D.	MEAN±S.D.	MEAN±S.D.	MEAN±S.D.
1		294.3 ± 18.7	298.9 ± 27.7 [22]b	289.2 ± 22.0	293.0 ± 21.1
4		300.0 ± 17.7	300.0 ± 23.1	291.9 ± 23.9 [19]c	301.2 ± 20.4 [19]c
7		309.4 ± 20.1	309.6 ± 18.9	296.7 ± 24.4 [19]c	309.7 ± 23.0 [19]c
10		314.0 ± 17.8	313.4 ± 22.4	307.2 ± 25.9 [19]c	319.9 ± 27.3 [19]c
14		325.3 ± 20.2	330.8 ± 28.5	323.6 ± 23.2 [19]c	341.0 ± 25.4 [19]c
21		349.2 ± 20.7 [20]b	352.6 ± 27.6	347.5 ± 23.0 [19]c	361.2 ± 26.8 [19]c

DAY = DAY OF LACTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage administration occurred on day 7 of gestation through day 20 of lactation.

b. Excludes values that were not recorded as well as those associated with interrupted water access.

c. Excludes values for dams that were sacrificed due to no surviving pups.

5. Absolute and relative feed consumption values during the gestation period were unaffected by dosages of ALX1-11 as high as 300 mcg/kg/day. Corresponding to reductions in body weight gains, transient reductions in absolute and relative feed consumption values occurred in the 1000 mcg/kg/day dosage group on DGs 7 to 10, as compared to the vehicle group values. Feed consumption values were variable during the lactation period; no test article-related changes occurred.
6. Pregnancy occurred in 22 (88%), 23 (92%), 20 (80%) and 20 (80%) of the 25 mated female rats in the 0 (Vehicle), 100, 300 and 1000 mcg/kg/day dosage groups, respectively.
7. Natural delivery, litter observations, clinical, necropsy, and physical development observations were unaffected by dosages of ALX1-11 at 100 mcg/kg/day, except for mild dehydration in 2/25F, and 1/25M euthanized because of a broken palate, and 1/25F found dead. One Dam in the 300 mcg/kg/day group was found with no Live Born Pups in the 300 mcg/kg/day group. A significant increase ($p \leq 0.01$ or $p \leq 0.05$) in the duration of gestation occurred in the 300 and 1000 mcg/kg/day dosage groups.

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TABLE A11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MCG/KG/DAY) ^a		I 0 (VEHICLE)	II 100	III 300	IV 1000
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N(%)	22 (88.0)	23 (92.0)	20 (80.0)	20 (80.0)
DELIVERED A LITTER	N(%)	21 (95.4)	23 (100.0)	20 (100.0)	20 (100.0)
DURATION OF GESTATION ^b	MEAN±S.D.	22.3 ± 0.5	22.4 ± 0.5	22.8 ± 0.6*	22.8 ± 0.7**
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	311 14.8 ± 2.4	337 14.6 ± 1.7	274 13.7 ± 3.0	278 13.9 ± 3.4
DAMS WITH STILLBORN PUPS	N(%)	4 (19.0)	1 (4.3)	4 (20.0)	5 (25.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)
GESTATION INDEX ^c	% N/N	95.4 21/ 22	100.0 23/ 23	95.0 19/ 20	100.0 20/ 20
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

a. Dosage administration occurred on day 7 of gestation through day 20 of lactation.

b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the day the first pup was delivered.

c. Number of rats with live offspring/number of pregnant rats.

* Significantly different from the vehicle control group value (p<0.05).

** Significantly different from the vehicle control group value (p<0.01).

F₁ physical development

There were test article-related mortalities in the F₁ generation male and female rats.

Two F₁ generation male rats in the 1000 mcg/kg/day maternal dosage group were euthanized on day 23 postpartum due to adverse clinical observations. Two other F₁ generation male rats in the 1000 mcg/kg/day maternal dosage group were found dead (days 28 and 98 postpartum) and one F₁ generation male rat in the 100 mcg/kg/day maternal dosage group was euthanized on day 126 postpartum because of a broken palate. In addition, one F₁ generation female rat in the 1000 mcg/kg/day maternal dosage group was euthanized on day 9 of gestation (DG 9) because of adverse clinical observations and one F₁ generation female rat in the 100 mcg/kg/day maternal dosage group was found dead on day 78 postpartum. None of these early deaths (found dead or unscheduled) can be ruled out as unrelated to the administration of ALX1-11 to F₀ generation rats.

There were no test article-related behavioral evaluation signs or clinical observations signs or at any dosage level, except for two male F₁ rats dosed at 1000 mcg/kg/day exhibited clinical observations of ↓ motor activity, impaired righting reflex, and ataxia. These two male F₁ rats were euthanized due to adverse clinical observations.

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TABLE B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MCG/KG/DAY)	I 0 (VEHICLE)	II 100	III 300	IV 1000
RATS TESTED	25	25	25	27
FOUND DEAD	0	0	0	2a,b
UNSCHEDULED SACRIFICE	0	1c	0	2d,e
TAIL BENT	263/ 3	255/ 3	231/ 3	376/ 6b
DEHYDRATION - TOTAL	2/ 1	5/ 2	0/ 0	15/ 6**
MILD	2/ 1	5/ 2	0/ 0	13/ 6**d,e
MODERATE	1/ 1	0/ 0	0/ 0	2/ 2d,e
SEVERE	0/ 0	0/ 0	0/ 0	2/ 2d,e
SOFT OR LIQUID FECES	11/ 5	1/ 1	5/ 5	9/ 4
SPARSE HAIR COAT: TOTAL	46/ 1	15/ 1	46/ 2	16/ 3
LIMB(S)	46/ 1	15/ 1	46/ 2	11/ 2b
NECK	0/ 0	0/ 0	0/ 0	5/ 1
SCAB f	29/ 1	47/ 6c	5/ 1	51/ 3
CHROMORRHOEAL	4/ 1	9/ 4c	9/ 5	2/ 2
PTOSIS	0/ 0	0/ 0	3/ 1	2/ 2d,e
IMPAIRED RIGHTING REFLEX	0/ 0	0/ 0	0/ 0	2/ 2d,e
ATAXIA	0/ 0	0/ 0	0/ 0	2/ 2d,e
PALE EARS	0/ 0	0/ 0	0/ 0	2/ 2d,e

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION

a. Rat 698 was found dead on day 28 postpartum.

b. Rat 698 was found dead on day 98 postpartum.

c. Rat 641 was sacrificed on day 126 postpartum due to adverse clinical observations.

d. Rat 683 was sacrificed on day 23 postpartum due to adverse clinical observations.

e. Rat 694 was sacrificed on day 23 postpartum due to adverse clinical observations.

f. Includes head, left or right ear, snout, mouth, left and/or right side of neck, neck, left and/or right side of back, back, tail and/or tip of tail.

** Significantly different from the vehicle control group value (p<0.01).

PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE B4 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MCG/KG/DAY)	I 0 (VEHICLE)	II 100	III 300	IV 1000
FOUND DEAD	0	1a	0	0
UNSCHEDULED SACRIFICE	0	0	0	1b
<u>PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):</u>				
RATS TESTED	25	25	25	25
TAIL BENT	57/ 2	64/ 2	52/ 2	79/ 4
DEHYDRATION - MILD	1/ 1	2/ 2	3/ 3	6/ 2b
SCAB c	9/ 1	0/ 0	0/ 0	16/ 2b
INCISOR(S): TOTAL	37/ 2	6/ 1	2/ 1	7/ 1
MISSING/BROKEN	17/ 2	0/ 0	0/ 0	7/ 1
MISALIGNED	23/ 2	6/ 1	2/ 1	0/ 0

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PRESUMED GESTATION:c

RATS TESTED	25	24	25	25
INCISOR(S): TOTAL	24/ 2	7/ 2	26/ 2	10/ 2
MISSING/BROKEN	2/ 1	3/ 1	26/ 2	9/ 1
MISALIGNED	22/ 1	4/ 1	22/ 1	1/ 1b
TAIL BENT	22/ 1	22/ 1	0/ 0	44/ 2
SPARSE HAIR COAT: TOTAL	47/ 3	23/ 2	29/ 3	3/ 1
LIMB(S)	47/ 3	23/ 2	29/ 3	3/ 1
NECK	22/ 1	0/ 0	0/ 0	0/ 0
UNDERSIDE	15/ 1	0/ 0	0/ 0	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	0/ 0	0/ 0	9/ 1	5/ 1
SOFT OR LIQUID FECES	0/ 0	0/ 0	0/ 0	1/ 1
HOLE IN PALATE	0/ 0	0/ 0	0/ 0	1/ 1b
LEFT SIDE OF MOUTH OR TAIL: SCAB(S)	4/ 1	10/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION

a. Rat 743 was found dead on day 78 postpartum.

b. Dam 782 was sacrificed on day 9 of gestation due to adverse clinical observations.

c. Restricted to rats with a confirmed mating date.

PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE B5 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MCG/KG/DAY)		0 (VEHICLE)	100	300	1000
RATS EXAMINED a	N	25	25	25	25
FOUND DEAD	N	0	1b	0	0
UNSCHEDULED SACRIFICE	N	0	0	0	1c
APPEARED NORMAL	N	24	25	25	24
KIDNEYS:					
RIGHT, PELVIS, MODERATE DILATION	N	1	0	0	0
BILATERAL, PELVIS, SLIGHT DILATION	N	0	0	0	1

a. Refer to the individual clinical observations table (Table B28) for external observations confirmed at necropsy.

b. Rat 743 was found dead on day 78 postpartum.

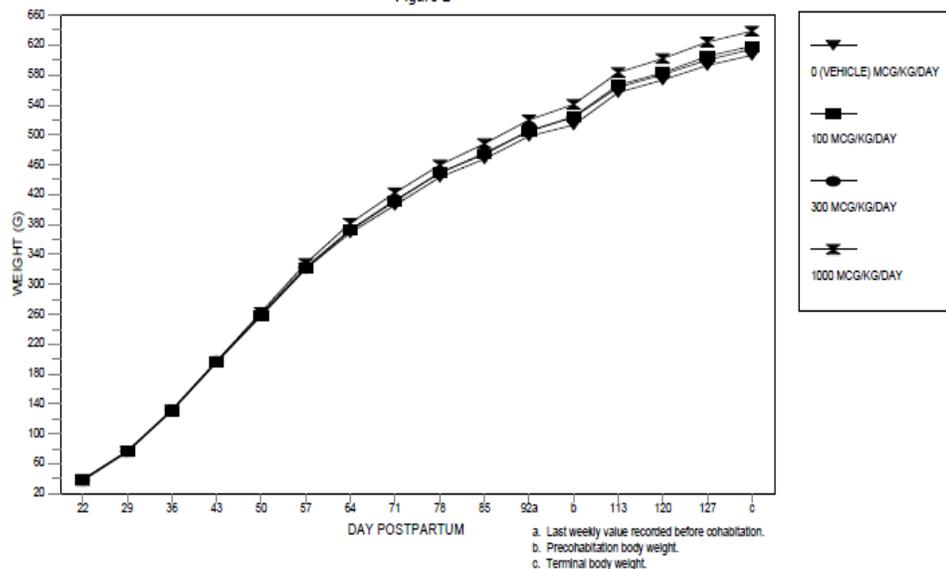
c. Dam 782 was sacrificed on day 9 of gestation due to adverse clinical observations.

Body weights, body weight gains and absolute and relative feed consumption values in the F1 generation male and female rats were unaffected by maternal dosages of ALX1-11 as high as 1000 mcg/kg/day.

PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

BODY WEIGHTS - F1 GENERATION MALE RATS

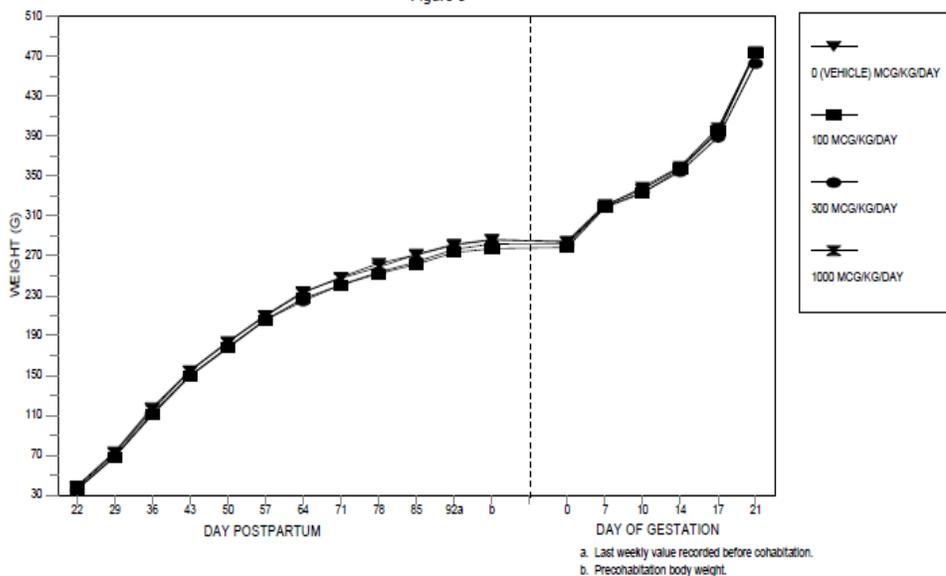
Figure 2



PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

BODY WEIGHTS - F1 GENERATION FEMALE RATS

Figure 3



F₁ behavioral evaluation

There were no test article-related behavioral evaluation signs at any dosage level, except for two male F1 rats dosed at 1000 mcg/kg/day which exhibited clinical observations of ↓ motor activity, impaired righting reflex, and ataxia. These two male F1 rats were euthanized due to adverse clinical observations.

F₁ reproductions

There were no effects of ALX1-11 on sexual maturation, behavior, male reproductive organ weights, mating and fertility or Caesarean-sectioning and litter parameters at any dosage level.

F1 Necropsy

There were no effects of ALX1-11 on necropsy findings at any dosage level, except at 300 mcg/kg/day, 2 male F1 offspring with slight dilation of R and/or L kidney pelvis, and 1 male with a large left testis.

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TABLE B2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 100	III 300	IV 1000
RATS EXAMINED a	N	25	25	25	27
FOUND DEAD	N	0	0	0	2b, c
UNSCHEDULED SACRIFICE	N	0	1d	0	2e, f
APPEARED NORMAL	N	21	25	22	23
LUNGS:					
ALL LOBES, MOTTLED RED AND DARK RED	N	0	0	0	1e
DIAPHRAGMATIC HERNIA:					
PORTION OF RIGHT LATERAL LOBE OF LIVER PROTRUDED INTO THORACIC CAVITY	N	0	0	0	1
KIDNEYS:					
RIGHT AND/OR LEFT, PELVIS, SLIGHT DILATION	N	1	0	2	1c
BILATERAL, PELVIS, MODERATE DILATION	N	0	0	0	1b
LEFT, LARGE	N	0	0	0	1c
RIGHT, MISSHAPEN	N	0	0	0	1c
RIGHT, NUMEROUS WHITE RAISED AREAS	N	0	0	0	1c

URETERS:					
LEFT, MODERATE DILATION	N	0	0	0	1c
URINARY BLADDER:					
NUMEROUS CALCULI	N	1	0	0	1c
WALLS THICK	N	1	0	0	0
DARK RED	N	0	0	0	1c
BROWN OPAQUE FLUID PRESENT	N	0	0	0	1c
EPIDIDYMIDES:					
RIGHT, SMALL	N	1	0	0	0
TESTES:					
RIGHT, SMALL	N	1	0	0	0
LEFT, LARGE	N	0	0	1	0

- a. Refer to the individual clinical observations table (Table B25) for external observations confirmed at necropsy.
 b. Rat 688 was found dead on day 28 postpartum.
 c. Rat 698 was found dead on day 98 postpartum.
 d. Rat 641 was sacrificed on day 126 postpartum due to adverse clinical observations.
 e. Rat 683 was sacrificed on day 23 postpartum due to adverse clinical observations.
 f. Rat 684 was sacrificed on day 23 postpartum due to adverse clinical observations.

PROTOCOL XGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE B5 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 100	III 300	IV 1000
RATS EXAMINED ^a	N	25	25	25	25
FOUND DEAD	N	0	1b	0	0
UNSCHEDULED SACRIFICE	N	0	0	0	1c
APPEARED NORMAL	N	24	25	25	24
KIDNEYS:					
RIGHT, PELVIS, MODERATE DILATION	N	1	0	0	0
BILATERAL, PELVIS, SLIGHT DILATION	N	0	0	0	1

- a. Refer to the individual clinical observations table (Table B28) for external observations confirmed at necropsy.
 b. Rat 743 was found dead on day 78 postpartum.
 c. Dam 782 was sacrificed on day 9 of gestation due to adverse clinical observations.

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F₂ findings

There were no fetal gross external alterations observed in the F₂ generation. Live fetal body weights were significantly lower ($p \leq 0.05$ to $p \leq 0.01$) for male and female fetuses (respectively) in the 300 mcg/kg/BW compared to the Vehicle Control Group.

PROTOCOL KGW00020: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF ALX1-11 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE B24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F₂ GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	
MATERNAL DOSAGE (MCG/KG/DAY)		0 (VEHICLE)	100	300	1000	
LITTERS WITH ONE OR MORE LIVE FETUSES		N	24	22	22	21
IMPLANTATIONS	MEAN±S.D.	14.8 ± 2.6	15.6 ± 1.5	14.5 ± 3.9	15.2 ± 1.6	
LIVE FETUSES	N	339	325	299	290	
	MEAN±S.D.	14.1 ± 2.5	14.8 ± 1.8	13.6 ± 3.8	13.8 ± 1.9	
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.2 ± 17.9	50.6 ± 16.2	50.4 ± 17.2	47.2 ± 10.7	
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.60 ± 0.35	5.56 ± 0.27	5.39 ± 0.40	5.64 ± 0.28	
MALE FETUSES	MEAN±S.D.	5.75 ± 0.36	5.69 ± 0.32	5.50 ± 0.40*	5.81 ± 0.30	
FEMALE FETUSES	MEAN±S.D.	5.45 ± 0.32	5.44 ± 0.27	5.20 ± 0.26** [21] ^a	5.48 ± 0.28	
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.6 ± 4.4	5.2 ± 6.4	6.0 ± 7.7	9.1 ± 8.2	

NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION

[] = NUMBER OF VALUES AVERAGED

^a. Litter 764 had no female fetuses.

* Significantly different from the vehicle control group value ($p \leq 0.05$).

** Significantly different from the vehicle control group value ($p \leq 0.01$).

Conclusions

Transient statistically significant reductions in body weight gains occurred between DGs 7 and 10 in the 300 and 1000 mcg/kg/day dosage groups. In addition, corresponding reductions in feed consumption occurred at 1000 mcg/kg/day during the first three days of the dosage period.

The reproductive NOAEL in the dams is <100 mcg/kg/day. There were no effects on reproduction (i.e., numbers of implantations or durations of gestation and parturition) at the highest dosage level tested (i.e., 1000 mcg/kg/day). A significant increase ($p \leq 0.01$ or $p \leq 0.05$) in the duration of gestation occurred in the 300 and 1000 mcg/kg/day dosage groups. One Dam had no live born pups at 300 mcg/kg/day.

The NOAEL for viability and growth in the offspring is <100 mcg/kg/day. This is based on the adverse findings on growth and development throughout the preweaning and postweaning periods and is summarized in the Table below:

Adverse Findings Reported in F0, F1, and F2 Generation Rats		
Natapara (mcg/kg/day)	In Life, Necropsy, and Pathology Findings	Incidence
100	Dehydration, mild F1 Offspring unscheduled sacrifice(broken palate) F1 Offspring found dead	2/25F 1/25M 1/25F
300	F0 Dams transient significant ↓ in body weight gains first 3 days of dosing (DGs 7-10) F0 Duration of Gestation significantly less than Vehicle Control Group F0 Dam with no Live Born Pups F0 Dam dehydration, mild F1 Kidneys, R and/or L Pelvis, slight dilation Testis, L, large F2 Fetal body weights significantly ↓ in M & F fetuses	 1/25F 3/25F 2/25M 1/25M
1000	Transient significant ↓ in body weight gains first 3 days of dosing (DGs 7-10) F0 Lactation Period, Urine-stained abdominal fur F0 Duration of Gestation Significantly less than Vehicle Control Group F0 Dams with all Pups Dying Days 1-4 Postpartum F1 Found dead ^b F1 Unscheduled sacrifice(adverse clinical observations) Dehydration, total F1 ↓ motor activity, impaired right reflex, and ataxia F1 Hole in palate F1 Lungs, all lobes mottled red and dark red F1 Urine-stained abdominal fur F1 Diaphragmatic Hernia (Portion of R Lateral Lobe of Liver protruded into Thoracic Cavity) F1 Kidneys, L, Large; R, Misshapen; R Pelvis, slight dilation; Numerous White Raised areas; Uterers, L, Moderate Dilation; Urinary Bladder, Dark Red, Brown Opaque Fluid Present, Kidneys, bilateral,	7/25 Dams 1/25 Dams 2/27M 2/27M, 1/25F 6/27M ^a , 2/25F 2/27M 1/25F(unscheduled sacrifice) 1/27M(unscheduled sacrifice) 1/27M 1/27M 1/27M

	pelvis, slight dilation	1/27M (found dead)
	F1 Kidneys, bilateral, pelvis, moderate dilation	1/27M (found dead)
	F1 Kidneys, bilateral, pelvis, moderate dilation	1/27F (unscheduled sacrifice)

^a Significantly different from the vehicle control group value ($p \leq 0.01$).

^b One male had slight dilation of a large left kidney pelvis, a missshapen right kidney, with numerous white raised areas on the right kidney, and left uterer dilation with dark red, brown opaque fluid present; the second male had moderate bilateral kidney pelvis dilation.

On the basis of these data, the maternal no-observable-adverse-effect-level (NOAEL) for ALX1-11 is <100 mcg/kg/day, and the NOAEL is <100 mcg/kg/day for viability and growth in offspring.

Study title: Subcutaneous Lactation Transfer Study of ALX1-11 in Rats

Study no.: (b) (4) Study No. 20008518

Sponsor Reference No. PAR-11-002

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: May 3, 2011

GLP compliance: Yes

QA statement: Yes

Drug, batch #, and % purity: ALX1-11 (Human PTH), G10261B201, % Purity by RP-HPLC 99.3% of total peak area; by CEC-HPLC 99.6% of total peak area

Key Study Findings

All rats survived until scheduled sacrifice. No clinical observations related to ALX1-11 occurred in the F0 generation dams or the F1 generation pups.

Maternal body weights, body weight gains, absolute and relative feed consumption values during the gestation and lactation periods were unaffected by dosages of ALX1-11 as high as 1000 µg/kg/day.

Natural delivery and litter observations were unaffected by dosages of ALX1-11 as high as 1000 µg/kg/day.

The results of the bioanalytical analysis were representative of the concentration of full length human PTH (1-84) in 20% and 100% rat K2EDTA plasma in casein-PBS for this study.

Based on the relatively low levels in the milk, the 2 to 42 fold ratio of plasma concentration to milk concentration and no discernible trends in the data, there was no apparent accumulation of ALX1-11 in the milk. The low levels detected in the milk were presumed to be contamination (picoliter amounts) during the milk collection

process due to the nature of the subcutaneous injection and the milk collection procedures and the necessary interaction of the technicians with the animals to complete these procedures. Therefore, it is unlikely that ALX1-11 was present in the milk during lactation; however based on these data the possibility of minimal maternal transfer of ALX1-11 through the milk to pups cannot be excluded.

On the basis of these data, the maternal no-observable-adverse-effect-level (NOAEL) for the test article is >1000 µg/kg/day. Subcutaneous administration of ALX1-11 to Crl:CD(SD) pregnant female rats did not result in apparent accumulation of ALX1-11 in the milk.

Methods

Doses 0 (Vehicle), 300 and 1000 µg/kg/day.

Species/Strain Crl:CD(SD) pregnant female rats.

Number/Sex/Group 38 Dams/group.

Route Subcutaneous

Formulation/Vehicle 10 mM Citrate Buffer with 5% Mannitol (pH 5.5)

Dosing Solution Analyses/Drug Stability and Homogeneity

The concentrations of the prepared formulations were within the acceptable limits ($\pm 15\%$ of the targeted concentration) with the exception of the 150 mcg/mL formulation on the last day of dose administration (17% lower than target).

SGS #	LOT#	SAMPLE DESCRIPTION	Content
181549	B-20008518-A (26May2011)	PTH (ALX1-11) from Protocol PAR-11-002 Day 1- Group 1, 0 mcg/mL	0 mcg/mL
181550	B-20008518-B (26May2011)	PTH (ALX1-11) from Protocol PAR-11-002 Day 1- Group 2, 150 mcg/mL	127 mcg/mL
181551	B-20008518-C (26May2011)	PTH (ALX1-11) from Protocol PAR-11-002 Day 1- Group 3, 500 mcg/mL	439 mcg/mL
181552	B-20008518-A (18Jun2011)	PTH (ALX1-11) from Protocol PAR-11-002 Last Day- Group 1, 0 mcg/mL	0 mcg/mL
181553	B-20008518-B (18Jun2011)	PTH (ALX1-11) from Protocol PAR-11-002 Last Day- Group 2, 150 mcg/mL	124 mcg/mL
181554	B-20008518-C (18Jun2011)	PTH (ALX1-11) from Protocol PAR-11-002 Last Day- Group 3, 500 mcg/mL	429 mcg/mL

Weight Day after Arrival 171 – 222g ; Study Assignment (DG 0) 212 – 259g

Observations Times and Results

Mortality All rats survived until scheduled sacrifice.

Clinical Signs

Clinical observations included localized alopecia or sparse hair coat on limb or underside, red perivaginal substance, rales and tail or incisor anomalies. These observed findings during the gestation and lactation periods were considered unrelated to ALX1-11, as they were not dosage dependent and/or occurred in only one rat in a given group.

Body Weights

Body weights and body weight gains during the gestation and lactation periods were unaffected by dosages of ALX1-11 as high as 1000 µg/kg/day. All values were comparable among the three dosage groups.

Food Consumption

Absolute and relative feed consumption values during the gestation and lactation periods were unaffected by dosages of ALX1-11 as high as 1000 µg/kg/day. All values were comparable among the three dosage groups.

Natural Delivery Observations

All rats were pregnant and all pregnant dams delivered a litter with the exception of one dam in the 1000 µg/kg/day dosage group. Natural delivery and litter observations were unaffected by dosages of ALX1-11 as high as 1000 µg/kg/day. Values for the numbers of dams delivering litters, the duration of gestation, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, viability and lactation indices, surviving pups per litter, percent male pups per number of pups sexed per litter, live litter size at weighing and pup weight per litter were comparable among the three dosage groups.

F1 Generation Clinical and Necropsy Observations

Clinical observations that occurred in the F1 pups included cold to touch, pale whole body, excess skin on the mouth, not nesting, not nursing, ulceration on the nose, scab on base of the tail, ungroomed coat, no milk band present, emaciation, mild to severe dehydration (based on skin turgor), macrophthalmia and tail anomalies. These

clinical observations were not considered related to ALX1-11 because the incidences were not dosage dependent and/or the observations are common in neonatal pups. No milk in stomach was observed in 1 pup in the 300 µg/kg/day dosage group and 5 pups in the 1000 µg/kg/day dosage group that were found dead and necropsied. All other pups that died before scheduled termination appeared normal at necropsy or autolysis precluded further evaluation.

Toxicokinetics

Mean final concentration of ALX-11 in 100% rat plasma and milk

Group	Dose (µg/kg/day)	Day	Time	Plasma Conc (pg/mL)		Milk Conc (pg/mL)		Ratio (Plasma/Milk)
				Mean	SD	Mean	SD	
1	0	2	30	658.60	480.55	3511.32	3314.86	NA
		2	120	<LLOQ	NA	331.85	409.33	
		16	30	<LLOQ	NA	<LLOQ	NA	
		16	120	<LLOQ	NA	46.14	NA	
2	300	1 or 2	30	44696.38	23841.15	1564.05	1036.53	28.6
		2	120	5780.63	3773.55	3118.80	1870.64	1.85
		16	30	11157.25	5619.98	2908.79	3428.61	3.84
		16	120	7432.19	5544.45	1425.24	306.17	5.21
3	1000	1 or 2	30	74587.42	49580.91	3431.20	1652.95	21.7
		1 or 2	120	20335.89	15227.88	10155.95	4386.96	2.00
		16	30	67939.42	12989.90	1627.46	197.55	41.7
		16	120	38235.90	5696.29	7985.24	1731.74	4.79

< LLOQ – Below lower limit of quantitation

NA – not applicable

(The control group samples (two out of 12 total), animals 401 and 402, Day 2, 30 minutes were analyzed and the results were >LLOQ concentration (20.00 pg/mL). These samples were considered to be anomalous and repeated for confirmation (at n = 2). Both duplicate repeats confirmed the original results, the original values are reported since the % difference between the original and the repeat values was <20%.)

10 Special toxicology studies

Study title: Validation of an ImmunoRadiometric assay (IRMA) for the Quantitative Determination of human PTH (1-84) in Rabbit K₂EDTA Plasma.

Study no.: (b) (4) Study No. 320292.

Study report location: (b) (4)

Conducting laboratory and location:



Date of study initiation: 25 January 2010

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity hPTH (1-84) Reference Standard Lot # 08PSA01,
Chromatographic purity 98.9%, Cation Exchange purity 99.6%

Key Study Findings

Background

The Whole PTH™ (1-84) specific immunoradiometric assay (IRMA) is a licensed *In-Vitro* Diagnostic device for assessment of this biomarker in human plasma samples and is manufactured by Scantibodies Laboratories (Product # 3KG056). In this procedure, components of the same kit are used for the assessment of administered whole human PTH in rabbit plasma (K₂EDTA). The method involves simultaneous incubation of sample (or standards or quality controls), ¹²⁵I labelled goat polyclonal antibodies specific for the n-terminal region of human PTH and goat polyclonal antibodies specific for the c-terminal region (amino acids 39-84) immobilized to a polystyrene bead. After a wash step to remove unbound reagents, the radioactivity associated with the bead is measured in a gamma counter.

The bound radioactivity is proportional to the concentration of whole PTH in the sample. The method is specific for whole PTH by virtue of the specificity of the labeled goat polyclonal antibody for only the n-terminal amino acids of PTH and is reported not to detect the major metabolites (including PTH 7-84). Samples (standards and quality controls) are diluted 2 fold in casein-PBS before the assay, as this measure was shown during method development to prevent losses of the analyte due to sticking.

Summary of Findings

The purpose of this study was to validate an immunoradiometric assay (IRMA) method for the quantitative determination of human PTH (1-84) in rabbit K₂EDTA plasma.

Validation of the method included: specificity (selectivity), dilution linearity, working calibration range, intra- and inter-assay precision and accuracy (including LLOQ and ULOQ assessments), and evaluation of the prozone effect. In addition, the stability of validation samples during storage at room temperature, at approximately 4°C and after freeze-thaw cycles at approximately -80°C were assessed. The long-term storage stability was assessed at approximately -80°C and reported by final report amendment.

No interference was observed with respect to the IRMA method for the quantitation of human PTH (1-84) when a test for selectivity and dilution linearity (up to 1,330,275-fold) was performed to evaluate specificity. A prozone effect* was noted when high concentration samples were analyzed using the method. The prozone effect was evident in concentrations

greater than 100,000 pg/mL in 50% rabbit plasma K2EDTA in Casein-PBS. The back-calculated concentrations of the calibration standards gave acceptable results over a range of 20.0 to 3,200 pg/mL using a 5 parameter-logistic curve-fit, with a weighting factor of 1/Y² (Statistical Ligand Immunoassay Analysis, (b) (4)). The results of the intra- and inter-batch precision and accuracy for quality control (QC) samples at 5 concentrations: 20.0, 80.0, 480, 2,400, and 3,200 pg/mL, in replicates of six are summarized below. The LLOQ and ULOQ were validated at 20.0 and 3,200 pg/mL, respectively.

*In an agglutination test, a person's serum (which contains antibodies) is added to a test tube, which contains a particular antigen. If the antibodies agglutinate with the antigen to form immune complexes, then the test is interpreted as positive. However, if too many antibodies are present that can bind to the antigen, then the antigenic sites are coated by antibodies, and few or no antibodies directed toward the pathogen are able to bind more than one antigenic particle. Since the antibodies do not bridge between antigens, no agglutination occurs. Because no agglutination occurs, the test is interpreted as negative. In this case, the result is a false negative. The zone of relatively high antibody concentrations within which no reaction occurs is called the prozone or the prezone.

Intra-assay Precision

Human PTH (1-84) Concentration (pg/mL)	Intra-batch (1 to 3) (n = 6)		
	CV (%)		
	Batch 1	Batch 2	Batch 3
LLOQ (20.0)	2.8	2.0	1.9
QC1 (80.0)	1.2	2.2	2.1
QC2 (480)	1.2	2.1	1.6
QC3 (2,400)	1.9	2.9	2.1
ULOQ (3,200)	1.4	4.3	1.9 *

* = n = 5 for this batch

Intra- assay Accuracy

Human PTH (1-84) Concentration (pg/mL)	Intra-batch (1 to 3) (n = 6)		
	Theoretical (%)		
	Batch 1	Batch 2	Batch 3
LLOQ (20.0)	97.8	105.0	100.3
QC1 (80.0)	99.3	98.6	101.2
QC2 (480)	98.8	105.3	98.0
QC3 (2,400)	103.7	99.1	100.6
ULOQ (3,200)	99.7	102.1	100.0*

* = n = 5 for this batch

Inter-assay Precision and Accuracy

Human PTH (1-84) Concentration (pg/mL)	Inter-batch	
	CV (%)	% Theoretical
LLOQ (20.0)	3.7	101.0
QC1 (80.0)	2.1	99.7
QC2 (480)	3.7	100.7
QC3 (2,400)	2.9	101.1
ULOQ (3,200)	2.7	100.5

Human PTH (1-84) was shown to be stable in 50% rabbit plasma K₂EDTA in Casein-PBS at room temperature for 18 hours and 19 minutes, at approximately 4°C for 17 hours and 22 minutes, and after 3 freeze-thaw cycles at approximately -80°C. Human PTH (1-84) was shown to be stable in rabbit plasma K₂EDTA at approximately 4°C for 18 hours and 6 minutes, and after 3 freeze-thaw cycles at approximately -80°C. However, the undiluted rabbit plasma stored at room temperature failed to meet the acceptance criteria. The long-term storage stability samples in rabbit plasma stored at approximately -80°C is currently ongoing and will be reported by final report amendment.

The above validation results demonstrated that the IRMA is suitable for the determination of human PTH (1-84) in rabbit plasma K₂EDTA.

Conclusions

The validation data demonstrated that the method is suitable for the analysis of human PTH (1-84) in rabbit plasma. The specificity met the acceptance criteria described for this method, indicating that the method is specific for human PTH (1-84). The accuracy and precision results were acceptable validating the calibration curve range from 20.0 (LLOQ) to 3,200 pg/mL (ULOQ). In addition, dilution linearity was established up to 1,330,275-fold with blank 50% rabbit plasma in Casein-PBS with the prozone effect observed at sample concentrations above 100,000 pg/mL with this method. The results indicated that human PTH (1-84) in rabbit plasma is stable when stored at a temperature set at 4°C for 18 hours and 6 minutes and after 3 freeze-thaw cycles at a temperature set at -80°C. However, the undiluted rabbit plasma stored at room temperature failed to meet the acceptance criteria. In addition human PTH (1-84) in 50% rabbit plasma in casein-PBS is stable when stored at room temperature for 18 hours and 19 minutes, when stored at a temperature set at 4°C for 17 hours and 22 minutes and after 3 freeze-thaw cycles at a temperature set at -80°C.

Study title: Validation of an Immunoradiometric Assay (IRMA) for the Quantitative Determination of Full Length Human PTH (1-84) in Rat Milk.

Study no.: (b) (4) Study No. 341608.

Study report location: (b) (4)

Conducting laboratory and location:



Date of study initiation: 16 September 2010

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity hPTH (1-84) Reference Standard Lot # 08PSA01,
Chromatographic purity 98.9%, Cation Exchange purity 99.6%

Key Study Findings

The purpose of this study was to validate an immunoradiometric (IRMA) method for the quantitative determination of full length human PTH (1-84) in rat milk.

Validation of the method included: specificity (selectivity), working calibration range, intra- and inter-assay precision and accuracy (including LLOQ and ULOQ assessments). In addition, the stability of validation samples during storage at ambient room temperature, in a refrigerator set to maintain 4°C, following 4 freeze-thaw cycles in a freezer set to maintain -80°C and following long-term storage in a freezer set to maintain -80°C were assessed.

The selectivity assessment failed to meet the predefined acceptance criteria, although the results suggest that there is no positive or negative interference in the matrix. The failure of the selectivity assessment is not considered to be due to a lack of selectivity in the method but suspected to be caused by the heterogeneity of the milk samples and the available aqueous volume in a given volume of sample. Alternatively the possibility of the PTH adhering to the fat globules in the milk sample may cause additional variability. Based on the results from the selectivity assessment it is considered likely that study samples may exhibit greater variability than the current validation precision and accuracy data would indicate. Therefore, based on the selectivity results, it was acknowledged that the accuracy of a determined concentration in study samples may be within a range of $\pm 40\%$ of the actual value.

The back-calculated concentrations of the calibration standards gave acceptable results over a range of 12.00 to 3200.00 pg/mL using a 5 parameter-logistic curve-fit, with a weighting factor of 1/Y² (Statistical Ligand Immunoassay Analysis,  (b) (4)). The results of the intra- and inter-batch precision and accuracy for quality control (QC) samples at 5 concentrations: 12.00, 60.00, 480.00, 2400.00 and 3200.00 pg/mL, in replicates of three are summarized below. The LLOQ and ULOQ were validated at 12.00 and 2400.00 pg/mL, respectively. The acceptance criteria for QC samples during sample analysis will be maintained at $\pm 20\%$ of the theoretical concentration.

Intra-assay Precision

Human PTH (1-84) Concentration (pg/mL)	Intra-batch (1 to 6) (n = 3)					
	CV (%)					
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
LLOQ (12.00)	4.4	1.4	7.6	2.6	7.4	13.4
QC1 (60.00)	5.6	1.5	12.2	3.0	2.3	4.4
QC2 (480.00)	2.8	3.3	7.6	2.3	2.0	2.8
QC3 (2400.00)	1.2	5.1	16.7	4.7	7.7	8.5
ULOQ (3200.00)	8.0	12.6	N/Ap*	N/Ap*	N/Ap*	5.2

* = %CV was not applicable, n < 2

Intra-assay Accuracy

Human PTH (1-84) Concentration (pg/mL)	Intra-batch (1 to 6) (n = 3)					
	Theoretical (%)					
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
LLOQ (12.00)	111.1	117.9	117.1	109.0	100.6	131.3**
QC1 (60.00)	110.2	108.9	109.1	110.7	121.3**	111.2
QC2 (480.00)	107.0	103.5	104.7	106.3	109.9	112.8
QC3 (2400.00)	111.7	113.3	111.0	117.9	140.4**	102.2
ULOQ (3200.00)	108.0	111.0	79.5*	118.7*	N/Ap	90.4

* = results based on n = 1

** = outside of acceptance criteria.

N/Ap = not applicable, no measurable results.

Inter-assay Precision and Accuracy

Human PTH (1-84) Concentration (pg/mL)	Inter-batch	
	CV (%)	% Theoretical
LLOQ (12.00)	9.4	114.3
QC1 (60.00)	6.3	111.9
QC2 (480.00)	4.4	107.4
QC3 (2400.00)	12.8	116.1
ULOQ (3200.00)	14.2	102.4

Human PTH (1-84) was shown to be stable in 50% rat milk in casein-PBS when stored at ambient room temperature for 4 hours and 2 minutes, following 4 freeze-thaw cycles in a freezer set to maintain -80°C combined with 4 hours and 41 minutes in a refrigerator set to maintain 4°C. Long-term storage stability of human PTH (1-84) in 50% rat milk in casein-PBS in a freezer set to maintain -80°C was demonstrated for up to 93 days. The stability human PTH (1-84) in 100% rat milk was demonstrated at ambient room temperature and in a refrigerator set to maintain 4°C for 1 hour and 49 minutes.

Validation data demonstrated that the IRMA is suitable for the determination of full length human PTH (1-84) in rat milk. However based on the selectivity data it is considered likely that the actual concentrations in study samples may be more variable than the precision and accuracy obtained during this validation would indicate. Therefore, based on the selectivity results a range of ±40% should be applied to measured concentrations of full length human PTH (1-84) in study samples.

Study title: Validation of an ImmunoRadiometric Assay (IRMA) Method for the Quantitative Determination of Full Length human PTH (1-84) in Rat

K₂EDTA Plasma.

Study no.: (b) (4) Study No. 341907

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: 30 May 2011

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity hPTH (1-84) Reference Standard Lot # 08PSA01,
Chromatographic purity 98.9%, Cation Exchange purity 99.6%

Key Study Findings

The purpose of this study was to validate an immunoradiometric (IRMA) method for the quantitative determination of full length human PTH (1-84) in rat plasma (K₂EDTA).

Validation of the method included: specificity (using interference check/selectivity and dilution linearity including prozone assessment), working calibration range, intra- and inter-assay precision and accuracy (including LLOQ and ULOQ assessments). In addition, the stability of validation samples during storage at ambient room temperature, in a refrigerator set to maintain 4°C and following 4 freeze-thaw cycles in a freezer set to maintain -80°C were assessed. The stability of validation samples during long-term storage was assessed following storage in a freezer set to maintain -80°C.

No interference was observed with respect to the IRMA method for the quantitation of full length human PTH (1-84) when a test for selectivity and dilution linearity (up to 773333-fold) was performed to evaluate specificity. A prozone effect was noted when high concentration samples were analyzed using the method. The prozone effect was evident in concentrations greater than 160,000 pg/mL in 20% rat plasma K₂EDTA in casein-PBS. The back-calculated concentrations of the calibration standards gave acceptable results over a range of 20.00 to 3,200.00 pg/mL using a 5 parameter-logistic curve-fit, with a weighting factor of $1/Y^2$ (Statistical Ligand Immunoassay Analysis, (b) (4)). The results of the intra- and inter-batch precision and accuracy for quality control (QC) samples at 5 concentrations: 20.00, 80.00, 480.00, 2,400.00, and 3,200.00 pg/mL, in replicates of three are summarized below. The LLOQ and ULOQ were validated at 20.00 and 3,200.00 pg/mL, respectively.

Intra-assay Precision

Human PTH (1-84) Concentration (pg/mL)	Intra-assay (1 to 6) (n = 3)					
	CV (%)					
	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6
LLOQ (20.00)	5.6	4.8	2.9	5.2	2.6	1.9
QC1 (80.00)	1.4	5.4	1.8	2.0	2.7	1.7
QC2 (480.00)	0.5	0.6	1.1	0.4	1.2	0.9
QC3 (2400.00)	3.4	3.2	2.9	1.0	1.5	2.0
ULOQ (3200.00)	1.2	2.9	1.0	2.0	0.9	1.8

Intra-assay Accuracy

Human PTH (1-84) Concentration (pg/mL)	Intra-assay (1 to 6) (n = 3)					
	Theoretical (%)					
	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6
LLOQ (20.00)	99.6	88.5	97.3	95.1	99.9	96.9
QC1 (80.00)	104.0	105.8	104.1	102.6	104.8	104.4
QC2 (480.00)	99.3	99.3	97.6	97.1	97.9	101.6
QC3 (2400.00)	103.8	100.6	101.0	100.7	98.7	102.4
ULOQ (3200.00)	95.2	95.3	94.3	96.4	92.2	94.1

Inter-assay Precision and Accuracy

Human PTH (1-84) Concentration (pg/mL)	Inter-assay	
	CV (%)	% Theoretical
	LLOQ (20.00)	5.3
QC1 (80.00)	2.6	104.3
QC2 (480.00)	1.7	98.8
QC3 (2400.00)	2.7	101.2
ULOQ (3200.00)	2.1	94.6

Human PTH (1-84) was shown to be stable in 20% rat plasma (K₂EDTA) in casein-PBS when stored in a refrigerator set to maintain 4°C for 25 hours and 39 minutes and following 4 freeze thaw cycles in a freezer set to maintain -80°C combined with 22 hours and 30 minutes at ambient room temperature. Long-term storage stability of human PTH (1-84) in 20% rat plasma (K₂EDTA) in casein-PBS in a freezer set to maintain -80°C was demonstrated for up to 97 days. The stability human of PTH (1-84) in 100% rat plasma (K₂EDTA) was demonstrated at ambient room temperature and in a refrigerator set to maintain 4°C for 1 hour and 20 minutes.

Validation data demonstrated that the IRMA was suitable for the determination of full length human PTH (1-84) in 20% rat plasma (K₂EDTA) in casein-PBS.

Study title: Validation of an ImmunoRadiometric (IRMA) Method for the Determination of Recombinant Human Parathyroid Hormone (hPTH 1-84) in Sprague Dawley Rat K₂EDTA Plasma.

Study no.: [REDACTED] (b) (4) Study No. AA74443.

Study report location: [REDACTED] (b) (4)

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: 17 November 2008 (Date of First Batch Performed)

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity

hPTH (1-84) Analytical Standard, lot # 08PSA01, and % purity/potency 100% (Assumed based on peak content, as per NPS Pharmaceuticals 26 August 2008 correspondence) (Note: Same Lot # used in previous bioanalytical reports.)

Key Study Findings

[REDACTED] (b) (4) established the following acceptance criteria for ligand-binding and related assays, based on the paper by Findlay et al*:

- The standard curve is reprocessed if the standard(s) deviate(s) more than $\pm 20\%$ from its/their respective nominal concentration(s).
- Quality control (QC) samples (low, medium and high) are within $\pm 20\%$ of their respective nominal concentration, with a %CV of replicate response (counts per minute (CPM)) $\leq 10\%$.
- The lower limit of quantitation (LLOQ) is within $\pm 25\%$ of its nominal concentration, with a %CV of replicate response (CPM) $\leq 10\%$.
- The upper limit of quantitation (ULOQ) is within $\pm 20\%$ of its nominal concentration, with a %CV of replicate response (CPM) $\leq 10\%$.

The Scantibodies Whole PTH™ (1-84) Specific kit is an immunoradiometric (IRMA) assay that uses a goat polyclonal anti-hPTH (1-84) antibody, which binds to the N-terminal region of hPTH (1-84) (label antibody) and the other polyclonal anti-hPTH (39-84) antibody, which binds to the C-terminal region of hPTH (1-84) (capture antibody). This method allows only whole hPTH (1-84) to be detected.

Standards, QC samples and plasma samples are incubated with capture antibody (beads) and label antibody (125I) in the polypropylene tubes. Following an appropriate incubation time, a wash step is performed to remove any unbound antibody. The concentration of hPTH (1-84) is directly proportional to the radioactivity bound to the beads after the separation. The amount of radioactivity is measured with a gamma counter. The concentration of hPTH (1-84) in unknown samples will be estimated by interpolation from a standard curve of hPTH (1-84) reference material (provided by the sponsor).

The immunoradiometric (IRMA) method for the determination of hPTH 1-84 in Sprague Dawley rat K₂EDTA plasma met the validation requirements. Stability was demonstrated for hPTH 1-84 in Sprague Dawley rat K₂EDTA plasma samples under varying conditions of storage.

*J.W.A. Findlay, W.C. Smith, J.W. Lee, G.D. Nordblom, I. Das, B.S. DeSilva, M.N. Khan and R.R. Bowsher. Validation of Immunoassays for Bioanalysis: A Pharmaceutical Industry Perspective. Journal of Pharmaceutical and Biomedical Analysis. 2000, 21.1249-1273.

Study title: NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers.

Study no.: (b) (4) No. 1150-003.

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: December 12, 2011

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: PTH Drug Substance, G10261B203, 99.4% by RP-HPLC, 99.6% by CEC-HPLC

PTH Drug Substance, 10PTF03, 99.1% by RP-HPLC, 99.2%
By CE-HPLC

Key Study Findings

Following 28 consecutive days of subcutaneous administration of NPSP558 (BI) or NPSP558 (SB) to male and female rats at dose levels of 0.050, 0.150, and 0.300 mg/kg/day, there appeared to be no significant difference in effects of test article formulations from Boehringer-Ingelheim RCV GmbH and Co KG or Synco Bio Partners B.V. The no-observed-adverse-effect-level (NOAEL) was considered to be 0.300 mg/kg/day NPSP558 (BI) and (SB). The corresponding Day 25 AUC values were 1688576 and 1466636 pg•min/mL for males and females administered the BI material and 1761826 and 1715506 pg•min/mL for males and females administered the SB material.

Methods

Doses

NPSP558 (BI) or NPSP558 (SB) at dose levels of 0.050, 0.150, or 0.300 mg/kg/day.

Six treatment groups of 10 male and 10 female experimentally naïve CD® [CrI:CD®(SD)] rats each were administered NPSP558 (BI) or NPSP558 (SB) at dose levels of 0.050, 0.150, and 0.300 mg/kg/day. One additional group of 10 animals per sex served as the control (0 mg/kg/day) and received the vehicle, 10 mM Citrate Buffer with 5% Mannitol (pH 5.5 ±0.2). The vehicle or test articles were administered to all groups via subcutaneous (bolus) injection, once daily for 28 consecutive days, at a dose volume of 1 mL/kg. Additionally, six groups of 10 animals per sex per group served as toxicokinetic (TK) animals and received NPSP558 (BI) or NPSP558 (SB) in the same manner as the main study groups at dose levels of 0.050, 0.150, and 0.300 mg/kg/day and a dose volume of 1 mL/kg for 25 consecutive days.

The two test articles were the same drug with one batch manufactured by Boehringer-Ingelheim RCV GmbH and Co KG (BI) and the other batch manufactured by Synco Bio Partners B.V. (SB).

Species/Strain CD® [CrI:CD®(SD)].

Number/Sex/Group (Main Study) 10/sex/group.

Route Subcutaneous (bolus) injection, once daily for 28 consecutive days.

Formulation/Vehicle 10 mM Citrate Buffer with 5% Mannitol (pH 5.5 ±0.2).

Dosing Solution Analyses/Drug Stability and Homogeneity
Test article formulations were analyzed on Days 1 and 25.

Concentration of NPSP558 in Dosing Formulations (Days 1 and 25)				
Dose Level (mg/kg/day)	Test Article Identification	Nominal Concentration (mg/mL)	Concentration Day 1 (µg/mL)	Concentration Day 25 (µg/mL)
0	Control Article	0.00	0	NA
0.050	NPSP558(BI)	0.05	32	32
0.150	NPSP558(BI)	0.15	134.40	133.96
0.300	NPSP558(BI)	0.30	278.14	276.28
0.050	NPSP558(SB)	0.05	29	31
0.150	NPSP558(SB)	0.15	126.91	126.44
0.300	NPSP558(SB)	0.30	270.15	272.13 ^a

^a Average of three assay values representing top, middle, and bottom strata for homogeneity analysis
NA – Not Applicable

Dose Volume/Infusion Rate 1 mL/kg/subcutaneous (bolus) injection

Satellite Groups Used For Toxicokinetics or Recovery

Six groups of 10 animals per sex per group served as toxicokinetic (TK) animals and received NPSP558 (BI) or NPSP558 (SB).

Age Approximately 8 weeks of age upon arrival, followed by 6 weeks of acclimation.

Weight Males, 426 to 565g and females 244 to 308g, respectively.

Unique Study Design or Methodology None

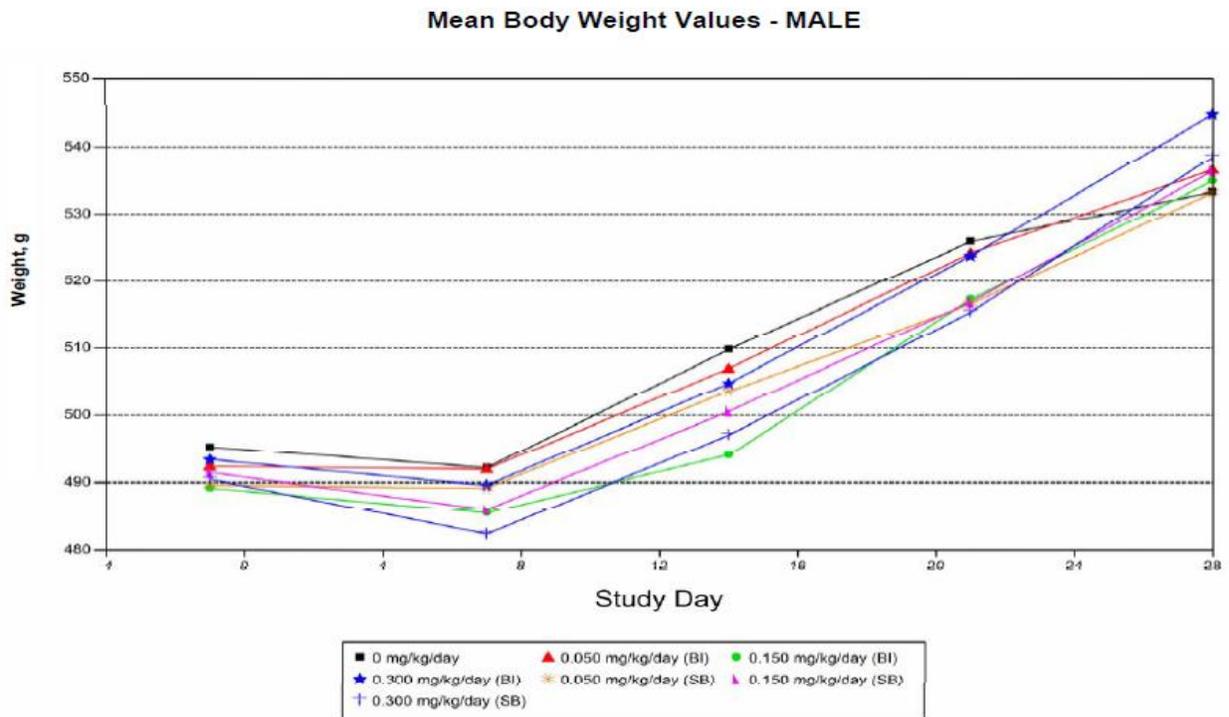
Observations Times and Results
Mortality

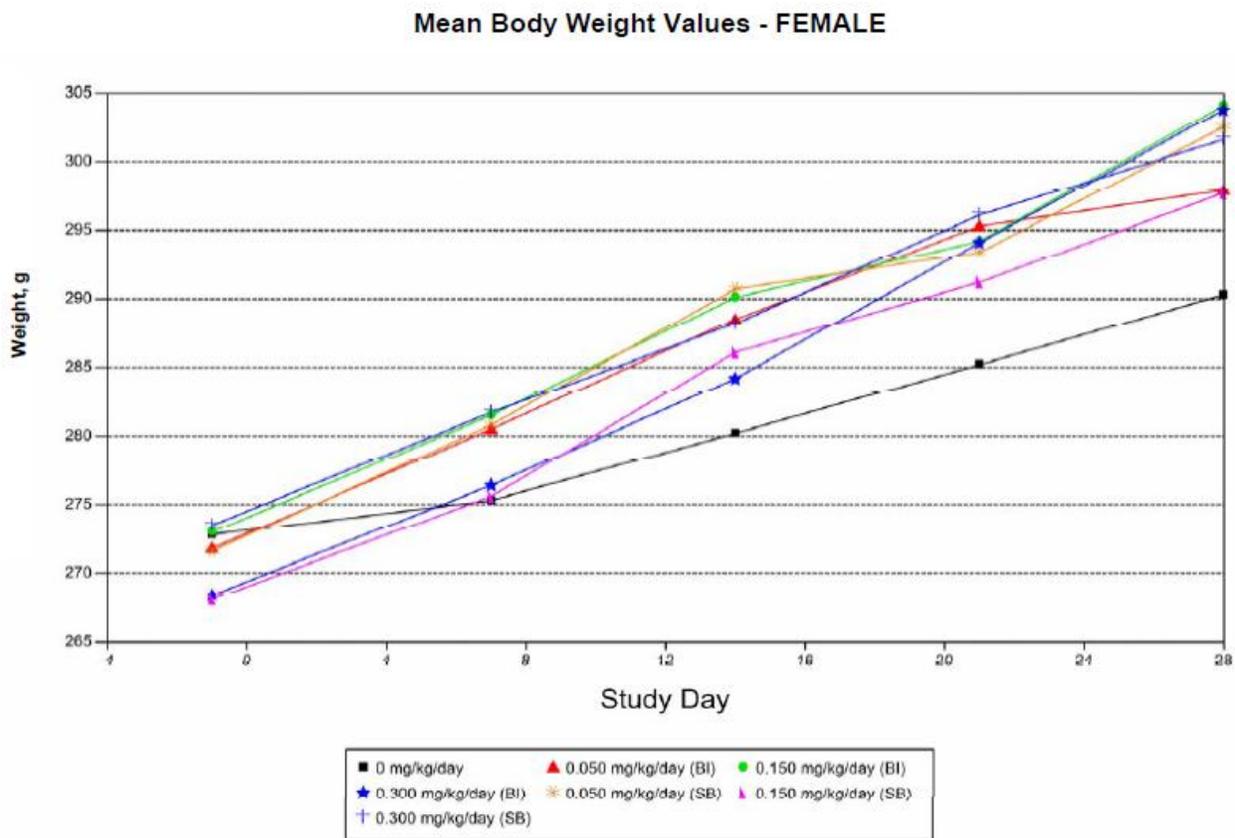
Clinical Signs

Clinical observations were performed twice daily. 5/10 male animals and 8/10 female animals at 0.300 mg/kg/day (SB), and 10/10 female animals at 0.300 mg/kg/day (BI) were observed with red, discolored skin on the ears.

Body Weights

There were no test article-related effects on body weight. Male and female animals in all groups gained or maintained weight compared to controls as measured on Day 28.





Food Consumption

There were no test article-related effects on food consumption.

Ophthalmoscopy

There were no test article-related ophthalmic effects.

Hematology

Boehringer-Ingelheim [NPSP558 (BI)]:

At termination in both sexes receiving ≥ 0.150 mg/kg/day, there was evidence of a mild regenerative anemia as illustrated by mild dose-dependent reductions in erythrocyte mass (erythrocytes, hemoglobin and hematocrit) (up to 16%) and mild dose-dependent increases in absolute reticulocytes (up to 1.3-fold) relative to controls.

At termination in both sexes receiving ≥ 0.150 mg/kg/day, there were minimal dose-dependent reductions in platelets (up to 14%) relative to controls. These changes were potentially test article related but not of biologic relevance based on their small magnitude.

At termination in males receiving ≥ 0.150 mg/kg/day, there were dose-dependent reductions in total leukocytes (up to 28%) relative to controls. Reductions in total leukocytes were primarily attributable to mild reductions in lymphocytes (up to 27%), neutrophils (up to 43%), and eosinophils (up to 70%) relative to controls.

Synco Bio Partners [NPSP558 (SB)]:

At termination in both sexes receiving ≥ 0.150 mg/kg/day, there was evidence of a mild regenerative anemia as illustrated by mild dose-dependent reductions in erythrocyte mass (erythrocytes, hemoglobin and hematocrit) (up to 12%) and mild dose-dependent increases in absolute reticulocytes (up to 1.5-fold) relative to controls.

At termination in all male treatment groups, and in females receiving ≥ 0.150 mg/kg/day, there were minimal dose-dependent reductions in platelets (up to 14%) relative to controls. These changes were potentially test article related but not of biologic relevance based on their small magnitude.

There were occasionally minor differences in the severity of hematologic changes between the Boehringer-Ingelheim and Synco Bio Partners test articles, however overall, differences were minor. There were other statistically significant fluctuations in hematology parameters that were not considered toxicologically or biologically relevant based on their sporadic nature or small magnitude.

Coagulation

Boehringer-Ingelheim [NPSP558 (BI)]:

At termination in both sexes and all treatment groups, there were mild to moderate dose-dependent elevations in fibrinogen (up to 1.9-fold) relative to controls, consistent with a test-article related inflammatory response. These changes however, were not supported by alterations among hematology parameters (increases in total leukocytes, neutrophils, etc.).

Synco Bio Partners [NPSP558 (SB)]:

At termination in both sexes and all treatment groups, there were mild to moderate dose dependent elevations in fibrinogen (up to 1.9-fold) relative to controls. Similar to the Boehringer-Ingelheim test-article; these changes were not supported by alterations among hematology parameters.

Clinical Chemistry

Boehringer-Ingelheim [NPSP558 (BI)]:

At termination in males receiving ≥ 0.150 mg/kg/day and females receiving 0.300 mg/kg/day, there were mild elevations in urea nitrogen (up to 1.3-fold) relative to controls. These changes were suggestive of mild dehydration and decreased glomerular filtration. These findings in conjunction with mild increases in urine volume and reductions in urine specific gravity (in males, discussed below) potentially indicated altered renal handling of water and solutes. These changes were

considered test article related but of limited biologic relevance based on their small magnitude.

At termination in all male treatment groups and females receiving ≥ 0.150 mg/kg/day, there were mild elevations in alkaline phosphatase (ALP) activity (up to 1.5-fold) relative to controls. These changes were dose-dependent and were likely related to test article induced bone remodeling, consistent with the known physiologic actions of parathyroid hormone.

At termination in both sexes and all treatment groups, there were minimal dose-dependent reductions in albumin/globulin ratios (up to 13%) that reflected minimal elevations in globulins (up to 1.1-fold) relative to controls. These changes coincided with elevations in fibrinogen, and supported the presence of an inflammatory response. They were however of questionable relevance to the test article based on their small magnitude.

Synco Bio Partners [NPSP558 (SB)]:

At termination in males receiving ≥ 0.150 mg/kg/day and females receiving 0.300 mg/kg/day, there were mild elevations in urea nitrogen (up to 1.3-fold) relative to controls. These changes were consistent with mild dehydration and decreased glomerular filtration. These findings in conjunction with mild increases in urine volume and reductions in urine specific gravity (in both sexes, discussed below) potentially indicated altered renal handling of water and solutes. These changes were considered test article related but of limited biologic relevance based on their small magnitude.

At termination in both sexes and all treatment groups, there were mild elevations in alkaline phosphatase (ALP) activity (up to 1.6-fold) relative to controls. These changes were considered test-article related.

At termination in both sexes and all treatment groups, there were minimal reductions in albumin/globulin ratios (up to 13%) that reflected mild elevations in globulins (up to 1.1-fold) relative to controls. These changes were generally dose-dependent and correlated with elevations in fibrinogen. They were of questionable relevance to the test article based on their small magnitude.

Urinalysis

Boehringer-Ingelheim [NPSP558 (BI)]:

At termination, males receiving ≥ 0.150 mg/kg/day (Boehringer-Ingelheim) had mildly increased urine volume (up to 2.1-fold) relative to controls. These changes correlated with a slight reduction in urine specific gravity, and indicated decreased urine concentration by the renal tubules.

Synco Bio Partners [NPSP558 (SB)]:

At termination, both sexes receiving 0.300 mg/kg/day (Synco Bio Partners) had mildly increased urine volume (up to 2.3-fold) relative to controls. These changes correlated with a mild reduction in urine specific gravity.

Overall, the changes among urinalysis parameters were of similar magnitudes between both the Boehringer-Ingelheim and Synco Bio Partners test-articles,

however relevant changes occurred at a lower dose in the animals receiving the Boehringer-Ingelheim test article.

Gross Pathology Unremarkable.

Organ Weights

Test article-related weight changes were present in the spleen of male and female animals. Spleen weights were statistically significantly increased in all parameters in males [except mean absolute weight in males dosed at 0.300 mg/kg/day (SB)] and females dosed at 0.300 mg/kg/day (BI) and 0.300 mg/kg/day (SB). Increased spleen weights were similar between NPSP558 BI and NPSP558 SB dosed groups: 28% (males) and 29% (females) dosed at 0.300 mg/kg/day (BI) compared to 20% (males) and 40% (females) dosed at 0.300 mg/kg/day (SB). Increased spleen weights were microscopically correlated to increased extramedullary hematopoiesis.

(b) (4) Study Number 1150-003
NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Organ Weight Values - MALE Terminal

Endpoint	0 mg/kg/day			0.050 mg/kg/day (BI)			0.150 mg/kg/day (BI)			0.300 mg/kg/day (BI)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Spleen g	0.887	0.115	10	0.923	0.146	10	1.008	0.100	10	1.134 ^b	0.121	10
Spleen/BWt %	0.1740	0.0133	10	0.1800	0.0217	10	0.1982	0.0217	10	0.2187 ^b	0.0181	10
Spleen/BrWt ratio	0.4292	0.0544	10	0.4451	0.0600	10	0.5043	0.0623	10	0.5522 ^b	0.0655	10

(b) (4) Study Number 1150-003
NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Organ Weight Values - MALE Terminal

Endpoint	0.050 mg/kg/day (SB)			0.150 mg/kg/day (SB)			0.300 mg/kg/day (SB)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Spleen g	0.843	0.068	10	0.925	0.104	10	1.068	0.248	10
Spleen/BWt %	0.1675	0.0237	10	0.1814	0.0237	10	0.2090 ^a	0.0458	10
Spleen/BrWt ratio	0.4146	0.0428	10	0.4482	0.0649	10	0.5339 ^b	0.1132	10

N - Number of measures used to calculate mean
SD - Standard Deviation

^a Significantly different from control; (p<0.05)
^b Significantly different from control; (p<0.01)

(b) (4) Study Number 1150-003
NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Organ Weight Values - FEMALE Terminal

Endpoint	0 mg/kg/day			0.050 mg/kg/day (BI)			0.150 mg/kg/day (BI)			0.300 mg/kg/day (BI)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N

Spleen g	0.564	0.140	10	0.565	0.103	10	0.683	0.084	10	0.729 ^a	0.073	10
Spleen/BWt %	0.2055	0.0413	10	0.2034	0.0376	10	0.2401	0.0228	10	0.2579 ^a	0.0159	10
Spleen/BrWt ratio	0.2937	0.0724	10	0.2995	0.0516	10	0.3555	0.0414	10	0.3713 ^a	0.0318	10

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

**Summary of Organ Weight Values - FEMALE
Terminal**

Endpoint	0.050 mg/kg/day (SB)			0.150 mg/kg/day (SB)			0.300 mg/kg/day (SB)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Spleen g	0.597	0.072	10	0.653	0.100	10	0.788 ^b	0.201	10
Spleen/BWt %	0.2141	0.0248	10	0.2345	0.0277	10	0.2799 ^b	0.0634	10
Spleen/BrWt ratio	0.3159	0.0288	10	0.3439	0.0423	10	0.4087 ^b	0.1118	10

N - Number of measures used to calculate mean
 SD - Standard Deviation

^b Significantly different from control; (p<0.01)

Histopathology

Test article-related microscopic changes were present in the bone and spleen of male and female rats. Hyperostosis in the femur and sternum and extramedullary hematopoiesis in the spleen were present in males and females at all NPSP558 BI and NPSP558 SB dose levels. The incidence and/or severity of hyperostosis in the femur and sternum and increased extramedullary hematopoiesis in the spleen were dose dependently increased in NPSP558 BI and NPSP558 SB dosed groups. There were no remarkable differences in magnitude or incidence of hyperostosis or increased extramedullary hematopoiesis between the NPSP558 BI and NPSP558 SB dosed groups. Hyperostosis was characterized by increased thickening of trabeculae in the marrow cavity. Hyperostosis was also present in sections of tibia that were included with the femur sections but was not separately recorded. Extramedullary hematopoiesis in the spleen was increased in NPSP558 BI and NPSP558 SB dosed animals when compared to controls. These changes were considered test article-related but not adverse. Hyperostosis is an anticipated pharmacological effect of treatment with NPSP558 and extramedullary hematopoiesis is an adaptive response.

No additional test article-related findings were noted by the pathologist, although minimal pyelitis and chronic progressive nephropathy were observed in several of the male NPSP558 SB 0.3 mg/kg/day male rats.

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Microscopic Observations - MALE

		Terminal			
		0 mg/kg/day	0.050 mg/kg/day (B1)	0.150 mg/kg/day (B1)	0.300 mg/kg/day (B1)
Tissue Observation	Severity				
bone, femur hyperostosis		(10)	(10)	(10)	(10)
		0	9	10	10
	- minimal	0	9	0	0
	- mild	0	0	10	6
within normal limits	- moderate	0	0	0	4
		10	1	0	0
bone, sternum hyperostosis		(10)	(10)	(10)	(10)
		0	7	10	10
	- minimal	0	7	10	6
	- mild	0	0	0	2
within normal limits	- moderate	0	0	0	2
		10	3	0	0
kidneys		(10)	(0)	(0)	(10)
	nephropathy, chronic progressive	3	0	0	0
	pyelitis	0	0	0	0
within normal limits		7	0	0	10

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Microscopic Observations - MALE

		Terminal		
		0.050 mg/kg/day (SB)	0.150 mg/kg/day (SB)	0.300 mg/kg/day (SB)
Tissue Observation	Severity			
bone, femur hyperostosis		(10)	(10)	(10)
		9	10	10
	- minimal	5	1	0
	- mild	4	8	9
within normal limits	- moderate	0	1	1
		1	0	0
bone, sternum hyperostosis		(10)	(10)	(10)
		9	9	10
	- minimal	9	8	8
	- mild	0	1	2
within normal limits	- moderate	0	0	0
		1	1	0
kidneys		(0)	(0)	(10)
	nephropathy, chronic progressive	0	0	3
	pyelitis	0	0	1
within normal limits		0	0	7

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Microscopic Observations - MALE

		Terminal			
		0 mg/kg/day	0.050 mg/kg/day (B1)	0.150 mg/kg/day (B1)	0.300 mg/kg/day (B1)
Tissue Observation	Severity				
spleen hematopoiesis, extramedullary, increased		(10)	(10)	(10)	(10)
		0	3	10	9
	- minimal	0	3	5	2
	- mild	0	0	5	7
within normal limits		10	7	0	1
kidneys		(10)	(0)	(0)	(10)
	nephropathy, chronic progressive	3	0	0	0
	pyelitis	0	0	0	0
within normal limits		7	0	0	10

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Microscopic Observations - MALE

		Terminal		
		0.050 mg/kg/day (SB)	0.150 mg/kg/day (SB)	0.300 mg/kg/day (SB)
Tissue Observation	Severity			

spleen		(10)	(10)	(10)
hematopoiesis, extramedullary, increased		2	10	10
	- minimal	2	8	4
	- mild	0	2	6
within normal limits		8	0	0
kidneys		(0)	(0)	(10)
nephropathy, chronic progressive	- minimal	0	0	3
pyelitis	- minimal	0	0	1
within normal limits		0	0	7

(b) (4) Study Number 1150-003
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substances from Two Manufacturers

Summary of Microscopic Observations - FEMALE
 Terminal

ISSUE	Severity	0 mg/kg/day	0.050 mg/kg/day (B1)	0.150 mg/kg/day (B1)	0.300 mg/kg/day (B1)
spleen		(10)	(10)	(10)	(10)
hematopoiesis, extramedullary, increased		0	3	9	10
	- minimal	0	3	8	2
	- mild	0	0	1	8
within normal limits		10	7	1	0
bone, femur		(10)	(10)	(10)	(10)
hyperostosis		0	3	10	10
	- minimal	0	3	2	0
	- mild	0	0	8	6
	- moderate	0	0	0	4
within normal limits		10	7	0	0
bone, sternum		(10)	(10)	(10)	(10)
hyperostosis		0	8	10	10
	- minimal	0	8	9	7
	- mild	0	0	1	3
within normal limits		10	2	0	0
kidneys		(10)	(0)	(0)	(10)
mineralization, pelvic	- minimal	1	0	0	2
mineralization, tubular	- minimal	1	0	0	2
nephropathy, chronic progressive	- minimal	1	0	0	0
kidneys		(10)	(0)	(0)	(10)
pyelitis	- minimal	1	0	0	1
within normal limits		8	0	0	6

Summary of Microscopic Observations - FEMALE
 Terminal

Tissue	Severity	0.050 mg/kg/day (SB)	0.150 mg/kg/day (SB)	0.300 mg/kg/day (SB)
Number of Animals Examined		10	10	10
bone, femur		(10)	(10)	(10)
hyperostosis		2	10	10
	- minimal	2	7	1
	- mild	0	3	8
	- moderate	0	0	1
within normal limits		8	0	0
bone, sternum		(10)	(10)	(10)
hyperostosis		3	10	10
	- minimal	3	10	9
	- mild	0	0	1
within normal limits		7	0	0
kidneys		(0)	(0)	(10)
mineralization, pelvic	- minimal	0	0	4
mineralization, tubular	- minimal	0	0	0
nephropathy, chronic progressive	- minimal	0	0	1
kidneys		(0)	(0)	(10)
pyelitis	- minimal	0	0	0
within normal limits		0	0	5
spleen		(10)	(10)	(10)
hematopoiesis, extramedullary, increased		1	10	9
	- minimal	1	7	5
	- mild	0	3	4
within normal limits		9	0	1

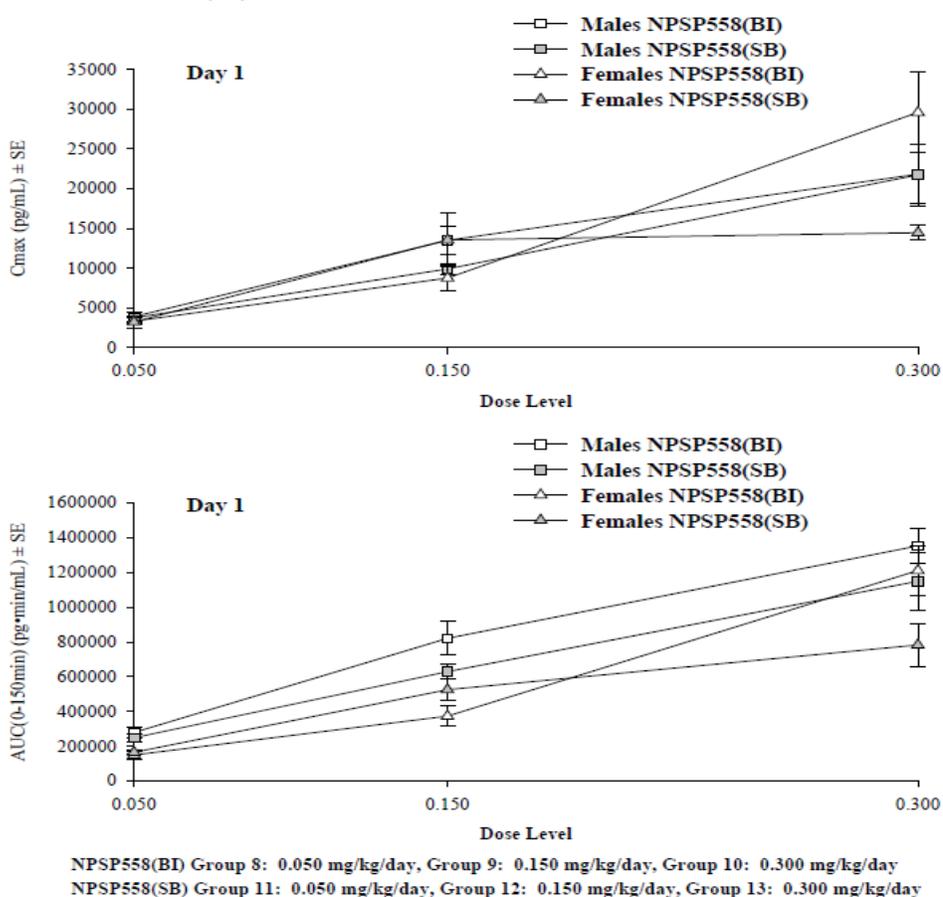
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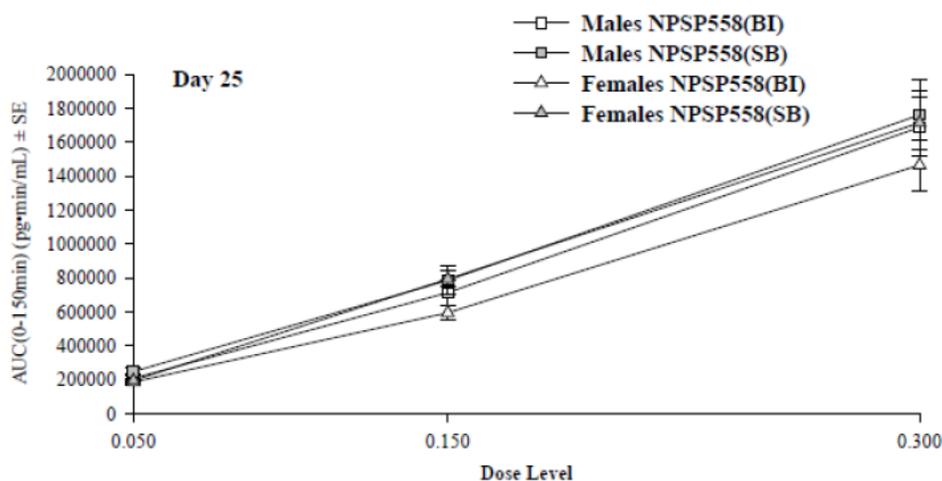
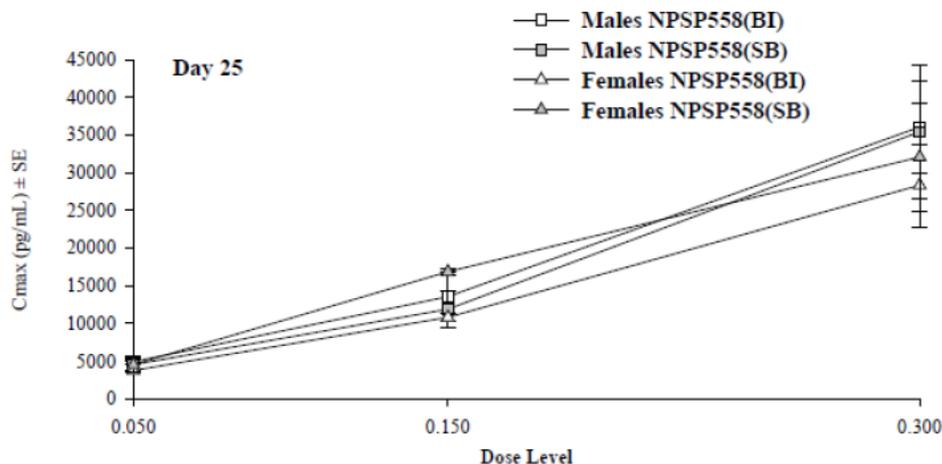
Peer Review No

Toxicokinetics

NPSP558 in plasma from the two manufacturers exhibited peak NPSP558 plasma concentrations between 5 and 30 min postdose followed by a monoexponential decline with T1/2 ranging from 12.9 to 43.0 min for all dose group levels on Days 1 and 25. Cmax and AUC values generally increased in a dose proportional manner on both sampling days. No gender differences were observed for either NPSP558 manufacturers. Exposure to NPSP558 on Day 25 did not change substantially from Day 1 following daily administration. There was no indication of accumulation; all predose samples on Day 25 were below the lower limit of quantitation (100 pg/mL). NPSP558 exposures from both manufacturers were comparable to each other.

Comparison of NPSP558 Exposure in CrI:CD[®](SD) Rat Plasma Following Subcutaneous Administration of NPSP558(BI) or NPSP558(SB)





NPSP558(BI) Group 8: 0.050 mg/kg/day, Group 9: 0.150 mg/kg/day, Group 10: 0.300 mg/kg/day
 NPSP558(SB) Group 11: 0.050 mg/kg/day, Group 12: 0.150 mg/kg/day, Group 13: 0.300 mg/kg/day

Study title: NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substance and Drug Product

Study no.: (b) (4) No. 1150-004

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: February 10, 2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: PTH Drug Substance, lot # G10261B203, % purity by RP-HPLC 99.4%, % purity by CEC-HPLC 99.6%

NPSP 558 (PTH) for sc injection, Lot # P11001D, % purity by RP-HPLC Comparable to PTH Reference

Key Study Findings

Non-adverse test article-related Day 1 FOB findings were limited to an increase in defecation in males in the Drug Substance groups compared to males administered the Drug Product.

Non-adverse test article-related findings in females on Day 1 included a decrease in body temperature at 0.050 and 0.300 mg/kg/day Drug Substance compared to Drug Substance controls, and an overall body temperature decrease in females administered the Drug Product compared to animals administered the Drug Substance. In addition, an increase in Day 1 hindlimb splay measurement in females at 0.150 (but not at 0.300) mg/kg/day Drug Substance and Drug Product was observed compared to the respective controls. It was concluded that neither NPSP558 Drug Substance nor NPSP558 Drug Product produced adverse effects on central nervous system function at doses up to and including 0.3 mg/kg/day.

Minor test article-related changes were noted in some hematology parameters (red blood cell parameters, reticulocytes, leukocytes). These were generally dose-dependent suggesting relation to test article; however, individual values remained within acceptable limits of physiologic variation and none were considered adverse. There were no adverse test article-related effects on coagulation parameters or meaningful differences between the two treatments evaluated. Individual fibrinogen values were minimally to mildly elevated at 0.150 and 0.300 mg/kg/day and were not considered adverse at the magnitudes observed.

There were no adverse effects on clinical chemistry analytes or meaningful differences between the two treatments evaluated. Dose-dependent changes and statistical differences that were observed were not considered toxicologically meaningful due to the small magnitude, sporadic nature, and/or direction of change.

There were no adverse effects or meaningful differences in urinalysis parameters between the two treatments evaluated.

Non-adverse test article-related weight changes were present in the spleen of male and female animals. Overall spleen weights were increased in all parameters in males and females at 0.150 and 0.300 mg/kg/day Drug Substance and Drug Product and were microscopically correlated to increased extramedullary hematopoiesis. Test article-related microscopic changes were present in the bone (hyperostosis) and spleen (extramedullary hematopoiesis) of male and female rats. The bone and spleen changes were considered test article-related, but not adverse based on the lack of adverse clinical pathology findings. Hyperostosis is an anticipated pharmacological effect of the test article and extramedullary hematopoiesis is an adaptive response.

Peak NPSP558 plasma concentrations were observed between 5 and 30 min postdose followed by a monoexponential decline with T_{1/2} ranging from 19.4 to 52.4 min for Drug Substance and 17.5 to 41.3 min for Drug Product on Days 1 and 28. NPSP558 exposure from both Drug Substance and Drug Product ranged from 171884 to 3236328 pg•min/mL and 199529 to 4392017 pg•min/mL, respectively, on

Days 1 and 28. NPSP558 systemic exposure appeared to increase in an approximately dose-proportional manner between 0.050 and 0.300 mg/kg/day. Male animals NPSP558 systemic exposure were generally higher than female animals and the exposure to NPSP558 increased by as much as 5.77-fold after 28 days of daily administration with Drug Product exhibiting higher accumulation ratios than Drug Substance.

Following 28 consecutive days of subcutaneous administration of NPSP558 Drug Substance or Drug Product to male and female rats at dose levels of 0.050, 0.150, and 0.300 mg/kg/day, there were no significant differences in the effects of the two test articles. The no-observed-adverse-effect-level (NOAEL) was considered to be 0.300 mg/kg/day Drug Substance and Drug Product, the highest dose administered.

Methods

Doses

Number/Sex/Group or Time Point (Main Study)

Satellite Groups Used For Toxicokinetics or Recovery

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study Groups			
1	0 ^a	10	10
2	0.050 ^b	10	10
3	0.150 ^b	10	10
4	0.300 ^b	10	10
5	0 ^c	10	10
6	0.050 ^d	10	10
7	0.150 ^d	10	10
8	0.300 ^d	10	10
Toxicokinetic Groups			
9	0.050 ^b	9 + 1 ^e	9 + 1 ^e
10	0.150 ^b	9 + 1 ^e	9 + 1 ^e
11	0.300 ^b	9 + 1 ^e	9 + 1 ^e
12	0.050 ^d	9 + 1 ^e	9 + 1 ^e
13	0.150 ^d	9 + 1 ^e	9 + 1 ^e
14	0.300 ^d	9 + 1 ^e	9 + 1 ^e
^a Administered vehicle only. ^b Administered NPSP558 Drug Substance ^c Administered NPSP558 Drug Product Placebo ^d Administered NPSP558 Drug Product ^e Additional animals included for use as possible replacements.			

Species/Strain CD® [CrI:CD®(SD)] rats

Route

Subcutaneous bolus injection between the skin and underlying layers of tissue in the scapular and caudal regions on the back of each animal.

Formulation/Vehicle 10 mM citrate buffer with 5% mannitol.

Dosing Solution Analyses/Drug Stability and Homogeneity

The concentrations of the prepared formulations were within the acceptable limits ($\pm 15\%$ of the targeted concentration) with the exception of the 50 $\mu\text{g/mL}$ (0.05 mg/mL) formulations and the 150 $\mu\text{g/mL}$ (0.150 mg/mL) drug substance formulation (Days 1 and 28). Based on the Sponsor's previous experience with this drug, the findings at 50 $\mu\text{g/mL}$ were not unexpected due to the tendency of the drug to adsorb to surfaces at low concentrations. The consistent results between the two low dose groups on Days 1 and 28 and between formulations from the Drug Substance (DS) and Drug Product (DP) suggest this was not due to technical error. This out of specification result did not adversely affect the outcome in the study because this occurred in the low dose groups and the NOAEL was established at the highest dose tested (0.300 mg/kg/day). Similarly, the out of specification result at 150 $\mu\text{g/mL}$ for the drug substance on Days 1 and 28 did not adversely affect the outcome of the study because the difference from acceptable limit was considered by the Study Director to be minimal.

Homogeneity analysis was not conducted from formulations prepared on Day 28 as specified in the protocol. This deviation was not expected to impact the study results since the test article at the highest concentration tested (300 $\mu\text{g/mL}$) in this study was found to be consistent between the top, middle, and bottom aliquots based on a previous evaluation of homogeneity ((b) (4) Study Number 1150-003). In addition, based on the interpretation of the text in the original protocol, the Sponsor's representative submitted a Sample Submission form to test only one rather than two aliquots of dose solution at each concentration level for Day 1. In addition, only one aliquot from the middle of the dose formulation samples at the 0.05, 0.15, and 0.30 mg/mL concentrations for Groups 2 through 4 and Groups 6 through 8 for Day 28 were tested. Stability analysis was not conducted since the formulations were prepared daily and used within 12 hours of formulation.

Concentration of NPSP558 in Dosing Formulations (Days 1 and 28)				
Dose Level (mg/kg/day)	Test Article Identification	Nominal Concentration (mg/mL)	Average Concentration Day 1 and 28 ^a (µg/mL)	Average %Recovery ^{a, b}
0	Control Article	0.0	0.00	NA
0.050	NPSP558(DS)	0.05	29 and 30	59.0
0.150	NPSP558(DS)	0.15	122.37 and 122.48	81.6
0.300	NPSP558(DS)	0.30	253.92 and 262.50	86.1
0	Placebo (DP)	0.00	0	NA
0.050	NPSP558(DP)	0.05	32 and 33	65.0
0.150	NPSP558(DP)	0.15	130.41 and 132.61	87.7
0.300	NPSP558(DP)	0.30	275.15 and 277.22	92.1

^a Results are the range of values determined on Days 1 and 28.
^b Average % recovery was calculated from the nominal concentration.
NA – Not Applicable

Dose Volume/Infusion Rate

0.50, 0.150, and 0.300 mg/kg/day NPSP558 Drug Substance or Drug Product and administered at a subcutaneous bolus injection volume of 1 mL/kg.

Age 8 weeks(+1 day) of age upon arrival, 13 to 17-day acclimation period.

Weight M and F, weighing 292 g to 332 g and 195 g to 246 g, respectively.

Observations Times and Results Twice daily observations.

Mortality

There was no test article-related effect on mortality. All animals survived through study termination.

Clinical Signs

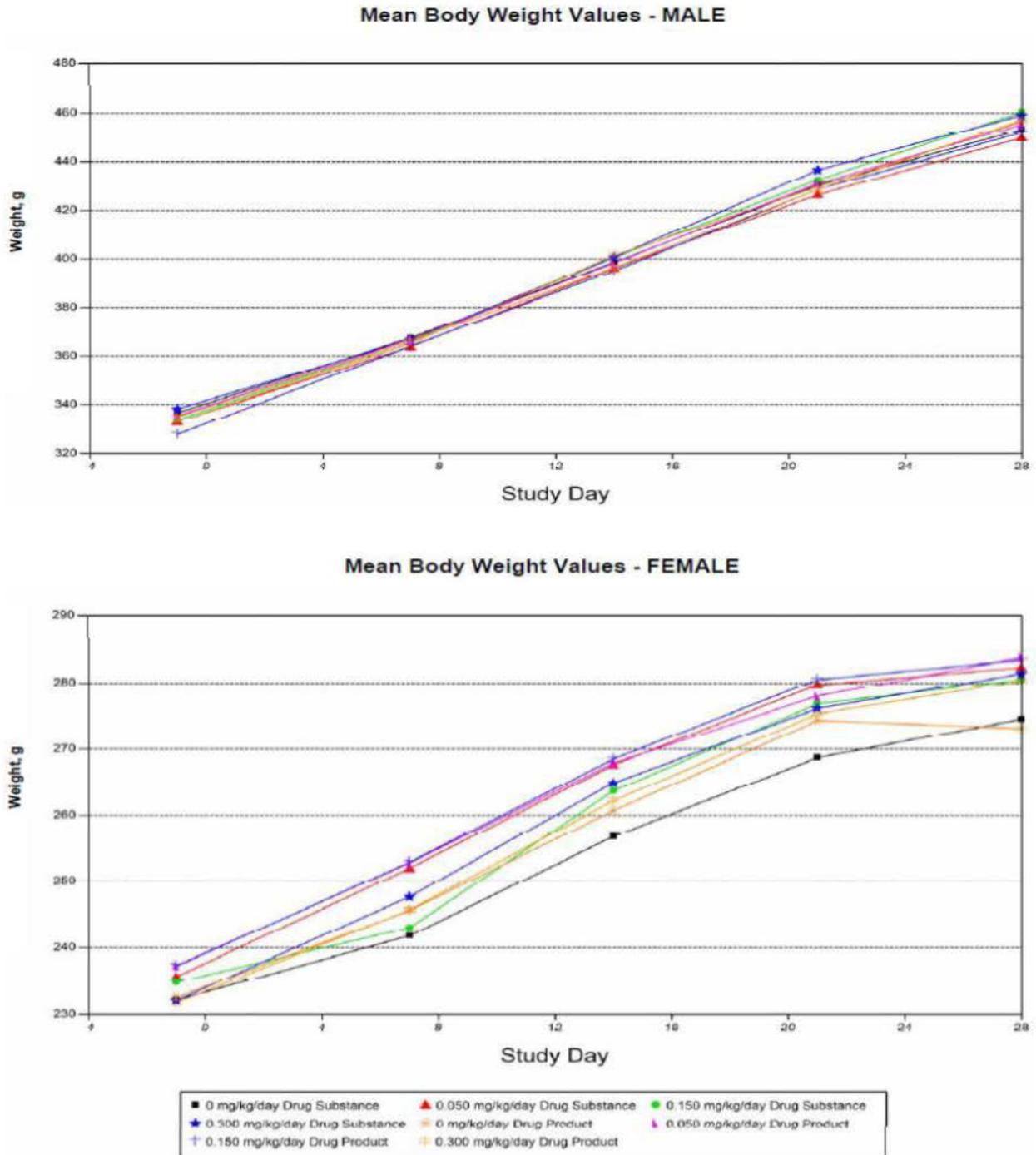
There were no test article-related effects noted during clinical examinations. All recorded observations are typical of rats of this age and strain.

Functional Observational Battery (FOB) evaluations were conducted without knowledge on the part of the testers of the treatment groups. FOB evaluations, including those conducted in the home-cage, during handling, in the open field, and others, were conducted on the first eight main study animals per sex per group prior to initiation of dosing [Day -3 (male animals) and Day -7 (female animals)] and at 1 hour (±15 minutes) postdose on Day 1.

The effects on FOB evaluations in male and female animals were not considered adverse.

Body Weights

There were no test article-related effects on body weight.



Summary of Group Mean Body Weights (At Day 28), g				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Respective Control Group	Mean	(%) Difference from Respective Control Group
0 DS	453.2	NA	274.5	NA
0.050 DS	449.8	(-0.8)	282.3	(+2.8)
0.150 DS	460.4	(+1.6)	280.5	(+2.2)
0.300 DS	459.1	(+1.3)	281.4	(+2.5)
0 DP	456.5	NA	273.1	NA
0.050 DP	455.3	(-0.3)	283.8	(+3.8)
0.150 DP	452.0	(-1.0)	283.4	(+3.8)
0.300 DP	457.1	(+0.1)	280.3	(+2.6)

DS – Drug Substance; DP – Drug Product; NA – Not applicable

Food Consumption

There were no test article-related effects on food consumption.

Average Food Consumption (Weeks 1 through 4); g/animal/day				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Respective Control Group	Mean	(%) Difference from Respective Control Group
0 DS	33.82	NA	24.18	NA
0.050 DS	33.52	(-0.9)	24.62	(+1.8)
0.150 DS	33.97	(+0.4)	23.40	(-3.2)
0.300 DS	33.10	(-2.1)	23.72	(-1.9)
0 DP	33.41	NA	23.41	NA
0.050 DP	31.97	(-4.3)	22.66	(-3.2)
0.150 DP	32.15	(-3.8)	23.91	(+2.1)
0.300 DP	33.13	(-0.8)	23.83	(+1.8)

DS – Drug Substance; DP – Drug Product; NA – Not applicable

Ophthalmoscopy

There were no test article related ophthalmic effects.

Hematology

There were no adverse effects on hematology/coagulation parameters or meaningful differences between the two treatments evaluated.

Clinical Chemistry

There were no adverse effects on clinical chemistry analytes or meaningful differences between the two treatments evaluated.

Urinalysis

There were no adverse effects on urinalysis parameters or meaningful differences between the two treatments evaluated.

Gross Pathology

There were no test article-related macroscopic observations noted in either sex.

Organ Weights

Test article-related weight changes were present in the spleen of male and female animals. Overall spleen weights were statistically significantly increased in all parameters in males and females at 0.150 and 0.300 mg/kg/day Drug Substance and Drug Product dose levels. Increased spleen weights were microscopically correlated to increased extramedullary hematopoiesis.

Histopathology

Test article-related microscopic changes were present in the bone (femur and sternum) and spleen of male and female rats.

Hyperostosis in the femur and sternum was present in males and females at all Drug Substance and Drug Product dose levels. Increased extramedullary hematopoiesis in the spleen was present in males at all Drug Substance and Drug Product dose levels and in females at 0.150 and 0.300 mg/kg/day Drug Substance and Drug Product dose levels. There were no remarkable differences in magnitude or incidence of hyperostosis or increased extramedullary hematopoiesis between the affected Drug Substance and Drug Product dosed groups.

The bone and spleen changes were considered test article-related but not adverse based on the lack of adverse clinical pathology findings. Hyperostosis is an expected pharmacological effect of the test article and extramedullary hematopoiesis is an adaptive response.

(b) (4) Study Number 1150-004
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substance and Drug Product

Summary of Microscopic Observations - MALE Terminal

Tissue Observation	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
bone, femur hyperostosis		(10)	(10)	(10)	(10)
	- minimal	0	6	10	10
	- mild	0	5	0	0
	- moderate	0	1	9	5
within normal limits		0	0	1	5
		10	4	0	0

Tissue Observation	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10
bone, femur hyperostosis		(10)	(10)	(10)	(10)
	- minimal	0	1	10	10
	- mild	0	1	1	0
	- moderate	0	0	9	4
within normal limits		0	0	0	6
		10	9	0	0

Tissue Observation	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
bone, sternum hyperostosis		(10)	(10)	(10)	(10)
	- minimal	0	7	10	10
	- mild	0	7	9	8
	- moderate	0	0	1	2
within normal limits		0	3	0	0
		10	3	0	0

Tissue Observation	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10
bone, sternum hyperostosis		(10)	(10)	(10)	(10)
	- minimal	0	9	10	10
	- mild	0	9	10	5
	- moderate	0	0	0	5
within normal limits		0	1	0	0
		10	1	0	0

Tissue Observation	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
spleen hematopoiesis, extramedullary, increased		(10)	(10)	(10)	(10)
	- minimal	1	6	10	10
	- mild	1	6	9	2
	- moderate	0	0	1	8
within normal limits		0	4	0	0
		9	4	0	0

Tissue Observation	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10
spleen hematopoiesis, extramedullary, increased		(10)	(10)	(10)	(10)
	- minimal	1	3	10	10
	- mild	1	3	9	5
	- moderate	0	0	1	5
within normal limits		0	7	0	0
		9	7	0	0

(b) (4) Study Number 1150-004
 NPSP558: A 4-Week Subcutaneous Toxicity Study in Rats Testing Drug Substance and Drug Product

Summary of Microscopic Observations - FEMALE

Terminal					
Tissue Observation	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
bone, femur hyperostosis		(10)	(10)	(10)	(10)
	- minimal	0	1	10	10
	- mild	0	1	2	1
	- moderate	0	0	8	8
within normal limits		0	0	0	1
		10	9	0	0
bone marrow, sternum within normal limits		(10)	(0)	(0)	(10)
		10	0	0	10

Tissue Observation	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10

bone, femur		(10)	(10)	(10)	(10)
hyperostosis		0	4	10	10
	- minimal	0	4	5	0
	- mild	0	0	5	6
	- moderate	0	0	0	4
within normal limits		10	6	0	0
bone marrow, sternum		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10

Summary of Microscopic Observations - FEMALE

Terminal

Tissue	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
bone, sternum		(10)	(10)	(10)	(10)
hyperostosis		0	5	10	7
	- minimal	0	5	10	7
	- mild	0	0	0	0
within normal limits		10	5	0	3

Tissue	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10
bone, sternum		(10)	(10)	(10)	(10)
hyperostosis		0	6	10	10
	- minimal	0	6	9	10
	- mild	0	0	1	0
within normal limits		10	4	0	0

Tissue	Severity	0 mg/kg/day Drug Substance	0.050 mg/kg/day Drug Substance	0.150 mg/kg/day Drug Substance	0.300 mg/kg/day Drug Substance
Number of Animals Examined		10	10	10	10
spleen		(10)	(10)	(10)	(10)
hematopoiesis, extramedullary, increased		0	0	6	10
	- minimal	0	0	6	7
	- mild	0	0	0	3
within normal limits		10	10	4	0

Tissue	Severity	0 mg/kg/day Drug Product	0.050 mg/kg/day Drug Product	0.150 mg/kg/day Drug Product	0.300 mg/kg/day Drug Product
Number of Animals Examined		10	10	10	10
spleen		(10)	(10)	(10)	(10)
hematopoiesis, extramedullary, increased		0	0	4	10
	- minimal	0	0	4	9
	- mild	0	0	0	1
within normal limits		10	10	6	0

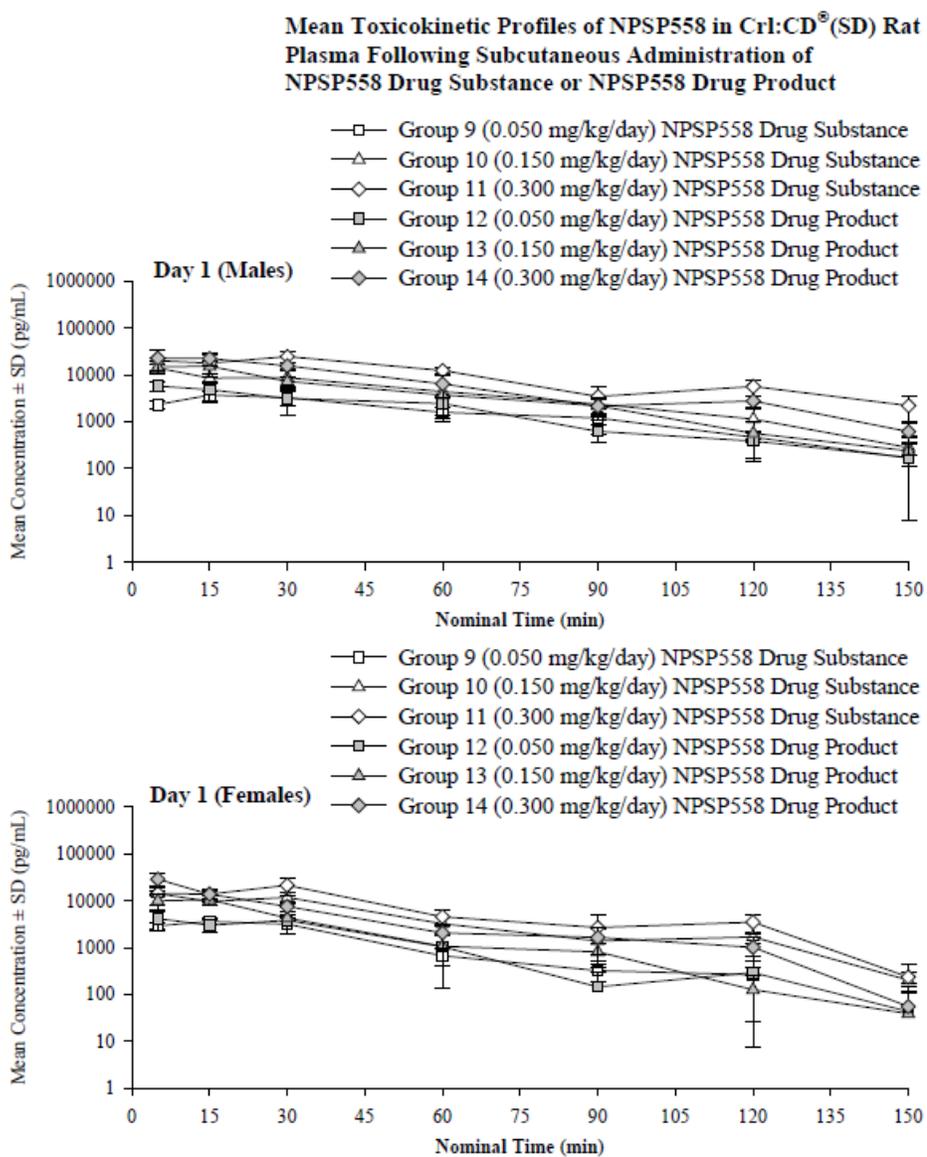
Adequate Battery Yes

Peer Review No

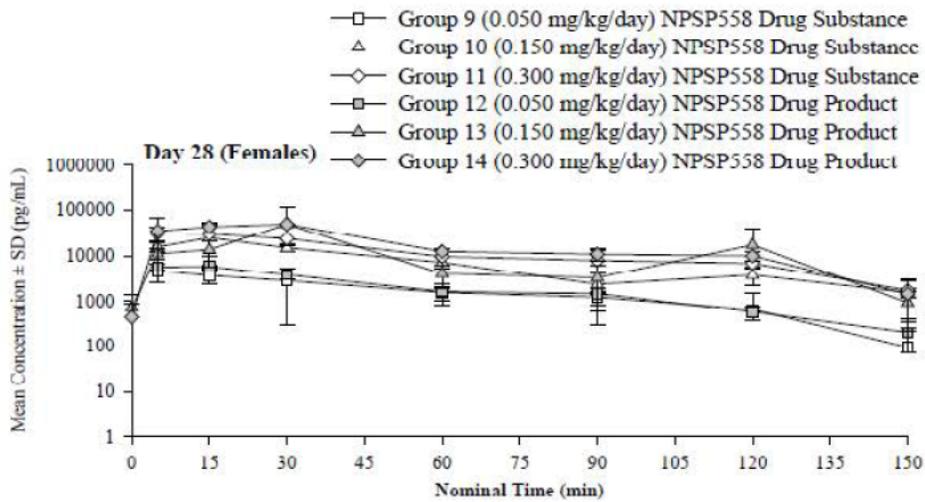
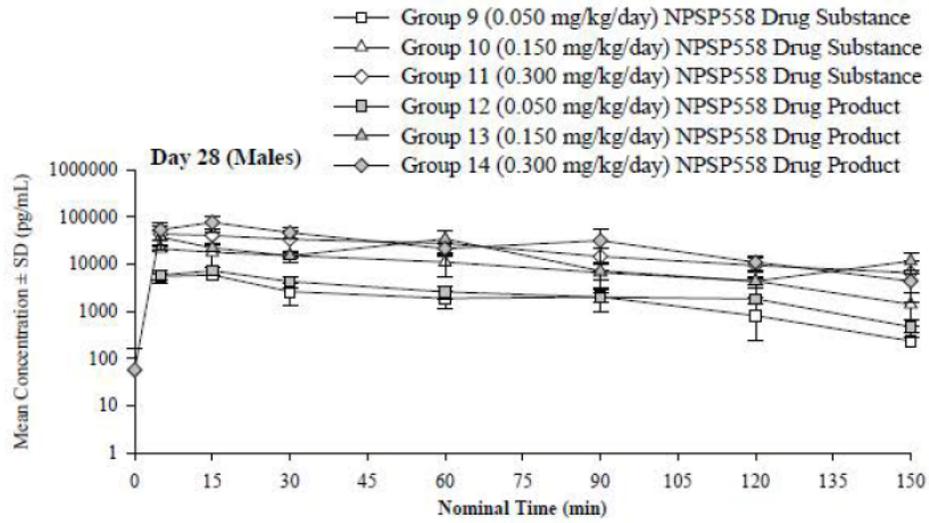
Toxicokinetics

Peak NPSP558 plasma concentrations were observed between 5 and 30 min postdose followed by a monoexponential decline with T_{1/2} ranging from 19.4 to 52.4 min for Drug Substance and 17.5 to 41.3 min for Drug Product on Days 1 and 28.

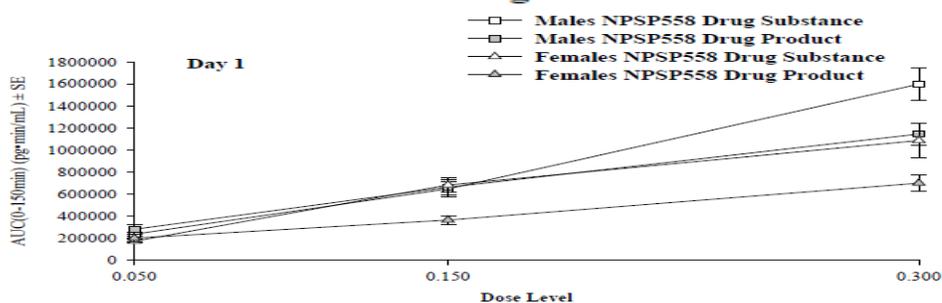
NPSP558 exposure from both Drug Substance and Drug Product ranged from 171884 to 3236328 pg•min/mL and 199529 to 4392017 pg•min/mL, respectively, on Days 1 and 28. NPSP558 systemic exposure appeared to increase in an approximately dose-proportional manner between 0.050 and 0.300 mg/kg/day. Male animals NPSP558 systemic exposure were generally higher than female animals and the exposure to NPSP558 increased by as much as 5.77-fold after 28 days of daily administration with Drug Product exhibiting higher accumulation ratios than Drug Substance.



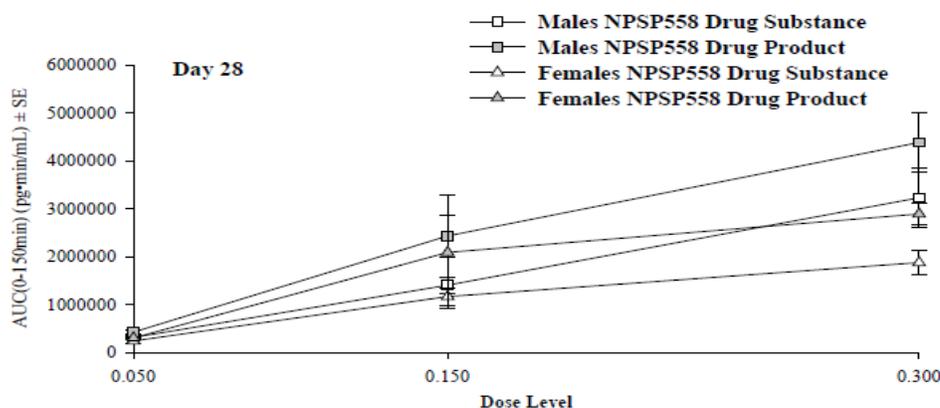
Mean Toxicokinetic Profiles of NPSP558 in CrI:CD[®](SD) Rat Plasma Following Subcutaneous Administration of NPSP558 Drug Substance or NPSP558 Drug Product



Comparison of NPSP558 Exposure in Crl:CD[®](SD) Rat Plasma Following Subcutaneous Administration of NPSP558 Drug Substance or NPSP558 Drug Product



NPSP558 Drug Substance Group 9: 0.050 mg/kg/day, Group 10: 0.150 mg/kg/day, Group 11: 0.300 mg/kg/day
 NPSP558 Drug Product Group 12: 0.050 mg/kg/day, Group 13: 0.150 mg/kg/day, Group 14: 0.300 mg/kg/day



NPSP558 Drug Substance Group 9: 0.050 mg/kg/day, Group 10: 0.150 mg/kg/day, Group 11: 0.300 mg/kg/day
 NPSP558 Drug Product Group 12: 0.050 mg/kg/day, Group 13: 0.150 mg/kg/day, Group 14: 0.300 mg/kg/day

7. Genetic Toxicology

PTH is not genotoxic in any of the following test systems: Bacterial reverse mutation (Ames) assay or the in vitro mammalian cell forward-gene mutation (AS52/XPRT) assay study with and without metabolic activation.

8. Carcinogenicity

The following summary is excerpted from Pharm/Tox Review, R. Wange, Serial No. 000, 02/03/2006.

In a 2-year carcinogenicity study in Fischer 344 rats, male and female rats were given daily subcutaneous Natapara injections of 10, 50 or 150 mcg/kg/day from 9-11 weeks of age. These doses resulted in systemic exposures that were, respectively, 4, 20 and 50 times higher than systemic exposure observed in humans following a subcutaneous dose of 100 mcg/day (based on AUC comparisons). PREOS resulted in a marked dose related increase in the incidence of osteosarcoma, rare, malignant bone tumors in male and female rats. Osteosarcomas were observed at doses ≥ 50 mcg/kg/day. The incidence reached 8-45% in the higher dose groups. Other bone neoplasms including osteoblastoma and osteoma were also seen in these dose groups. PREOS given at 10 mcg/kg/day did not show an increased incidence of osteosarcomas compared to control incidence in the 2-year

carcinogenicity study in rats. This dose results in systemic exposure to PTH that is 4 times higher than systemic exposure observed in humans following a subcutaneous dose of 100 mcg/day (based on AUC comparison). The clinical relevance of this finding is unknown in that no species' specific mechanism has provided an explanation.

Table 1 -- Incidence (Animals Affected) of Bone Neoplasms in Male F344 Rats: Comparison of ALX1-11 to Teriparatide

	ALX1-11					Teriparatide			
	C1	C2	LD	MD	HD1*	C	LD	MD	HD
Number examined	60	60	60	60	60	60	60	60	60
Dose Group	C1	C2	LD	MD	HD1*	C	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	0	10	50	150	0	5	30	75
Exposure Ratio ($\text{AUC}_{\text{rat}}/\text{AUC}_{\text{human}}$)	-	-	3	19	51	-	3	21	58
Osteoma (n)	0	0	0	1	2	0	0	2	1
(%)	0	0	0	1.67	3.33	0	0	3.33	1.67
Osteoblastoma (n)	0	0	0	2	4	0	0	2	7
(%)	0	0	0	3.33	6.67	0	0	3.33	11.67
Osteosarcoma (n)	0	0	1	13	27	0	3	21	31
(%)	0	0	1.67	21.67	45.00	0	5.00	35.00	51.67
All Bone Neoplasms (n)	1	0	2	17	28	0	3	24	36
(%)	1.67	0	3.33	28.33	46.67	0	5.00	40.00	60.00

*Male HD1 only dosed for 94 weeks and necropsied after 101 weeks.

11 Integrated summary and safety evaluation

The following summary includes excerpts from Pharm/Tox Review, R. Wange, Serial No. 000, 02/03/2006.

The toxicologic profile of ALX1-11 associated with chronic sc dosing has been assessed in the rat and monkey animal model systems. In both models the primary toxicological targets were the kidney and bone marrow, and the toxic responses likely arose out of exaggerated pharmacodynamics. Post-dose transient hypercalcemia was apparent out to 3-6 hours in the monkey at all tested doses, with excursions as high as 1.6 mg/dL (~17%) at 10 mcg/kg/day (~2x the MRHD, based on AUC ratios). Transient serum calcium excursions were not assessed in the rat in the chronic sc dosing study, but can be inferred from the dose-dependent calciuria that was observed. At toxic doses of ALX1-11, repetitive kidney exposures to high serum calcium levels resulted in the formation of renal calculi, mineralization damage to the renal tubules and occasionally the parenchyma. This was associated with increases in serum alkaline phosphatase and BUN. At the highest dose tested in the rat, 1000 mcg/kg/day (~100x the MRHD, based upon a mg/m^2 comparison), 30% of the male rats died or were sacrificed moribund. The cause of death/morbidity was determined to be severe kidney damage. All had moderate to severe renal tubular mineralization, and most had metastatic calcification of the major vessels, heart and/or stomach. The other consistent toxicological finding with ALX1-11 was a

dose-dependent reduction in the level of blood cells (all types) that occurred as a consequence of an exaggerated bone-anabolic effect, which lead to osteosclerosis and occlusion of the marrow space, causing a reduction in blood cell precursors.

A rat fertility study and both rat and rabbit embryo-fetal developmental toxicity studies found no significant effects outside of the laboratory's range of the historical control variation for measures of fertility, early embryonic development and development of the embryo and fetus following exposure of the pregnant dam from implantation to closure of the hard palate. In a pre- and post-natal development study, pregnant rats were administered Natpara during organogenesis until weaning without any significant effects on the F1 generation, but there were test article-related mortalities in the F1 generation male and female rats. A total of seven early deaths (found dead or euthanized) occurred during the postweaning period (1 and 4 males in the 100 and 1000 mcg/kg/day maternal dosage group, respectively, and 1 female in each of the 100 and 1000 mcg/kg/day maternal dosage group).

All nonclinical test species developed antibodies to ALX1-11. However, only a small percentage of test animals developed detectable levels of antibody, and these were generally not neutralizing. Being a native human protein, ALX1-11 is not expected to be highly immunogenic in humans, and animal immunoreactivity is not necessarily predictive.

In a 2-year rat carcinogenicity study, daily sc dosing of F344 rats with doses of ALX1-11 > 50 mcg/kg/day were associated with significant increases in the incidence of bone neoplasms, especially osteosarcoma. The incidence of osteosarcoma in males was 22% at 50 mcg/kg/day (~20x the MRHD, based on AUC ratios) and 45% at 150 mcg/kg/day (~50x the MRHD, based on AUC ratios), and somewhat lower in females at 8.3% and 22%, respectively. There was no increase in the incidence of osteosarcoma or other bone neoplasms at a dose of 10 mcg/kg/day (~4x the MRHD, based on AUC ratios). These findings are qualitatively similar to what was seen in the initial 2-year carcinogenicity study conducted by Eli Lilly & Company to support the marketing of teriparatide. The clinical significance of this class effect of PTH bone anabolic agents in rats remains unclear, as does the significance of the Sponsor's analysis showing a lower incidence of osteosarcoma in ALX1-11-treated F344 rats, as compared to teriparatide-treated F344 rats when the results are normalized on the basis of human AUC ratios. Since it is likely that the molecular events that are associated with the bone anabolic effects of PTH peptides are the same that, when exaggerated, cause osteoblast neoplasia, the differences in the outcomes of the carcinogenicity studies of ALX1-11 and teriparatide may be explainable by the drug-related increase in bone formation rate, there is little difference between the two peptides.

Safety margins for major toxicity findings

PTH Margins of Safety Based on Exposure in Pivotal Toxicology Studies					
Study Type	Study Number (Species)	Observation	PTH Dose Level($\mu\text{g}/\text{kg}$)	Mean AUC(0-24) ^a (ng.hr/mL)	Margin of Safety ^b
Chronic Toxicity					
	PH 460-ALX-001	Renal histopathology	300	71.5	77 ^c
	93(Rat)	Hematology/Clinical Chemistry			
		NOAEL	50	8.7	9.4
	CTBR	NOAEL (Sponsor's)	30 ^d	15.5	16.8
	(Monkey)	NOAEL (CDER)	2	0.4	0.4 ^e
Carcinogenicity					
	CTBR 89712	Osteosarcoma	50	21.5	23 ^c
	(Rat)				
		NOAEL	10	3.7	4

^aMale and female combined on last day of sampling for a given study. ^bAnimal AUC/human AUC (human AUC = 0.924 ng-hr/mL, from the Phase III fracture study, TOP). ^cLowest Observed Adverse Effect Level (LOAEL). ^dHighest dose tested. ^eThis safety margin is not considered to be clinically dose limiting, since the minimal-slight changes in kidney histopathology associated with higher doses were a consequence of longstanding untreated hypercalcemia, which would be clinically monitorable and treatable.

Suggested Labeling:

(b) (4)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ROBERT W MAHER
07/31/2014

KAREN L DAVIS BRUNO
07/31/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA 125511

BLA Number: 125511
(IND 076514)

Applicant: NPS Pharmaceuticals, Stamp Date: October 24, 2013
Inc.

Drug Name: Natpara®
(rhPTH[1-84]) for Injection

BLA Type: 1

On initial overview of the BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		The nonclinical development program addressing the toxicity of rhPTH(1-84) is comprised of studies including single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, antigenicity, local tolerance, and special studies.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Formulations of rhPTH(1-84) used in the toxicology studies contained varying concentrations in buffer (5% mannitol in 10 mM citrate buffer, pH 5.5 or 6.0), ^{(b) (4)} 
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		The disposable medication cartridge is designed for use with a reusable Natpara Mixing Device for product reconstitution and a reusable Natpara Q-Cliq™ pen injector for subcutaneous (SC) drug delivery. As once-daily SC administration is the intended regimen for humans, this was the predominant regimen used in most nonclinical testing. Intravenous administration was also investigated in rats to gain an understanding of the systemic pharmacokinetics of rhPTH(1-84) and to

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
BLA 125511**

	Content Parameter	Yes	No	Comment
				establish absolute bioavailability following SC administration.
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		All of the toxicology studies performed on rhPTH(1-84) have been conducted in compliance with United States (US) Food and Drug Administration (FDA) Good Laboratory Practices (GLPs), except for the 21-day rat study and the antigenicity studies in rats, dogs and monkeys. The toxicology studies followed standard, currently accepted study designs with respect to group sizes and parameters evaluated, were conducted in accordance with the study protocols and laboratory standard operating procedures, and met the requirements of the International Conference on Harmonisation (ICH) guidelines for the conduct of these studies, with one exception . The chronic monkey study included smaller group sizes for the control and high-dose groups at study termination than are currently employed (ie, 3 animals/sex/group versus 4 animals/sex/group).
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	X		Refer to Section 1.14.1 Draft Labeling.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurity Study Reports 1150-003 & 1150-004 in Section 4.2.3.7.6 Impurities.
11	Has the applicant addressed any abuse potential issues in the submission?	X		Applicant states that rhPTH(1-84) is not a controlled substance, and there is no abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
BLA 125511**

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION
FILEABLE? Yes**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. **BLA is fileable.**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **There are no potential review issues at this time.**

Reviewing Pharmacologist Robert W. Maher, PhD, DABT	Date
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Acting Team Leader/Supervisor Jessica Hawes, PhD	Date
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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ROBERT W MAHER
12/23/2013

JESSICA J HAWES
12/23/2013